Volume 299, number 3, 251–254 FEBS 10826 March 1992

Time course of K^+ current inhibition by low oxygen in chemoreceptor cells of adult rabbit carotid body

Effects of carbon monoxide

J.R. López-López and C. González

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

Received 17 January 1992

K^+ currents recorded from adult rabbit chemoreceptor cells are reversibly inhibited on lowering the pO_2 in the bathing solution. Bath application of a hypoxic TTX-containing solution revealed that inhibition of K^+ current by low pO_2 proceeds faster than TTX inhibition of Na^+ currents, the apparent $t_{1/2}$ being 3.68 and 7.14 s, respectively. Addition of carbon monoxide to the hypoxic gas mixture used to equilibrate the bathing solution reversed the inhibition of K^+ currents by approx. 70%.

Carotid body; Chemoreceptor cell; K^+ current; Low pO_2; Carbon monoxide; Oxygen sensor

1. INTRODUCTION

Carotid body (CB) chemoreceptors respond to a decrease in arterial blood pO_2 with an increase in action potential frequency in the sensory fibers innervating the organ, the response developing in a few seconds [1]. Chemoreceptor cells of the CB are the O_2 sensing structures, releasing neurotransmitters in proportion to the pO_2 decrease and producing an electrical discharge of parallel intensity in the sensory nerve fibers of the carotid sinus nerve [2,3]. Recently, it has been shown that chemoreceptor cells possess an O_2-sensitive K^+ current that is reversibly inhibited by decreasing pO_2 in the bathing solution. The suggestion was made that this inhibition can lead to cell depolarization and activation of voltage-dependent Ca^{2+} channels [4,5]; the entry of Ca^{2+} through these channels, that are dihydropyridine-sensitive, would trigger the release of neurotransmitters [6,7]. To accept this sequence, the inhibition of the K^+ current must precede the activation of the sensory nerve discharges [8], that is, the inhibition must develop within the initial 3 s after lowering pO_2, the time elapsing between a decrease in arterial pO_2 and the increase in the carotid sinus nerve action potential frequency [1]. On the other hand, the suggestion has been made that the O_2-sensor in chemoreceptor cells should be a hemoglobin-like hemoprotein, but no direct evidence to support this suggestion is available [1]. The present study compares the time course of the inhibition of the K^+ current by low pO_2 and that of Na^+ current inhibition by TTX, which is known to occur in a few hundred ms [9]. It is shown that the former inhibition is faster. It is also shown that carbon monoxide, a very inert gas that in biological systems only reacts with hemoproteins, prevents the low pO_2-induced inhibition of chemoreceptor cells K^+ current.

Correspondence address: C. González, Departamento de Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, Universidad de Valladolid, 47005 Valladolid, Spain. Fax: (34) (83) 42 30 85.

2. MATERIALS AND METHODS

CBs from adult New Zealand rabbits were enzymatically dissociated and maintained in primary culture as previously described [10]. Whole cell-clamp recordings were made using electrodes (resistance 1.6–4 MΩ) filled with (in mM): KCl, 130; $MgCl_2$, 2; HEPES, 10; EGTA, 10; ATP, 3; pH adjusted to 7.20 with KOH. Cells were superfused by gravity from a reservoir containing (in mM): NaCl, 140; KCl, 5.4; $MgCl_2$, 2; $CaCl_2$, 1.8; HEPES, 10; Glucose, 5.5; pH adjusted to 7.40 with NaOH. The solutions were delivered to the 0.5 ml recording bath at a flow rate of 3 ml/min using plastic tubing of very low permeability to oxygen as assessed with an oxygen electrode. The presence of TTX in the bathing solutions as well as the equilibrating gas mixtures used in specific experiments are given in section 3. In all experiments, the holding potential was −80 mV. Pulse generation, acquisition and analysis of the data were made by computer using VCAN software kindly provided by J. Dempster (Strathclyde, UK).

3. RESULTS

Fig. 1A shows a series of records of the currents elicited by pulse depolarizations to +20 mV, given at 3 s intervals, during the perfusion either with standard bathing solution (TTX-free; pO_2 ≈150 mmHg; labeled −3 in the figure) or with a nitrogen-equilibrated TTX

