Effects of D600 on hypoxic suppresssion of K^+ currents in isolated type I carotid body cells of the neonatal rat

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Received 24 July 1990

K^+ currents recorded from isolated type I carotid body cells were reversibly suppressed by hypoxia (pO_2 25 Torr). Inhibition of Ca^{2+} influx using high Mg^{2+}, low Ca^{2+} solutions abolished this effect, indicating hypoxia selectively inhibits the Ca^{2+}-activated component of K^+ currents. The Ca^{2+} channel blockers D600 and verapamil reversed the suppressive effects of hypoxia. These drugs also partially inhibited Ca^{2+}-independent K^+ currents, but this effect was relieved by hypoxia, causing apparent reversal of hypoxic suppression of the K^+ currents seen under control conditions.

Carotid body; Hypoxia; Patch-clamp; K^+ current; Phenylalkylamine; D600; Verapamil

1. INTRODUCTION

Type I carotid body cells play a key role in chemotransduction, releasing transmitter substances in response to the stimuli which alter afferent sinus nerve discharge [1]. Electrophysiological studies have shown hypoxia depolarizes type I cells, and selectively suppresses K^+ channels [2]. This presumably leads to opening of voltage-dependent Ca^{2+} channels and transmitter release, as such release is inhibited by removing extracellular Ca^{2+} or applying organic Ca^{2+} channel blockers [3]. Type I cell K^+ currents are composed of Ca^{2+}-activated and Ca^{2+}-insensitive components (respectively $I_{K(Ca)}$ and I_{Kv}) and the latter can be isolated by inhibiting the Ca^{2+} influx which activates $I_{K(Ca)}$ [4]. The present study compares the effects of hypoxia on K^+ currents under control conditions, and during inhibition Ca^{2+} influx using organic and inorganic Ca^{2+} channel blockade.

2. MATERIALS AND METHODS

Carotid bodies from 8- to 11-day-old rats were enzymatically dissociated into single-cell preparations and maintained in primary culture as previously described [4,5]. Whole-cell patch-clamp recordings from type I cells were made using electrodes (resistance 2 to 6MΩ) filled with (in mM): KCl, 107; $CaCl_2$, 1; $MgSO_4$, 2; NaCl, 10; K-EGTA, 11; Hepes, 11; ATP, 2; pH 7.20. Cells were perfused by gravity from a reservoir containing (in mM): NaCl, 135; KCl, 5; $MgSO_4$, 1.2; $CaCl_2$, 2.5; Hepes, 5; 21–23°C, pH 7.40. The reservoir was made hypoxic by N_2 equilibration and adding 1 mM sodium dithionite, giving a *bath* pO_2 of 25 Torr (measured with an oxygen electrode; Strathkelvin Instruments). Outward K^+ currents were evoked by 50 ms test depolarizations (V_{test}s) applied from a holding potential of −70 mV at 0.2 Hz and amplitudes measured by computer (VCAN software, J. Dempster). Statistical comparisons were made using the paired, two-tailed Student's *t*-test.

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3. RESULTS

Fig. 1A shows that outward K^+ currents in type I cells activate at V_{test}s above approximately −30 mV, and a shoulder in the $I-V$ relationship is apparent at low, positive V_{test} values. Hypoxia (pO_2 25 Torr) reversibly suppressed K^+ currents at all activating V_{test}s, but the effect was maximal at +20 mV where the shoulder of the $I-V$ relationship was greatest, as previously reported [6]. As this shoulder arises from Ca^{2+} influx activating $I_{K(Ca)}$ [4], and hypoxia does not affect Ca^{2+} currents directly [7,8], this suggests that hypoxia selectively inhibits $I_{K(Ca)}$ in type I cells.

