

Potassium Channel Types in Arterial Chemoreceptor Cells and Their Selective Modulation by Oxygen

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ABSTRACT Single K^+ channel currents were recorded in excised membrane patches from dispersed chemoreceptor cells of the rabbit carotid body under conditions that abolish current flow through Na^+ and Ca^{2+} channels. We have found three classes of voltage-gated K^+ channels that differ in their single-channel conductance (γ), dependence on internal Ca^{2+} (Ca_i^{2+}), and sensitivity to changes in O_2 tension (PO_2). Ca^{2+} -activated K^+ channels (K_{Ca} channels) with $\gamma \sim 210$ pS in symmetrical K^+ solutions were observed when $[Ca^{2+}]_i$ was $>0.1 \mu M$. Small conductance channels with $\gamma = 16$ pS were not affected by $[Ca^{2+}]_i$ and they exhibited slow activation and inactivation time courses. In these two channel types open probability (P_{open}) was unaffected when exposed to normoxic ($PO_2 = 140$ mmHg) or hypoxic ($PO_2 \approx 5-10$ mmHg) external solutions. A third channel type (referred to as K_{O_2} channel), having an intermediate γ (~ 40 pS), was the most frequently recorded. K_{O_2} channels are steeply voltage dependent and not affected by $[Ca^{2+}]_i$; they inactivate almost completely in <500 ms, and their P_{open} reversibly decreases upon exposure to low PO_2 . The effect of low PO_2 is voltage dependent, being more pronounced at moderately depolarized voltages. At 0 mV, for example, P_{open} diminishes to $\sim 40\%$ of the control value. The time course of ensemble current averages of K_{O_2} channels is remarkably similar to that of the O_2 -sensitive K^+ current. In addition, ensemble average and macroscopic K^+ currents are affected similarly by low PO_2 . These observations strongly suggest that K_{O_2} channels are the main contributors to the macroscopic K^+ current of glomus cells. The reversible inhibition of K_{O_2} channel activity by low PO_2 does not desensitize and is not related to the presence of F^- , ATP, and GTP- γ -S at the internal face of the membrane. These results indicate that K_{O_2} channels confer upon glomus cells their unique chemoreceptor properties and that the O_2 - K^+ channel interaction occurs either directly or through an O_2 sensor intrinsic to the plasma membrane closely associated with the channel molecule.

INTRODUCTION

Although it has been known for decades that the mammalian carotid bodies participate in the regulation of breathing by adjusting the ventilatory rate to the level

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of oxygen tension (PO_2) in arterial blood (De Castro, 1926; Heymans, Bouckaert, and Dautrebande, 1930; Fidone and González, 1986; Fitzgerald and Lahiri, 1986) the mechanisms involved in the process of O_2 sensing have remained unknown. There has been a general consensus that type I (or glomus) cells, the most numerous in the carotid body, are the elements responsible for chemotransduction since they make synapses with afferent nerve endings, have cytosolic granules containing catecholamines, and secrete dopamine in response to hypoxia and high external K^+ (Fidone, González, and Yoshizaki, 1982; Fishman, Greene, and Platika, 1985; Almaraz, González, and Obeso, 1986; Rigual, González, González, and Fidone, 1986; Obeso, Fidone, and González, 1987). Direct proof for the chemoreceptive properties of type I cells has come, however, from recent electrophysiological experiments. It has been shown that type I cells can fire action potentials repetitively and, as in other electrically excitable cells, they generate voltage-dependent Na^+ , Ca^{2+} , and K^+ currents (Duchen, Caddy, Kirby, Patterson, Ponte, and Biscoe, 1988; López-Barneo, López-López, Ureña, and González, 1988; Ureña, López-López, González, and López-Barneo, 1989a). Furthermore, it has also been found that the voltage-gated K^+ current of type I cells is reversibly attenuated by lowering environmental PO_2 , whereas Na^+ and Ca^{2+} currents remain unaltered (López-Barneo et al., 1988). These findings, confirmed by the parallel work of other investigators on several mammalian species (Delpiano and Hescheler, 1989; Hescheler, Delpiano, Acker, and Pietruschka, 1989; Peers, 1990; Stea and Nurse, 1991), have provided a framework for understanding the basic mechanisms underlying sensory transduction in the carotid body. Inhibition of the O_2 -sensitive K^+ current under hypoxic conditions produces an increase in the firing frequency of glomus cells (López-López, González, Ureña, and López-Barneo, 1989), which could lead to Ca^{2+} influx, enhanced transmitter release, and activation of the afferent fibers of the sinus nerve. This basic scheme is also supported by work on type I cells loaded with fluorescent Ca^{2+} indicators showing an increase in cytosolic Ca^{2+} in response to low PO_2 (Biscoe and Duchen, 1990a; Benot, A., J. Ureña, and J. López-Barneo, unpublished observations).

The macroscopic K^+ current recorded in type I cells has a small Ca^{2+} -dependent component that disappears after wash-out of Ca^{2+} channels and when internal solutions with high Ca^{2+} -buffering capacity are used. In addition, the Ca^{2+} -independent component of the current inactivates almost entirely in 200 ms but the degree of inactivation varies among different cells (Ureña et al., 1989a). Therefore, it can be expected that, as in other excitable cells (Marty and Neher, 1985; Hoshi and Aldrich, 1988), glomus cells possess several classes of K^+ channels with specific biophysical properties (for review, see Rudy, 1988). The present research was undertaken to establish a first classification of K^+ channels in type I cells and to ascertain whether a specific K^+ channel class is responsible for the O_2 sensitivity of the cells' electrical properties. These questions are of critical importance for elucidating the molecular mechanisms underlying O_2 sensing. The identification of the primary site involved in O_2 detection is also of interest because it has been argued that the attenuation of the macroscopic K^+ current on lowering PO_2 could be a secondary phenomenon rather than an initial step in the process of chemotransduction (Biscoe and Duchen, 1989, 1990a, b).

In this paper we present a systematic analysis of the single K^+ channel types found in glomus cells. We show that in excised membrane patches there are three different

K⁺ channel types and that only one of them (referred to as "K_{O₂} channel") is selectively and reversibly modulated by hypoxia. The characteristics of these channels fully account for the properties of the macroscopic O₂-sensitive K⁺ current. Our experimental results indicate that K_{O₂} channels are directly regulated by O₂ and strongly suggest that the O₂ sensor of chemoreceptor cells is in, or closely associated with, the plasma membrane. In the following article we focus on a more detailed description of the kinetic properties of the K_{O₂} channel and propose a minimal model that explains the effects of hypoxia on channel gating.

A brief report of part of the work presented in this paper has been published (Ganformina and López-Barneo, 1991).

METHODS

Cell Preparation

Experiments were performed on type I cells isolated from rabbit carotid bodies. The procedures followed for enzymatic cell dispersion and culture were the same as described

TABLE I
Composition of Solutions

	External					
	NaCl	KCl	CaCl ₂	MgCl ₂	HEPES	Glucose
Standard Na	140	2.7	2.5–5	2	10	5
140 K	—	140	2.5–5	2	10	5
80 K	60	80	2.5–5	2	10	5
	Internal					
	KCl	K-glutamate	KF	HEPES	EGTA	MgCl ₂
Standard K	30	80	20	10	10	2.58
130 K	130	—	—	10	10	2.58

All values are given in millimolar. Tetrodotoxin at a concentration of 0.6 μM was added to the standard Na and 80 K solutions. Solutions with variable [Ca²⁺] were made by mixing CaCl₂, MgCl₂, and EGTA. The final free [Ca²⁺] was calculated with a computer program that takes into account the affinity constants of EGTA for Ca²⁺ and Mg²⁺ at different pH (Tabares, Ureña, and López-Barneo, 1989). In those solutions with 10 mM EGTA and no Ca²⁺ added the estimated [Ca²⁺] was <10⁻⁹ M.

previously (López-Barneo et al., 1988; Ureña et al., 1989a). Cells were plated on slivers of glass coverslips treated with poly-*l*-lysine and used for recording between 12 h and 2 d after dissociation. During the experiments a coverslip was transferred to a small chamber of ~0.2 ml volume with continuous flow of solutions that could be replaced in 10–15 s.

Solutions

The composition of solutions used in the experiments is shown in Table I. Solutions were adjusted to a pH of 7.3 (internal) or between 7.35 and 7.4 (external) and had an osmolality of 290–300 mosmol/kg. In the text and in the figure legends solutions are given as external//internal with specification of the final [Ca²⁺] or [EGTA] used. Experiments were performed at

room temperature (22–25°C). During the experiments the external solutions were equilibrated with either air, N₂, or a mixture of both, in order to obtain the desired PO₂ in the recording chamber (see below).

Recording Techniques

The data presented in this article are mainly based on single K⁺ channel currents recorded from membrane patches of glomus cells using the patch clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981). In some experiments whole-cell K⁺ currents were studied before establishing the outside-out patch configuration following the methodology previously reported (Ureña et al., 1989a). For single-channel recording we used fire-polished glass pipettes fabricated from borosilicate glass (Kimax 51) that once filled with solution had a resistance of 4–10 MΩ. The recording bandwidth of the amplifier was 10 kHz, however its output signal was low-pass filtered by an 8-pole Bessel filter (model 902; Frequency Devices Inc., Haverhill, MA) with cutoff frequencies between 1 and 2 kHz, giving an effective cutoff frequency of 0.95–1.29 kHz (Colquhoun and Sigworth, 1983). The time resolution of our recording system and its influence on the measurement of the amplitude and duration of single-channel events is further explained in the accompanying paper.

