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PROPERTIES OF A TRANSIENT K^+ CURRENT IN CHEMORECEPTOR CELLS OF RABBIT CAROTID BODY

BY JOSE R. LÓPEZ-LÓPEZ, DANIEL A. DE LUIS AND CONSTANCIO GONZALEZ

From the Departamento de Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, Universidad de Valladolid, 47005 Valladolid, Spain

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SUMMARY

1. Adult rabbit carotid body chemoreceptor cells, enzymatically dispersed and short-term cultured, exhibit an inactivating outward K^+ current that is reversibly inhibited by low P_{O_2}. In the present work we have characterized the biophysical and pharmacological properties of this current using the whole-cell voltage clamp recording technique.

2. Inactivating current was recorded after blockage of Ca^{2+} currents with extracellular Co^{2+}, Cd^{2+}, or after complete washing out of Ca^{2+} channels.

3. The threshold of activation of this inactivating current was about -40 mV. Current activated very quickly (mean rise time $4{\cdot}8 \pm 0{\cdot}42$ ms at $+60$ mV) but inactivated more slowly. Inactivation was well fitted by two exponentials with time constants of $79{\cdot}7 \pm 6{\cdot}6$ and $824 \pm 42{\cdot}8$ ms (at $+40$ mV). The inactivation process showed a little voltage dependence.

4. The steady-state inactivation was well fitted by a Boltzman function. Inactivation was fully removed at potentials negative to -80 mV and was complete at voltages near -10 mV; 50% inactivation occurred at -41 mV.

5. Recovery from inactivation had several components and was voltage dependent. Initial recovery was fast, but full recovery, even at -100 mV, required more than 30 s.

6. Inactivating current was selectively blocked by 4-aminopyridine (4-AP), in a dose-dependent manner (IC_{50}, 0·2 mM). The duration of chemoreceptor cells action potentials was augmented by 1 mM 4-AP from $2{\cdot}3 \pm 0{\cdot}36$ to $7{\cdot}0 \pm 0{\cdot}25$ ms at 0 mV. Tetraethylamonium (TEA), at concentrations above 5 mM, blocked inactivating and non-inactivating components of the whole K^+ current.

7. Inactivating current was modulated by cyclic AMP (cAMP). Bath application of 2 mM dibutyryl cAMP reduced peak amplitude by $18{\cdot}7 \pm 2{\cdot}9$% (at $+30$ mV) and slowed down the rise time of the current. The effect was not voltage dependent. Forskolin (10–20 μM) also affected inactivating current, by accelerating the inactivation process. In the same preparations neither dibutyryl cAMP nor forskolin affected Ca^{2+} currents.

8. It is concluded that modulation of K^+ channels by cAMP might play a physiological role potentiating the low P_{O_2} inhibition of K^+ channels.

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INTRODUCTION

Mammalian carotid bodies (CBs) are sensory organs sensitive to blood P_{O_2}, P_{CO_2} and pH. When P_{O_2} decreases and P_{CO_2} or $[H^+]$ increase, the activation of CBs gives place to reflex hyperventilation which is directed to correct the blood gases or $[H^+]$ variation (Heymans, Bouckaert & Dautrebande, 1930; Fitzgerald & Lahiri, 1986). The sensing structures in the CBs are chemoreceptor cells which are derived from the neural crest and form synaptic contacts with the sensory nerve endings of the carotid sinus nerve. On natural (or pharmacological) stimulation, chemoreceptor cells release neurotransmitters in proportion to the intensity of stimulation, the release response being paralleled by the action potential frequency simultaneously recorded in the carotid sinus nerve (Fidone, Gonzalez & Yoshizaki, 1982; Obeso, Almaraz & Gonzalez, 1989; Rigual, López-López & Gonzalez, 1991). Low P_{O_2}-induced release of neurotransmitters was Ca^{2+} dependent and sensitive to dihydropyridines (Obeso, Fidone & Gonzalez, 1987; Shaw, Montague & Pallot, 1989; Obeso, Rocher, Fidone & Gonzalez, 1992).

In recent electrophysiological experiments it has been found that freshly dissociated or short-term cultured chemoreceptor cells from adult rabbits are excitable cells possessing voltage-dependent Na^+, Ca^{2+} and K^+ channels, and are able to produce action potentials (Duchen, Caddy, Kirby, Patterson, Ponte & Biscoe, 1988; López-Barneo, López-López, Ureña & Gonzalez, 1988; Urena, López-López, Gonzalez & López-Barneo, 1989). In addition, chemoreceptor cells from adult rabbits possess an inactivating component of the whole K^+ current that is reversibly inhibited on lowering P_{O_2} in the bathing solution (López-Barneo *et al.* 1988; López-López, Gonzalez, Ureña & López-Barneo, 1989). These findings lead to the proposal of a mechanism for low P_{O_2} chemoreception in which the inhibition of the K^+ current would depolarize chemoreceptor cells. Activation of voltage-operated Ca^{2+} channels, increase in $[Ca^{2+}]_i$, release of neurotransmitters and activation of sensory nerve endings would follow (López-Barneo *et al.* 1988; López-López *et al.* 1989).

Whole-cell patch recordings performed on chemoreceptor cells isolated from rabbit embryos (Hescheler, Delpiano, Acker & Pietruchka, 1989) or neonatal rats (Peers, 1990*a*; Stea & Nurse, 1991) have shown some differences in the basic electrical properties of the cells, but nevertheless in all studies a partial inhibition of K^+ currents by low P_{O_2} was observed. In neonatal rats, the component inhibited by low P_{O_2} was a Ca^{2+}-activated K^+ current, probably carried by charybdotoxin-sensitive high-conductance K^+ channels (Peers, 1990*a*). Studies on chemoreceptor cells from adult rabbits, however, have shown that the O_2-sensitive component of the K^+ current was inactivating and not Ca^{2+} dependent (López-Barneo *et al.* 1988; López-López *et al.* 1989), and this has been confirmed in a recent study in isolated membrane patches (Ganfornina & López-Barneo, 1991).

In the present work, performed on adult rabbit chemoreceptor cells, we studied the inactivation properties of the transient component of the whole K^+ current and its sensitivity to 4-aminopyridine (4-AP). We have also studied the modulation of K^+ currents by cAMP, the synthesis of which is known to be increased in chemoreceptor cells by low P_{O_2} stimulation (Pérez-García, Almaraz & Gonzalez, 1990). The results show that the inactivating O_2-sensitive component of the K^+ current has some

properties typical of a delayed rectifier current (Hodgkin & Huxley, 1952) and also some others which are typical of an A-current (I_A) (Connor & Stevens, 1971). cAMP is shown to inhibit the inactivating component, suggesting that part of the actions of this nucleotide on the response of chemoreceptor cells (Pérez-García, Almaraz & Gonzalez, 1991) could be produced at this level.

