Low pO$_2$ Selectively Inhibits K Channel Activity in Chemoreceptor Cells of the Mammalian Carotid Body

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ABSTRACT   The hypothesis that changes in environmental O$_2$ tension (pO$_2$) could affect the ionic conductances of dissociated type I cells of the carotid body was tested. Cells were subjected to whole-cell patch clamp and ionic currents were recorded in a control solution with normal pO$_2$ (pO$_2$ = 150 mmHg) and 3–5 min after exposure to the same solution with a lower pO$_2$. Na and Ca currents were unaffected by lowering pO$_2$ to 10 mmHg, however, in all cells studied (n = 42) exposure to hypoxia produced a reversible reduction of the K current. In 14 cells exposed to a pO$_2$ of 10 mmHg peak K current amplitude decreased to 35 ± 8% of the control value. The effect of low pO$_2$ was independent of the internal Ca^{2+} concentration and was observed in the absence of internal exogenous nucleotides. Inhibition of K channel activity by hypoxia is a graded phenomenon and in the range between 70 and 120 mmHg, which includes normal pO$_2$ values in arterial blood, it is directly correlated with pO$_2$ levels. Low pO$_2$ appeared to slow down the activation time course of the K current but deactivation kinetics seemed to be unaltered. Type I cells subjected to current clamp generate large Na- and Ca-dependent action potentials repetitively. Exposure to low pO$_2$ produces a 4–10 mV increase in the action potential amplitude and a faster depolarization rate of pacemaker potentials, which leads to an increase in the firing frequency. Repolarization rate of individual action potentials is, however, unaffected, or slightly increased. The selective inhibition of K channel activity by low pO$_2$ is a phenomenon without precedents in the literature that explains the chemoreceptive properties of type I cells. The nature of the interaction of molecular O$_2$ with the K channel protein is unknown, however, it is argued that a hemoglobin-like O$_2$ sensor, perhaps coupled to a G protein, could be involved.

INTRODUCTION

Type I, or glomus, cells of the mammalian cartoid body are thought to be the primary chemoreceptors mediating the hyperventilation produced in response to a

decrease in arterial O_2 tension (pO_2). Hypoxia ultimately induces in these cells the release of transmitters which, in turn, set the level of electrical activity in the afferent fibers of the carotid sinus nerve (see for reviews Eyzaguirre and Zapata, 1968; Fidone and González, 1986). Although several models of chemotransduction have been postulated (Belmonte and González, 1983), the basic mechanisms involved in the detection of the hypoxic stimulus remain unknown.

It has recently been shown in taste and olfactory cells that chemical stimuli interact with membrane receptors and, by altering ionic conductances, induce modifications in the electrical properties of the cells (Nakamura and Gold, 1987; Avenet et al., 1988; Kinnamon and Roper, 1988; Tonosaki and Funakoshi, 1988), therefore it is logical to hypothesize that similar phenomena could occur in type I cells. In previous electrophysiological studies in type I cells, performed with intracellular microelectrodes, it was found that they were unexcitable and that their electrical parameters were unaltered by changes in environmental pO_2 (Acker and Pietruschka, 1977; Eyzaguirre et al., 1983). However, in these studies the cells were most probably damaged by the microelectrode since, as was shown in the preceding article (Ureña et al., 1989), type I cells subjected to voltage and current clamp have an appreciable density of Na, Ca, and K channels and they can generate large action potentials. These results explain previous experiments indicating that transmitter release in type I cells induced by hypoxia and high external potassium was blocked by Ca channel antagonists (Almaraz et al., 1986; Obeso et al., 1987). The electrophysiological properties of these cells are further documented in this article; it is shown that a K conductance is selectively and reversibly inhibited by a decrease in pO_2. This is a mechanism of ion channel modulation without precedents in the literature, and which is not present in other cells tested, but which indicates that in type I cells membrane ionic channels directly participate in chemotransduction. Some of these results have appeared in a short report (López-Barneo et al., 1988).

METHODS

Experiments were performed on type I cells enzymatically dispersed from rabbit carotid bodies. In a few experiments currents were also recorded from type II cells and from dispersed guinea pig septal neurons. Procedures of cell dissociation and culturing, the concentrations of the solutions, and recording technique were as described in the preceding paper (Ureña et al., 1989). In the experiments designed to record potassium tail currents an external solution with high K concentration was used. The composition of this solution is given in the figure legends. During the experiments cells were transferred to a small chamber that had a continuous flow of solutions that could be changed in 15–20 s. Solutions were equilibrated with air, N_2, or a mixture of both, and O_2 tension in the chamber was monitored with an O_2 electrode (model 53; Yellow Springs Instruments, Yellow Springs, OH). The protocol that was used to study the effect of altering pO_2 on the different ionic currents of type I cells was as follows: a cell was patch clamped and the whole-cell recording mode was initiated with an external solution equilibrated with air ($pO_2 = 150$ mmHg). After a period of 3–5 min, during which control current traces were acquired, the same cell was exposed to a test solution of the same composition but equilibrated with a gas mixture of lower pO_2. In most experiments reversibility was checked and current recordings were also obtained 3–5 min after switching to the control solution.

