ciently competed with sonicated and denatured calf thymus DNA (5 μg/ml) and therefore this nonspecific competitor was substituted for poly(dI-dC) · poly(dI-dC) in all the screens described.
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18. The mRNA was subjected to electrophoresis through a formaldehyde-containing 1.3% agarose gel and transferred to a nitrocellulose filter (*19*). After prehybridization, the filter was hybridized at high stringency with a labeled 1.2-kb cDNA segment from phage 3 (*19*). The filter was washed in 0.2× saline sodium citrate and 0.1% SDS at 68°C and autoradiographed with an intensifying screen at −70°C for 24 hours. The filter was stripped by washing in 50% formamide, 10 m*M* tris (*p*H 7.4), and 1 m*M* EDTA at 68°C for 1 hour, and then rehybridized with a ^{32}P-labeled rat α-tubulin cDNA probe (*20*) to control for the amount of mRNA loaded.
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Chemotransduction in the Carotid Body: K^+ Current Modulated by P_{O_2} in Type I Chemoreceptor Cells

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The ionic currents of carotid body type I cells and their possible involvement in the detection of oxygen tension (P_{O_2}) in arterial blood are unknown. The electrical properties of these cells were studied with the whole-cell patch clamp technique, and the hypothesis that ionic conductances can be altered by changes in P_{O_2} was tested. The results show that type I cells have voltage-dependent sodium, calcium, and potassium channels. Sodium and calcium currents were unaffected by a decrease in P_{O_2} from 150 to 10 millimeters of mercury, whereas, with the same experimental protocol, potassium currents were reversibly reduced by 25 to 50 percent. The effect of hypoxia was independent of internal adenosine triphosphate and calcium. Thus, ionic conductances, and particularly the O_2-sensitive potassium current, play a key role in the transduction mechanism of arterial chemoreceptors.

The mammalian carotid bodies are arterial chemoreceptors responsible for the hyperventilation observed in physiological and pathological conditions that produce a decrease in blood P_{O_2} (*1*). The chemoreceptor, or type I, cells transduce the decrease in P_{O_2} and produce a neurosecretory response. The neurotransmitters released by these cells set the level of electrical activity in the afferent fibers of the carotid sinus nerve (*2*).

The mechanism involved in the transduction of the hypoxic stimulus has been elusive (*3*). Either low P_{O_2} or high external K^+ can induce release of dopamine from type I cells, which is dependent on external Ca^{2+} and is inhibited by Ca^{2+} channel blockers such as nitrendipine and nisoldipine. On the basis of these data, it has been suggested that membrane depolarization may play a part in the response of type I cells to hypoxia (*4*).

The electrophysiological characteristics of type I cells are not well known. It has been reported that these cells are nonexcitable because, after impalement with microelectrodes, action potentials have not been recorded from them (*5*). However, these data cannot be considered definitive because, possibly as a result of cell damage, the average resting potential (−19.8 mV) was low enough to produce a complete inactivation of the ionic conductances that produce depolarization of the membrane. We have now found that type I cells have voltage-dependent Na^+, Ca^{2+}, and K^+ channels and that the K^+ channel activity is inhibited by low P_{O_2}.

Our experiments were performed on cultured type I cells isolated from rabbit carotid bodies. These cells were easily recognized by their small diameter (10 to 14 μm) and their birefringent appearance under the light microscope. The dispersed cells released dopamine in response to hypoxia. Clean carotid bodies were incubated for 30 min in a Ca^{2+}- and Mg^{2+}-free Tyrode solution with collagenase (3 mg/ml) and trypsin (1 mg/ml). After incubation, cells were mechanically dispersed with a fire-polished Pasteur pipette and washed twice to remove the enzymes. The final pellet was resuspended in 5 ml of minimum essential medium supplemented with glutamine (1%) and bovine fetal serum (5%). Cells were plated on sliv-