METHODS

Preparation of isolated chemoreceptor cells

Chemoreceptor cells from adult rabbit CBs were obtained as previously described (Ureña *et al.* 1989). The cells were plated on small glass coverslips coated with poly-L-lysine (0·1 mg ml^{-1}; Sigma), and cultured for 4–48 h in a CO_2–O_2 incubator (5%–20%, pH 7·4), at 37 °C, in Dulbecco's minimum essential medium, F12 (1:1, Gibco) supplemented with glutamine (2 mM, Sigma) and fetal calf serum (5%, Flow).

Electrophysiological recordings

Ionic currents were recorded using the whole-cell variant of the patch clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Experiments were performed at room temperature ($\approx$ 20 °C). The coverslips with the attached cells were placed in a small recording chamber (0·5 ml) and perfused by gravity at a flow rate of 3 ml min^{-1}. Recordings were referenced to a Ag–AgCl electrode placed in an additional chamber located down stream to the recording compartment. Chemoreceptor cells were identified by their spherical shape, bright appearance under phase contrast, diameter between 10 and 14 μm and a nucleus-to-cytoplasm ratio greater than two. Pipettes of borosilicate glass (Clark Electromedical Instruments) were double pulled (Narishige PP-83) and polished (Narishige MF-83) to resistances between 1·6 and 3·3 MΩ. In most of the experiments, which required long-lasting stable recordings, the pipettes were filled with (mM):KF, 100; KCl, 30; $MgCl_2$, 2; Hepes, 10; EGTA, 10; pH was adjusted to 7·2 with KOH (*ca* steady $[Ca^{2+}]_i$ below 10^{-9} M). Fluoride is commonly included in the internal solution to optimize the stability of the recording conditions (Kostyuk, Krishtal & Pidoplichko, 1975); in our preparation fluoride allowed us to obtain frequent stable recordings for up to 3 h without significant differences with those obtained with chloride as the only internal anion. The bathing solution was (mM): NaCl, 140; KCl, 5·4; $CaCl_2$, 1; $MgCl_2$, 2; Hepes, 10; glucose, 5; pH was adjusted to 7·4 with NaOH. In the experiments shown in Fig. 1*B*, and in those where dibutyryl cyclic AMP or forskolin (Fig. 8) were tested on K^+ currents, the internal solution contained 3 mM MgATP as a substrate for adenylate cyclase and cAMP-dependent protein kinase A, and fluoride was replaced by chloride to avoid influencing adenylate cyclase activity (Gilman, 1987). In these experiments the control bathing solution contained 1·8 mM $CaCl_2$ and 0·18 mM $MgCl_2$. When Ca^{2+} currents were recorded, KCl was replaced by CsCl in the internal solution to block potassium currents, and the bathing solution was Mg^{2+} free and contained 10 mM $CaCl_2$ and 50 nM tetrodotoxin (TTX). Tetraethylammonium (TEA), TTX, MgATP, dibutyryl cAMP, forskolin and 4-AP were obtained from Sigma.

The currents were recorded using a List EPC-7 patch clamp amplifier and filtered at 3 kHz corner frequency, −3dB, with an 8-pole Bessel filter (Frequency Devices 902LPF2B). Capacitative transients were electronically compensated. Seal resistances achieved in our recording conditions were over 10 GΩ. Cells with series resistances over 10 MΩ were discarded. Pulse generation, data acquisition, leakage substraction and record analyses were performed with a PC/AT computer using the CED1401 interface (Cambridge Electronic Design) commanded with VCAN software, kindly provided by Dr J. Dempster (University of Strathclyde). The analysis of some experiments that involved record subtraction and function fitting was performed with Sigmaplot® software package (Jandel Scientific). Usually, the records were stored in the computer on-line, but in some instances they were stored on a videotape (Neuro Data, Neuro-corder DR-890) before analysis. Protocols of pulse generation are described for every experiment in the Results section. The holding potential was usually −80 mV. In all the experiments the intervals between test pulses were selected to prevent accumulation of inactivation. Two hyperpolarizing pulses to −120 mV were applied between test pulses in order to subtract leakage and capacitative currents.

RESULTS

Whole-cell voltage clamp recordings were made on short-term cultured chemoreceptor cells. On long depolarizing steps the outward K^+ currents rise to a peak amplitude, which increases with depolarization, and then declines slowly along the duration of the pulse (Fig. 1*A*). The morphology of the outward K^+ currents was variable from cell to cell, but an inactivating component was always present. In most of the cells it represented the largest part of the outward current. As previously described (Duchen *et al.* 1988; Ureña *et al.* 1989), some cells displayed a prominent Ca^{2+}-activated component. We have studied outward currents in cells where this component was either not apparent, or after its disappearance due to washing out of Ca^{2+} channels (Ureña *et al.* 1989). Figure 1*A* shows the current elicited by 1 s depolarizing steps to −10, +30 and +70 mV from a holding potential of −80 mV, obtained 1 min after establishing the whole-cell recording and 20 min later, when the Ca^{2+} current was washed out. Figure 1*B* shows the complete peak current–voltage relationship at the two different times. peak current diminished with cell dialysis and the shoulder in the *I–V* curve around +20 mV disappeared after Ca^{2+} channels wash-out. Cobalt ions in the bathing solution (1 mM) also eliminated the shoulder without affecting the K^+ current *I–V* curve at voltages where Ca^{2+} currents are expected to be minimal or not present at all (Fig. 1*C* and *D*). Cobalt was tested in three additional cells with the same results.

Time course of activation

The time course of K^+ current activation was estimated by measuring their rise time (10 to 90% of the peak current) at each pulse potential. Figure 2*A* shows rise time means (± S.E.M., $n = 10$) as a function of test potential. The inset shows a typical example of the time course of activation of K^+ currents during depolarizing pulses to −30 mV and up to +60 mV (10 mV steps) from a holding potential of −80 mV. Activation was sigmoidal at lower voltages, but turned exponential with increasing depolarizations. Although these estimated rise times refer to the whole K^+ current, they should reflect fairly well the rise times of the inactivating component, because it represented over 90% of the whole current in these experiments (see below and Fig. 3*B*). In three of the cells, one of which is presented in Fig. 2*B*, the rise time was also studied in the presence of Co^{2+} (1 mM) or Cd^{2+} (0·5 mM). It can be seen that the full blockage of Ca^{2+} currents, and hence of the Ca^{2+}-dependent component of the K^+ current, did not modify the form of the rise time curves. The only observed effect was a shift of K^+ current activation to more depolarized potentials, a well-known effect of divalent cations (Hille, 1984).