Data Acquisition and Analysis

An IBM-PC-AT computer interfaced to the analog electronics was used for data acquisition, display, and analysis. In most experiments we recorded ionic currents in response to voltage steps. In these cases the current signal was digitized on-line by an input-output interface built in our laboratory (Ureña, Mateos, and López-Barneo, 1989b). A sweep was defined by either 500 or 1,000 digital points. Single-channel currents generated in response to long-lasting (>220 ms) or stationary depolarizations were initially stored on video tape. The segments of the signal required for figures or analysis were replayed on a chart recorder or converted into digital form using a GPIB-PC card plugged into the computer expansion slots. The sample frequency varied according to the experimental protocol and is given in the figure legends. Leakage and uncompensated capacity currents were digitally subtracted using scaled templates constructed by fitting smooth functions to either records with no openings or to the average of 20 consecutive current sweeps generated by 20-mV hyperpolarizations from a holding potential of –80 mV. Ensemble averages were obtained from original traces after capacity and leakage subtraction.

Single-channel current amplitude was measured by averaging values obtained from 20–40 well-resolved single events. We used a 50% amplitude criterion to detect opening and closing transitions (Colquhoun and Sigworth, 1983). Unless otherwise noted, no correction for unresolved events was performed. The number of active channels in a patch (N) was determined by observing for long time periods the maximum number of simultaneous current steps that appeared at strongly depolarized voltages. In patches where one or two simultaneous openings were observed, the probability that this number could be smaller than the actual value of N was statistically tested by the binomial distribution method as indicated by Patlak and Horn (1982). In those recordings where the estimated value of N was one, average channel open probability (P_{open}) was calculated by dividing the time spent in the open state by the total duration of the recording. In patches with more than one channel, P_{open} was estimated by the formula:

$$P_{\text{open}} = (N \cdot i \cdot T)^{-1} \cdot \int_0^T I(t) \cdot dt \quad (1)$$

where i is the single-channel current amplitude, T is the duration of the pulse or of the observation period in stationary conditions, and $I(t)$ is the net current during the recording

period. In channels activated during depolarizing pulses, we obtained an ensemble current average ($I(a)$) given by:

$$I(a) = N \cdot i \cdot P_o(t) \quad (2)$$

where $P_o(t)$ is the open probability as a function of time. Significance of differences between mean values obtained (e.g., for different PO_2) was determined with a Student's t test for paired samples. Unless otherwise indicated, the level of significance (α) of the test was set at 0.05.

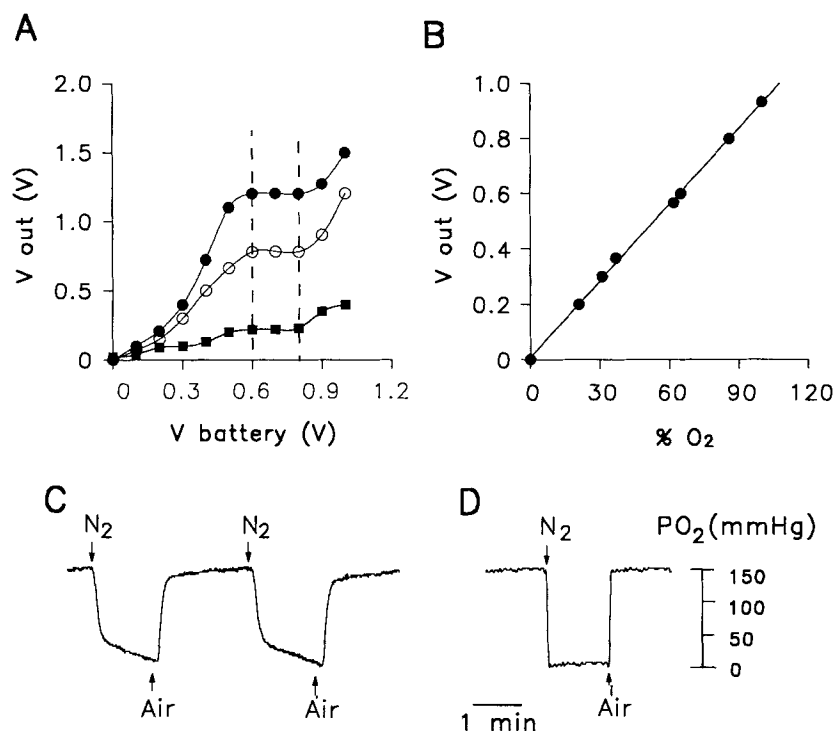


FIGURE 1. Performance and calibration of the O₂-sensing electrode. (A) Output voltage of the current-to-voltage converter as a function of the negative polarizing potential and O₂ tension. 150 mmHg (filled circles), 87 mmHg (open circles), and 0–5 mmHg (filled squares). At a polarizing voltage between -0.6 and -0.8 V the output voltage is linearly related to the PO_2 in the solution (range between dashed lines). (B) Output voltage vs. %O₂ relative to air at a polarizing voltage of -0.7 V. (C and D) Responses of the O₂ electrode in the chamber (C) and during instantaneous immersions in solutions equilibrated with N₂ and air (D).

Measurement of Oxygen Tension

Because some experimental protocols required repetitive exposure of a membrane patch to external solutions with a reproducible PO_2 value, we built an O₂-measuring electrode to estimate PO_2 values in the vicinity of the current-recording pipette. We used a negatively polarized 100- μ m-thick platinum wire insulated by an O₂-impermeant enamel except at the end section (Tsacopoulos and Lehmenkühler, 1977; Tsacopoulos, Poitry, and Borsellino, 1981). Current generated in the wire in response to variable PO_2 values was recorded by an

operational amplifier wired as an I/V converter. The negative pole of a d.c. battery was connected to the noninverting input of the I/V converter to maintain the polarizing voltage at a constant value. The major characteristics as well as the performance of the O_2 -measuring electrode are illustrated in Fig. 1. Plot *A* shows the changes in the output voltage of the I/V converter (V_{out}) as a function of the negative potential applied to the platinum electrode ($V_{battery}$) for three different PO_2 values. In the $V_{battery}$ range between -0.6 and -0.8 V, V_{out} was proportional to the O_2 concentration; thus we used -0.7 V as the most appropriate value to polarize the platinum electrode. At this polarizing voltage, which was used in all the experiments, the output voltage of the recording electrode is linearly related to the O_2 concentration and therefore a calibration curve could be done using solutions equilibrated with known concentrations of O_2 (Fig. 1 *B*). During the experimental protocol V_{out} was continuously monitored and stored on tape. The response of the electrode is illustrated in Fig. 1, *C* and *D*. When immersed in solutions equilibrated with N_2 or air, the change in voltage was almost instantaneous (*D*), switching to similar solutions when placed in the experimental chamber produced a fast change ($\sim 80\%$ of the maximum) in a few seconds but complete equilibration required >40 s (*C*). The voltage signal from the O_2 -sensing electrode was unaffected by changes in pH or by modifications in the ionic composition of the solutions. Most of the experiments reported here and in the accompanying report were based on repetitive exposure of the K^+ channels to a reproducible PO_2 value. Given that our recording chamber is in contact with the air, we found that the most easily reproducible PO_2 level in the vicinity of the cells ($PO_2 = \sim 5\text{--}10$ mmHg) was obtained by bubbling the test solution with N_2 .

RESULTS

There Are Three Major K^+ Channel Types in Glomus Cells

Single K^+ channel currents recorded from membrane patches with well-resolved single-channel events allowed the classification of the K^+ channels of type I cells into three major classes. To facilitate comparison, single-channel currents representative of the various K^+ channel types are shown in isolation in Fig. 2. Table II summarizes the major properties of each channel population. The traces of Fig. 2 are from three different inside-out patches with two functional channels. In all cases the membrane was exposed to asymmetrical K^+ solutions and the current was recorded at various membrane potentials (V_m). The three sets of recordings display openings and closures of the channels that appear as current steps of fixed amplitude. Fast transitions are partially filtered due to the limited recording bandwidth. Opening of each channel type produced current steps of clearly different amplitude, but in all cases channel opening was favored by membrane depolarization. It will be shown below that besides distinct single-channel conductance values the three channel populations also differ in their kinetic properties, Ca^{2+} dependence, and sensitivity to changes in PO_2 (see Table II). For the sake of clarity abbreviations are used for each channel type. K_{Ca} denotes the large Ca^{2+} -activated K^+ channels, SK are channels of small conductance, and K_{O_2} refers to the O_2 -sensitive K^+ channels.

Ca^{2+} -dependent K^+ Channels (K_{Ca} Channels)

K_{Ca} channel activity was clearly observed in inside-out membrane patches (a total of 24 patches) when $[Ca^{2+}]$ in the solution facing the cytosolic side of the membrane was >0.1 μM . These channels were active during maintained depolarizations and

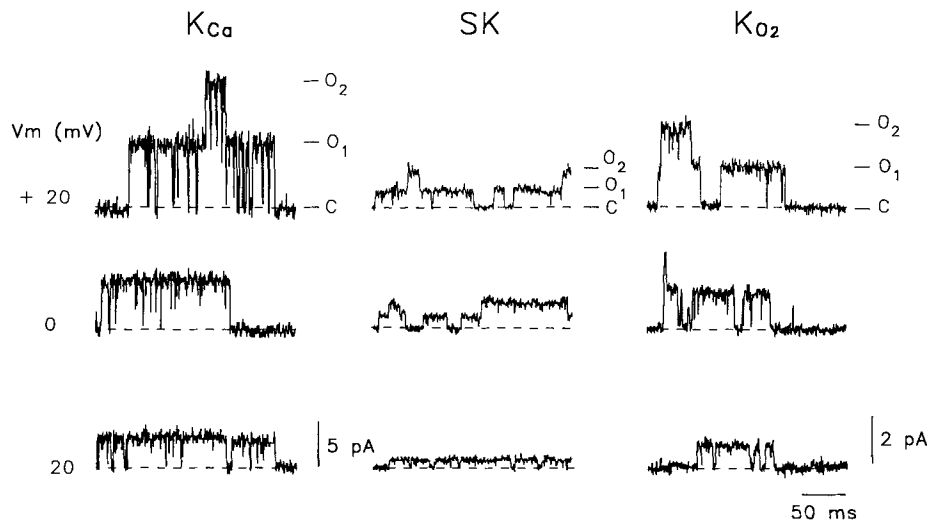


FIGURE 2. Representative recordings of the activity of the three major K^+ channel types found in type I cells. The data were obtained from different inside-out excised patches, containing two channels each, depolarized at the indicated membrane potentials. K_{Ca} = Ca^{2+} -activated channels; SK = small conductance channels; K_{O_2} = O_2 -sensitive channels. In all figures upward deflections from the zero current level (c) indicate outward current. K_{Ca} and SK channels were recorded under steady depolarizations and K_{O_2} channels upon 200-ms step depolarizations from -80 mV. Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μ s. Solutions: standard Na, TTX//130 K, 10 EGTA. K_{Ca} channels were recorded with an internal solution containing 1 μ M free Ca^{2+} .

