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Evidence for a P_{O_2}-sensitive K^+ channel in the type-I cell of the rabbit carotid body

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Type-I cells of rabbit carotid bodies were studied with the patch-clamp technique in the whole-cell and on the cell-attached configuration. Cells exhibiting resting potentials of about −40 mV under normoxic conditions (P_{O_2}: 20 kPa), depolarized during hypoxia (P_{O_2}: 3.7 kPa). Hypoxia did not affect inward Ca^{2+} currents but inactivated outward K^+ currents in voltage-clamp experiments. Single-channel currents recorded for the cell-attached mode showed a slope conductance of about 137 pS and a 0 mV reversal potential under symmetrical K^+ concentration (140 mM). The open-probability (P_o) of the single channel was dependent on the extracellular P_{O_2}. These data demonstrate the existence of a P_{O_2}-sensitive K^+ channel in type-I cells, which may account for cell depolarization and the resulting chemosensory response.

Hypoxia; Patch-clamp; Oxygen partial pressure; Single-channel current; (Carotid body, Type-I cell)

1. INTRODUCTION

The carotid body, a peripheral chemoreceptor located in the bifurcation of the carotid arteries, responds to hypoxia with an increase in nerve excitation to regulate ventilation and circulation [9]. The P_{O_2}-sensory unit [13] of this organ, is composed of cells rich in catecholamines (type-I), in contact with nerve endings and surrounded by glia-like (type-II) cells. Since the chemosensory nerve response induced by hypoxia is closely related to both transmitter release from glomus cells [5] and depolarization of the nerve endings [7], it has been postulated that type-I cells may be the primary site where the chemosensory response is initiated. The intrinsic mechanism by which changes in P_{O_2} may disturb the metabolism of these cells resulting in transmitter release has not yet been explained. Several recent studies using the patch-clamp technique [6] have shown that type-I cells may possess different ionic channels [4,8,11] that could be involved in the P_{O_2}-sensing mechanism, as some of the ionic currents are affected by hypoxia [8,11]. Here, we demonstrate the presence of a P_{O_2}-sensitive K^+ channel that may be related to the hypoxia-induced transmitter release in type-I cells.

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2. MATERIALS AND METHODS

Patch-clamp experiments were carried out on carotid body type-I cells from rabbit embryos (primary cell culture). The organs were dissected and enzymatically dissociated according to Pietruschka [12]. Cells (no older than 4 days in culture) were seeded at a density of about 10 cell/mm^2 on small, polylysine-coated glass cover-slips. These cells were transferred into a small chamber and superfused with modified Locke's solution containing (in mM): 128 NaCl, 5.6 KCl, 1.8 $CaCl_2$, 5.5 glucose, 10 Hepes (pH 7.40) at 35°C. The solution was equilibrated with different gas mixtures (20% O_2, 3% CO_2; remainder: N_2) in two reservoirs using two gas-mixing pumps (Wösthoff, Bochum). The P_{O_2} of about 20.0 kPa flowing in the chamber from one reservoir was referred to as normoxia. Fast hypoxic stimulation was produced by quickly re-routing the flow into the chamber from the other reservoir containing a low P_{O_2} of about 3.0 kPa. The P_{O_2} and pH were respectively monitored with thin P_{O_2} and pH electrodes, and the P_{CO_2} of about 3.1 kPa was controlled by a gas analyser (AVL, Biomed. Inst., FRG) as described elsewhere [2,3]. For whole-cell as well as single-channel recording patch electrodes were prepared from pyrex glass capillaries.

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Their resistance amounted to 3–7 MΩ when filled with a solution containing (in mM): 80 K^+-aspartate, 50 KCl, 1 $MgCl_2$, 3 MgATP, 0.1 EGTA, 10 Hepes (pH 7.40) at 35°C. The cell-attached configuration was achieved by forming a seal using negative pressure (−30 mmH_2O). In the whole-cell recordings, the patch membrane was disrupted by increasing suction (−70 mmH_2O). The recording conditions for voltage control and monitoring of the currents were achieved as in [8]. In some experiments (see fig.1A), conventional microelectrodes (resistance 20–30 MΩ) were used for measuring the membrane potential.

3. RESULTS AND DISCUSSION

Fig.1 compares the membrane potential and its modulation by hypoxia, recorded in aggregated (A) as well as isolated type-I cells (B). In both cases, the membrane potential was around −40 mV and showed small fluctuations. In isolated cells, lowering the extracellular P_{O_2} from 20.0 to 3.7 kPa (constant P_{CO_2} and pH) induces an initial flickering of the membrane potential followed by a delayed (about 2 min) depolarization to 0 mV (fig.1B).