The effects of hypoxia on I_{Kv} were tested while blocking $I_{K(Ca)}$ by lowering extracellular $[Ca^{2+}]$ to 0.1 mM and raising extracellular $[Mg^{2+}]$ to 6 mM, to inhibit Ca^{2+} influx. $I-V$ relationships obtained under these conditions were almost linear, with no appearance of the shoulder seen under control conditions (Fig. 1B), and exposure of type I cells to hypoxia was always ($n=6$) without effect throughout the range of activating V_{test}s studied (e.g. Fig. 1B). Similar observations have been made using 100 μM Cd^{2+} or 5 μM nifedipine to inhibit Ca^{2+} influx [6]. This further indicated that hypoxia selectively inhibits $I_{K(Ca)}$ in these cells (Fig. 1A), as I_{Kv} remaining in this high Mg^{2+}, low Ca^{2+} solution was unaffected.

The phenylalkylamine D600 (methoxyverapamil), a Ca^{2+} channel blocker, reduces type I cell K^+ current amplitudes, and inhibits the shoulder in $I-V$ relationships [4], and so its effects on the hypoxic response of K^+ currents were tested. In the presence of 5 μM D600

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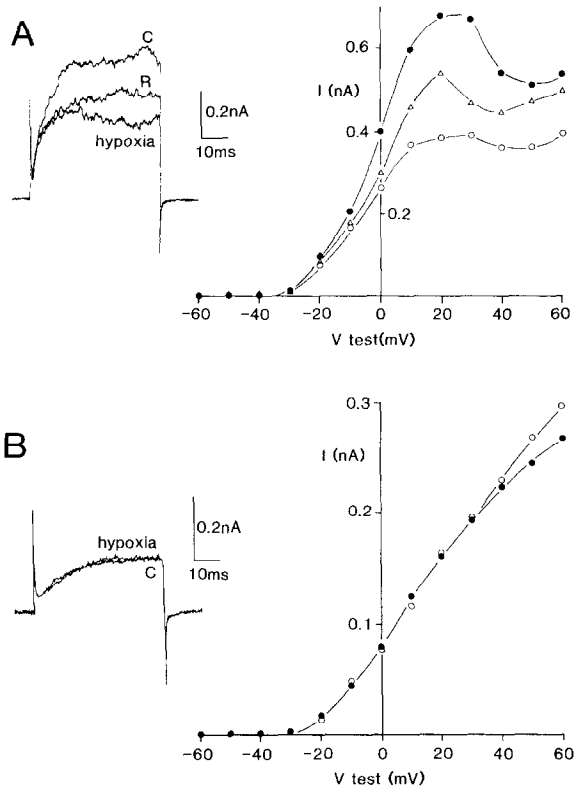

Fig. 1. (A) $I-V$ relationship obtained from a type I carotid body cell, before (●), during (○) and after (△) exposure of the cell to a hypoxic solution (pO_2 25 Torr). Inset, example traces under these conditions, V_{test} +10 mV. C, control; R, recovery. (B) $I-V$ relationship obtained in high Mg^{2+} (6 mM), low Ca^{2+} (0.1 mM) solution under normoxic (●) and hypoxic (○) conditions. Inset; example traces under these conditions, V_{test} +20 mV. C, control.