therefore their single-channel current-voltage ($i-V_m$) relation and P_{open} were studied after steady-state changes in the membrane potential. Single K_{Ca} channel activity as a function of the membrane potential is illustrated in Fig. 3A. The traces are from a patch bathed in symmetrical K^+ solutions that contained at least three active channels. Single-channel current amplitude varied in parallel with the electrochemical driving force for K^+ ions, and the number of active channels as well as the time

TABLE II
Classification of K^+ Channels in Glomus Cells

Channel type	Conductance in 130 K//130 K		Conductance in 2.7 K//130 K		Ca dependence	O_2 sensitivity
	Inside-out	Outside-out	Inside-out	Outside-out		
K_{Ca}	206.7 (10)	—	83.7 (3)	—	Yes	No
SK	16 (6)	—	6 (2)	—	No	No
K_{O_2}	41.5 (1)	41.5 (2)	17.6 (18)	20.1 (14)	No	Yes
			21 (10)*	19.5 (5)*		

Average slope conductance values are given in picosiemens. The number of patches is in parentheses. K_{Ca} = Ca-activated channel; SK = small conductance channel; K_{O_2} = oxygen-sensitive channel. For the K_{O_2} channel values are given in control and hypoxic (*) conditions. Potassium concentrations are in millimolar and indicated as external/internal.

spent in the open state increased with depolarization. The average i - V_m relations obtained from data pooled from several patches exposed to symmetrical and asymmetrical K^+ concentrations ($[K^+]$) are plotted in Fig. 3 *B*. In symmetrical $[K^+]$ (filled symbols) the i - V_m plot is linear between -50 and $+60$ mV, and the reversal potential was 0 mV, as expected for K^+ -selective channels. A linear regression fit to the data points yields a value of 206.7 pS ($n = 10$ patches) as the average single-

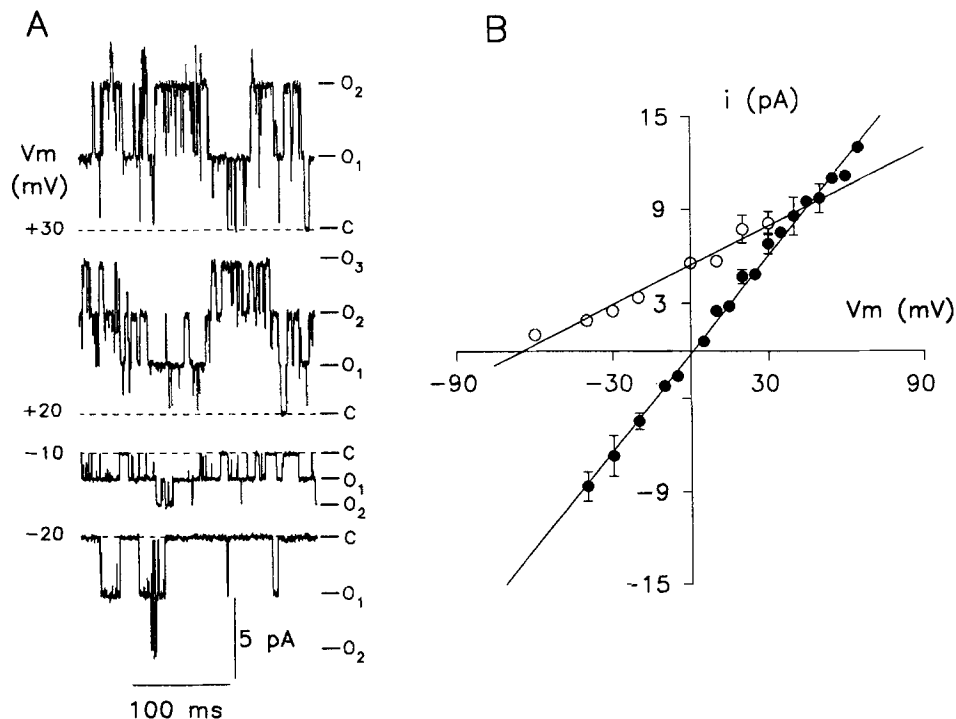


FIGURE 3. Single-channel current-voltage relation of the K_{Ca} channels. (*A*) Unitary events recorded in an inside-out excised patch with at least three simultaneously open channels at various stationary membrane potentials. Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μ s. Solutions: 130 K, 0.01 μ M Ca^{2+} //130 K, 1 μ M Ca^{2+} . (*B*) Plot of single-channel current (i) as a function of the membrane potential (V_m) measured in symmetrical (filled symbols, $n = 10$ inside-out patches) and asymmetrical (open symbols, $n = 3$ inside-out patches) K^+ concentrations. The points indicate the mean \pm SD values and the straight lines the linear regression fits to the data. Slope conductances are: 206.7 pS (filled symbols, $r = 0.99$) and 83.7 pS (open symbols, $r = 0.98$). Solutions: 130 K, 0.01 μ M Ca^{2+} //130 K, 1 μ M Ca^{2+} (symmetrical $[K^+]$); standard Na, TTX//130 K, 1 μ M Ca^{2+} (asymmetrical $[K^+]$).

channel conductance. In asymmetrical $[K^+]$ (open symbols) the average slope conductance, measured between -30 and $+50$ mV, is 83.7 pS ($n = 3$ patches).

The possible modulatory effect of PO_2 on the activity of K_{Ca} channels was investigated in inside-out excised patches that were initially exposed to variable internal Ca^{2+} concentrations ($[Ca^{2+}]_i$) to test the Ca^{2+} dependence of channel activation, and thereafter to various PO_2 levels keeping $[Ca^{2+}]_i$ unaltered. An example

of this experimental protocol is illustrated in Fig. 4. The membrane was bathed in symmetrical high K⁺ solutions with a [Ca²⁺]_i of 1 μM and held at a potential of +20 mV. Under these conditions opening of the two active channels included in the patch produced an outward current that disappeared completely after switching to a solution with 0.01 μM Ca²⁺. This effect was perfectly reversible on reintroduction of 1 μM Ca²⁺ in the chamber (Fig. 4A). When at a fixed [Ca²⁺]_i of 1 μM the same patch was exposed to low PO₂, no appreciable changes in single-channel activity or unitary

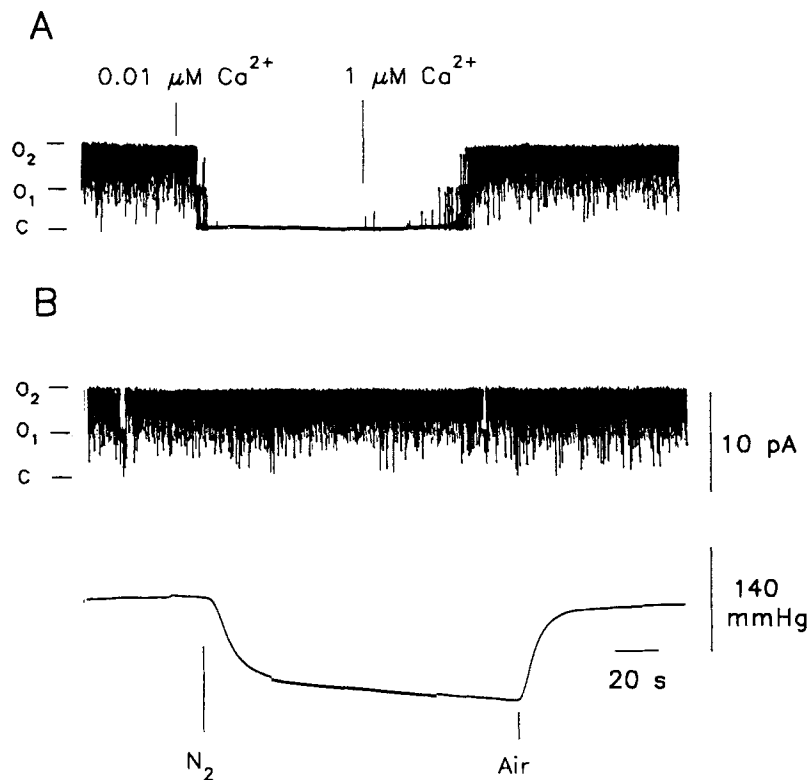


FIGURE 4. Dependence of K_{Ca} channels on internal Ca²⁺ and lack of effect of changes in PO₂. (A) Activity at +20 mV of two K_{Ca} channels in an inside-out patch exposed to 1 μM internal Ca²⁺ and reversible inhibition when [Ca²⁺]_i is decreased to 0.01 μM. (B) At the same membrane potentials and [Ca²⁺]_i = 1 μM channel activity was unaffected by lowering PO₂. The signal from the O₂-measuring electrode is shown in the lower panel. Effective cutoff frequency = 0.95 kHz. Solutions: 130 K, 0.01 μM Ca²⁺//130 K, 1 μM or 0.01 μM Ca²⁺.

current amplitude were observed (Fig. 4B). The signal from the PO₂-measuring electrode is shown in the lower panel. Following this same experimental procedure, but using various [Ca²⁺]_i (between 0.04 and 1 μM) and V_m values (between -30 and +30 mV), exposure to hypoxia did not alter K_{Ca} channel open probability (P_{open}) in the 16 patches tested (paired *t* test). Fig. 5 summarizes the effects of [Ca²⁺]_i and low PO₂ on K_{Ca} channel activity. In Fig. 5A, single-channel P_{open} as a function of V_m is plotted at two [Ca²⁺]_i. In both cases the data points are the mean ± SE values from

two experiments. At +20 mV and with a $[Ca^{2+}]_i$ of 1 μM , P_{open} is ~ 0.2 , a value quite comparable to previous estimations for this channel type in other cells (Barrett, Magleby, and Pallotta, 1982). The dependence of P_{open} on $[Ca^{2+}]_i$ together with the lack of effect of low PO_2 at a V_m of +20 mV is further illustrated in Fig. 5 B. Thus, the results indicate that type I cells have Ca^{2+} - and voltage-activated maxi- K^+ channels with properties that resemble those reported for the same channel type in other preparations (see Blatz and Magleby, 1987). The channel P_{open} was unaffected by hypoxia, which indicates that the channels are not directly involved in the modulation by O_2 of the macroscopic K^+ current of glomus cells (López-Barneo et al., 1988).