Steady-state inactivation

To study steady-state inactivation we used conventional two pulse protocols from a holding potential of −80 mV (Fig. 3*A*). First, a prepulse of 40 s duration displaced the membrane potential to the desired voltage (range −100 to −10 mV; 10 mV steps) and then a 1 s test pulse depolarized the cell to +40 mV. The inactivating prepulse was 40 s in duration in order to reach a steady-state of inactivation. Shorter

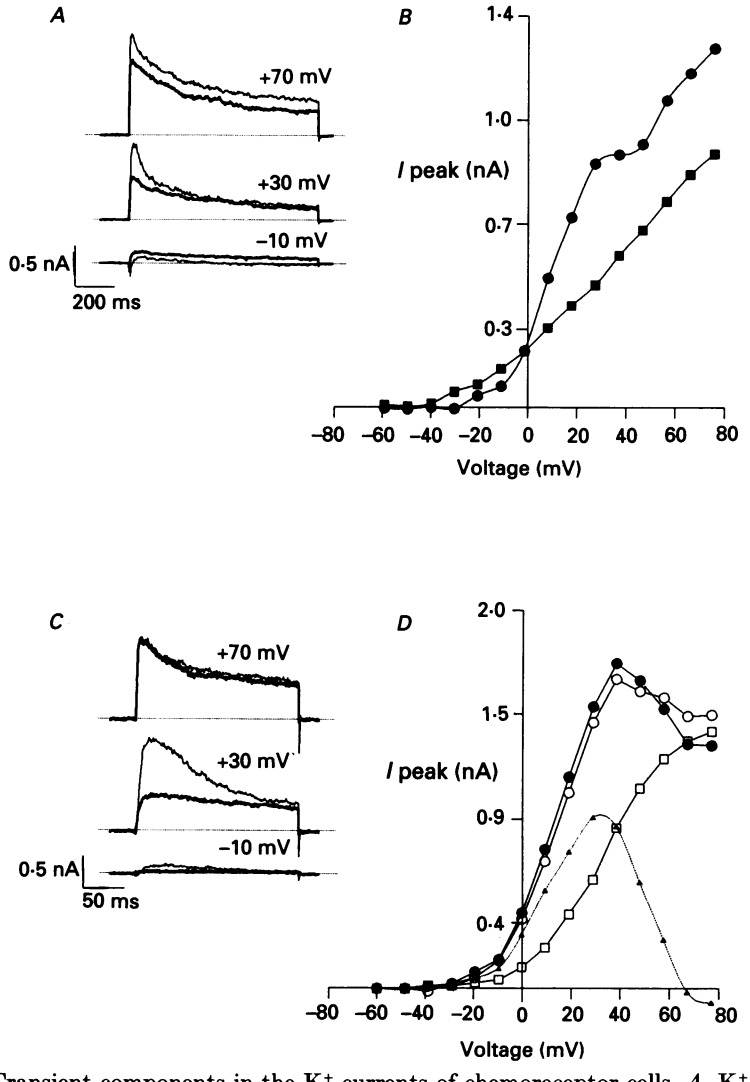

Fig. 1. Transient components in the K^+ currents of chemoreceptor cells. *A*, K^+ currents evoked in a chemoreceptor cell by 1 s depolarizing pulses from −80 mV to the indicated test potentials, 1 min after establishing whole-cell recording (thin lines) and 20 min later (thick lines), when Ca^{2+} channels are washed out. *B*, peak *I–V* relationships obtained from the same cell before (●) and after (■) Ca^{2+} channel wash-out. *C*, K^+ current records obtained in a different cell by 200 ms depolarizing pulses from −80 mV to the indicated potentials, in control conditions (thin lines) and in presence of 1 mM Co^{2+} (thick lines). *D*, peak *I–V* relationships obtained from the same cell before (●) during (○) and after (□) cell perfusion with cobalt. Dotted line results from subtracting cobalt *I–V* relationship from the control one.

prepulses resulted in an apparent increase in the non-inactivating component (I_{min}, see below) and shifted the inactivation curve to more depolarized potentials. Between each set of prepulse–pulse protocols the cell was maintained for more than

1 min at the holding potential to allow full recovery of inactivation. Inactivation was measured as the ratio of peak currents, I/I_{max}, where I_{max} was the largest current recorded during the test pulse following the −100 mV prepulse. Figure 3*B* shows the mean inactivation curve obtained in eight different cells. Inactivation was fully

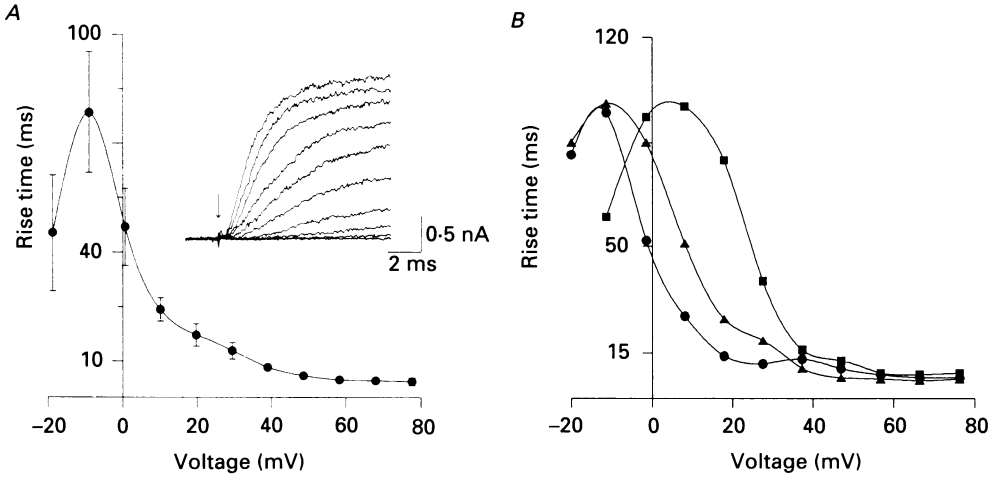

Fig. 2. Rise time of transient K^+ current. *A*, shows the rise times of K^+ currents (10 to 90% of peak current) plotted as a function of the membrane potential. Data are means ± S.E.M., $n = 10$. The inset shows the onset of the K^+ currents in sample records obtained in a typical cell at different imposed depolarizations (−30 to +60 mV, 10 mV steps) from a holding potential of −80 mV. *B*, shows the rise times measured in one of the cells included in *A*, in control solution (●), in presence of 1 mM Co^{2+} (▲), and in presence of 0·5 mM Cd^{2+} (■).

removed at −80 mV and was virtually complete at −10 mV. Data fitted well to a Boltzman distribution, considering a non-inactivating component (I_{min}),

$$I/I_{max} = (1-I_{min})/\{1+\exp[(V-V_{0\cdot5})/k_j]\}+I_{min}, \quad (1)$$

where $V_{0\cdot5}$ is the membrane potential for half-inactivation ($I/I_{max} = 0\cdot5$), and k_j is the slope factor at $V_{0\cdot5}$. For the data shown in Fig. 3*B*, the best fit to eqn (1) gave $V_{0\cdot5} = -41$ mV, $k_j = 10\cdot2$ and $I_{min} = 0\cdot05$. Similar results were obtained with a different protocol in which the holding potential was moved at the different voltages and a depolarizing pulse was then applied.