RESULTS

Low pO2 Reversibly Reduces K Current Amplitude in Type I Cells

Fig. 1 illustrates the effect of decreasing pO_2 from 150 to 10 mmHg on the different ionic current components previously identified in type I cells (Ureña et al., 1989). The currents were recorded during depolarization to +40 mV that lasted 8 ms. These traces clearly show that the inward current and the tail was unaffected by low pO_2 but the outward current was reversibly reduced in amplitude.

The effect of hypoxia on the different ionic currents in isolation is shown in Fig. 2. *A* shows Na and Ca currents recorded in a cell where K conductance was blocked by internal Cs. The traces were obtained during 7-ms depolarizations to 0 mV from a holding potential of −80 mV in the control solution and 3 min after switching to low pO_2. The recordings are perfectly superimposable, which indicates that neither sodium nor calcium currents were affected by hypoxia. Na currents recorded in a different cell after blockade of Ca channels by bath application of 0.5 mM Cd are shown in Fig. 2 *B*. Control and low pO_2 traces, recorded with a time interval of 5 min. were also almost identical. We have tested the effect of low pO_2 on Na and Ca
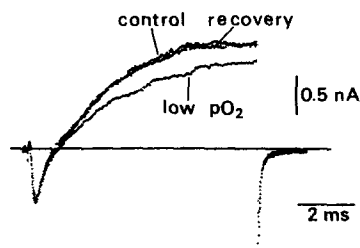

FIGURE 1. Effect of lowering pO_2 from 150 (control and recovery) to 10 mmHg (low pO_2) on the different current components recorded in type I cells. Currents were elicited by depolarization to 40 mV from a holding potential (HP) of −80 mV. Solutions (in millimolar): 140 Na, 5 Ca//130 K, 3 Mg-ATP, and 0.1 μM Ca (3 mM Ca and 5 mM EGTA). Experiment MZ2188J.

currents in 18 cells with similar results. In some of these cells, as in the example shown in Fig. 1, K currents were also simultaneously recorded and therefore the effect of hypoxia on the different ionic currents could be compared in the same cell. A decrease of pO_2 had, however, a marked effect on K currents (Fig. 2 *C*). In this cell exposure to a pO_2 of 10 mmHg produced a 45% reduction in the amplitude of the K current recorded by a depolarization to 40 mV, which reverted almost completely after we reintroduced a solution with normal O_2 content. Changes in the current amplitude occurred roughly with the time course of bath exchange and could be repeated several times in the same cell. It has been previously shown that during a maintained depolarization the K current in type I cells reaches a maximal value in ∼20 ms, and thereafter inactivates almost completely (Ureña et al., 1989). The effect of low pO_2 on the time course of the K current is shown in Fig. 2 *D* by current traces recorded during voltage pulses lasting 200 ms.

These results indicate that K channels of type I cells are selectively modulated by environmental pO_2. The reversible reduction of K current amplitude in response to lowering pO_2 was observed in every cell tested ($n = 42$). In 14 cells subjected to a

pO_2 of 10 mmHg, the reduction of the peak K current amplitude ranged between 25 and 50% with an average value of 35 ± 8% (mean ± SD).

Effects of Hypoxia Are Independent of Internal Concentrations of Calcium and Adenosine Triphosphate

During the initial experiments, which were designed to test the effects of low pO_2 on K currents, we used pipette solutions with Ca concentrations between 0.1 and 0.5 μM, and with 3 mM Mg-ATP added to retard wash-out of Ca channels (Kostyuk, 1984; Forscher and Oxford, 1985). Subsequent experiments were performed using internal solutions without any ATP but with 10 mM EGTA added (estimated Ca concentration, $<10^{-9}$ M) to check the possibility that either of these two variables could affect the response of K channels to hypoxia. All these experiments were
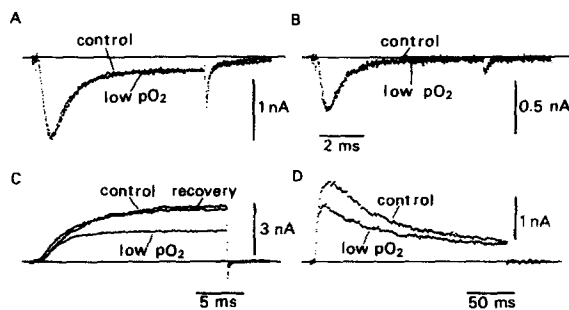
FIGURE 2. Effect of lowering pO_2 on the different ionic currents in isolation. (A) Sodium and calcium currents recorded during voltage steps to 0 mV from a HP of −80 mV in the control solution (pO_2 = 150 mmHg) and 3 min after exposure to low pO_2 (10 mmHg). Solutions (in millimolar): 140 Na, 2.5 Ca//130 Cs, 3 Mg-ATP, and 10 EGTA. (B) Sodium currents recorded in the control solution and 5 min after switching to the low pO_2 solution. The test pulse was to 10 mV with a return to a HP of −80 mV. Solutions (in millimolar): 140 Na, 0.5 Cd//130 Cs, 3 Mg-ATP, and 10 EGTA. (C) Potassium currents recorded during pulses to 40 mV with a return to −80 mV in the control solution and during exposure to low pO_2. The recovery trace was obtained 3 min after the solution with normal pO_2 was introduced again in the chamber. Solutions (in millimolar): 140 Na, 5 Ca, TTX//130 K, 3 Mg-ATP, and 0.5 μM ionic Ca (4.44 Ca and 5 EGTA). (D) Potassium currents elicited by 200-ms pulses to 40 mV with a return to −80 mV in the control solution and during exposure to low pO_2. Solutions (in millimolar): 140 Na, 5 Ca, TTX//130 K, 3 Mg-ATP, and 10 EGTA. Experiments (A) FE0288K, (B) FE0388K, (C) FE0588J, (D) FE0588K.