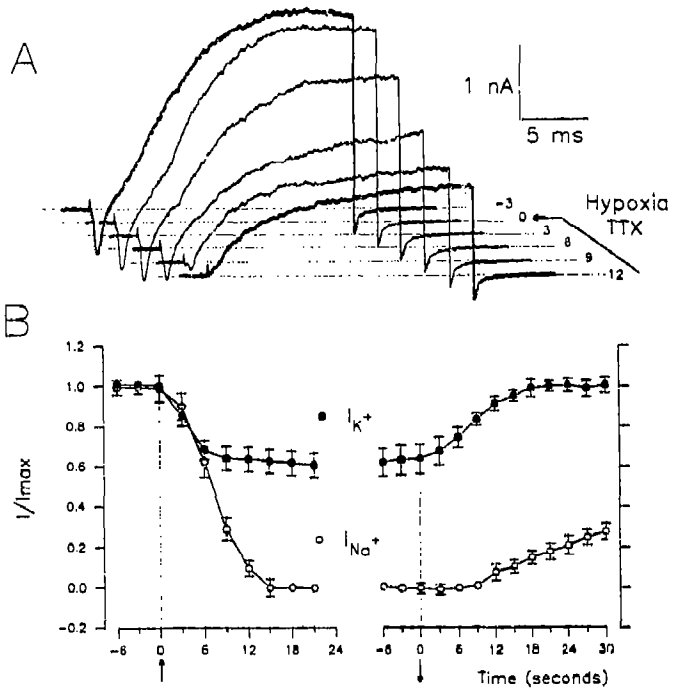

Fig. 1. Time course of the inhibition of the Na^+ and K^+ currents in hypoxic TTX-containing solution. (A) Whole-cell clamp records of Na^+ and K^+ currents from a chemoreceptor cell before and after the application of a hypoxic N_2-equilibrated solution (pO_2 in the bath ≈5 mmHg) containing 0.1 μm TTX. The currents were elicited by test pulses (20 ms; +20 mV) applied every 3 s. Leak substraction pulses to −120 mV were applied between test pulses. (B) Average time courses of the inhibition of K^+ and Na^+ currents by the hypoxic TTX-containing solution (left), and of their recovery on returning to control solution (right). Solutions were changed as indicated by arrows. The amplitudes of both currents were normalized. Values are means ± S.E.M. of 6 individual data.

containing solution (0.1 μM TTX; pO_2 ≈5 mmHg; labeled 0 to 12 in the figure). On perfusion with the hypoxic TTX-containing solution it can be seen that the amplitude of the inward-directed Na^+ currents remained unchanged for at least 3 s, were reduced by about one-third at 6 s, and disappeared completely at 12 s. A reduction in the outward-directed K^+ currents was already noticeable 3 s after the onset of the perfusion with the hypoxic solution and maximum inhibition was achieved at 9 s.

Fig. 1B shows the average time courses of the inhibition of Na^+ and K^+ currents during perfusion with the hypoxic TTX-containing solution (left), and their recovery after returning to standard solution (right). The maximum inhibition observed for the K^+ current (about 40%) was reached after about 9 s of perfusion with the hypoxic solution, with a $t_{1/2}$ of 3.68 s. The inhibition of Na^+ current by TTX occurred with a $t_{1/2}$ of 7.14 s and was completed 15 s after the onset of the perfusion. Potassium current recovered completely on returning to standard solution, but with a slower time course than the inhibition; full recovery was obtained after 21 s. The recovery of the Na^+ currents on returning to standard perfusion was very slow; only 23% of the current was recovered after 30 s, and full recovery was only completed after several minutes.

Fig. 2 shows the effect of carbon monoxide (CO) on the inhibition of the K^+ current by low PO_2. Fig. 2A shows a set of K^+ current records obtained in a cell while perfusing with standard solution (pO_2 150 mmHg; record 1), with a hypoxic solution equilibrated with 5%O_2 in N_2 (pO_2 ≈40 mmHg; record 2), with a hypoxic solution equilibrated with a gas mixture containing 5%O_2 and 10%CO in N_2 (pO_2 ≈40 mmHg; pCO estimated 70 mmHg; record 3) and after returning to standard solution (record 4). All the solutions contained TTX at a concentration of 0.1 μM. It is evident that in this cell CO reduced by about 60% the inhibition of the K^+ current produced by low pO_2. Fig. 2B shows the peak amplitudes of the currents recorded every 5 s during the course of the experiment, the numbers indicating the currents shown in part A of the figure. The data of Fig. 2C and D were obtained from other cells using different protocols of CO application. In this cell, CO completely reversed or prevented the inhibition of the K^+ current by low pO_2.