Figure 3*B* also shows the peak current evoked by steps from −80 mV to a series of potentials between −60 and +80 mV, expressed as a fraction of the peak current at +80 mV for each chemoreceptor cell (means ± S.E.M. $n = 6$). The threshold activation of the K^+ currents was around −40 mV.

Time course of inactivation

The time course of inactivation was studied according to the protocol depicted in Fig. 4*A*. Current elicited during a 1 s depolarizing pulse from a holding potential of −10 mV, where the inactivating current was not present, was subtracted from the current elicited during a pulse from a holding potential of −80 mV, where both inactivating and non-inactivating components were present. The inset of Fig. 4*A*

shows the currents elicited during 1 s pulses from holding potentials of −80 mV and −10 to +30 mV. Subtracted currents (Fig. 4*A*) had two phases in their decay. Current relaxations fitted well (at voltages above 0 mV) to the equation:

$$I_t = A\exp(-t/\tau_1) + B\exp(-t/\tau_2), \quad (2)$$

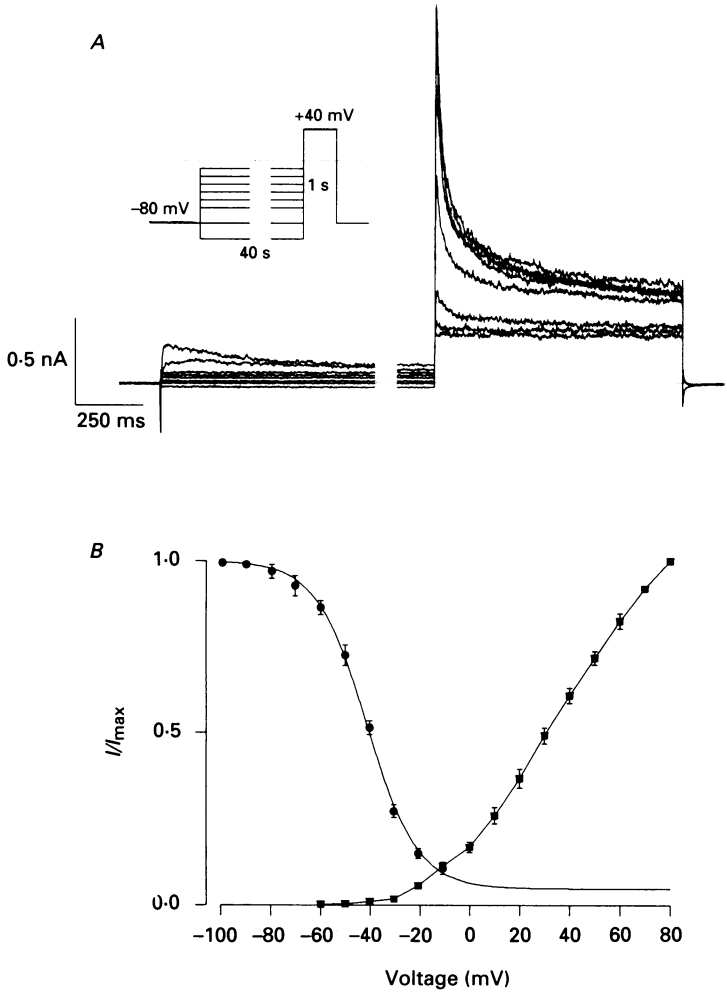

Fig. 3. Steady-state inactivation of transient K^+ current. *A*, records obtained with the two-step voltage protocol (inset) in a chemoreceptor cell. The holding potential was −80 mV, and the paired pulses were applied at more than 1 min intervals. Leakage currents were not subtracted. *B*, membrane potential dependence of transient K^+ current inactivation (●). The peak current evoked during the test pulses was expressed as the fraction of the maximum peak current and plotted against the potential of the prepulse. Data are means ± S.E.M., $n = 8$, and continuous line was drawn according to eqn (1) (see text). A similar relationship was obtained when the currents were measured at the end of the pulse. The figure also shows (■) the peak current evoked by steps from −80 mV to a series of potentials between −60 and +80 mV expressed as a fraction of the peak current at +80 mV. Data are means ± S.E.M., $n = 6$. Line was drawn by eye.

where A and τ_1 are the initial amplitude and time constant of the fast component, respectively, and B and τ_2 are the corresponding parameters for the slow component. Time constants of inactivation showed small voltage dependence (Fig. 4*B*) at voltages above +30 mV, where the activation process should be essentially complete

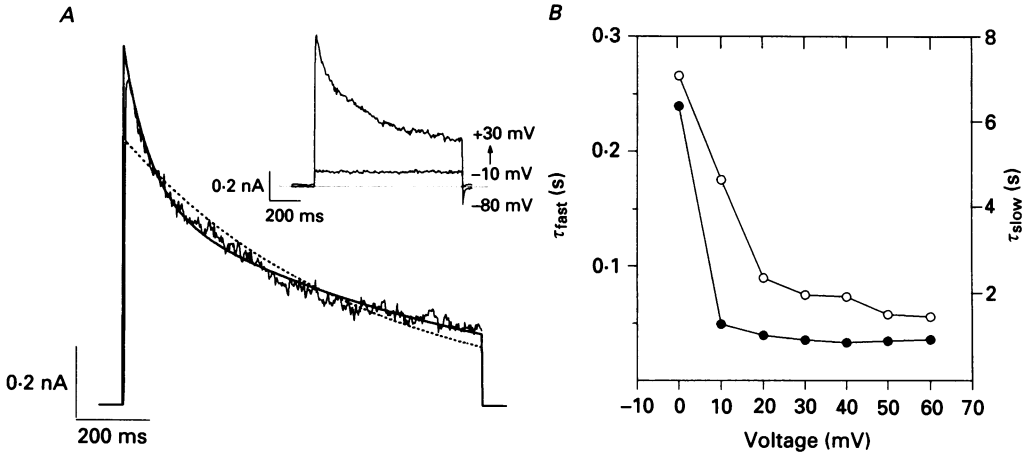

Fig. 4. Time course of inactivation of transient K^+ current. *A*, shows isolated K^+ transient current, obtained by subtraction of the records in the inset, and its fit to one (dashed line) or two exponentials (solid line; eqn (2), see text). The records in the inset were obtained by 1 s depolarizing pulses from −80 and from −10 mV to +30 mV. *B*, shows the inactivation constants (τ_{fast} ○; τ_{slow} ●) obtained in the same cell at different test potentials using the subtraction protocol shown in *A*.

(see Fig. 2). The mean (±S.E.M., $n = 9$) time constants measured at +40 mV were $\tau_1 = 79{\cdot}7 \pm 6{\cdot}6$ and $\tau_2 = 825 \pm 42{\cdot}8$ ms.