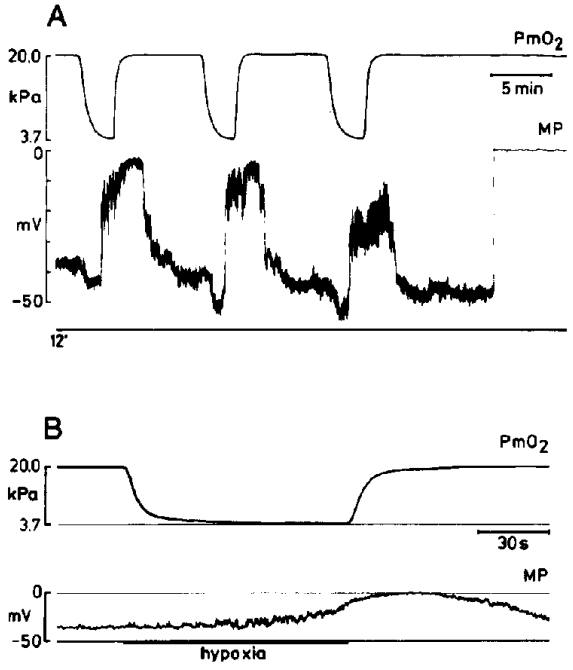

Fig.1. Changes in membrane potential of aggregated (A) and single (B) type-I cells stimulated by hypoxia. Upper part (A,B): P_{O_2} in the bath (P_{mO_2}) monitored by a catheter electrode; lower part (A,B): resting potential (MP). The right-hand side of A shows the 0 mV level after removing the electrode from the cell.

This depolarization outlasted the hypoxic period but recovered after normoxia. In the case of aggregated cells the depolarization was preceded by a slight (about 10 mV) hyperpolarization (fig.1A). The electrical response in isolated cells was observed in most cases ($n = 50$). In no case did we observe action potentials as been described for cells from adult animals [4]. Since depolarization of the membrane potential can be produced by either an increase in the inward or a decrease of the outward currents, it seems that for type-I cells the latter accounts for this effect. As illustrated in fig.2A, the inward current was not affected by hypoxia. In contrast, hypoxia inactivated the outward current (fig.2B). These results agree with those from previous voltage-clamp studies where these components were characterized using pharmacological tools as Ca^{2+} and K^+ currents, respectively [4,8,11]. Since the input resistance of type-I cells is high (about 4 GΩ) at the resting potential of about −40 mV [4,8], it was expected that small changes in currents at this level may determine clear changes in the membrane potential. Therefore, we examined the cell membrane with respect to single channels using the cell-attached mode at this negative potential. As illustrated in fig.3, single-

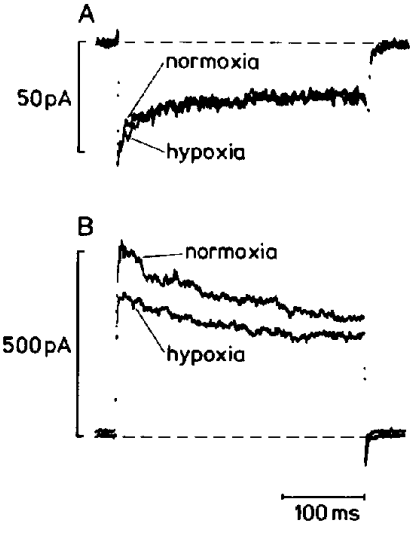

Fig.2. Voltage-dependent Ca^{2+} inward (A) and K^+ outward (B) currents of type-I cells measured in the whole-cell configuration. (A,B) Voltage-clamp pulses from −40 to 0 mV and from −50 to 50 mV were applied, respectively. Bath containing TTX (3 μM) (for details see [8]).