done 5–8 min after the beginning of the whole-cell recording mode to allow for a complete equilibration of the pipette solution with the intracellular milieu (Fernández et al., 1984). In the absence of exogenous ATP we have observed an almost complete disappearance of the Ca current in type I cells (see Fig. 10 of the preceding paper), which is possibly due to dilution of nucleotides and other cytosolic components (Forscher and Oxford, 1985; Cota, 1986). Fig. 3 illustrates that in these experimental conditions K current amplitude was also decreased by lowering pO_2 to 88 mmHg, which indicates that internal Ca and ATP does not significantly change the response of K channels to hypoxia. Control and low pO_2 traces were recorded during depolarizations to (A) 20, (B) 40, and (C) 60 mV from a holding potential of −80 mV. The plot in D illustrates the current-voltage relations in the control solution (squares) and during exposure to hypoxia (dots).

K Current Amplitude Is Modulated by O_2 Tension

Fig. 4 *A* shows K current records elicited by pulses to 40 mV in the control solution and during exposure to a pO_2 of 88 mmHg. Current amplitude in low pO_2 decreased to ~42% of the control value and during recovery it reached an amplitude even larger than before exposure to hypoxia (see below). In the same cell lowering pO_2 to 132 mmHg (Fig. 4 *B*) produced a reduction of the current to only 65% of the control value. Reversibility in this second case was almost perfect. Thus, there is a correlation between the amplitude of the K current and pO_2 levels. This relationship is further illustrated in Fig. 4 *C* where the fraction of the current inhibited by low pO_2 (ordinate) is plotted as a function of O_2 tension (abscissa). Each point of
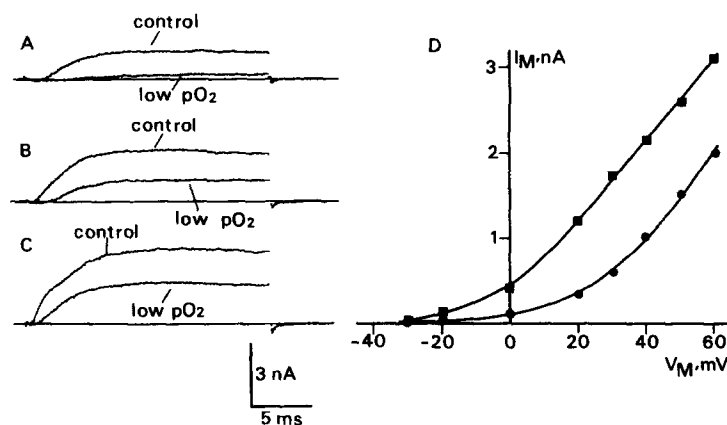

FIGURE 3.   Reduction in potassium current amplitude by hypoxia in the absence of internal calcium and exogenous ATP. (*A–C*) Potassium currents recorded during depolarizations to (*A*) 20, (*B*) 40, and (*C*) 60 mV with a return to −80 mV in a solution with normal O_2 content (control, pO_2 = 150 mmHg) and 3–5 min after switching to low pO_2 (88 mmHg). (*D*) Peak current amplitude vs. voltage curves in the control solution (squares) and during exposure to hypoxia (dots). The experiment was done 8–10 min after the beginning of the whole-cell recording to allow for a complete equilibration of the pipette solution with the cytosol and wash-out of calcium channels. Solutions (in millimolar): 140 Na, 5 Ca, TTX//130 K, and 10 EGTA (estimated free Ca, $<10^{-9}$ M); no ATP was added. Experiment AB0888J.

the plot is from a different cell and in all cases the effect of hypoxia was reversible. In these experiments currents were recorded during depolarizations to 40 mV and the internal solution contained 10 mM EGTA but no ATP. The data show that between 80 and 150 mmHg, which is a range that includes the normal pO_2 values in arterial blood, reduction of the K current is more or less linearly related to the decrease in environmental O_2. The shape of the line between 10 and 70 mmHg is for the moment difficult to explain; it may have some unknown physiological significance. These results indicate that inhibition of K channel activity by hypoxia is a graded phenomenon, directly correlated with pO_2 levels, and probably due to a modulatory effect of O_2 on K channels.