Recovery from inactivation

The time course of recovery from inactivation was studied with the two pulse protocol illustrated in the inset of Fig. 5*A*. The cell was maintained at a holding potential of −100 mV and two pulses to +40 mV (P_1, 1 s; P_2, 400 ms) were applied at different intervals (t). The interval between pulses varied between 10 ms and 50 s. To prevent accumulation of inactivation the time between pairs of pulses was 1 min, and during this period two 1 s hyperpolarizing pulses to −120 mV were applied. Figure 5*A* shows a family of current records obtained in a typical experiment. While the amplitude of the currents in all first pulses was identical, that of the second pulse decreased for short intervals to recover with increasing time between pulses. Figure 5*B* represents the ratio of the amplitude of the K^+ current in the first pulse to that in the second (I_{P_2}/I_{P_1}) as a function of the time interval between pulses. The three relationships in the figure were obtained with holding potentials of −100, −80 and −60 mV. At −100 mV the time course of recovery was initially fast so that at a time interval between pulses of 2 s the recovery was about 80%, but thereafter the recovery from inactivation slowed down and full recovery was only achieved with intervals greater than 30 s. The recovery rate was not too different at −80 mV, but at −60 mV it was clearly slower, especially at the shorter intervals.

Pharmacology of the inactivating outward current

Inactivating current was selectively blocked by the external application of 4-AP at concentrations below 1 mM. Figure 6 shows a family of K^+ currents obtained during 1 s depolarizing steps from a holding potential of −80 mV to −60 and up to

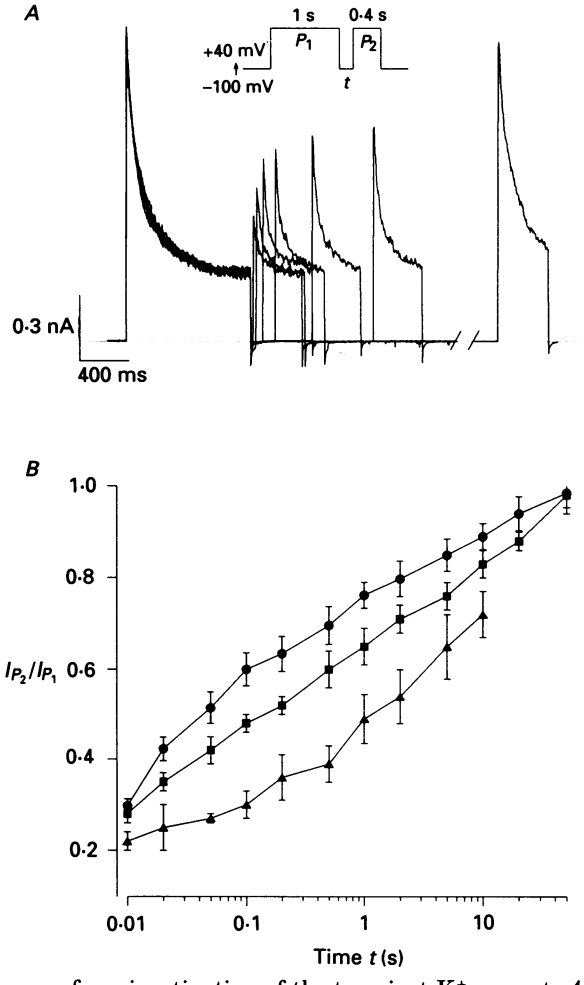

Fig. 5. Recovery from inactivation of the transient K^+ current. *A*, currents elicited in a cell by pairs of pulses (P_1 and P_2) with intervals (t) of 20, 50, 100, 200 and 500 ms and 1 and 50 s. The currents obtained during the first pulses are superimposed. Holding and interpulse potentials were −100 mV. Leakage currents were not subtracted. The two pulse protocol used is shown in the inset. *B*, plots of the ratio of peak K^+ current amplitudes in the second pulse to that of the first one as a function of the interpulse interval. Holding potentials were −100 mV (●, $n = 5$), −80 mV (■, $n = 4$) and −60 mV (▲, $n = 3$). Data are means ± S.E.M.

+40 mV (10 mV steps) in control conditions (*A*) and with 1 mM 4-AP in the bath (*B*). Figure 6*C* shows the difference between the two current families. Only the inactivating current was blocked by 4-AP the non-inactivating component being

unaffected. Figure 6*D* represents the peak *I–V* relationship of the records shown in *A* and *B*. Action potentials evoked under current clamp are shown in Fig. 6*E*. The cell was first in control conditions, then with 1 mM 4-AP and finally in control solution again for 5 min. Membrane potential of the cell was maintained at

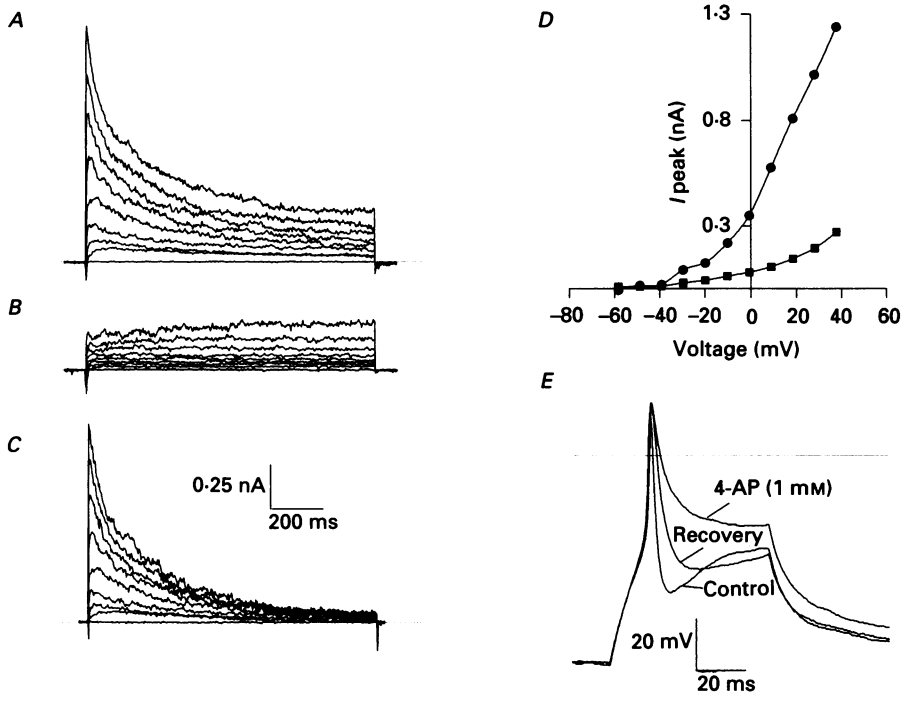

Fig. 6. Effects of 4-AP on K^+ currents and action potentials of chemoreceptor cells. *A* and *B* show K^+ currents obtained during 1 s depolarizations to different potentials (−60 to +40 mV; 10 mV steps) from a holding potential of −80 mV in control conditions (*A*) and with 1 mM 4-AP in the bath (*B*). *C*, represents the difference between *A* and *B* showing that 4-AP selectively inhibited the transient current. *D*, shows the peak *I–V* relationships of currents presented in *A* and *B*. *E*, action potentials elicited under current clamp in control conditions, in the presence of 1 mM 4-AP and 5 min after returning to control conditions.

