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 Ca2+ channels. We have found three classes of voltage-gated K+ channels that differ in their single-channel conductance (γ), dependence on internal Ca2+ ([Ca2+]i), and sensitivity to changes in O2 tension (Po2). Ca2+-activated K+ channels (Kca channels) with γ ~ 210 pS in symmetrical K+ solutions were observed when [Ca2+]i > 0.1 μM. Small conductance channels with γ = 16 pS were not affected by [Ca2+]i and they exhibited slow activation and inactivation time courses. In these two channel types open probability (Popen) was unaffected when exposed to normoxic (Po2 = 140 mmHg) or hypoxic (Po2 = 5–10 mmHg) external solutions. A third channel type (referred to as Ko2 channels), having an intermediate γ (~40 pS), was the most frequently recorded. Ko2 channels are steeply voltage dependent and not affected by [Ca2+]i, they inactivate almost completely in <500 ms, and their Popen reversibly decreases upon exposure to low Po2. The effect of low Po2 is voltage dependent, being more pronounced at moderately depolarized voltages. At 0 mV, for example, Popen diminishes to ~40% of the control value. The time course of ensemble current averages of Ko2 channels is remarkably similar to that of the O2-sensitive K+ current. In addition, ensemble average and macroscopic K+ currents are affected similarly by low Po2. These observations strongly suggest that Ko2 channels are the main contributors to the macroscopic K+ current of glomus cells. The reversible inhibition of Ko2 channel activity by low Po2 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 Ko2 channels confer upon glomus cells their unique chemoreceptor properties and that the O2-K+ channel interaction occurs either directly or through an O2 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
of oxygen tension (Po2) 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 O2 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 Yoshizald, 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+, Ca2+, 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 Po2, whereas Na+ and Ca2+ 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 O2-sensitive K+ current under hypoxic conditions produces an increase in the firing frequency of glomus cells (López-López, González, Ureña, and González, 1989), which could lead to Ca2+ 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 Ca2+ indicators showing an increase in cytosolic Ca2+ in response to low Po2 (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 Ca2+-dependent component that disappears after wash-out of Ca2+ channels and when internal solutions with high Ca2+-buffering capacity are used. In addition, the Ca2+-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 O2 sensitivity of the cells’ electrical properties. These questions are of critical importance for elucidating the molecular mechanisms underlying O2 sensing. The identification of the primary site involved in O2 detection is also of interest because it has been argued that the attenuation of the macroscopic K+ current on lowering Po2 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 "Kₒ₂ 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 Kₒ₂ 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 Kₒ₂ 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 (Ganfornina 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 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 osmolarity 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, Marry,
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:

\[ P_{\text{open}} = \frac{\langle N \cdot i \cdot T \rangle^{-1} \int_0^T I(t) \, dt}{T} \]  

(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:

\[
I(a) = N \cdot i \cdot P_o(t)
\]

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

\[\text{FIGURE 1. Performance and calibration of the } \text{O}_2\text{-sensing electrode. (A) Output voltage of the current-to-voltage converter as a function of the negative polarizing potential and } \text{O}_2\text{ tension.} 150\text{ mmHg (filled circles), 87 mmHg (open circles), and 0–5 mmHg (filled squares). At a polarizing voltage between } -0.6 \text{ and } -0.8 \text{ V the output voltage is linearly related to the } \text{O}_2\text{ in the solution (range between dashed lines). (B) Output voltage vs. } \% \text{O}_2 \text{ relative to air at a polarizing voltage of } -0.7 \text{ V. (C and D) Responses of the } \text{O}_2\text{ electrode in the chamber (C) and during instantaneous immersions in solutions equilibrated with } \text{N}_2\text{ and air (D).}}\]