Effects of Hypoxia on K Current Kinetics

Although the effects of low pO_2 on K current kinetics were not studied in detail it was commonly observed that during hypoxia, the activation time course was somewhat slower than in the control solution. An opposite effect was observed in the recovery currents, which often had larger amplitudes and faster activation kinetics than the control recordings (see Figs. 2 C and 4 A). The effect of lowering pO_2 on activation and deactivation kinetics was studied in two cells bathed with a high K solution. In Fig. 5 A it is shown that upon depolarization to 40 mV an outward current was elicited and then, at the instant of the repolarization, it was followed by a large tail K current. Lowering pO_2 to 84 mmHg produced the typical reversible decrease in current amplitude. Expanded current traces recorded before and dur-
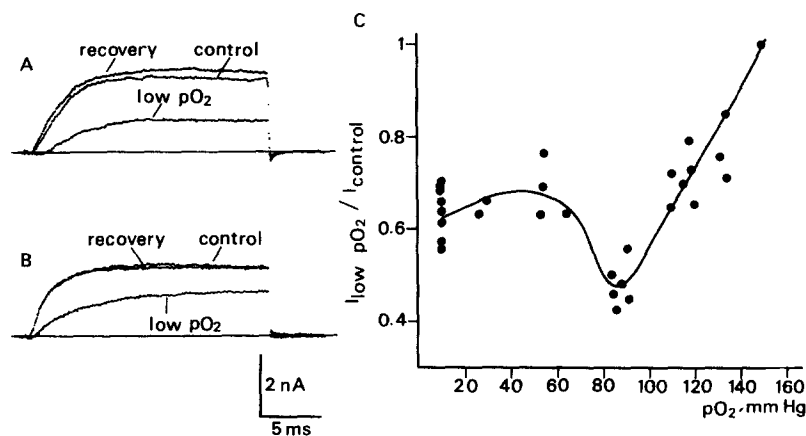

FIGURE 4.   Relative effects of different pO_2 levels. Reversible reduction in potassium current amplitude by a decrease in pO_2 from 150 (control) to 88 (low pO_2, A) and 132 (low pO_2, B) mmHg. Recordings are from the same cell and in all cases were obtained during voltage steps to 40 mV with a return to −80 mV. Experiment AB0888J. (C) Relative peak current amplitude (ordinate) at different pO_2 levels (abscissa). Each data point is from a different experiment but in all cases the current amplitude was measured during depolarizations to 40 mV. The line was drawn by eye. Solutions used in all experiments were (in millimolar): 140 Na, 5 Ca, TTX//130 K, and 10 EGTA; no ATP was added.

ing hypoxia, scaled to the same amplitude, are compared in Fig. 5 B, which shows the slower activation time course of the recording in low pO_2. By contrast, deactivation kinetics appear to be unaltered by hypoxia since, as illustrated in Fig. 5 C, the time course of tail currents recorded in both situations are almost indistinguishable.

Low pO_2 Changes the Firing Pattern in Type I Cells

It was shown in the preceding article that, as expected from the voltage-clamp recordings, current-clamped type I cells were able to generate action potentials spontaneously. The shape of the action potentials varied from cell to cell, which is due, at least partially, to variabilities in the magnitude of the different ionic conduc-

tances. In this section it is illustrated that by altering K conductance low pO_2 also modifies the firing properties of the cells.

Fig. 6, A and C are examples of action potentials recorded in type I cells. They have been chosen because they illustrate the typical fast (A) and slow (C) spikes recorded in our experiments, although in most occasions action potentials had intermediate characteristics between these two extreme examples. Ionic currents recorded in these same cells during depolarizations to 0 and 40 mV are shown in B and D, respectively. The figure clearly shows that in cells with large inward and outward currents the action potentials lasted 4–5 ms and had fast depolarizing and repolarizing rates (A and B). The overshoot potential (36 mV) was near the Na equilibrium potential. In this cell Ca current density was small as indicated in (Fig. 6 B) by the small size of the tails at the instant of repolarization (see also the preceding
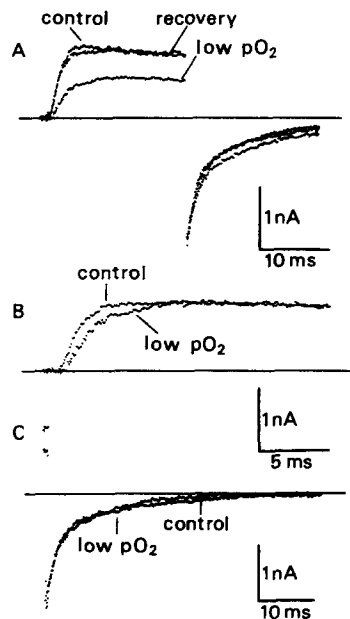