≈ −80 mV and a depolarizing current pulse (≈ 60 pA) of 100 ms was applied to elicit an action potential. 4-AP considerably increased action potential duration in all the cells tested. Values measured at 0 mV were 2·3 ± 0·36 ms in control conditions and 7·0 ± 0·25 ms with 4-AP ($n = 4$).

The effect of 4-AP was dose dependent. This dependence was studied in five different cells at +40 mV with increasing 4-AP from 0·05 to 1 mM. Figure 7*A* shows the dose-dependent inhibitory effect of 4-AP for a typical experiment. Note that 40 mM TEA blocked wholly the inactivating current component, but also reduced considerably the non-inactivating component. Other cells tested with 5 mM TEA showed it effectively blocked both components (not shown). Figure 7*B* shows the mean results obtained in the five cells studied. The effect of 4-AP was expressed as the ratio of inactivating current present at each concentration of 4-AP to that

present in control solutions ($I_{K^+, 4\text{-AP}}/I_{K^+, \text{Control}}$). In these experiments, the inactivating current was estimated as the difference between the peak current and that remaining at the end of the pulse. Results are plotted against the log of 4-AP concentration and fitted to a straight line. The apparent IC_{50} was $\approx 0{\cdot}2$ mM.

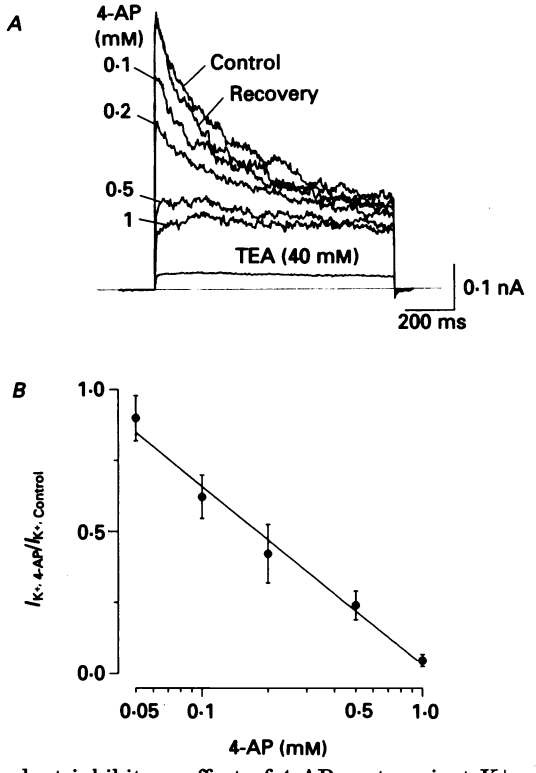

Fig. 7. Dose-dependent inhibitory effect of 4-AP on transient K^+ current. *A*, shows K^+ currents elicited in a cell during 1 s pulses to +40 mV from −80 mV in control conditions and at four different concentrations of 4-AP in the bath. The current obtained after returning to control solution is labelled as recovery. The effect of 40 mM TEA on K^+ currents is also shown. *B*, average blockages of transient K^+ current, at differing concentrations of 4-AP, expressed as the ratio of the test to control transient currents ($I_{K^+, 4\text{-AP}}/I_{K^+, \text{Control}}$). Transient currents were estimated as the difference between the peak current and that at the end of the pulse. Data are means ± S.E.M. ($n = 5$). Correlation coefficient, $r = 0{\cdot}992$.

Modulation by cAMP

The modulation of K^+ currents by cAMP was studied by bath application of forskolin and dibutyryl cAMP. Both agents were tested soon after starting cell dialysis. Dibutyryl cAMP, a permeant analogue of the natural nucleotide, diminished the peak current and slowed down the time course of activation (records in Fig. 8*A*). We tested the nucleotide in ten different cells, and this effect was clearly seen in eight cells. The inhibition produced by 3 mM dibutyryl cAMP on the peak current did not appear to be voltage dependent and it amounted to 35·7 % at +30 mV (Fig. 8*A*). In the other seven responding cells dibutyryl cAMP was tested at a concentration of

2 mM. The inhibition of K^+ currents produced by the nucleotide did not show voltage dependence, as is shown in Figure 8*B*. This voltage independence excludes an effect of the nucleotide on Ca^{2+}-dependent K^+ channels, otherwise a large inhibition should be expected at those voltages at which Ca^{2+} currents are maximal. Forskolin (10 or