**Measurement of Oxygen Tension**

Because some experimental protocols required repetitive exposure of a membrane patch to external solutions with a reproducible \( P_o \) value, we built an \( O_2 \)-measuring electrode to estimate \( P_o \) values in the vicinity of the current-recording pipette. We used a negatively polarized 100-\( \mu \)m-thick platinum wire insulated by an \( O_2 \)-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 \( P_o \) 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 $P_{O_2}$ 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 $P_{O_2}$ value. Given that our recording chamber is in contact with the air, we found that the most easily reproducible $P_{O_2}$ level in the vicinity of the cells ($P_{O_2} = \sim 5-10$ 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 $P_{O_2}$ (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 $>0.1$ $\mu$M. 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. $K_{Ca} = \text{Ca}^{2+}$-activated channels; $SK = \text{small conductance channels}; K_{O2} = \text{O}_2$-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 $K_{O2}$ channels upon 200-ms step depolarizations from −80 mV. Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μs. Solutions: standard Na, TTX/130 K, 10 EGTA. $K_{Ca}$ channels were recorded with an internal solution containing 1 μM free Ca²⁺.

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

| Channel type | $\text{Conductance in 130 K/130 K}$ | $\text{Conductance in 2.7 K/130 K}$ | $\text{Ca}$ dependence | $\text{O}_2$ sensitivity |
|--------------|---------------------------------|---------------------------------|------------------------|------------------------|
|              | $\text{Inside-out}$ | $\text{Outside-out}$ | $\text{Inside-out}$ | $\text{Outside-out}$ | |
| $K_{Ca}$     | 206.7 (10)       | —                     | 83.7 (3)               | —                     | Yes | No |
| SK           | 16 (6)           | —                     | 6 (2)                  | —                     | No  | No |
| $K_{O2}$     | 41.5 (1)         | 41.5 (2)              | 17.6 (18)              | 20.1 (14)             | No  | Yes |

Average slope conductance values are given in picosiemens. The number of patches is in parentheses. $K_{Ca} = \text{Ca-activated channel};SK = \text{small conductance channel}; K_{O2} = \text{oxygen-sensitive channel.}$ For the $K_{O2}$ 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. 3B. 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-

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 P$O_{2}$ 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$^{2+}$]) to test the Ca$^{2+}$ dependence of channel activation, and thereafter to various P$O_{2}$ 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 [Ca\(^{2+}\)]\(_i\) of 1 \(\mu\)M 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 \(\mu\)M Ca\(^{2+}\). This effect was perfectly reversible on reintroduction of 1 \(\mu\)M Ca\(^{2+}\) in the chamber (Fig. 4 A). When at a fixed [Ca\(^{2+}\)]\(_i\) of 1 \(\mu\)M the same patch was exposed to low PO\(_2\), no appreciable changes in single-channel activity or unitary current amplitude were observed (Fig. 4 B). The signal from the PO\(_2\)-measuring electrode is shown in the lower panel. Following this same experimental procedure, but using various [Ca\(^{2+}\)]\(_i\) (between 0.04 and 1 \(\mu\)M) and \(V_m\) values (between -30 and +30 mV), exposure to hypoxia did not alter \(K_{Ca}\) channel open probability (\(P_{\text{open}}\)) in the 16 patches tested (paired \(t\) test). Fig. 5 summarizes the effects of [Ca\(^{2+}\)]\(_i\) and low PO\(_2\) on \(K_{Ca}\) channel activity. In Fig. 5 A, single-channel \(P_{\text{open}}\) as a function of \(V_m\) is plotted at two [Ca\(^{2+}\)]\(_i\). In both cases the data points are the mean ± SE values from

![Diagram of experimental protocol](image-url)
two experiments. At +20 mV and with a \([\text{Ca}^{2+}]\) of 1 \(\mu\text{M}\), \(P_{\text{open}}\) 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 \(P_{\text{open}}\) on \([\text{Ca}^{2+}]\) together with the lack of effect of low \(\text{PO}_2\) at a \(V_m\) of +20 mV is further illustrated in Fig. 5 B. Thus, the results indicate that type I cells have \(\text{Ca}^{2+}\)- 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 \(P_{\text{open}}\) 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).