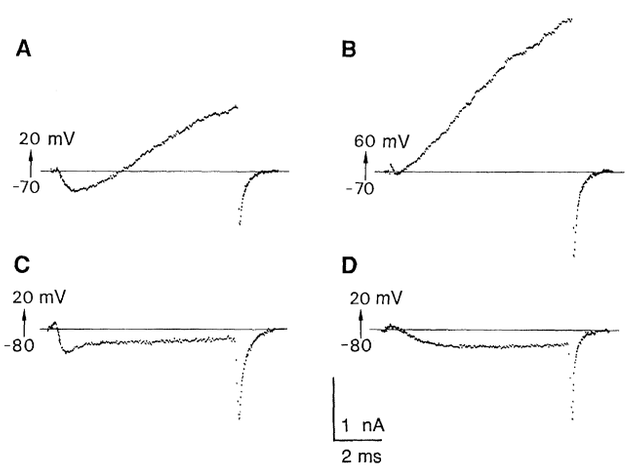

Fig. 1. Voltage-dependent ionic currents recorded in type I chemoreceptor cells of the carotid body. (**A** and **B**) Inward and outward currents recorded by voltage steps to 20 mV (A) and 60 mV (B) from a holding potential of −70 mV. External solution: 140 m*M* NaCl, 10 m*M* $CaCl_2$, 2.7 m*M* KCl, and 10 m*M* Hepes; internal solution: 80 m*M* potassium glutamate, 30 m*M* KCl, 20 m*M* KF, 2 m*M* $MgCl_2$, 10 m*M* Hepes, and 1 m*M* EGTA. (**C** and **D**) Na^+ and Ca^{2+} currents recorded after depolarization to 20 mV from a holding potential of −80 mV; K^+ currents were abolished by replacement of internal K^+ with Cs^+ (C), and Na^+ currents were blocked by bath application of TTX (1 μg/ml) (D). External solution as in (A) and (B); internal solution: 110 m*M* CsCl, 20 m*M* CsF, 2 m*M* $MgCl_2$, 10 m*M* Hepes, 5 m*M* EGTA, and 2 m*M* Mg^{2+}-ATP. The *p*H of all solutions was 7.3, and P_{O_2} was equilibrated to 150 mmHg. Linear ionic and capacitance currents were subtracted with a P/4 procedure (*10*). Temperature was 20° to 22°C.

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ers of glass cover slips treated with poly(L-lysine) and kept in a CO_2 incubator at 37°C until use (4 to 24 hours after plating). Type I cells were subjected to whole-cell patch clamp (*6*). We used low-resistance electrodes (between 0.8 and 2 megohms), electronic compensation of access resistance, and a low-feedback (100 megohms) resistance to improve the frequency response of our recording amplifier. During the experiments each cover slip was transferred to a small chamber that had a continuous flow of solution that could be changed within about 15 to 20 s. Solutions were equilibrated with air, N_2, or a mixture of both, and P_{O_2} in the chamber was monitored with an O_2 electrode.

A summary of the ionic currents recorded in type I cells is shown in Fig. 1. With high internal K^+ (Fig. 1, A and B) depolarization evoked a fast inward current followed by a larger and slower outward current. At the end of the 8-ms pulse a prominent tail current was observed. The outward current disappeared after all K^+ in the pipette solution was replaced with Cs^+ (Fig. 1C). The fast component of the inward current in this situation was abolished by introduction of external tetrodotoxin (TTX), but a slow inward current component followed by a large tail remained unaltered (Fig. 1D). The slow inward current and the tail represent the activity of Ca^{2+} channels (these features disappeared after external Ca^{2+} was replaced by Mg^{2+} and were blocked by 0.2 to 0.5 m*M* external Cd^{2+}). These electrophysiological properties were found in every cell studied ($n > 100$), and they indicate that arterial chemoreceptor cells have voltage-dependent K^+, Na^+, and Ca^{2+} channels.