The effect of low pO_2 on the K^+ current are seen at all test voltages above the activation threshold (≈−30 mV) [5], therefore it was of interest to see if CO prevented the effects of low pO_2 at all test voltages. Fig. 3A shows the I–V relationships obtained in a cell in control conditions, during perfusion with a solution equilibrated with 5%O_2, during perfusion with a solution equilibrated with 5%O_2 plus 10%CO, and again with control solution (recovery). It is evident that CO prevented the effects of low pO_2 at all test potentials. The I–V curves were similar in another seven cells studied. The inset at the left of Fig. 3A shows records of the K^+ currents obtained in control (C), hypoxic (H), and hypoxic plus CO conditions (H+CO) and at three different test voltages, −10 (upper), +10 (middle) and +30 mV (lower). The recovery traces (not shown) were almost identical to the controls. Fig. 3B shows the inhibition of the K^+ currents (mean ± S.E.M.; n = 8) obtained at +40 mV during perfusion with solutions equilibrated with 5%O_2 or 5%O_2 plus 10%CO. The inhibition obtained with 5%O_2 (pO_2 ≈ 40 mmHg) amounted to 32.32 ± 4.11% whereas in the presence of CO it was 10.18 ± 3.21% (P < 0.001); i.e. CO reversed by about 70% the inhibition induced by low pO_2.

4. DISCUSSION

The results presented here indicate that the inhibition of K^+ currents by low pO_2 in the chemoreceptor cells of the CB proceeds at a faster rate than the inhibition of Na^+ currents by TTX, which, on the other hand, is known to occur within a few hundred ms at the concentrations of TTX used in this study [10]. Therefore we

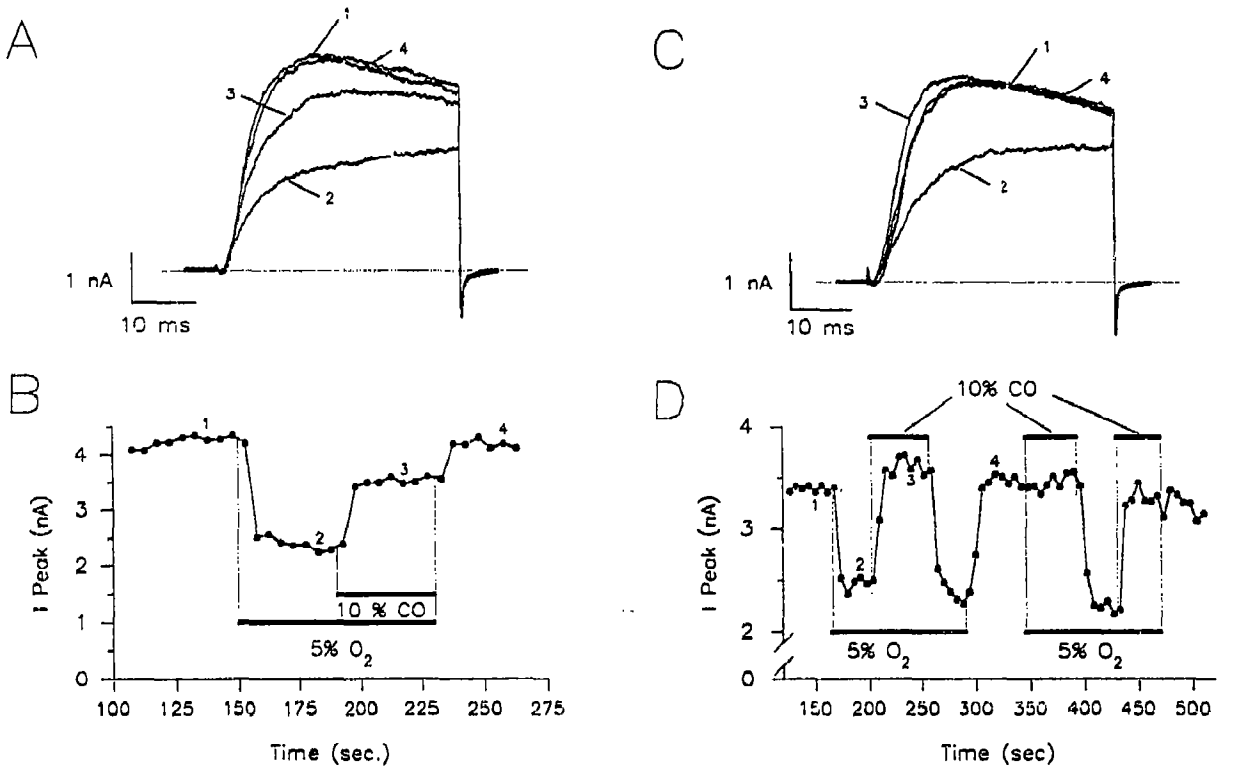

Fig. 2. Effects of carbon monoxide on the inhibition of the K^+ current by low pO_2. (A) Sample records of K^+ currents elicited by 40 ms pulses to +40 mV while perfusing with air-equilibrated control solution (pO_2 150 mmHg) (1), with 5%O_2-equilibrated hypoxic solution ($pO_2 \approx 40$ mmHg) (2), with 5%O_2 + 10% CO-equilibrated solution (estimated pCO 70 mmHg) (3), and on returning to control solution (4). (B) Peak amplitudes of the K^+ currents recorded every 5 s throughout the experiment. Solutions were changed as labeled in the figure. Numbers correspond to the traces shown in A. (C) and (D) correspond to a similar experiment performed in a different cell.