**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.

![Diagram](image-url)
conductance of 6 pS (Fig. 6 B, open symbols). Activation of SK channels did not require intracellular Ca$^{2+}$ 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 [Ca$^{2+}$] did not produce appreciable changes in the average $P_{\text{open}}$ 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 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 O$_2$ 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 $P_{\text{open}}$ ($\sim 0.21$ at $+20$ mV and $\sim 0.05$ at $-70$ mV) was not significantly affected by low PO$_2$ (paired t test).
Interestingly, $P_{\text{open}}$ 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).

**O$_2$-sensitive Channels (K$_{O_2}$ 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 $P_{\text{open}}$ is reversibly decreased by lowering PO$_2$ (see Table II).

![Figure 7](image)

**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. 8 A 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.

Since the K$_{O_2}$ 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 ± 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 ($P_{\text{open}}$) increases with membrane depolarization. The normalized $P_{\text{open}}$ (ordinate) as a function of the membrane potential (abscissa) is plotted in Fig. 9, indicating that the $P_{\text{open}}$ of $K_{\text{O2}}$ channels is a steep function of membrane voltage. The values of $P_{\text{open}}$ 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 ± SD; n = 2).

Low $P_{\text{O2}}$ decreases channel $P_{\text{open}}$. It was shown in Fig. 8A that on depolarization $K_{\text{O2}}$ 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 during a maintained depolarization channel activity ceases almost completely in <500 ms. Recovery from inactivation of the $K_{\text{O2}}$ 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 $P_{\text{O2}}$ that could produce nonspecific irreversible changes in membrane properties and to correct for the possible run down of the preparation, the effect of $P_{\text{O2}}$ levels on $K_{\text{O2}}$ channels was studied by alternating short exposures to hypoxic and normoxic solutions.

The major effects of low $P_{\text{O2}}$ 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 $P_{\text{O2}}$ 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 C1 to C6) and during exposure to hypoxia (periods H1 to H6) 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
Reversible inhibition of \(K_{o2}\) channel activity by lowering \(P_{O2}\). 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 (\(P_{O2} = 150\) mmHg) and hypoxic (\(P_{O2} < 5\) 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 \(P_{O2}\) 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 (\(n = 24, P_{open} = 0.31\)) and low \(P_{O2}\) (\(n = 15, P_{open} = 0.18\)) solutions are grouped together (\(EC/n\) and \(EH/n\), 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 μs. Solutions: standard Na, TTX//130 K, 10 EGTA.
recordings (panel B) show a peak $P_{\text{open}}$ at the beginning of the depolarization and a progressive decrease during the pulse. Single-channel $P_{\text{open}}$ integrated throughout the pulse duration (see Eq. 1) is 0.31 in the control solution but only 0.18 during exposure to low Po2. 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 Po2 on the $P_{\text{open}}$ of K$_2$ channels at different membrane potentials is shown in Fig. 11. The average $P_{\text{open}}$ value during 200-ms pulses was measured in patches with one or two functional K$_2$ channels. Low Po2 produced a decrease in single-channel $P_{\text{open}}$ that was statistically significant at all membrane voltages (paired t test, $\alpha < 0.01$). At +30 mV, for example, $P_{\text{open}}$ is ~0.43 in the control solution and ~0.31 in low Po2, 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 accompanying paper). For simplicity, in this set of experiments solutions were bubbled with either N$_2$ or air and thus the low Po2 values in the vicinity of membrane patches were ~5–10 mmHg (see Methods). With this protocol the decrease of $P_{\text{open}}$ by hypoxia is underestimated since it is known that on exposure to extremely low Po2 the inhibition of K$^+$ channel activity is relatively less pronounced than when the channels are exposed to moderately low Po2 values (between 60 and 80 mmHg; López-López et al., 1989; Ganfornina and López-Barneo, 1991).