We designed experiments to test the effect of low P_{O_2} on the ionic currents recorded in type I cells. The whole-cell recording mode was initiated with an external solution equilibrated with air (P_{O_2} = 150 mmHg), and, after a period of 3 to 5 min for acquisition of control current traces, the cell was exposed to a test solution of the same composition but equilibrated with a gas mixture of lower P_{O_2}. Figure 2 illustrates the effect of decreasing P_{O_2} from 150 to 10 mmHg on the different ionic currents. The Na^+ and Ca^{2+} currents were almost identical after 3-min exposure to hypoxia (A and B). We also recorded Na^+ currents in a different cell after blockade of Ca^{2+} channels by bath application of 0.5 m*M* Cd^{2+} (C and D); the two current traces, elicited by voltage steps to 10 mV with a time interval of about 5 min, were superimposable, indicating that low P_{O_2} does not alter the amplitude and time course of Na^+ currents. We tested the effect of hypoxia on Na^+ and Ca^{2+} currents in two more cells with similar results. Exposure to hypoxia had, however, a marked effect on K^+ currents (Fig. 2, E and F). Low P_{O_2} in this cell caused a 45% reduction of the peak K^+ current recorded by a depolarization to 40 mV that reverted almost completely after we switched to the solution with normal P_{O_2}. Changes in the current amplitude occurred roughly with the time course of bath exchange and could be repeated several times in the same cell. The reversible reduction of peak K^+ current amplitude in response to lowering environmental P_{O_2} was observed in every cell tested (n = 42). In cells subjected to a P_{O_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, n = 14).

The standard internal solution (inside the pipette and the cell) used in the initial experiments designed to record K^+ currents at various P_{O_2} levels had a Ca^{2+} concentration of 0.5 μ*M* and a Mg^{2+}–adenosine triphosphate (ATP) concentration of 3 m*M*. We checked the possibility that either of these two variables could affect the response of K^+ channels to hypoxia by utilizing an internal solution without ATP and with 10 m*M* EGTA added (estimated Ca^{2+} concentration < 10^{-9}*M*). The effect of lowering