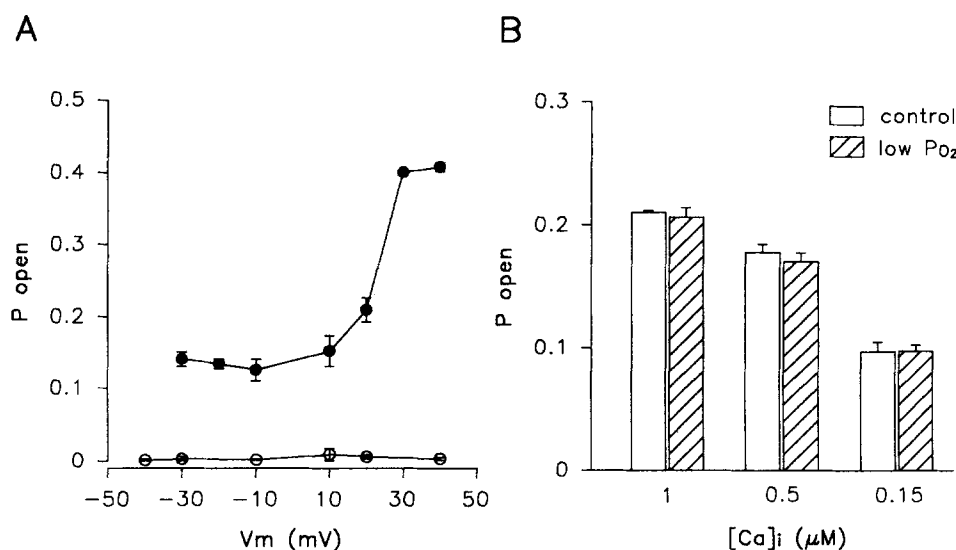


FIGURE 5. (A) P_{open} of K_{Ca} channels (ordinate) as a function of the membrane potential (abscissa) and $[Ca^{2+}]_i$. Each data point is the mean \pm SE from two experiments. Solutions: 130 K, 0.01 Ca^{2+} /130 K, either 1 (filled symbols) or 0.01 (open symbols) μM Ca^{2+} . (B) P_{open} of K_{Ca} channels (ordinate) at different $[Ca^{2+}]_i$ (abscissa) in patches exposed to control (150 mmHg) and hypoxic (0–5 mmHg) solutions. Membrane potential in all experiments was +20 mV. Error bars are the mean \pm SE. The number of experiments were three (1 μM Ca), four (0.5 μM Ca), and four (0.15 μM Ca). Differences were not statistically significant (paired t test). Solutions as in A with the indicated $[Ca^{2+}]_i$.

Small Conductance Channels (SK Channels)

SK channels were recorded in isolation in 16 inside-out patches. Fig. 6 A illustrates an example of SK channel activity during 200-ms depolarizing pulses to the indicated membrane potentials applied from a holding potential of -80 mV. The patch was bathed in symmetrical 130 mM K^+ solutions and contained one open channel. In the range between -50 and $+50$ mV the average $i-V_m$ relation (Fig. 6 B, filled symbols) is linear, the current reverses at 0 mV, and the mean slope conductance is 16 pS ($n = 6$). In asymmetrical $[K^+]$ the $i-V_m$ relation in the range between -40 and $+40$ mV can be reasonably well fitted by a linear regression with an average slope

conductance of 6 pS (Fig. 6 B, open symbols). Activation of SK channels did not require intracellular Ca²⁺ since in most experiments they were recorded with internal solutions containing 10 mM EGTA. In two experiments exposure of SK channels to a solution with 1 μM [Ca²⁺]_i did not produce appreciable changes in the average P_{open} value (not shown). Since SK channels do not inactivate even when the membrane is maintained at positive potentials for several minutes, the effect of hypoxia on channel

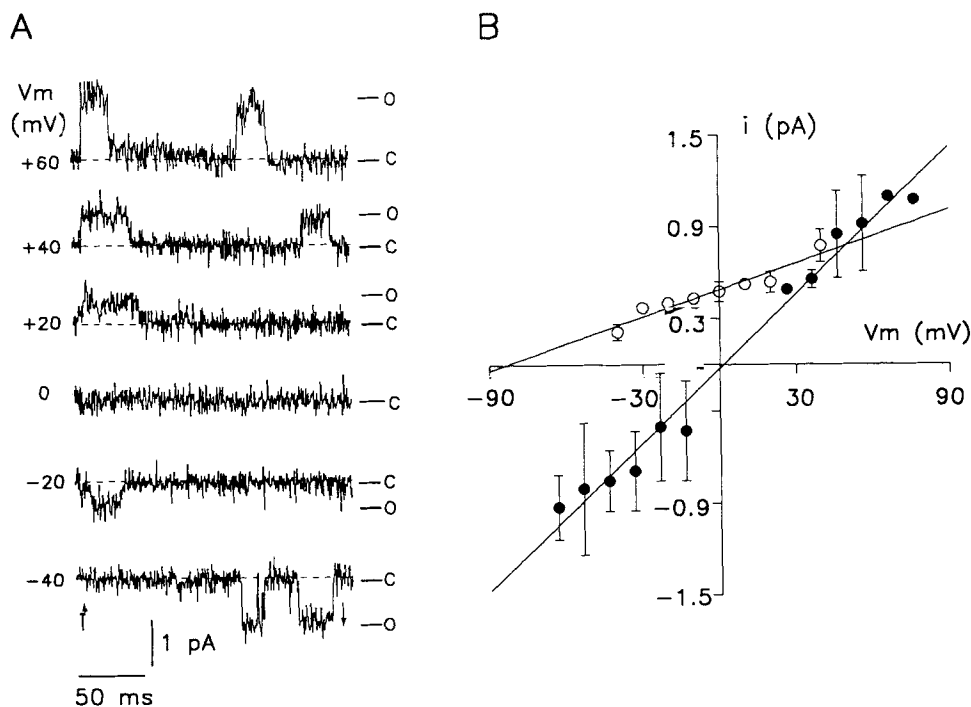


FIGURE 6. Single-channel current–voltage relation of SK channels. (A) Single-channel currents from an inside-out patch containing one observable open channel during 200-ms depolarizations from -80 mV to the indicated membrane potentials. Command pulse duration is represented by the arrows. Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μs. Solutions: 130 K, 0.01 μM Ca²⁺//130 K, 10 EGTA. (B) Single-channel current amplitude (*ordinate*) as a function of the membrane potential (*abscissa*) in inside-out patches bathed in symmetrical (*filled symbols*, $n = 6$) and asymmetrical (*open symbols*, $n = 2$) K⁺ concentration. Values are the mean \pm SD. Linear regression fits yield slope values of 16 pS (*filled symbols*, $r = 0.98$) and 6 pS (*open symbols*, $r = 0.97$). Solutions: 130 K, 0.01 μM Ca²⁺//130 K, 10 EGTA (symmetrical [K⁺]); standard Na, TTX//130 K, 10 EGTA (asymmetrical [K⁺]).

activity was tested during steady-state depolarizations. Fig. 7 A shows the current recorded at a V_m of -70 mV from a patch with two functional SK channels bathed in symmetrical high K⁺ solutions. The simultaneous measurement of O₂ tension in the chamber is shown in the lower part of the figure. Fig. 7 B summarizes the results obtained from seven different experiments. SK channel P_{open} (~ 0.21 at $+20$ mV and ~ 0.05 at -70 mV) was not significantly affected by low PO₂ (paired t test).

Interestingly, P_{open} of SK channels at membrane voltages as negative as -70 mV is relatively high; therefore, they may contribute to the regulation of the resting potential of type I cells. Although in excised patches these channels do not seem to be influenced by O_2 , there exists the possibility that in situ they could be modulated by some cytosolic mediator (see Discussion).

O₂-sensitive Channels (K_{O₂} Channels)

K_{O_2} channels were the most numerous channels found in cell-attached, inside-out, or outside-out membrane patches. These K^+ channels are voltage dependent, their activation is independent of internal Ca^{2+} , and, in contrast to K_{Ca} and SK channels, their P_{open} is reversibly decreased by lowering PO_2 (see Table II).