**Figure 11.** Decrease of K$_2$ channel $P_{\text{open}}$ by hypoxia. Average open probability during 200-ms pulses (ordinate) was measured from either inside-out or outside-out patches at various membrane potentials. $P_{\text{open}}$ values measured in the control and in the low (<5 mmHg) Po2 solutions are represented by the mean ± 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.

Direct modulation of K$_2$ channels accounts for the properties of the macroscopic O$_2$-sensitive K$^+$ current. The kinetic and pharmacological properties of the K$_2$ 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$_2$ 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_o channels (Ganfornina and López-Barneo, 1991). The parallel time courses of K_o 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 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_open = 0.29), during a 9-min exposure to hypoxia (PO_2 < 5 mmHg; n = 18 consecutive pulses; P_open = 0.15), and after recovery in the normal PO_2 solution (n = 18 consecutive pulses; P_open = 0.27). Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μs. (C) Whole-cell currents recorded with the same experimental protocol. Effective cutoff frequency = 10 kHz and sampling interval = 500 μs. 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 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 channels can be estimated by dividing the peak K + current by the value of the single K_o 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 ± 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 MΩ).

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

![Image](https://example.com/image.png)

**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 K02 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_open = 0.52) and hypoxic (n = 16 sweeps, P_open = 0.28) conditions. Effective cutoff frequency = 0.95 kHz and sampling interval = 500 μs. Solutions: standard Na, TTX//130 K, 10 EGTA.

of K02 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 K02 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 K02 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 (Lópezen-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 K02 channels excised from a cell that had been preincubated in extreme hypoxia (Po2 = 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 K02 channel activity by low Po2 can be observed repeatedly after long-lasting exposure to extreme hypoxia, further suggesting that the O2-K02 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 K02 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 (KCa, SK, and K02 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 \( P_{O_2} \). 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 \( P_{O_2} \) (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 \( K_{Ca} \) 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 (Ganfornina, 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 \( P_{O_2} \), 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 Kₒ₂ 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). Kₒ₂ 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 Kₒ₂ 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 Kₒ₂ 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 (Určíka et al., 1989a) and strongly suggest that the Kₒ₂ 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 Kₒ₂ channels.

Modulation of Kₒ₂ Channels by O₂ Tension

A distinct property of Kₒ₂ channels is that their P_open decreases on exposure to low PO₂. The inhibition of channel activity by hypoxia is reversible and concentration dependent (Ganfornina 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 Kₒ₂ 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 $P_{O_2}$ 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 (Ganfornina, 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 Clapham, 1987; Brown and Birnbaumer, 1988), with negative results. Both GTP-$\gamma$-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 $P_{O_2}$. 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 \( P_{O_2} \) 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 \( P_{\text{open}} \) of \( K_{O_2} \) channels is reversibly modified by changes of \( P_{O_2} \) in a physiological range (Ganfornina and López-Barneo, 1991). The lack of appreciable desensitization in the \( O_2-K_{O_2} \) channel interaction may ensure that changes of the physico-chemical variable (\( O_2 \) 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 \( K_{O_2} \) 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.

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REFERENCES

Acker, H., and F. Pietruschka. 1977. Meaning of the type I cell for the chemoreceptive process: an electrophysiological study on cultured type I cells of the carotid body. In Chemoreception in the Carotid Body. H. Acker, S. Fidone, D. Pallot, C. Eyzaguirre, D. W. Lübbers, and R. W. Torrance, editors. Springer-Verlag, Berlin. 92–98.

Adams, D. J., and W. Nonner. 1989. Voltage-dependent potassium channels: gating, ion permeation and block. In Potassium Channels: Structure, Classification, Function and Therapeutic Potential. D. L. Cook, editor. Ellis Horwood Limited, New York. 40–69.