FIGURE 5. Effects of hypoxia on potassium current kinetics. (A) Reversible reduction in potassium current amplitude by a decrease in pO_2 from 150 (control and recovery) to 84 (low pO_2) mmHg in the presence of high external potassium. B shows traces scaled to the same amplitude to illustrate the slower activation time course of the potassium current during exposure to low pO_2. Scaled tail currents in C show that the deactivation kinetics were almost unaltered. Solutions (in millimolar): 60 NaCl, 80 KCl, 5 $CaCl_2$, 10 HEPES, TTX//130 K, and 10 EGTA. Experiment AB1288J.

paper). By contrast, in cells with a Ca current larger than the Na current the action potentials had a smaller amplitude and slower time course (C and D). Regardless of the density of the different current components in all type I cells exposed to low pO_2, action potentials became larger and the firing frequency increased.

Fig. 7 shows voltage- and current-clamp recordings alternately obtained from the same cell. Fig. 7 A shows superimposed current traces elicited by depolarization to 40 mV before, during, and after exposure to hypoxia (pO_2 = 10 mmHg). As shown in previous figures the inward Na current and the Ca tail were unaltered by lowering pO_2, however the outward K current was reversibly reduced in amplitude. Fig. 7 B illustrates that switching from voltage to current clamp (V-clamp off) produced an almost instantaneous depolarization, followed by a slow pacemaker potential which preceded the action potential upstroke that was initiated at ~ − 38 mV. Termination of the spike was followed by another slow pacemaker depolarizing potential.
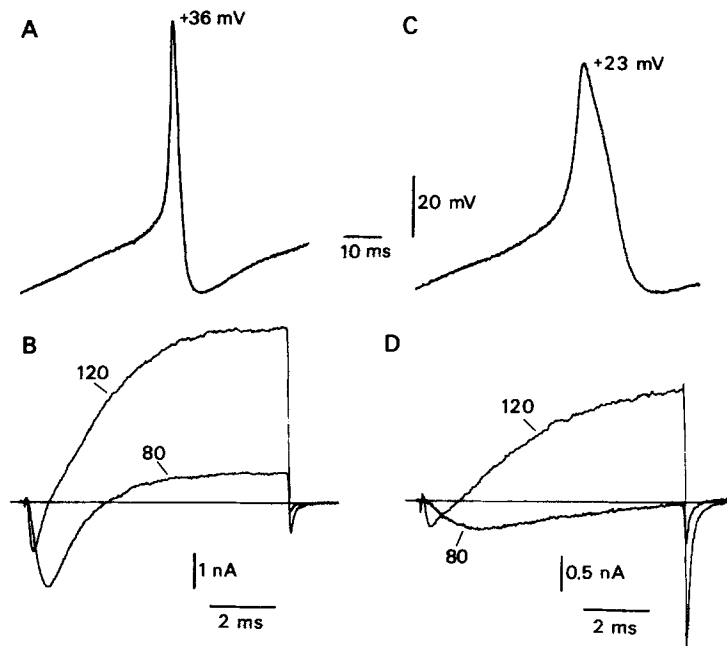
FIGURE 6. Fast and slow action potentials in type I cells. Current (A and C) and voltage (B and D) clamp recordings obtained alternately from two cells. The amplitudes of the voltage steps are indicated next to each voltage-clamp recording. HP, -80 mV. Solutions (in millimolar): 140 Na, 5 Ca//130 K, 3 Mg-ATP, and 0.1 μM ionic calcium (3 Ca and 5 EGTA). Experiments (A and B) MZ1888J and (C and D) MZ2188J.
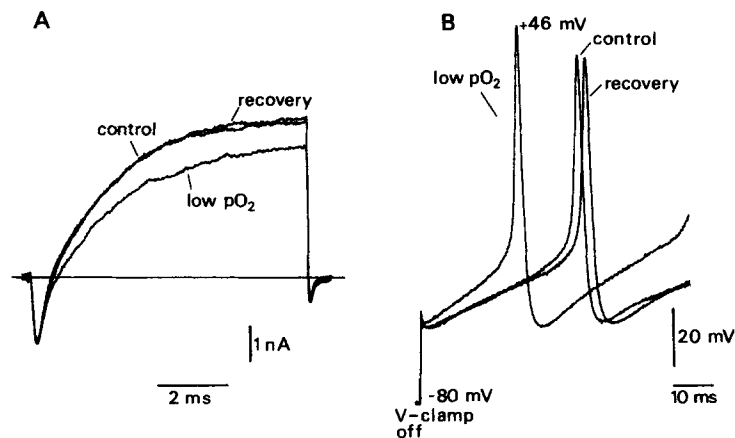
FIGURE 7. Effect of lowering pO_2 on (A) whole-cell current and (B) voltage. (A) Ionic currents recorded with a pO_2 of 150 (control and recovery) and 10 (low pO_2) mmHg. Voltage steps were to 40 mV with a return to -80 mV. (B) Current-clamp recordings from the same cell and in the same experimental conditions. Transition from voltage to current-clamp modes is indicated in the figure (V-clamp off). Solutions (in millimolar): 140 Na, 5 Ca//130 K, 3 Mg-ATP, 0.1 μM ionic calcium. Experiment MZ1888J.