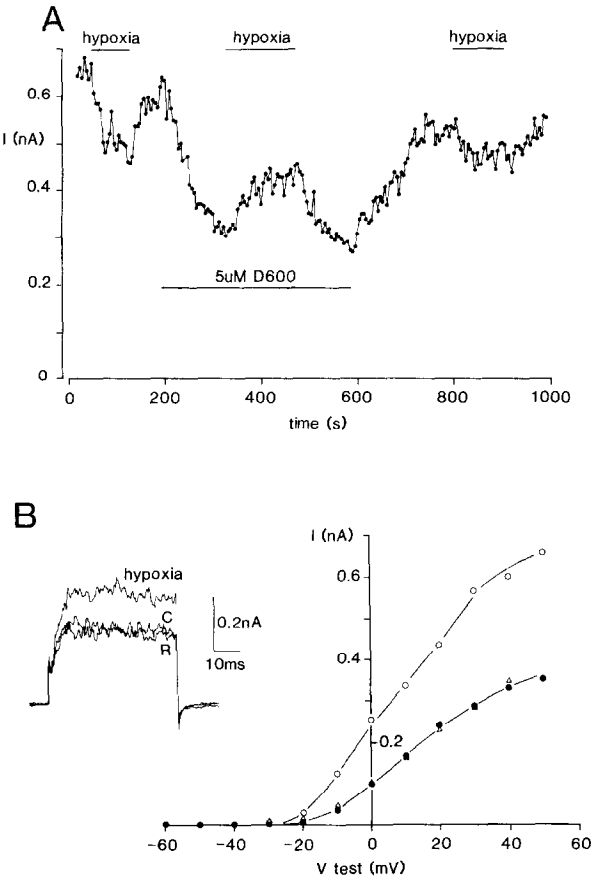

Fig. 2. (A) Time series plot of K^+ current amplitudes, each evoked by a 50 ms step depolarization to +20 mV. Exposures of the cell to hypoxic solutions and D600 (5 μM) indicated by horizontal bars. (B) $I-V$ relationships obtained in the presence of D600 throughout, recorded before (●), during (○) and after (△) a change to a hypoxic solution. Inset; example traces recorded under these conditions (V_{test} of +20 mV). C, control: R, recovery.

the residual IKv reversibly *increased* in amplitude when exposed to hypoxia (Fig. 2A). The $I-V$ relationship for this effect (Fig 2B), shows that, in the presence of D600, hypoxia reversibly enhanced K^+ currents at all activating V_{test}s studied ($P<0.05$ to $P<0.005$, $n=11$). No reappearance of the shoulder in the $I-V$ curve was ever apparent. The mean effect of hypoxia in the presence of D600 was to enhance K^+ currents by $55.4\pm9.3\%$ at a V_{test} of +20 mV. Similar effects were found using verapamil instead of D600 (not shown): in normoxia verapamil (5 μM) reduced K^+ currents at all activating V_{test}s, and in its presence hypoxia significantly ($P<0.02$) enhanced K^+ current amplitudes (e.g. by $54.6\pm14.0\%$ at V_{test} +20 mV; $n=8$).

These findings for D600 and verapamil were surprising, given the results shown in Fig. 1 which suggested that hypoxia selectively *inhibits* IK(Ca) in type I carotid body cells. However the phenylalkylamines do not only block Ca^{2+} channels, they also modulate K^+ currents directly [9,10], in particular in murine T lymphocytes, where they cause an apparent inactivation of Ca^{2+}-independent, delayed rectifier-type K^+ currents [10]. A possible direct effect of D600 on IKv in type I cells (and a modulation of the effect by hypoxia) was therefore investigated. To do this, K^+ currents were recorded in solutions containing raised Mg^{2+} (6 mM) and lowered Ca^{2+} (0.1 mM), so outward currents arose exclusively from activation of IKv (see Fig. 1B). K^+ currents recorded in this solution were inhibited by 5 μM D600 (Fig. 3A). This effect was voltage-dependent; at negative V_{test}s blockade was not statistically significant (<8%), but between V_{test}s of 0 mV and +60 mV currents were significantly reduced, by $21.4\pm4.6\%$ at 0 mV and by $36.5\pm4.7\%$ at +60 mV ($P<0.01$ to $P<0.002$, $n=8$). D600 also caused an apparent time-dependent inactivation of these currents, which were normally sustained during 50 or 100 ms step depolarizations.

In the absence of D600, IKv recorded in high Mg^{2+}, low Ca^{2+} solutions was also inhibited by 2 mM 4-aminopyridine (4-AP; Fig. 3A), to between $30.8\pm4.4\%$ and $37.6\pm2.3\%$ of control values over the V_{test} range −10 mV to +60 mV ($n=5$). This suggests that the sustained IKv found under these conditions in