Almaraz, L., C. González, and A. Obeso. 1986. Effects of high potassium on the release of \(^{3}H\) dopamine from the cat carotid body in vitro. Journal of Physiology. 379:293–307.

Barrett, J. N., K. L. Magleby, and B. S. Pallotta. 1982. Properties of single calcium-activated potassium channels in cultured rat muscle. Journal of Physiology. 331:211–230.

Bigay, J., P. Deterre, C. Pfister, and M. Chabre. 1985. Fluoroaluminates activate transducin-GDP by mimicking the gamma-phosphate of GTP in its binding site. FEBS Letters. 191:181–185.

Biscoe, T. J., and M. R. Duchen. 1989. Electrophysiological responses of dissociated type I cells of the rabbit carotid body to cyanide. Journal of Physiology. 413:447–468.

Biscoe, T. J., and M. R. Duchen. 1990a. Responses of type I cells dissociated from the rabbit carotid body to hypoxia. Journal of Physiology. 428:39–59.

Biscoe, T. J., and M. R. Duchen. 1990b. Cellular basis of transduction in carotid chemoreceptors. American Journal of Physiology. 258:L271-L278.

Blat, A. L., and K. L. Magleby. 1987. Calcium-activated potassium channels. Trends in Neurosciences. 10:463–467.
Brown, A. M., and L. Birnbaumer. 1988. Direct G protein gating of ion channels. *American Journal of Physiology*. 254:H401-H410.

Colquhoun, D., and F. L. Sigworth. 1983. Fitting and statistical analysis of single channel records. In *Single Channel Recordings*. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 191–265.

Conti, F., B. Hille, and W. Nonner. 1984. Non-stationary fluctuations of the potassium conductance at the node of Ranvier of the frog. *Journal of Physiology*. 353:199–230.

Cooper, E., and A. Shrier. 1985. Single-channel analysis of fast transient potassium currents from rat nodose neurones. *Journal of Physiology*. 369:199–208.

Cooper, E., and A. Shrier. 1989. Inactivation of A currents and A channels on rat nodose neurons in culture. *Journal of General Physiology*. 94:881–910.

Cross, A. R., L. Henderson, O. T. G. Jones, M. A. Delpiano, J. Hentschel, and H. Acker. 1990. Involvement of an NAD(P)H oxidase as a Po2 sensor protein in the rat carotid body. *Biochemical Journal*. 272:743–747.

De Castro, F. 1926. Sur la structure et l'innervation de la glande intercarotidienne (glomerus caroticum) de l'homme et des mammifères, et sur un nouveau système d'innervation autonome du nerf glosso-faryngien. *Trabajos del Laboratorio de Investigaciones Biologicas de la Universidad de Madrid*. 24:365–432.

Delpiano, M. A., and J. Hescheler. 1989. Evidence for a Po2-sensitive K+ channel in the type-I cell of the rabbit carotid body. *FEBS Letters*. 249:195–198.

Dubois, J. M. 1983. Potassium currents in the frog node of Ranvier. *Progress in Biophysics and Molecular Biology*. 42:1–20.

Duchen, M. R., K. W. T. Caddy, G. C. Kirby, D. L. Patterson, J. Ponte, and T. J. Biscoe. 1988. Biophysical studies of the cellular elements of the rabbit carotid body. *Neuroscience*. 26:291–311.

Eyzaguirre, C., R. S. Fitzgerald, S. Lahiri, and P. Zapata. 1983. Arterial chemoreceptors. In *Handbook of Physiology: The Cardiovascular System*. J. T. Sheperd, and F. Abboud, editors. American Physiological Society, Bethesda, MD. 557–621.

Fidone, S. J., and C. González. 1986. Initiation and control of chemoreceptor activity in the carotid body. In *Handbook of Physiology: The Respiratory System II*. A. P. Fishman, editor. American Physiological Society, Bethesda, MD. 247–312.