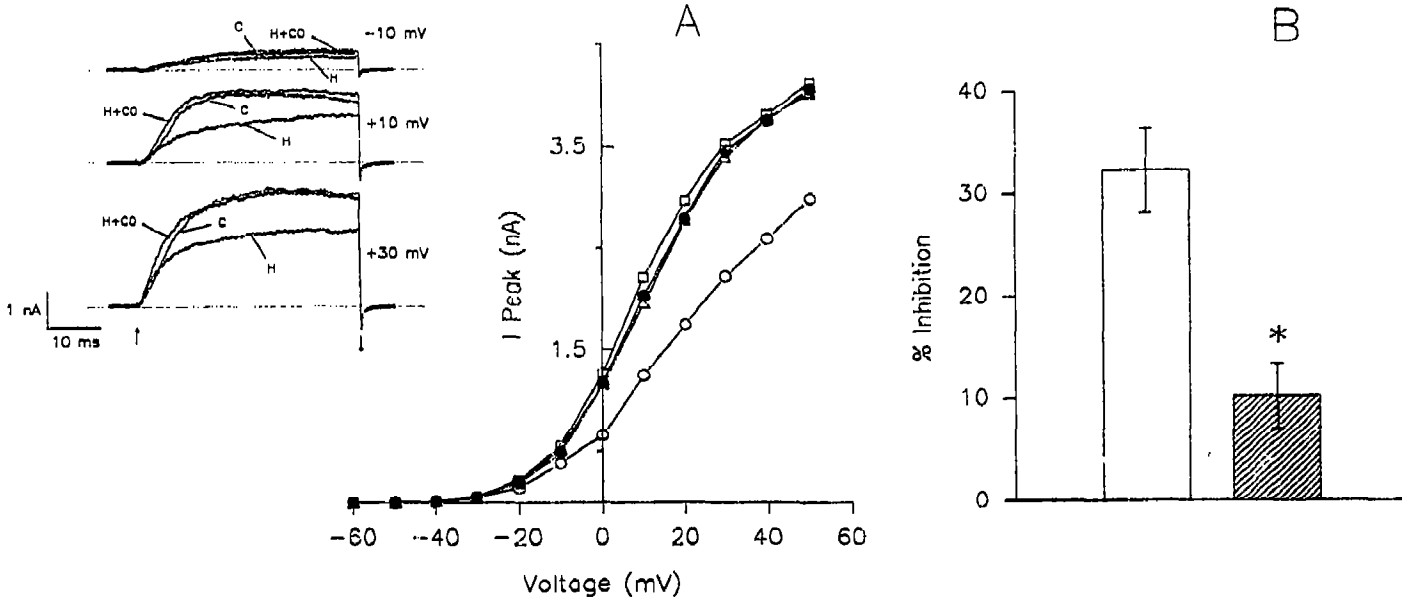

Fig. 3. (A) *I–V* relationships for K^+ currents in a carotid body chemoreceptor cell in control conditions (●), during hypoxic perfusion ($pO_2 \approx 40$ mmHg) (○), during perfusion with the same hypoxic solution containing carbon monoxide (estimated pCO 70 mmHg) (□) and after returning to control solution (△). The inset represents currents recorded at −10, +10 and +30 mV, in control (C), hypoxic (H) and hypoxic plus carbon monoxide conditions (H+CO). (B) Inhibition of the K^+ current at +40 mV during bath perfusion with the same hypoxic solution (pO_2 = 40 mmHg; empty bar) and during perfusion with the same hypoxic solution containing 10% CO (dashed bar). Data are means ± S.E.M.; n = 8, $P < 0.001$.

can state that the effect of low pO_2 on K^+ currents occurs almost instantaneously, the delay observed (<3 s) being due to the dead time of our recording bath (time for mixing of solutions and gas diffusion). This finding indicates that the inhibition of K^+ currents by low pO_2 occurs fast enough in order to be considered the primary electrical event in the transductive cascade of the hypoxic stimulus in chemoreceptor cells of the CB.