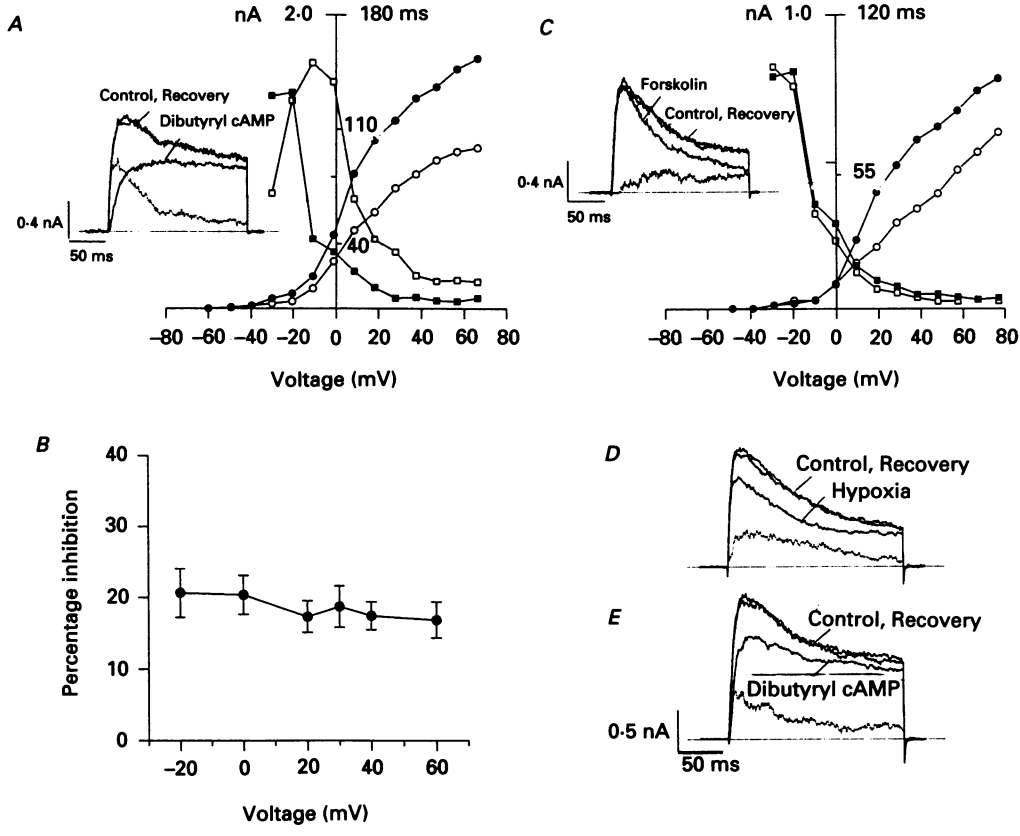

Fig. 8. Effects of dibutyryl cAMP and forskolin on K^+ currents of chemoreceptor cells. *A*, shows rise times (squares) and peak *I–V* relationship (circles) obtained in a chemoreceptor cell in control conditions (filled symbols) and during bath perfusion with 3 mM dibutyryl cAMP (open symbols). Inset, sample records at +30 mV; dotted trace represents the difference between control and test records. *B*, shows the percentage of inhibition produced by 2 mM dibutyryl cAMP on peak K^+ currents at different voltages. Data are means ± S.E.M. ($n = 7$). *C*, as in *A*, but using 20 μM forskolin in a different cell. Currents were measured at the end of the pulse. *D* and *E* compare the effect of low P_{O_2} ($\approx$ 5 mmHg, *D*) and dibutyryl cyclic AMP (2 mM, *E*) in two different cells at +40 mV.

20 μM) was tested in six different cells. A clear effect was seen in four of them. Forskolin affected minimally peak current amplitude and rise time but increased clearly the inactivation rate of the current (Fig. 8*B*). For comparison, Fig. 8*C* and *D* show the effect of low P_{O_2} ($\approx$ 5 mmHg) and of dibutyryl cAMP (2 mM) on K^+ currents in two different cells. Note that striking similarity between the effects of dibutyryl cAMP and hypoxia.

Due to the fact that in these experiments functional Ca^{2+} channels were present, the possibility exists that the described effects on K^+ currents were to some extent

distorted by a concomitant modulation of Ca^{2+} currents. To disclose this possibility we have tested the effect of forskolin and dibutyryl cAMP on Ca^{2+} current in nine additional cells, in which both K^+ and Na^+ currents were blocked with Cs^+ in the internal solution and 50 nM TTX in the bathing solution, respectively. Figure 9*A*

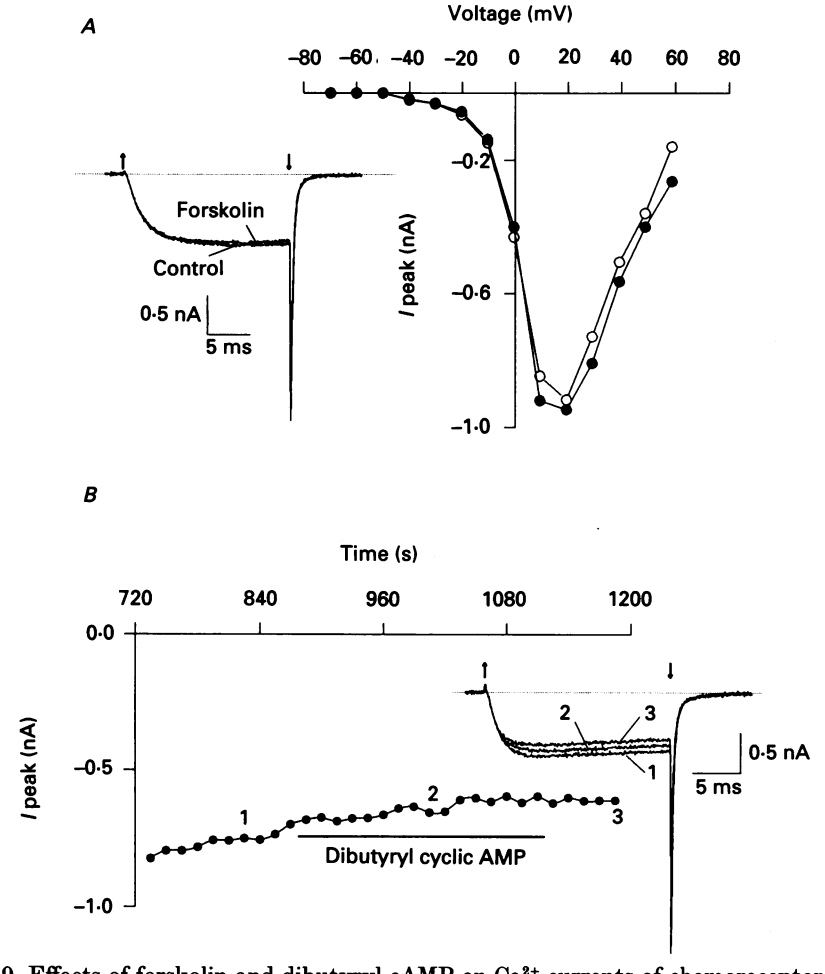

Fig. 9. Effects of forskolin and dibutyryl cAMP on Ca^{2+} currents of chemoreceptor cells. *A*, peak *I–V* relationships obtained in control conditions (●) and during perfusion with 10 μM forskolin (○). The inset shows sample records obtained at +20 mV. *B*, represents the peak amplitudes of Ca^{2+} currents obtained on 20 ms depolarization pulses to +20 mV applied every 15 s. Dibutyryl cAMP was present in the perfusing solution for the time indicated by the horizontal bar. Time scale indicates time after establishing whole-cell recording. The inset shows sample records corresponding to marked points in the main figure.

shows that forskolin (10 μM) did not produce any significant change either in the shape or in the peak amplitude of Ca^{2+} currents, the same result was obtained in five additional cells. Dibutyryl cAMP (2 mM) was tested in three cells with identical results in all of them. Figure 9*B* shows that the nucleotide analogue was also ineffective in modifying Ca^{2+} currents.

DISCUSSION

The results presented in this paper demonstrate the presence of a fast-activating and slow-inactivating outward current in CBs chemoreceptor cells. The blockade of this current by internal Cs^+, external TEA and 4-AP indicates that it is carried by K^+. The current can be recorded with very low $[Ca^{2+}]_i$ and when Ca^{2+} currents are blocked or washed out. This inactivating current, like many described in other preparations (Rudy, 1988), share characteristics of both the delayed rectifier (I_K) and the A-type (I_A) K^+ currents. For example, the inactivating current of chemoreceptor cells has an activation voltage range similar to that of I_K in rat sympathetic neurones (Belluzi, Sacchi & Wanke, 1985*b*), but it shows inactivation as the I_A described also in sympathetic neurones (Belluzi, Sacchi & Wanke, 1985*a*). The inactivating current here described is sensitive to 4-AP like a typical I_A (Rogawski, 1985), but also to low concentrations of TEA as the I_K (Rudy, 1988).