Fidone, S. J., C. González, and K. Yoshizaki. 1982. Effects of low oxygen on the release of dopamine from the rabbit carotid body in vitro. *Journal of Physiology*. 333:93–110.

Fishman, M. C., W. L. Greene, and D. Platika. 1985. Oxygen chemoreception by carotid body cells in culture. *Proceedings of the National Academy of Sciences, USA*. 82:1448–1450.

Fitzgerald, R. S., and S. Lahiri. 1986. Reflex responses to chemoreceptor stimulation. In *Handbook of Physiology: The Respiratory System II*. A. P. Fishman, editor. American Physiological Society, Bethesda, MD. 513–562.

Ganfornina, M. D. 1991. Estudio electrofisiológico del canal K O2 de las células quimiorreceptoras del cuerpo carotídeo. Doctoral thesis. Universidad de Sevilla, Sevilla, Spain. 183 pp.

Ganfornina, M. D., and J. López-Barneo. 1991. Single K+ channels in membrane patches of arterial chemoreceptor cells are modulated by O2 tension. *Proceedings of the National Academy of Sciences, USA*. 88:2927–2930.

Gilles-González, M. A., G. S. Ditta, and D. R. Helinski. 1991. A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. *Nature*. 350:170–172.

Gilman, A. G. 1987. G proteins: transducers of receptor-generated signals. *Annual Review of Biochemistry*. 56:615–649.
Goldberg, M. A., S. P. Dunning, and H. F. Bunn. 1988. Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. *Science.* 242:1412–1415.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.

Hertzberg, T., S. Hellström, H. Lagercrantz, and J. M. Pequignot. 1990. Development of the arterial chemoreflex and turnover of carotid body catecholamines in the newborn rat. *Journal of Physiology.* 425:211–225.

Hescheler, J., M. A. Delpiano, H. Acker, and F. Pietruschka. 1989. Ionic currents on type-I cells of the rabbit carotid body measured by voltage-clamp experiments and the effect of hypoxia. *Brain Research.* 486:79–88.

Heymans, C., J. J. Bouckaert, and L. Dautrebande. 1930. Sinus carotidien et réflexes respiratoires. II. Influences respiratoires réflexes de l'acidose, de l'alcalose, de l'anhydride carbonique, de l'ion hydrogène et de l'anoxémie: sinus carotidiens et échanges respiratoires dans les poumons et au delà des poumons. *Archives Internationales de Pharmacodynamie et de Thérapie.* 39:400–408.

Hille, B. 1984. Ionic channels of excitable membranes. Sinauer Associates, Inc., Sunderland, MA. 426 pp.

Hoshi, T., and R. W. Aldrich. 1988. Voltage-dependent K+ currents and underlying single K+ channels in pheochromocytoma cells. *Journal of General Physiology.* 91:73–106.

Kasai, H., M. Kameyama, K. Yamaguchi, and J. Fukuda. 1986. Single transient K channels in mammalian sensory neurons. *Biophysical Journal.* 49:1243–1247.

Llano, I., C. K. Webb, and F. Bezanzilla. 1988. Potassium conductance of the squid giant axon. *Journal of General Physiology.* 92:179–196.

Logothetis, D. E., Y. Kurachi, J. Galper, E. J. Neer, and D. E. Claphan. 1987. The β subunits of GTP-binding proteins activate the muscarinic K+ channel in heart. *Nature.* 325:321–326.

López-Barneo, J., J. R. López-López, J. Ureña, and C. González. 1988. Chemotransduction in the carotid body: K+ current modulated by PO2 in type I chemoreceptor cells. *Science.* 241:580–582.

López-López, J., C. González, J. Ureña, and J. López-Barneo. 1989. Low PO2 selectively inhibits K channel activity in chemoreceptor cells of the mammalian carotid body. *Journal of General Physiology.* 93:1001–1015.