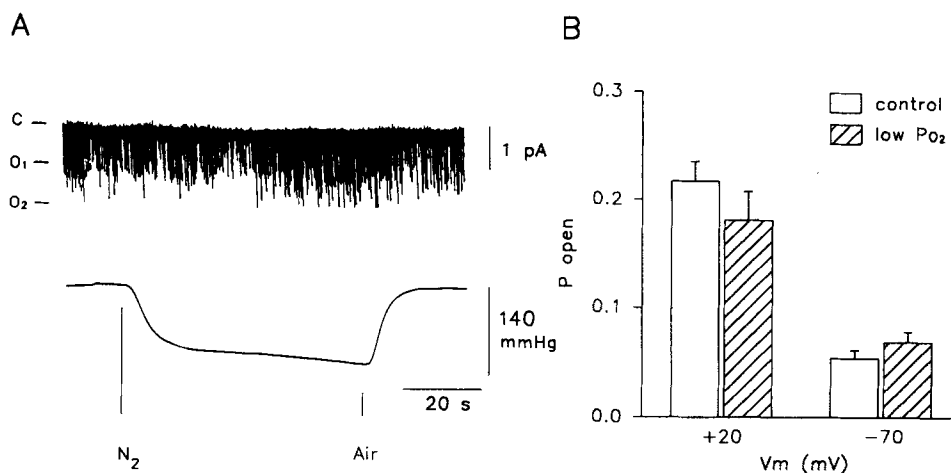


FIGURE 7. Lack of effect of changes in PO_2 on the P_{open} of SK channels. (A) Continuous recording of single-channel events from an inside-out patch with two functional SK channels held at -70 mV and exposed to low PO_2 . The lower panel represents the simultaneous monitoring of the PO_2 in the chamber. (B) Average P_{open} (ordinate) at two different membrane potentials in patches exposed to control ($\text{PO}_2 = 150$ mmHg) and hypoxic ($\text{PO}_2 < 5$ mmHg) solutions. Values are the mean \pm SE of seven experiments. Differences were not statistically significant (paired t test). Solutions: 130 K, 0.01 μM Ca^{2+} //130 K, 10 EGTA.

Current-voltage relation and voltage dependence. The voltage-dependent activation of K_{O_2} channels is illustrated in Fig. 8 by recordings from an inside-out patch containing two observable open channels. Between the arrows 200-ms pulses were applied from -80 mV to the indicated membrane potentials. In this experiment the membrane was bathed in asymmetrical K^+ solutions with 10 mM EGTA added to the internal solution. In two patches that did not contain K_{Ca} channels, we found that exposure of the channels to 1 μM internal Ca^{2+} did not alter their activity (not shown). Fig. 8A indicates that depolarization increases the amplitude of the outward single-channel current steps and favors the occurrence of simultaneous openings at the beginning of the pulse. No substates of conductance longer than our time resolution were observed. The i - V_m relation is linear between V_m values of -40 and

+40 mV (Fig. 8 B, open circles) and the unitary conductance is 18.5 pS (average of 32 measurements from 18 inside-out and 14 outside-out patches). On depolarizations to potentials more positive than +40 mV, however, a slight rectification and a decrease in the amplitude of single-channel current can be observed. In symmetrical [K⁺] the single-channel conductance raised to 41.5 pS (average of three measurements from one inside-out and two outside-out patches; Fig. 8 B, filled symbols), and the reversal potential changed from ~ -75 to 0 mV.

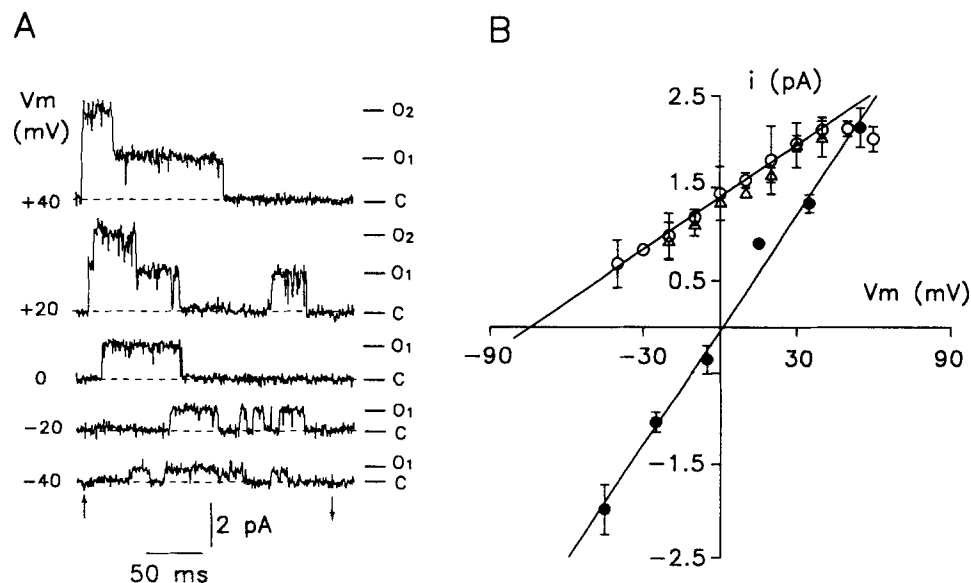


FIGURE 8. Single-channel current-voltage relation of K_{O₂} channels. (A) Single-channel activity in an inside-out patch with at most two open channels during 200-ms depolarizations to the indicated membrane potentials (V_m) from a holding potential of -80 mV. Pulse duration is represented by the arrows. Effective cutoff frequency = 1 kHz and sampling interval = 500 μ s. Solutions: standard Na, TTX//130 K, 10 EGTA. (B) Single K_{O₂} channel current amplitude (ordinate) as a function of the membrane potential (abscissa) in symmetrical (filled symbols) and asymmetrical (open symbols) K⁺ concentrations. Data from either inside-out or outside-out patches are pooled together and expressed as mean \pm SD. Filled symbols, 3 patches and control PO₂ solution; open circles, 32 patches and control PO₂ solution; open triangles, 15 patches and low PO₂ solution (PO₂ < 5 mmHg). Straight line fits ($r > 0.99$ in all cases) yield slope values of 41.5 pS (filled circles), 18.9 pS (open circles), and 20.4 pS (open triangles). The two open circles at +50 and +60 mV were not included in the fit. Solutions: 140 K//130 K, 10 EGTA (symmetrical [K⁺]); standard Na, TTX//130 K, 10 EGTA (asymmetrical [K⁺]).

Since the K_{O₂} channels are highly selective for K⁺ ions, the comparison of the i - V_m relations obtained in cell-attached patches and after forming an inside-out patch was used to estimate the resting potential of intact cultured type I cells. The cytosolic [K⁺] of type I cells is probably near the value used in our internal solution (between 130 and 140 mM) since the slope conductance was the same in the two experimental

conditions. The average value estimated for the resting membrane potential of glomus cells with this method (-54 ± 8 mV; mean \pm SD, $n = 6$) is clearly larger than the reported previously with intracellular microelectrodes (~ -20 mV; Acker and Pietruschka, 1977; Eyzaguirre, Fitzgerald, Lahiri, and Zapata, 1983) and is compatible with the electrical excitability exhibited by type I cells.

Fig. 8A shows that the latency to the first channel opening becomes shorter and that the probability of a channel being open (P_{open}) increases with membrane depolarization. The normalized P_{open} (ordinate) as a function of the membrane potential (abscissa) is plotted in Fig. 9, indicating that the P_{open} of K_{O_2} channels is a steep function of membrane voltage. The values of P_{open} were obtained as indicated in Methods (Eq. 1) and are fitted by a Boltzmann distribution function (see figure legend). The values of $V_{1/2}$ and k were, respectively, 6.61 ± 3.6 and 7.95 ± 0.04 mV (mean \pm SD; $n = 2$).

Low PO_2 decreases channel P_{open} . It was shown in Fig. 8A that on depolarization K_{O_2} channel openings are grouped at the onset of the pulse. After a few tens of milliseconds these channels tend to enter a nonconducting inactivated state and

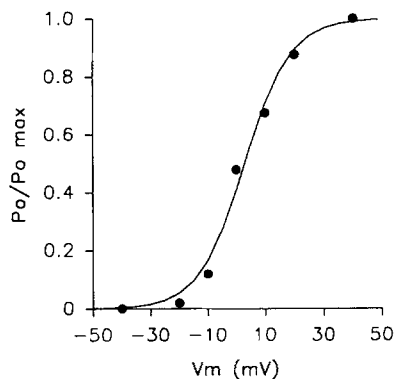


FIGURE 9. Average P_{open} of K_{O_2} channels as a function of the membrane potential. Normalized open probability (ordinate) was measured as the mean value during 200-ms depolarizations in an outside-out patch containing one channel. Data points are fitted by a Boltzmann distribution function of the form: $P_o/P_{o(\text{max})} = [1 + \exp \{(V_{1/2} - V)/k\}]^{-1}$, where $V_{1/2}$ is the V_m at which P_{open} is half-maximal and k is a slope factor.

during a maintained depolarization channel activity ceases almost completely in < 500 ms. Recovery from inactivation of the K_{O_2} channels is particularly slow and thus command pulses must be delivered with an interval > 30 s to obtain a statistically valid sample of single-channel events. To avoid long-lasting exposures to low PO_2 that could produce nonspecific irreversible changes in membrane properties and to correct for the possible run down of the preparation, the effect of PO_2 levels on K_{O_2} channels was studied by alternating short exposures to hypoxic and normoxic solutions.