The rate of repolarization of the action potential recorded in the control solution was 26 V/s and the slope of the pacemaker potential was 0.6 V/s. The action potential generated during exposure to hypoxia had an overshoot potential (46 mV) that was ~10 mV more positive than in the control recording, and a slightly faster repolarizing rate (30 V/s), however, the most apparent effect was an almost twofold increase (from 0.6 to 1 V/s) in the slope of the depolarizing pacemaker potentials before and after the spike. Recovery of all electrical parameters was, as occurred with the voltage-clamp recordings, almost perfect. Identical qualitative results are illustrated in Fig. 8, which shows recordings from a cell in which, as shown before in Fig. 6 D, the inward current was mainly carried by Ca ions. In this experiment the repolarizing rates of the first action potential recorded in both experimental condi-
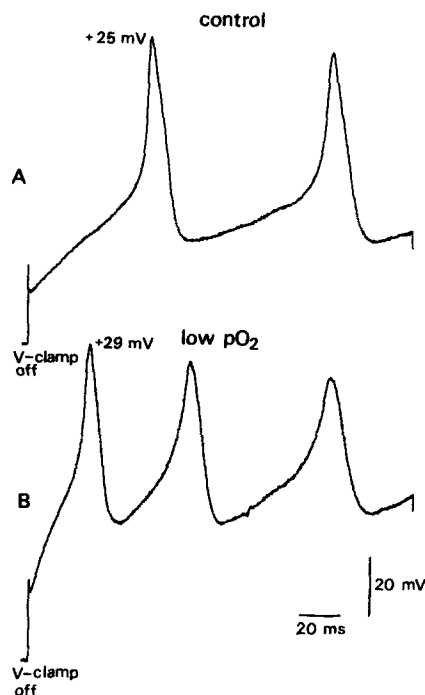

FIGURE 8. Increase in firing frequency by exposure to low pO_2. Current-clamp recordings in a cell bathed in a solution with normal O_2 content (A, control, $pO_2 = 150$ mmHg) and during exposure to a pO_2 of 10 mmHg (B, low pO_2). Transitions from voltage to current-clamp mode are indicated in the figure (V-clamp off). Solutions (in millimolar): 140 Na, 5 Ca//130 K, 3 Mg-ATP, and 0.1 μM ionic calcium. Experiment MZ2188J.

tions were only slightly different (4.6 and 5.4 V/s in control and low pO_2, respectively), whereas the rate of depolarization of pacemaker potentials in low pO_2 (2 V/s) was almost three times larger than in the control solution (0.7 V/s). These results indicate that low pO_2, by reducing the K conductance of type I cells, produces an increase in the action potential amplitude and in the slope of pacemaker potentials that determine the firing frequency of the cells. These two effects probably result in an increase of Ca entry into the cytosol, which in turn enhances transmitter release.

The O_2-sensitive K Current Is also Reduced by Lowering External pH

Although the strongest response in the carotid body is obtained by a decrease in O_2 tension, it is known that other stimuli, for example the acidification of the external
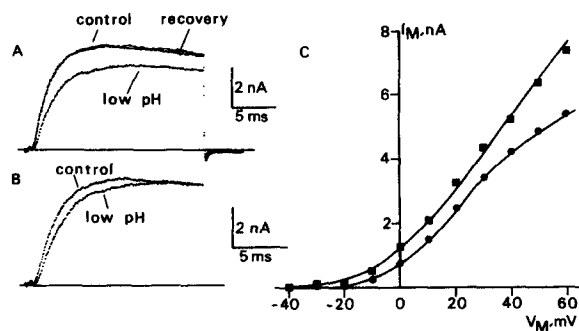
FIGURE 9. Effect of lowering external pH on the O_2-sensitive potassium current. (A) Potassium currents recorded during voltage steps to 40 mV with return to −80 mV in solutions with pH 7.4 (control and recovery) and 7.0 (low pH). (B) Control and low pH traces are scaled to the same amplitude to compare the activation time course of the current in both experimental situations. (C) Peak current vs. voltage relation in normal (squares) and low pH (dots). The lines were drawn by eye. Solutions (in millimolar): 140 Na, 5 Ca, TTX//130 K, and 10 EGTA. Experiment AB1588K.