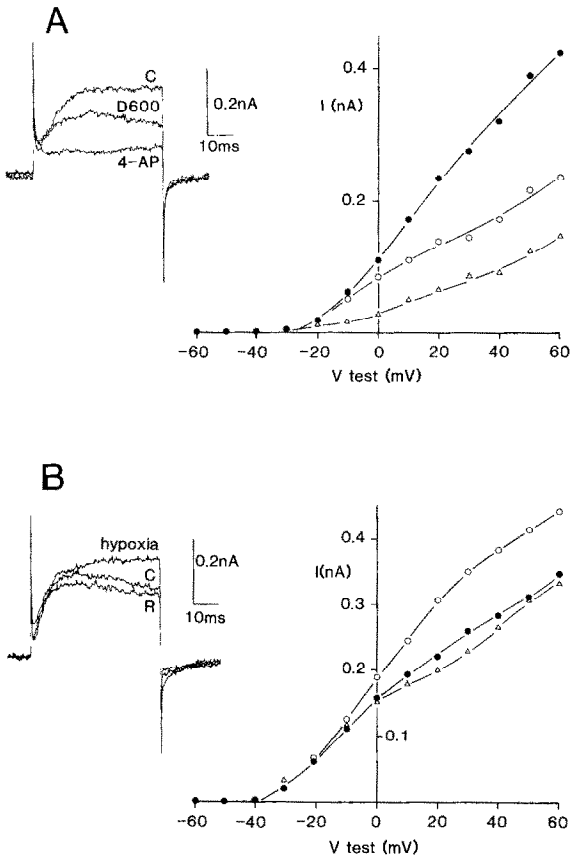

Fig. 3. (A) $I-V$ relationship obtained in high Mg^{2+}, low Ca^{2+} solution, in the absence of drugs (●), and in the presence of 5 μM D600 (○) and 4-aminopyridine (Δ; following recovery from D600). Inset; example traces under these 3 conditions, V_{test} +20 mV. C, control; 4-AP, 4-aminopyridine. (B) $I-V$ relationship recorded in high Mg^{2+}, low Ca^{2+} solution containing 5 μM D600 throughout, before (●), during (○) and after (Δ) exposure to a hypoxic solution. Inset; example traces, V_{test} +20 mV. C, control; R, recovery.

type I cells arise from activation of delayed rectifier-type K^+ channels seen in other cell types [11].

Hypoxia enhanced K^+ currents recorded in the presence of D600 (5 μM) in the high Mg^{2+}, low Ca^{2+} solutions, an effect similar to that in control solutions (Fig. 3B). This enhancement was reversible, and the K^+ currents which showed a time-dependent decay in the presence of D600 under normoxic conditions, appeared more sustained in hypoxic conditions (Fig. 3B), as they do under normoxic conditions in the absence of D600 (e.g. Fig. 1B). The hypoxic enhancement of K^+ currents in the presence of D600 in these high Mg^{2+}, low Ca^{2+} solutions was voltage-dependent; at V_{test}s of 0 mV or below, the effect of hypoxia was negligible, but at V_{test}s between +20 mV and +60 mV the effect of hypoxia was significant ($P<0.03$ to $P<0.002$), and currents were increasingly enhanced, by $27.5\pm6.5\%$ at +20 mV to $38.3\pm5.8\%$ at +60 mV ($n=9$). This voltage-dependent effect was similar to the voltage-dependence of D600 inhibition of the currents in normoxia (see above).

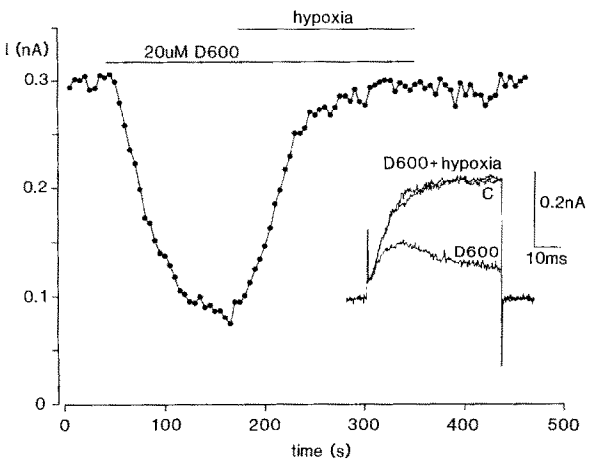

Fig. 4. Time series plot of K^+ current amplitudes, each evoked by a 50 ms step depolarization to +20 mV, recorded in high Mg^{2+}, low Ca^{2+} solution. Horizontal bars show periods of exposure to 20 μM D600 and hypoxia. Inset; example traces under the 3 conditions, V_{test} +20 mV. C, control.