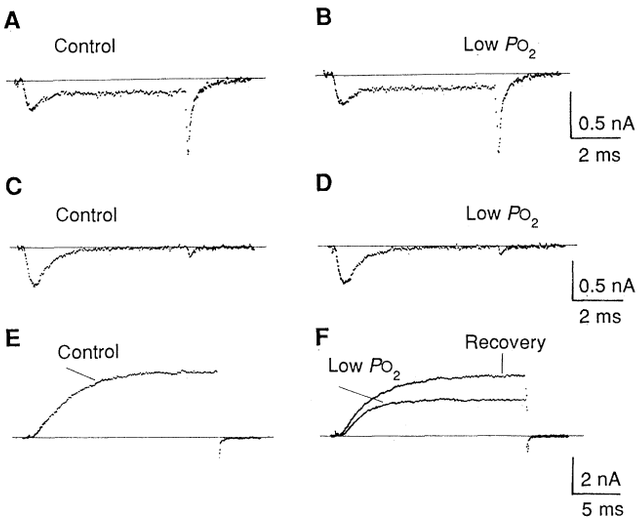

Fig. 2. Effect of low P_{O_2} on the voltage-dependent ionic currents of chemoreceptor cells. (**A** and **B**) Na^+ and Ca^{2+} currents recorded by voltage steps to 20 mV from a holding potential of −80 mV in an external solution with a P_{O_2} of 150 mmHg (control) (A) and 3 min after switching to a solution of the same composition but equilibrated with a P_{O_2} of 10 mmHg (low P_{O_2}) (B). Solutions were as in Fig. 1C except that the internal solution contained 3 m*M* ATP. (**C** and **D**) Na^+ currents recorded after depolarization to 10 mV from a holding potential of −80 mV at a P_{O_2} of 150 and 10 mmHg. Solutions were as in (A) and (B) except for the different external Ca^{2+} concentration (1.5 m*M*) and the addition of 0.5 m*M* $CdCl_2$ to block Ca^{2+} channels. (**E** and **F**) K^+ currents during 20-ms voltage steps to 40 mV from a holding potential of −80 mV recorded in an external solution with 150 mmHg P_{O_2} and 10 mmHg P_{O_2}. A reduction in current amplitude of about 45% was observed, which reverted after the reintroduction of the control solution (recovery). Solutions were as in Fig. 1, A and B, but with 5 m*M* $CaCl_2$ and TTX (1 μg/ml) externally. The internal solution had 3 m*M* ATP and 0.5 μ*M* Ca^{2+} (5 m*M* EGTA plus 4.44 m*M* $CaCl_2$). Other experimental conditions as in Fig. 1.

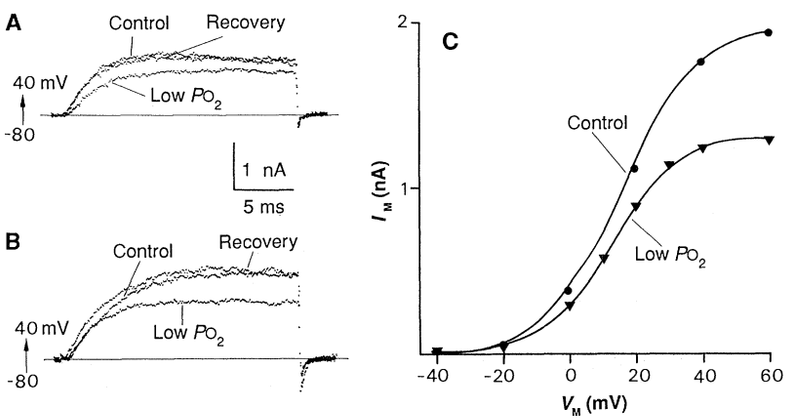

Fig. 3. Effect of lowering P_{O_2} from 150 mmHg to two different levels (110 and 10 mmHg) on K^+ currents in the absence of internal ATP and Ca^{2+}. Response to test solutions with 110 mmHg (low P_{O_2}) (**A**) and 10 mmHg (low P_{O_2}) (**B**). Experimental protocol and solutions were as in Fig. 2, E and F, except that the internal solution had 0 m*M* ATP, 0 m*M* Ca^{2+}, and 10 m*M* EGTA (estimated Ca^{2+} concentration < 10^{-9}*M*). Recovery traces were recorded 3 min after restoration of normal (150 mmHg) P_{O_2}. (**C**) Current-voltage (I_M-V_M) curves for the control (●, P_{O_2} = 150 mmHg) and test (▼, P_{O_2} = 10 mmHg) solutions. Current amplitude was measured at the end of the 20-ms voltage steps.

P_{O_2} on K^+ currents in this experimental condition is shown in Fig. 3, which also illustrates the relative potency of test solutions with two different P_{O_2} values. A decrease of P_{O_2} from 150 to 110 mmHg caused a reduction of peak current amplitude of 19% (Fig. 3A), whereas decreasing P_{O_2} to 10 mmHg inhibited the current by about 32% (Fig. 3B). Recovery in both cases was almost complete. Peak K^+ current as a function of voltage in 150 and 10 mmHg P_{O_2} is shown in Fig. 3C. Although we did not study the K^+ current kinetics in detail, we observed that after recovery from extremely low P_{O_2}, the K^+ current had a faster activation time course than it did before exposure to hypoxia.