milieu, can induce transmitter release in type I cells (Rigual et al., 1984). In two cells we tested the effect of lowering pH from 7.4 to 7.0 on the O_2-sensitive K current of type I cells. Fig. 9 illustrates K currents recorded during depolarizations to 40 mV following a protocol similar to that used while testing the effect of alterations in pO_2. Fig. 9 A shows that low external pH produces an ~20% reduction in current amplitude with a complete reversibility. In Fig. 9 B control and low pH traces are scaled to the same amplitude to illustrate that acidification of the external solution also slows down potassium activation kinetics. The plot in Fig. 9 C is the peak current vs. voltage relation in the control solution (squares) and during exposure to low pH (dots). These results indicate that the O_2-sensitive K current of type I cells is also reduced by lowering pH within a physiological range. This phenomenon could contribute to type I cell activation by low pH although other mechanisms may be also involved. The effect of pH on the O_2-sensitive K current of the carotid body is not totally specific since it is known that acidification, using a much larger pH change than in our experiments, blocks voltage-dependent ionic currents from other preparations (Hille, 1968).

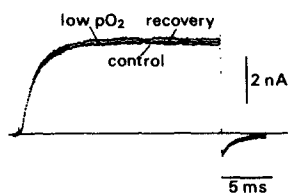FIGURE 10. Potassium currents recorded in a septal neuron during exposure to pO_2 tensions of 150 (control and recovery) and 10 (low pO_2) mmHg. Currents were elicited by 20-ms depolarizations to 60 mV from a holding potential of −80 mV. Solutions (in millimolar): 130 Na, 10 Ca, TTX//130 K, 3 Mg-ATP, and 10 EGTA. Experiment MA0488F.

Low pO_2 and K Current in Other Cells

The effect of lowering O_2 tension on the K channels of type I cells is a phenomenon with a clear physiological significance, however it is important to know whether this is a mechanism of ion channel modulation specific to the carotid body or if environmental pO_2 also interacts with K channels in other cells. We designed experiments to test the effect of pO_2 on the K current of type II cells which, as shown previously (Ureña et al., 1989), lack Na and Ca channels and only have a small population of K channels. Lowering pO_2 did not seem to affect the outward current of type II cells. In most type II cells, however, a measurable current is only recorded with very large depolarizations, and at these membrane potentials current amplitude has large fluctuations, which prevents the recording of stable control traces and the detection of small changes in current amplitude. A second test was performed on dispersed guinea pig septal neurons which, as shown in Fig. 10, can generate a large outward K current. The traces were obtained during depolarizations to 60 mV from a holding potential of -80 mV with an experimental protocol similar to that followed in the experiments on type I cells. The figure illustrates that neither current amplitude nor the activation kinetics are significantly altered by lowering pO_2 to 10 mmHg; current during hypoxia is even slightly larger that in the control solution. Thus, these results suggest that the effect of hypoxia reported in this article may be specific to the K channels of type I cells.

DISCUSSION

The results show the existence in type I cells of the carotid body of a K conductance modulated by environmental pO_2. This finding and the discovery in these cells of voltage-dependent ionic conductances (Ureña et al., 1989) shed light on the mechanism of sensory transduction in the carotid body and indicate that membrane ionic conductances are critically involved in chemoreception.

Interaction of O_2 with K Channels

The modulatory action of pO_2 on K conductance is a phenomenon without precedents in the literature and is probably specific to the K channels of type I cells. In this same preparation Na and Ca channels are unaltered by hypoxia and we have also observed that, using the same experimental protocol, both the K current of dispersed mammalian septal neurons and the small outward current of type II cells seem to be unaffected by changes in pO_2. The number of different cell types tested so far is, however, very limited and therefore it cannot be discounted that O_2 could interact with K channels of other tissues, either in normal physiological situations or during adaptation to changes in environmental conditions.

The effect of pO_2 on K conductance of type I cells could be a result of a direct action on the channels, perhaps coupled to an O_2 sensor, or it may require the action of soluble intracellular mediators. In a number of preparations, including taste and olfactory chemoreceptor cells, it is known that different substances turn on and off K and other channels, by increasing cytosolic concentrations of cyclic AMP and other nucleotides, which, in turn, alter the channel's conducting state either directly (Fesenko et al., 1985; Nakamura and Gold, 1987) or by inducing

phosphorylation of the channel protein (Levitan, 1985; Avenet et al., 1988). Our experiments suggest that soluble intracellular mediators do not participate in the response of K channels to hypoxia since it was unaffected by altering intracellular Ca and ATP and was observed in cells dialyzed for >15–20 min, long enough to produce a large dilution of cytosolic components due to the equilibration of the pipette solution with the intracellular milieu (Fernández et al., 1984; Forscher and Oxford, 1985; Cota, 1986).