These findings for hypoxia in the presence of D600 suggested that hypoxia relieved the blocking effect of D600 on I_{Kv}. This was further supported by results obtained using a higher dose of D600 (Fig. 4). Bath application of 20 μM D600 caused substantial reductions and a time-dependent decay of K^+ currents, but when the perfusing solution was made hypoxic *in the continued presence of D600*, K^+ currents returned to the same amplitudes and time-courses as were seen under normoxic conditions in the absence of the drug (Fig. 4). The currents recorded under normoxic conditions in the absence of D600 and under hypoxic conditions in its presence were indistinguishable (Fig. 4). These observations were found in all 5 cells where 20 μM D600 was tested.

4. DISCUSSION

The results presented here indicate that hypoxia selectively inhibits $I_{K(Ca)}$ in type I carotid body cells (Fig. 1), suggesting the channels underlying this current play a key role in chemotransduction, as hypoxia causes type I cell depolarization, Ca^{2+} influx and ultimately Ca^{2+}-dependent transmitter release from these cells [2,3]. Others have shown a high conductance (140 pS) K^+ channel that is inhibited by hypoxia [2] and it should be noted that channels underlying $I_{K(Ca)}$ reported here are of high conductance as they are inhibited by charybdotoxin, and not apamin [4].

The apparent reversal of hypoxic suppression caused by D600 (Fig. 2; and also presumably by verapamil) arose because hypoxia relieved a non-specific blocking effect of these Ca^{2+} channel antagonists on I_{Kv} (Figs. 3 and 4). Thus a second effect of hypoxia was revealed, but it is not known at present whether hypoxia acts on the drug itself, or renders it inactive on I_{Kv} in some

other manner. It is unlikely that hypoxia was affecting the channels underlying I_{Kv} as it was ineffective in high Mg^{2+}, low Ca^{2+} solutions in the absence of the drugs. The findings suggest that phenylalkylamines are unsuitable tools for investigating effects of hypoxia on Ca^{2+}-dependent mechanisms underlying chemotransduction in the intact carotid body.

Acknowledgement: This work was supported by the Wellcome Trust.

REFERENCES

[1] Fidone, S.J. and Gonzalez, C. (1986) in: Handbook of Physiology (Fishman, A.P., Cherniack, N.S., Widdicombe, J.G. and Geiger, S.R. eds) The Respiratory System, vol. 2 pp. 247–312, Am. Phys. Soc., Bethesda, USA.

[2] Delpiano, M.-A. and Hescheler, J. (1989) FEBS Lett. 249, 195–198.

[3] Obeso, A., Fidone, S. and Gonzalez, C. (1987) in: Chemoreceptors in Respiratory Control (Ribeiro, J.A. and Pallot, D.J. eds) pp. 91–97, Croom Helm, London.

[4] Peers, C. (1990) J. Physiol. 422, 381–395.

[5] Peers, C. and O'Donnell, J. (1990) Brain Res. 522, 259–266.

[6] Peers, C. (1990) Neurosci. Lett. (in press).

[7] Hescheler, J., Delpiano, M.A., Acker, H. and Pietruschka, F. (1989) Brain Res. 486, 79–88.

[8] Lopez-Barneo, J., Gonzalez, C., Urena, J. and Lopez-Barneo, J. (1988) Science 241, 580–582.

[9] Kass, R.S. and Tsien, R.W. (1975) J. Gen. Physiol. 66, 169–192.

[10] DeCoursey, T.E., Chandy, K.G., Gupta, S. and Cahalan, M.D. (1984) Biophys. J. 45, 144a.

[11] Hille, B. (1984) Ionic Channels of Excitable Membranes, Sinauer, Sunderland, USA.