The major effects of low PO_2 on single-channel activity are shown in Fig. 10, A and B. The inset in Fig. 10C illustrates the experimental protocol by a recording of the variations of PO_2 in the chamber with indication of the time at which pulses to +20 mV were applied. Representative current sweeps recorded in the control solution (periods C_1 to C_n) and during exposure to hypoxia (periods H_1 to H_n) are shown in panel A. In control and hypoxic conditions flipping of the channel between open and closed states appears as unitary events of 1.6 ± 0.17 pA of amplitude occurring more frequently at the onset of the pulses. The ensemble averages of the two sets of

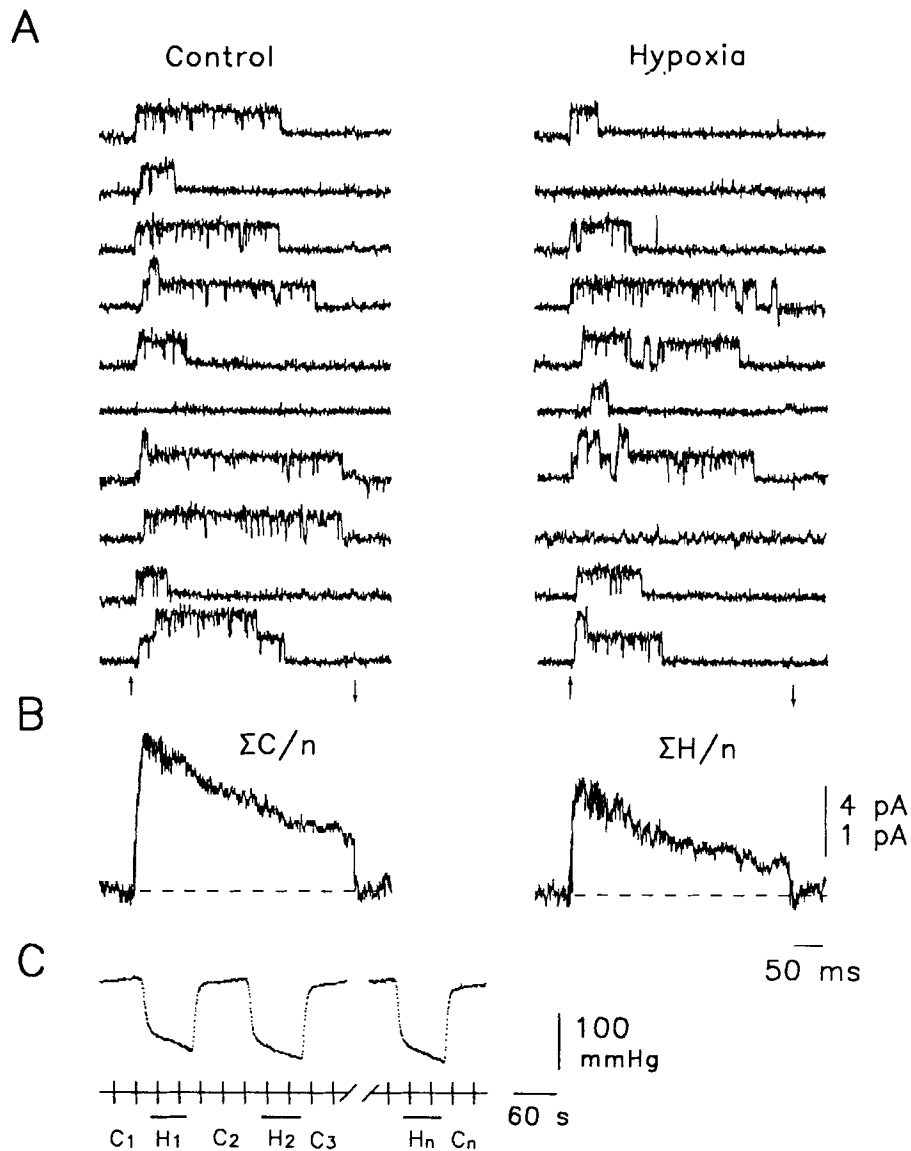


FIGURE 10. Reversible inhibition of K_{O₂} channel activity by lowering PO₂. Single-channel currents were recorded from an inside-out patch with three K_{O₂} channels during 350-ms depolarizations to +20 mV. (A) Representative sweeps in control (PO₂ = 150 mmHg) and hypoxic (PO₂ < 5 mmHg) solutions. The arrows indicate the onset and the end of the pulse. Command pulses were applied every 30 s and the instant of delivery in relation to the changes of PO₂ in the chamber are represented by the vertical lines in C. (B) Ensemble averages of single-channel currents recorded in the two experimental conditions. Sweeps recorded during alternating exposure (for 1–2 min) to control ($n = 24$, $P_{\text{open}} = 0.31$) and low PO₂ ($n = 15$, $P_{\text{open}} = 0.18$) solutions are grouped together ($\Sigma C/n$ and $\Sigma H/n$, respectively). Current calibration bar is 4 pA for the single-channel traces and 1 pA for the average traces. Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μ s. Solutions: standard Na, TTX//130 K, 10 EGTA.

recordings (panel *B*) show a peak P_{open} at the beginning of the depolarization and a progressive decrease during the pulse. Single-channel P_{open} integrated throughout the pulse duration (see Eq. 1) is 0.31 in the control solution but only 0.18 during exposure to low PO_2 . Thus, hypoxia produces a reversible decrease in channel open probability but, as shown in Fig. 8 *B* (triangles), it does not modify either the i - V_m relation or the single-channel conductance (see Table II).

A summary of the effect of low PO_2 on the P_{open} of K_{O_2} channels at different membrane potentials is shown in Fig. 11. The average P_{open} value during 200-ms pulses was measured in patches with one or two functional K_{O_2} channels. Low PO_2 produced a decrease in single-channel P_{open} that was statistically significant at all membrane voltages (paired t test, $\alpha < 0.01$). At +30 mV, for example, P_{open} is ~ 0.43 in the control solution and ~ 0.31 in low PO_2 , but at 0 mV these values are, respectively, ~ 0.2 and ~ 0.07 . This indicates that the inhibition of K^+ channel activity by hypoxia is more marked at less depolarized membrane potentials (see also

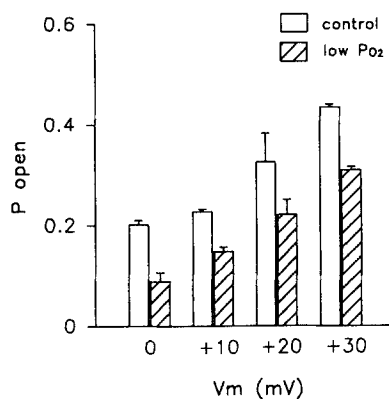


FIGURE 11. Decrease of K_{O_2} channel P_{open} by hypoxia. Average open probability during 200-ms pulses (*ordinate*) was measured from either inside-out or outside-out patches at various membrane potentials. P_{open} values measured in the control and in the low (< 5 mmHg) PO_2 solutions are represented by the mean \pm SE. Number of experiments were: $n = 5$ (0 mV); $n = 3$ (+10 mV); $n = 23$ (+20 mV); and $n = 2$ (+30 mV). At all voltages the differences were statistically significant (paired t test, $\alpha < 0.01$). Solutions: standard Na^+ , TTX//130 KCl, 10 EGTA or standard K, 10 EGTA.

accompanying paper). For simplicity, in this set of experiments solutions were bubbled with either N_2 or air and thus the low PO_2 values in the vicinity of membrane patches were ~ 5 – 10 mmHg (see Methods). With this protocol the decrease of P_{open} by hypoxia is underestimated since it is known that on exposure to extremely low PO_2 the inhibition of K^+ channel activity is relatively less pronounced than when the channels are exposed to moderately low PO_2 values (between 60 and 80 mmHg; López-López et al., 1989; Ganformina and López-Barneo, 1991).

Direct modulation of K_{O_2} channels accounts for the properties of the macroscopic O_2 -sensitive K^+ current. The kinetic and pharmacological properties of the K_{O_2} channels indicate that they are the main channels responsible for the macroscopic O_2 -sensitive K^+ current of type I cells. The ensemble average currents shown earlier (see Fig. 10 *B*) illustrate that the time course exhibited by K_{O_2} channels during a depolarization closely resembles the kinetics of the macroscopic K^+ current which turns on in a few milliseconds and inactivates almost completely in 200–300 ms

(López-López et al., 1989; see also Fig. 12). In addition, TEA⁺ reversibly blocks the whole-cell K⁺ current (Ureña et al., 1989a) as well as the K_{O₂} channels (Ganforina and López-Barneo, 1991). The parallel time courses of K_{O₂} channel P_{open} and the macroscopic K⁺ current were clearly evident when recordings in the whole-cell mode and in outside-out multichannel patches were obtained following the same experimental protocol. Fig. 12 illustrates the reversible inhibition of the macroscopic K⁺ current (traces in C) and the decrease in K⁺ channel P_{open} in an outside-out patch with at least five channels (traces in A and B) during a transient exposure to hypoxia. The ensemble averages of Fig. 12 B, which represent the behavior of a few channels, have a time course comparable to that of the whole-cell currents.