Our observations that type I cells have voltage-dependent Na^+, Ca^{2+}, and K^+ channels are of interest because type I cells, which are of neuroectodermal origin (*7*), have been thought to be nonexcitable (*5*), and this has influenced earlier models of the transduction mechanism in the carotid body. Thus, any explanation of transduction in this arterial chemoreceptor must now take into account the electrical properties of type I cells. The discovery in type I cells of a K^+ current sensitive to environmental P_{O_2} could be a direct result of the action of O_2 on K^+ channels, perhaps coupled to an O_2 sensor, or, as in other chemoreceptors (*8*), it may require an intracellular mediator. The fact that the inhibition of K^+ currents by low P_{O_2} was seen in dialyzed cells and appeared to be unaffected by intracellular Ca^{2+} and ATP favors the idea of a direct effect of molecular O_2 on the channels.

The ionic conductances of type I cells are well suited for a major contribution to stimulus-secretion coupling. The activation of Na^+ and Ca^{2+} channels causes the generation of action potentials and fast injection of Ca^{2+} into the cytosol, whereas the K^+ conductance sensitive to P_{O_2}, which has a relatively slow activation kinetic, may be critical for determining the firing frequency of the cells and thus may translate a decrease in P_{O_2} into the appropriate secretory response. We have recently observed that, as predicted by the voltage clamp recordings, type I cells fire action potentials repetitively after they are switched from voltage to current clamp mode and that hypoxia decreases only slightly the rate of repolarization of individual action potentials but it greatly increases the firing frequency of the cells (*9*).

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Selective Activation of Transcription by a Novel CCAAT Binding Factor

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A novel CCAAT binding factor (CBF) composed of two different subunits has been extensively purified from rat liver. Both subunits are needed for specific binding to DNA. Addition of this purified protein to nuclear extracts of NIH 3T3 fibroblasts stimulates transcription from several promoters including the α2(I) collagen, the α1(I) collagen, the Rous sarcoma virus long terminal repeat (RSV-LTR), and the adenovirus major late promoter. Point mutations in the CCAAT motif that show either no binding or a decreased binding of CBF likewise abolish or reduce activation of transcription by CBF. Activation of transcription requires, therefore, the specific binding of CBF to its recognition sites.

EUKARYOTIC GENES CONTAIN A COMplex array of cis-regulatory elements that mediate induced, repressed, or basal transcription rates (*1, 2*). One of these elements contains a conserved CCAAT sequence and is often present at about 80 to 120 bp upstream from the transcriptional start site. Studies with several promoters have indicated that the integrity of this CCAAT sequence is required for optimal promoter activity (*3, 4*). Several different DNA binding proteins can interact with CCAAT-containing promoter elements. Among these are a factor from murine erythroleukemia cells (*5*), CCAAT binding transcription factor/nuclear factor–1 (CTF/NF-1) from HeLa cells (*6*), CCAAT binding protein from rat liver cells (*4*), and nuclear factor–Y from B-lymphoma line M12 (*7*). We previously identified a factor that binds to a segment containing a CCAAT motif in the −80 region of the mouse α2(I) collagen promoter and CCAAT-containing sequences in a number of other promoters (*8, 9, 10*). This factor consists of two different protein components; both are required for DNA binding and are present in the DNA-protein complex (*10*). Point mutations in the −80 CCAAT motif of the α2(I) collagen promoter caused a decrease in promoter activity to 1/8 to 1/12 of normal, as assayed in DNA transfection of NIH 3T3 fibroblasts in culture. These same mutations also strongly inhibited interaction with a CCAAT binding factor (CBF) present in nuclear extracts of NIH 3T3 fibroblasts (*11*). CBF is distinguished from CTF/NF-1 by chromatographic properties and molecular size (*10*). In addition, direct binding and competition experiments indicate that this factor and CTF/NF-1 bind to different DNA segments (*9*). This suggests that sequence determinants outside the CCAAT motif help discriminate between these factors.

We report here that the new heterodimeric CBF activates transcription from several promoters when the purified protein is added to NIH 3T3 nuclear extracts. The activation of transcription is specific since point mutations in the CCAAT motif which

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