Marty, A. 1981. Ca-dependent K channels with large unitary conductance in chromaffin cell membranes. *Nature.* 291:497–500.

Marty, A., and E. Neher. 1985. Potassium channels in cultured bovine adrenal chromaffin cells. *Journal of Physiology.* 367:117–141.

Nielsen, A. M., G. E. Bisgard, and E. H. Vidruk. 1988. Carotid chemoreceptor activity during acute and sustained hypoxia in goats. *Journal of Applied Physiology.* 65:1796–1802.

Obeso, A., S. Fidone, and C. González. 1987. Pathways for calcium entry into type I cells: significance for the secretory response. In *Chemoreceptors in Respiratory Control.* J. A. Ribeiro and D. J. Pallot, editors. Croom Helm, London. 91–97.

Patlak, J., and R. Horn. 1982. Effect of N-bromoacetamide on single sodium channel currents in excised membrane patches. *Journal of General Physiology.* 79:333–351.

Peers, C. 1990. Effects of D600 on hypoxic suppression of K+ currents in isolated type I carotid body cells of the neonatal rat. *FEBS Letters.* 271:37–40.

Pérez-García, M. T., L. Almaraz, and C. González. 1990. Effects of different types of stimulation on cyclic AMP content in the rabbit carotid body: functional significance. *Journal of Neurochemistry.* 55:1287–1293.

Quandt, F. N. 1988. Three kinetically distinct potassium channels in mouse neuroblastoma cells. *Journal of Physiology.* 395:401–418.
Rigual, R., E. González, C. González, and S. Fidone. 1986. Synthesis and release of catecholamines by the cat carotid body in vitro: effects of hypoxic stimulation. Brain Research. 374:101–109.

Rudy, B. 1988. Diversity and ubiquity of K channels. Neuroscience. 25:729–749.

Stea, A., and C. A. Nurse. 1989. Chloride channels in cultured glomus cells of the rat carotid body. American Journal of Physiology. 257:C147–C181.

Stea, A., and C. A. Nurse. 1991. Whole-cell and perforated-patch recordings from O₂-sensitive rat carotid body cells grown in short- and long-term culture. Pflügers Archiv. 418:93–101.

Stebbens, W. C., C. H. Brown, and M. R. Peterson. 1984. Sensory function in animals. In Handbook of Physiology. Section I. American Physiological Society, Bethesda, MD. 3:123–184.

Sternweis, P. C., and A. G. Gilman. 1982. Aluminum: a requirement for activation of the regulatory component of adenylate cyclase by fluoride. Proceedings of the National Academy of Sciences, USA. 79:4888–4891.

Tabares, L., J. Ureña, and J. López-Barneo. 1989. Properties of calcium and potassium currents of clonal adrenocortical cells. Journal of General Physiology. 93:495–519.

Tsacopoulos, M., and A. Lehmenkühler. 1977. A double-barrelled Pt-microelectrode for simultaneous measurements of PO₂ and bioelectrical activity in excitable tissues. Experientia. 33:1337–1338.

Tsacopoulos, M., S. Poitry, and A. Borsellino. 1981. Diffusion and consumption of oxygen in the superfused retina of the drone (Apis mellifera) in darkness. Journal of General Physiology. 77:601–628.

Ureña, J., J. López-López, C. González, and J. López-Barneo. 1989a. Ionic currents in dispersed chemoreceptor cells of the mammalian carotid body. Journal of General Physiology. 93:979–999.

Ureña, J., J. C. Mateos, and J. López-Barneo. 1989b. Low-cost system for automated acquisition, display and analysis of transmembrane ionic currents. Medical and Biological Engineering and Computing. 27:94–98.

Wary, W. J., C. F. Cheng, B. G. Dinger, and S. Fidone. 1989. Effect of hypoxia on cyclic nucleotide formation in rabbit carotid body in vitro. Neuroscience Letters. 105:164–168.