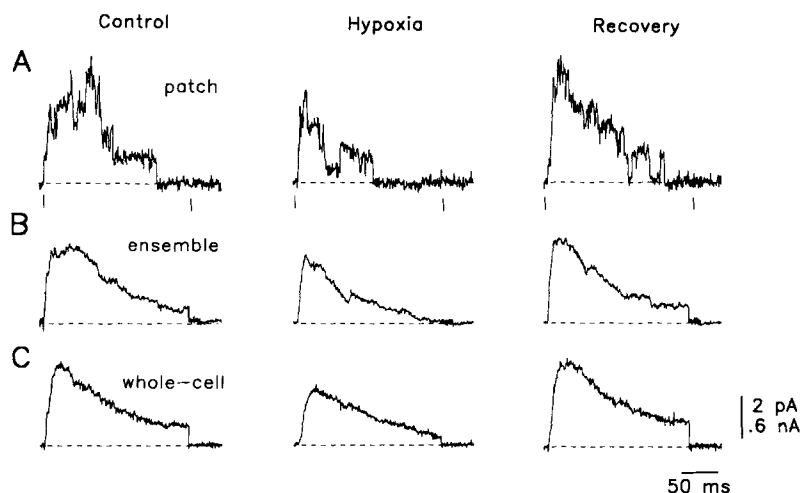


FIGURE 12. Comparison of the effect of lowering PO_2 on single K_{O_2} channel and macroscopic K^+ currents elicited by depolarization to 0 mV from a holding potential of -80 mV. (A and B) Representative single sweeps and ensemble averages of current recorded from an outside-out patch, containing at least five channels, in the control solution ($PO_2 = 150$ mmHg; $n = 13$ consecutive sweeps; $P_{\text{open}} = 0.29$), during a 9-min exposure to hypoxia ($PO_2 < 5$ mmHg; $n = 18$ consecutive pulses; $P_{\text{open}} = 0.15$), and after recovery in the normal PO_2 solution ($n = 18$ consecutive pulses; $P_{\text{open}} = 0.27$). Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μ s. (C) Whole-cell currents recorded with the same experimental protocol. Effective cutoff frequency = 10 kHz and sampling interval = 500 μ s. Current calibration bar is 2 pA for A and B, and 0.6 nA for C. Solutions: standard Na, TTX//130 K, 10 EGTA.

With a high Ca^{2+} buffer capacity at the internal solution, most of the macroscopic K^+ current is due to the activity of K_{O_2} channels since K_{Ca} channels cannot be activated and, in addition, the density of SK channels is low and their unitary conductance is small. Therefore, the number of K_{O_2} channels can be estimated by dividing the peak K^+ current by the value of the single K_{O_2} channel current amplitude at the same voltage and correcting for the peak channel P_{open} (~ 0.8 at $+20$ mV). Our estimate gives values of 720 ± 80 (mean \pm SD, $n = 18$) channels per cell, which corresponds to two to four channels per square micrometer. This relatively

high density may explain why we obtained multichannel patches in ~50% of the experiments (87 of 168) even though we used relatively high resistance pipettes (>8 M Ω).

It was shown in a previous report that the modulation by O₂ of the macroscopic K⁺ current of type I cells is independent of internal Ca²⁺ or the presence of exogenous nucleotides (López-Barneo et al., 1988; López-López et al., 1989). Our results at the single-channel level confirm and extend these observations since reversible inhibition

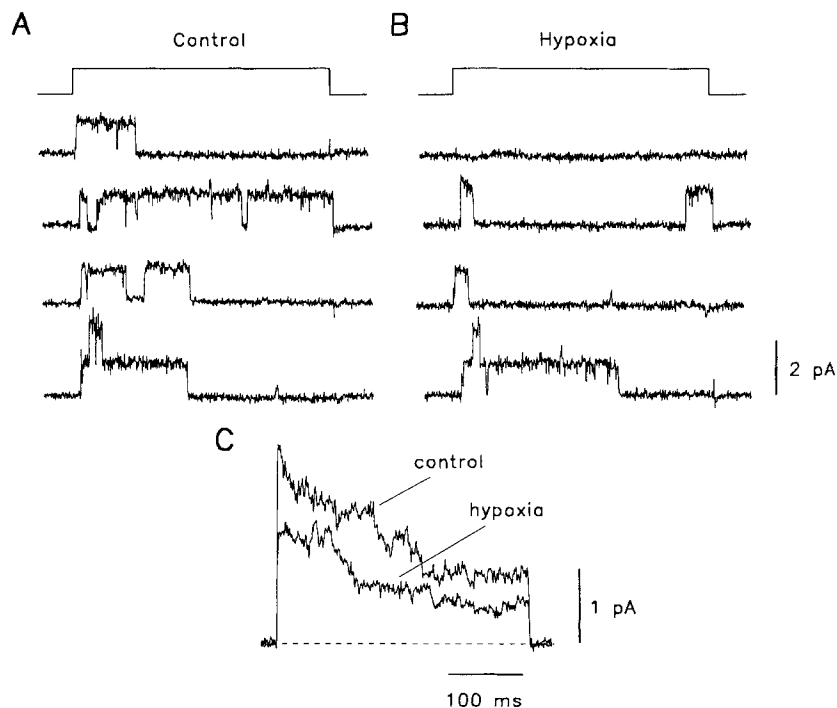


FIGURE 13. Lack of desensitization after maintained exposure to low PO₂. (A and B) Single-channel currents recorded from an inside-out patch with two K_{O₂} channels during 350-ms depolarizations to +20 mV from -80 mV. The cell was exposed for 30 min to low PO₂ (<5 mmHg) before excising the patch from which the sweeps were obtained during alternating exposures to control and hypoxic solution as explained in Fig. 10. (C) Superimposed ensemble averages of traces recorded in control ($n = 25$ sweeps, $P_{\text{open}} = 0.52$) and hypoxic ($n = 16$ sweeps, $P_{\text{open}} = 0.28$) conditions. Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μ s. Solutions: standard Na, TTX//130 K, 10 EGTA.

of K_{O₂} channel activity was observed in excised patches without Ca²⁺ or nucleotides added to the internal solution. We also tested whether O₂ could act through the activation of a membrane-bound G protein, a family of proteins that are irreversibly activated by GTP- γ -S (Gilman, 1987) or by AlF₄⁻ formed from fluoride (a normal component of some of our internal solutions) and aluminum (which could be released from the micropipette glass) (Sternweis and Gilman, 1982; Bigay, Deterre, Pfister, and Chabre, 1985). The reversible modulation of K_{O₂} channels by changes in PO₂ was

unaltered in solutions free of F⁻ ($n = 51$) or when up to 200 μM GTP- γ -S was added to the internal solution ($n = 22$). Thus, the results suggest that soluble cytosolic mediators or membrane-bound G proteins do not participate in the effect of O₂ on the K_{O₂} channel, and that O₂ may interact with an intrinsic sensor closely associated with the channel protein.

Repetitive exposure to low PO₂ does not produce desensitization. In many examples of ligand-receptor interaction, repeated or permanent exposure to the agonist produces an attenuation of the physiological response. This phenomenon, called "desensitization," has been typically studied in some ligand-activated channels and it is well known that, after withdrawal of the agonist, recovery to the resting conditions is slow (see Hille, 1984). Although desensitization is a term applicable to ligand-receptor interactions, it is a phenomenon that could play a part in the physiological adaptation observed in some sensory receptors (Stebbens, Brown, and Peterson, 1984).

It was described in our previous work that chemosensory transduction in the type I cell is a nonadapting, or slowly adapting, process since reversible attenuation of the macroscopic K⁺ current can be repeatedly observed in a given cell (López-Barneo et al., 1988; see also Ganfornina, 1991). Fig. 13 shows single-channel current sweeps recorded during alternating exposure to control (A) and low PO₂ (B) solutions in a patch with two K_{O₂} channels excised from a cell that had been preincubated in extreme hypoxia (PO₂ \approx 5 mmHg) for 30 min. To facilitate comparison, ensemble averages in the two experimental conditions are shown superimposed in Fig. 13 C. Reversible inhibition of K_{O₂} channel activity by low PO₂ can be observed repeatedly after long-lasting exposure to extreme hypoxia, further suggesting that the O₂-K_{O₂} channel interaction does not desensitize.

DISCUSSION

In this article we describe the properties of three types of K⁺ channels in chemoreceptor cells of the carotid body that can be distinguished by their biophysical characteristics. We also demonstrate that in excised membrane patches only the activity of a specific K⁺ channel class, the K_{O₂} channel, is reversibly inhibited by lowering environmental PO₂. Our findings explain the modulation by O₂ of the macroscopic K⁺ current of glomus cells and strongly suggest that the O₂-sensing mechanism resides in the plasma membrane.

K⁺ Channel Types in Glomus Cells

Both cell-attached and excised membrane patches of rabbit glomus cells contain three major K⁺-selective channels (K_{Ca}, SK, and K_{O₂} channels). A Ca²⁺-independent and high-conductance Cl⁻ channel encountered in rat type I cells (Stea and Nurse, 1989) was not studied. The three classes of K⁺ channels differ in their single-channel conductance and kinetics as well as in their dependence on internal Ca²⁺ and O₂ sensitivity. K⁺ channels are extraordinarily diverse (see for reviews Rudy, 1988; Adams and Nonner, 1989) and it is well known that different subpopulations coexist in a given membrane (Dubois, 1983; Conti, Hille, and Nonner, 1984; Marty and Neher, 1985; Hoshi and Aldrich, 1988; Llano, Webb, and Bezanilla, 1988). In this respect, the single K⁺ channels identified in type I cells share most of their properties

with those classified in bovine chromaffin (Marty and Neher, 1985) and mouse neuroblastoma (Quandt, 1988) cells. Equivalent single-channel currents are also present in pheochromocytoma cells (Hoshi and Aldrich, 1988). Interestingly, all these cell types have a close embryological origin.

The K_{Ca} channels have, in symmetrical high K^+ solutions, an average conductance of 206.7 pS. These channels are similar to the maxi- K^+ Ca^{2+} -dependent channels of other preparations (Marty, 1981; Barrett et al., 1982; Quandt, 1988), and, in excised patches, they are unaffected by changes in PO_2 . These observations confirm our previous experiments showing that part of the macroscopic K^+ current, presumably a Ca^{2+} -dependent component, disappears after wash-out of Ca^{2+} channels (Ureña et al., 1989a) and that under these conditions, and with 10 mM EGTA added to the internal solution, the K^+ current is still reversibly attenuated by lowering PO_2 (López-Barneo et al., 1988; López-López et al., 1989). It has been reported that hypoxia specifically inhibits the Ca^{2+} -dependent component of the macroscopic K^+ current recorded in dialyzed carotid body cells from newborn rats (Peers, 1990). The discrepancy between these data and our whole-cell and single-channel results may reflect a difference between animal species; however, we also believe that the conclusion reached by Peers (1990) may have been biased by the experimental protocol used in the isolation of the Ca^{2+} -activated K^+ current. We know, for example, that millimolar concentrations of Cd^{2+} and Co^{2+} , which could produce a decrease of the Ca^{2+} -activated K^+ current due to blockade of Ca^{2+} channels, can also produce a large and reversible inhibition of the Ca^{2+} -independent and O_2 -sensitive component of the K^+ current (Ganforina, M.D., and J. López-Barneo, unpublished results).