A Ca^{2+}-activated transient K^+ current has been described in some preparations (Siegelbaum & Tsien, 1980; Zbicz & Weight, 1985; Mayer & Sugiyama, 1988; reviewed in Rudy, 1988). From the data of our Fig. 1 it appears that the washing out of Ca^{2+} channels or the addition of Co^{2+} to the bathing solution reduces preferentially an inactivating component of the K^+ current, suggesting that this current could be modulated by Ca^{2+}. Since chemoreceptor cells have high-conductance Ca^{2+}-activated K^+ channels (Ganfornina & López-Barneo, 1991), the Ca^{2+}-activated transient component could result also from variations of Ca_i^{2+} (entry and buffering) during the test pulse and then transient activation of high-conductance Ca^{2+}-dependent K^+ channels (Brown, Constanti & Adams, 1982). In addition, inactivating K^+ channels could be recorded in isolated membrane patches after buffering Ca^{2+} below 10^{-9} M (Ganfornina & López-Barneo, 1991). These inactivating channels are in all probability responsible for the macroscopic transient K^+ current characterized in the present work.

As it has been reported for transient K^+ currents in other preparations (Clark, Giles & Imaizumi, 1988; Oxford & Wagoner, 1989; Cooper & Shrier 1989; Thorn, Wang & Lemos, 1991), chemoreceptor cell transient current inactivation could be fitted by two exponentials, but their time constants are rather slower. As in the above studies, inactivation process shows weak voltage dependence, especially at those voltages where activation is complete (see Fig. 4*B*).

The range of voltages for activation and steady-state inactivation are more positive than those of the typical I_A (Rudy, 1988), but similar $V_{0.5}$ of inactivation have been reported also in some preparations (De Coursey 1990; Lynch & Barry 1991). The overlap of activation and inactivation curves indicates that a macroscopic steady current will be recorded over a membrane potential range of ≈ -40 mV to ≈ -10 mV.

Complete recovery from inactivation of the transient K^+ current requires tens of seconds in chemoreceptor cells while typical I_A needs less than 1 s (Cooper & Shrier 1989; Solc & Aldrich 1990). The recovery process has several components and is voltage dependent; the most rapid component is much slower at -60 than at -100 mV. A similar behaviour of the recovery process has been described for inactivating currents in GH_3 pituitary cells (Oxford & Wagoner, 1989) and rat

olfactory receptor neurones (Lynch & Barry, 1991). In the latter study it was suggested that the presence of a fast component at the onset of inactivation and its slow recovery would produce accumulation of inactivation during repetitive firing, leading to the characteristic decrement of spike amplitude in olfactory neurones. In chemoreceptor cells repetitive test pulses also produce accumulation of inactivation. Therefore, the inactivating properties of their transient K^+ current could produce a pattern of action potential generation similar to that found in olfactory neurones. It should be noted, however, that although dialysed chemoreceptor cells produce trains of action potentials (López-López *et al.* 1989), the pattern of generation of action potentials of these cells in physiological conditions is not known.

4-AP selectively and reversibly blocked the inactivating current in the sub-millimolar range (IC_{50} = 0·2 mM) and expanded the action potential, suggesting a role of inactivating current in the repolarization process. Both kinetic components of the inactivation have similar sensitivity to 4-AP, and also very similar voltage dependence of steady-state inactivation (see Fig. 3, legend), suggesting that an unique channel population with at least two inactivation states is the responsibility of the whole inactivating current. Single channel analysis is required to verify this possibility. In chemoreceptor cells from neonatal rats, it has been reported also that 4-AP (2 mM) reduced by about 70% whole K^+ currents; no further characterization of the inhibition was done (Peers, 1990*b*).

Low P_{O_2} inhibits the inactivating K^+ current in whole-cell recordings (López-Barneo *et al.* 1988; López-López *et al.* 1989) and lowers the opening probability of an inactivating K^+ channel in isolated membrane patches (Ganfornina & López-Barneo, 1991). As already mentioned, these K^+ channels seem to be responsible for the macroscopic, low P_{O_2}-inhibited, inactivating current. It has been proposed that its inhibition by low P_{O_2} may increase the pacemaker activity in chemoreceptor cells (López-López *et al.* 1989). Such function would be expected from a typical I_A. However, the voltage dependence of the inactivating current in chemoreceptor cells is shifted to more depolarized potentials than I_A in other systems. But, on the other hand, this shift does not exclude a role in pacemaker activity if it represents an adaptation to the particular resting membrane potential and threshold of action potentials of chemoreceptor cells (see Rudy 1988). In fact, inactivating K^+ current activates in a voltage range near the threshold for action potentials initiation (threshold for Na^+ channels is about −40 mV, Ureña *et al.* 1989), so its inhibition could affect the cell excitability and then increase the probability of cell firing.

Forskolin slightly reduces the peak of the inactivating current and accelerates the inactivation process. This effect has been described in other publications (Strong & Kaczmarek, 1987), and recently it has been reported that it could be due to a direct action of forskolin on K^+ channels, independent of the activation of the adenylate cyclase. Other analogues of forskolin that lack adenylate cyclase activating properties produced the same effects on K^+ currents (Hoshi, Garber & Aldrich, 1988). In addition, we found that bath application of dibutyryl cAMP, a permeant analogue of cAMP, clearly diminishes the peak of the inactivating current and slows down the activation process, indicating that in chemoreceptor cells as in other preparations forskolin possesses cAMP-independent effects on K^+ channels. Contrary to these effects on K^+ currents, neither forskolin nor dibutyryl cAMP seem to affect Ca^{2+}

currents, which are mediated by L-type channels (Ureña *et al.* 1989). Since modulation of L-type Ca^{2+} channels by cAMP-dependent phosphorylation is well established in many cell types (Hofmann, Nastainczyk, Röhrkasten, Schneider & Sieber, 1987; Catterall, Seagar, Takahashi & Nunoki, 1989), our findings would indicate that cAMP-dependent kinase A has been dialysed and then K^+ channels would be modulated directly by cAMP. An alternative possibility would be that L-type Ca^{2+} channels in chemoreceptor cells are not modulated by kinase A-dependent phosphorylation, as has been recently suggested on the basis of neurochemical data (Pérez-García *et al.* 1991). In this case the modulation of K^+ channels by cAMP-dependent phosphorylation could not be excluded.

The modulation of inactivating K^+ current by cAMP may have a very significant physiological role. It has been shown that low P_{O_2} increases cAMP levels in the CBs, the maximum increase being observed at a P_{O_2} near 40 mmHg (Pérez-García *et al.* 1990). It has been also shown that different experimental manoeuvres that increase the nucleotide levels potentiate the release of neurotransmitters induced by low P_{O_2} (Pérez-García *et al.* 1991; Wang, Cheng, Yoshizaki, Dinger & Fidone, 1991). The suggestion was made (Pérez-García *et al.* 1991) that cAMP would be acting on the transduction machinery for the hypoxic stimulus, a suggestion that gains direct support from the findings presented here. Then, although cAMP is not necessary for low P_{O_2} to inhibit the transient K^+ current (López-Barneo *et al.* 1988; Ganfornina & López-Barneo, 1991), it may potentiate the low P_{O_2}-induced inhibition especially at those P_{O_2} levels at which cAMP production is maximum.

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