Although we know of no experimental evidence indicating the existence of O_2-binding proteins in plasma membranes, there are some observations in the literature that suggest a role for hemoglobin in the regulation of ion transport in erythrocytes. Hemoglobin binds to the inner surface of the erythrocyte membrane and it has been hypothesized that according to its degree of oxygenation it could interact with membrane integral proteins and influence specific transport functions (cf. Motais et al., 1987). Hemoglobin has not been reported to be present in type I cells although the possible existence in the carotid body of a hemoglobin-like chromophore with low O_2 affinity has been postulated (Lloyd et al., 1968; Lahiri, 1977). Another possibility is that the putative O_2 sensor of type I cells could be linked to K channels through the action of G proteins, messenger molecules that diffuse within the surface membrane and are involved in the regulation of K channel permeability in several preparations (Brown and Birnbaumer, 1988; Neer and Claphan, 1988). None of these hypotheses have for the moment experimental support but they may serve as guides for future research work.

The nature of the effect of changes in pO_2 on K channel protein is also unknown but it probably requires strong molecular interactions. The effect of hypoxia on K current amplitude is reversible but, although not studied in detail, in most experiments we observed modifications in K current kinetic properties during and after exposure to hypoxia. We do not know whether the reduction in K current amplitude induced by low pO_2 is a result of a decrease in the probability of channel opening or of a reduction of single-channel conductance. These and other kinetic aspects may be partially answered by studying the effects of hypoxia on single K channel recordings.

Physiological Significance of K Channel Modulation by pO_2

Our experiments clearly show that the reduction of K current amplitude is directly dependent on the degree of hypoxia in the pO_2 range between 70 and 120 mmHg, which is close to the values of O_2 tension in arterial blood. This observation has therefore an unequivocal physiological significance although the curve relating K current reduction and pO_2 is displaced 20 to 30 mmHg to higher pO_2 levels with respect to the curve that relates firing frequency in afferent fibers of the sinus nerve and arterial pO_2 in vivo (Hornbein, 1968). This last relation represents the integration of a number of cellular processes, including chemoreception, transmitter release, and depolarization of nerve terminals. This may explain the differences referred to above. It is, however, also possible that the O_2 tensions measured in the bulk of the solution during our experiments are different from the pO_2 levels in the relatively unstirred layer of solution surrounding type I cells.

The decrease in K current amplitude induced by hypoxia produces dramatic

changes in the firing properties of type I cells that are most probably of importance for stimulus-secretion coupling. In all experiments action potentials during exposure to hypoxia reached an overshoot potential 4–10 mV more positive than in the control solution despite the fact that the repolarization rate was unaffected, or even slightly increased. Low pO_2, however, had a marked effect on the slope of pacemaker potentials determining the interspike intervals. These results suggest that the K current modulated by pO_2 does not have a significant role in spike repolarization, but that it participates in regulating the firing frequency of the cells. As shown in the preceding article and in this report, the O_2-sensitive K current inactivates in the range of 100–200 ms and has properties similar to transient K currents that participate in pacemaking in other excitable cells (Connor and Stevens, 1971). Low pO_2 may also induce the closure of some K channels that are open at the resting potential and produce an increase in the input resistance of the cells, which would contribute to the larger amplitude of the action potentials during hypoxia. Apart from other possible mechanisms, the increase in action potential amplitude and, more critically, in the firing frequency of type I cells during exposure to low pO_2 would enhance Ca entry through voltage-dependent Ca channels and would thus explain the well known increase in the secretory rate induced by hypoxia (Fidone and González, 1986).

Effects of Other Chemical Stimuli on the O_2-sensitive K Current

It is known that other chemical stimuli, such as acidification of the external solution and an increase in CO_2 levels, also induce transmitter release in type I cells and increase the firing frequency of fibers in the carotid sinus nerve. These stimuli are however less potent than hypoxia (Biscoe et al., 1970, Rigual et al., 1984; Fidone and González, 1986). The effect of CO_2 was not tested in our experiments but we have shown that the O_2-sensitive K current is also reduced by lowering external pH from 7.4 to 7.0. These values are within the range of physiological pH changes and fall below pH 7.56, which is considered to be within normoxic conditions the threshold for activation of the carotid body in vivo (Fitzgerald and Parks, 1971). The effect of pH has a clear physiological significance in the carotid body but it is not totally specific to type I cells since other voltage-dependent currents are also blocked by protons (Hille, 1968). It is, however, important to note that the pH change required for blocking ionic currents in nerve is larger than the one used in our experiments, which suggests that the K current of type I cells could also be particularly sensitive to pH. Future experiments must be conducted to further characterize the effect of pH on the ionic currents of type I cells and to study in this preparation the possible interactions between pO_2, pCO_2, and pH.

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