We followed the terminology of Marty and Neher (1985) to denote a second population of K^+ -selective channels that have a small conductance (SK channels). In asymmetrical K^+ solutions the unitary conductance of carotid body SK channels (~ 6 – 7 pS) is similar to the values reported in chromaffin and neuroblastoma cells (Marty and Neher, 1985; Quandt, 1988). Hoshi and Aldrich (1988) have also found in pheochromocytoma cells two populations of K^+ channels (K_y and K_x) with the same unitary conductance value. Although we did not study in detail the kinetic properties of SK channels, in accord with previous work (Marty and Neher, 1985; Quandt, 1988), they behaved as Ca^{2+} -independent and slowly activating channels. In glomus cells inactivation of SK channels, if any, must be also very slow since channel activity could be recorded for minutes at depolarized membrane potentials. SK channels are only moderately voltage dependent ($P_{open} = 0.2$ at $+20$ and 0.05 at -70 mV) and their P_{open} at negative voltages suggests that they may contribute to the generation of the resting potential of the cells. In excised patches SK channels were unaltered by changes in PO_2 , but we cannot discount that in situ they could be subjected to modulation. This idea is based on the fact that in some inside-out patches the activity of SK channels appeared abruptly several minutes after excision of the membrane, which could be explained by the dilution of some soluble mediator that blocks the channels or that favors their closed conformation.

We have coined the term K_{O_2} to designate the K^+ -selective and O_2 -sensitive channels of type I cells. These channels were the most frequently observed, probably because they are densely packed in the glomus cell membrane. Our estimate is two to

four channels per square micrometer. In asymmetrical K⁺ solutions the unitary conductance of the K_{O₂} channel is ~20 pS. This value is in excellent agreement with the conductance of fast activating (FK) channels in chromaffin, neuroblastoma, and pheochromocytoma cells (Marty and Neher, 1985; Hoshi and Aldrich, 1988; Quandt, 1988) as well as of delayed rectifier and A-type K⁺ channels described in a number of preparations (Cooper and Shrier, 1985, 1989; Kasai, Kameyama, Yamaguchi, and Fukuda, 1986; Llano et al., 1988). K_{O₂} channels are not influenced by changes in internal Ca²⁺ but they are steeply dependent on membrane voltage; the activation threshold is at ~-50 to -40 mV, and at +20 mV the peak P_{open} is 0.8. This last parameter is similar to values reported for other mammalian inactivating K⁺ channels (Marty and Neher, 1985; Cooper and Shrier, 1989).

Although the activation and inactivation kinetics are studied in more detail in the accompanying article, here we show that K_{O₂} channels have a fast activation. As the membrane is more depolarized the number of active channels increases and the latency to the first opening decreases. During maintained depolarizations K_{O₂} channels inactivate completely in a few hundred milliseconds. These properties are perfectly compatible with the characteristics of the macroscopic K⁺ current of type I cells (Ureña et al., 1989a) and strongly suggest that the K_{O₂} channels are the main contributors to this current. This is also supported by the close parallelism existing between the time courses of the whole-cell K⁺ current and the ensemble averages from patches containing only K_{O₂} channels.

Modulation of K_{O₂} Channels by O₂ Tension

A distinct property of K_{O₂} channels is that their P_{open} decreases on exposure to low PO₂. The inhibition of channel activity by hypoxia is reversible and concentration dependent (Ganformina and López-Barneo, 1991), and is a process that does not undergo desensitization. These properties fit perfectly with those encountered in the modulation of the macroscopic K⁺ current by PO₂ (López-Barneo et al., 1988; López-López et al., 1989). The correspondence between the O₂ modulation of whole-cell and single-channel currents is quite remarkable and can be clearly seen when ensemble averages from patches containing K_{O₂} channels in isolation are compared with the O₂-sensitive K⁺ current (see, for example, Fig. 12). The major effect of lowering PO₂ is a decrease in the P_{open} of the channels leaving unaltered unitary conductance. Interestingly, the magnitude of the decrease of P_{open} in hypoxic conditions in the voltage range between +10 and +30 mV (~25% of the control value) is the same as the inhibition of the macroscopic K⁺ current by low PO₂ (López-Barneo et al., 1988). The action of low PO₂ is more pronounced at less depolarized membrane voltages, which is an observation that, as discussed in the accompanying paper, could be expected if the lack of O₂ favors closed or inactivated conformations of the channels.

Delpiano and Hescheler (1989) have reported in cell-attached patches of glomus cells from rabbit embryos the existence of a K⁺ channel reversibly inhibited by lowering PO₂. This channel was recorded during stationary membrane depolarizations and the unitary conductance value was 137 pS with high K⁺ in the pipette solution. These results are difficult to compare with our own since in Delpiano and Hescheler's work the O₂-sensitive channel was not characterized and it does not

include data about other possible channel types. The kinetics and conductance of Delpiano and Hescheler's channel are comparable to those of the K_{Ca} channel, which, as shown before, is insensitive to changes in PO_2 in excised patches. Although we have observed in situ the same types of K^+ channels as in excised patches, we did not attempt an initial classification of K^+ channels in the cell-attached configuration because the membrane potential, cytosolic Ca^{2+} concentration, and other variables are unknown. In addition, we have also observed that type I cells are electrically very compact and therefore current flowing through a cell-attached patch can induce modifications in the membrane potential of the cell (Ganformina, 1991). Nevertheless, we cannot eliminate the possibility that there could be a change in the O_2 -sensing mechanisms of carotid body cells during development (Hertzberg, Hellström, Lagercrantz, and Pequignot, 1990). In this respect it is interesting to note that whereas embryonic glomus cells seem to lack Na^+ channels (Hescheler et al., 1989), in the adult tissue large Na^+ currents can be recorded (Ureña et al., 1989a).

The fact that the modulation of K_{O_2} channels by O_2 is maintained for long periods of time in excised patches strongly suggests that the O_2 - K_{O_2} channel interaction occurs through an intrinsic sensor of the plasma membrane which may be part of the channels or a molecule closely associated with them. This interaction seems to be direct without the participation of soluble cytosolic mediators. We have sought for the possible involvement of membrane-diffusible G proteins, which after being activated can directly regulate ionic channel activity (Logothetis, Kurachi, Galper, Neer, and Claphan, 1987; Brown and Birnbaumer, 1988), with negative results. Both GTP- γ -S and F^- , agents that irreversibly activate G proteins (Gilman, 1987), were ineffective in preventing the reversibility of the inhibition of K_{O_2} channels by lowering PO_2 . Nonetheless, we cannot discount that the K_{O_2} or other channels of glomus cells might be modulated by cytosolic mediators in situ. Exposure to hypoxia alters the content of cGMP and cAMP in the carotid body (Wary, Cheng, Dinger, and Fidone, 1989; Pérez-García, Almaraz, and González, 1990) and these agents are known to regulate a broad number of ionic channels.

The K_{O_2} channel may belong to a family of O_2 sensors broadly distributed in nature. Apart from the well-known O_2 transport functions of heme proteins, there are, from bacteria to mammalian cells, examples of heme-linked enzymes, the activity of which is regulated by environmental O_2 (Goldberg, Dunning, and Bunn, 1988; Gilles-González, Ditta, and Helinski, 1991). Cross, Henderson, Jones, Delpiano, Hentschel, and Acker (1990) have recently proposed that the activity of a NADPH-oxidase in glomus cells (containing a *b*-type cytochrome) could be regulated by O_2 , and that this enzyme could determine the redox state of thiol groups of proteins and influence the properties of ionic channels.

Physiological Significance of the K_{O_2} Channel

The K_{O_2} channel represents the first known example of an ionic channel regulated by O_2 . A similar type of regulation has been sought, but not found, in septal neurons (López-López et al., 1989) and in the small dopaminergic interneurons of the sympathetic ganglia (Stea and Nurse, 1991), which are developmentally related to glomus cells. Our results demonstrate the specificity of the K_{O_2} channels located in an O_2 -responsive cell and strongly suggest that they represent the initial step in

chemotransduction and thus confer upon glomus cells their unique chemoreceptor properties. Due to our experimental requirements (see Methods), the PO₂ values of the hypoxic solutions used in this and the accompanying paper are much lower than the ones that can be attained under physiological conditions; however, we have shown before that the P_{open} of K_{O₂} channels is reversibly modified by changes of PO₂ in a physiological range (Ganformina and López-Barneo, 1991). The lack of appreciable desensitization in the O₂-K_{O₂} channel interaction may ensure that changes of the physico-chemical variable (O₂ tension) would be translated into a maintained electrophysiological response. This characteristic could be related to the fact that single-fiber chemoreceptor afferent discharges can be maintained for long periods during sustained hypoxia (Nielsen, Bisgard, and Vidruk, 1988).

The significance of K_{O₂} channels for respiratory physiology is obvious but they may have a broader functional, and perhaps pathophysiological, relevance. Similar types of channels may exist in lung alveolus and in the fine branches of the pulmonary artery and participate in the regulation of regional pulmonary perfusion, or in small vessels of brain and heart tissues where they may contribute to the autoregulation of blood flow.

The authors wish to thank Drs. R. Aldrich, T. Hoshi, and W. Zagotta (Stanford University) for comments on the manuscript.

This research was supported by a grant from the Dirección General de Investigación Científica y Técnica (PB86-0250).

Original version received 18 December 1991 and accepted version received 16 April 1992.

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