The results shown in Figs. 2 and 3 would indicate that CO interacts with the O_2 sensor, replacing O_2 and preventing the inhibition of the K^+ current. Since CO is a very inert gas and reduced hemoproteins with accessible iron sites are its only known targets in biological systems [11,12], our experiments strongly suggest that the O_2 sensor in chemoreceptor cells is a hemoprotein. Due to the explosive nature of some O_2/CO mixtures, we have not carried out a detailed study on the relative affinities of this putative hemoprotein for O_2 and CO. In spite of that, our data suggest that both affinities are similar (5%O_2+10%CO substitute the 20%O_2 of the control solution by a 70%), a situation completely different from the well-known case of hemoglobin. This difference is not surprising taking into account that it is the protein moiety of hemoproteins which determines the relative affinities for both gases [13].

Another question in relation to this presumptive O_2-sensor refers to its location in chemoreceptor cells. Since in a recent study in isolated patches of membranes from chemoreceptor cells it has been found that low pO_2 decreases the opening probability of K^+ channels [14], it would appear that the O_2-sensor is located in the cell membrane. It should be mentioned that Cross et al. [15] have suggested that an heme-linked NADPH-dependent oxidase could be the O_2 sensor in chemoreceptor cells. It is conceivable that this, as well as any other oxygen-using enzyme, are affected by lowering pO_2 in proportion to their affinities for oxygen itself. However, the experiments carried out in isolated patches exclude the participation in the inhibition of the K^+ currents of any enzyme requiring intracellular cofactors. Then, the experiments with CO presented here provide the first direct evidence for the many times [1] suggested hemoproteic nature of the O_2-sensor in CB chemoreceptor cells. In this context, it should be mentioned that in other oxygen-sensing cell type, the erythropoietin-producing cells, strong experimental evidence has been presented [16] in favor of a hemoproteic O_2 sensor.

Acknowledgements: We would like to thank Prof. B. Herreros for his advice in preparing the manuscript. This work was supported by grants DGICYT 89/0358 and Junta de Castilla y León 1101/89.

REFERENCES

[1] Fidone, S.J. and Gonzalez, C. (1986) in: Handbook of Physiology, The Respiratory System (A.P. Fishman, Ed.) Vol. II, American Physiological Society, Bethesda, pp. 247–312.
[2] Fidone, S.J., Gonzalez, C. and Yoshizaki, K. (1982) J. Physiol. 333, 93–110.
[3] Fishman, M.C., Greene, W.L. and Platika, D. (1985) Proc. Natl. Acad. Sci. USA 82, 1448–1450.
[4] López-Barneo, J., López-López, J.R., Ureña, J. and Gonzalez, C. (1988) Science 241, 580–582.
[5] López-López, J.R., Gonzalez, C., Ureña, J. and López-Barneo, J. (1989) J. Gen. Physiol. 93, 1001–1014.
[6] Obeso, A., Fidone, S.J. and Gonzalez, C. (1987) in: Chemoreceptors in Respiratory Control (J.A. Ribeiro and D.J. Pallot, Eds.) Croom Helm, London, pp. 91–97.
[7] Shaw, K., Montagne, V. and Pallot, D.J. (1989) Biochim. Biophys. Acta 1013, 42–46.
[8] Biscoe, T.J. and Duchen, M.R. (1990) Am. J. Physiol. 258, L271–L278.
[9] Ulbricht, W., Wagner, H.H. and Schmidtmayer, J. (1986) Ann. NY Acad. Sci. 479, 68–83.
[10] Ureña, J., López-López, J.R., Gonzalez, C. and López-Barneo, J. (1989) J. Gen. Physiol. 93, 979–1001.
[11] Caughey, W.S. (1970) Ann. NY Acad. Sci. 174, 148–153.
[12] Coburn, R.F. and Forman, H.J. (1986) in: Handbook of Physiology, The Respiratory System (L.E. Fahri and S.M. Tenney, Eds.) Vol. IV, American Physiological Society, Bethesda, pp. 439–456.
[13] Haab, P.E. and Durand-Arczynska, W.Y. (1991) in: The Lung: Scientific Foundations (R.G. Crystal and J.B. West, Eds.) Vol. II, pp. 1267–1275.
[14] Ganfornina, M.D. and López-Barneo, J. (1991) Proc. Natl. Acad. Sci. USA 88, 2927–2930.
[15] Cross, A.R., Henderson, L., Jones, O.T.J., Delpiano, M.A., Heutschel, J. and Acker, H. (1990) Biochem. J. 272, 743–747.
[16] Goldberg, M.A., Dunnind, S.P. and Bunn, H.F. (1988) Science 242, 1412–1415.