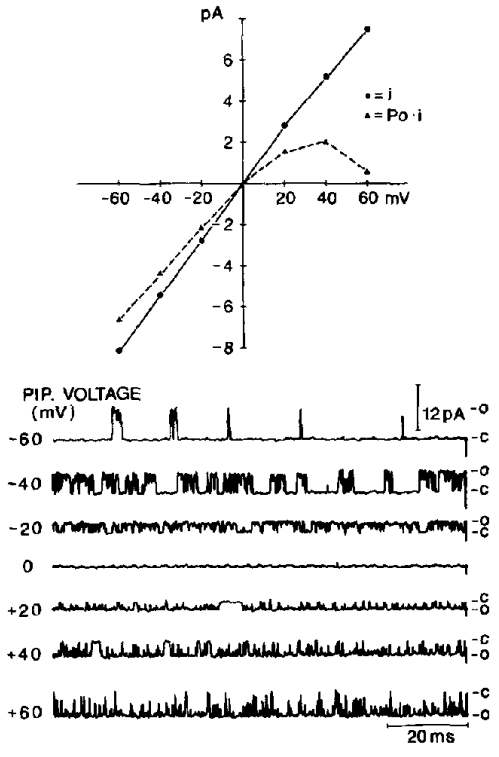

Fig.3. Single-channel recordings from chemoreceptor type-I cells. (Lower panel) Single-channel current at different pipette potentials (indicated on the left). (Upper panel) Data points (filled circles) in the i-V curve with a single-channel conductance of 137 pS and a 0 mV reversal potential. The iP_o-V relationship for macroscopic currents exhibits inward rectification (filled triangles).

channel events recorded under symmetrical K^+ conditions were characterized by their voltage dependency. On variation of the pipette potential from -60 to 60 mV the single channel amplitude (i) changed with respect to the membrane potential in a linear fashion. A plot for the i-V curve (filled circles) could be fitted by a straight line with a slope conductance of 137 pS, which reversed at 0 mV. This channel behaviour accounts for the bidirectional permeation through the channel pore and the reversal potential of 0 mV denotes high K^+ selectivity. Similarly to others [4], no activity was observed when K^+ was replaced by Cs^+ in the extra- and intracellular solutions (not shown). As illustrated in the lower part of fig.3, the channel open probability (P_o), estimated from the total time in the open state divided by the time of the recording, was voltage-dependent. This is clearly depicted in the iP_o-V curve (fig.3, dark triangles), where at positive membrane potentials the P_o was low (e.g. P_o: 0.07 at 60 mV) and high at negative values (e.g. P_o: 0.82 at -60 mV). The iP_o-V curve for the whole-cell current was obtained from the relationship: $I_{MAC} = P_o \cdot i \cdot N$ [1], where I_{MAC} represents macroscopic currents and N the number of channels, with i and P_o being as defined above. This iP_o-V curve displays inward rectification [10], i.e. higher conductance at negative potentials (resting potential of type-I cell) and lower above 0 mV. Therefore, this single K^+ channel may correspond only to a partial component of the outward-rectifying K^+ currents observed in the total membrane recordings [4,8,11]. Such outward current had a slope conductance of 11 nS positive to -20 mV and 0.8 nS below this threshold [8].

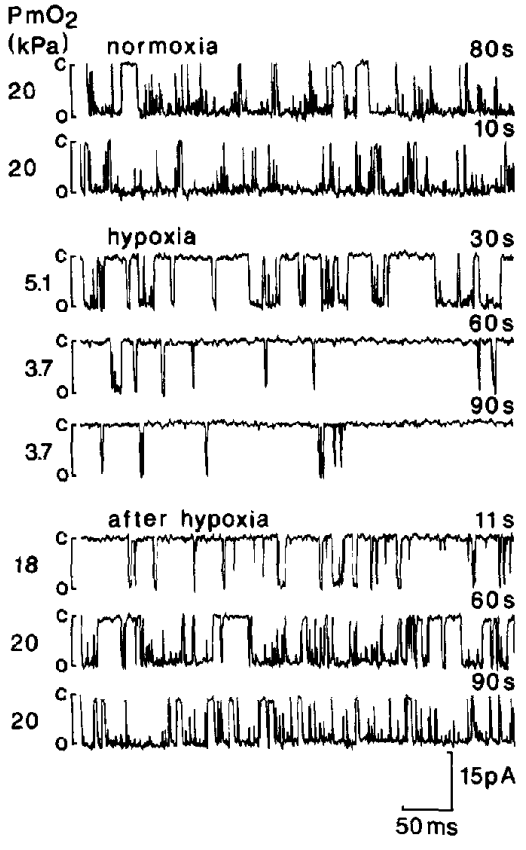

Fig.4. Single-channel recordings at a membrane potential of -50 mV under normoxia (P_{mO_2} 20 kPa) and hypoxia (P_{mO_2} 3.7 kPa). Channel closing is shown by an upward deflection. Under hypoxia there was a drastic decrease in open probability (P_o), which was restored after 90 s of normoxia.

Thus, due to its iP_o-V relationship this K^+ channel may be physiologically relevant in the influence of hypoxia on the resting potential of type-I cells. This assumption is supported by the behaviour of this channel during hypoxia. As shown in fig.4, at a membrane potential of -50 mV, P_o amounts to 0.86 under normoxic conditions (P_{O_2} 20.0 kPa, 1st trace) and decreases to 0.11 under hypoxia (P_{O_2} 3.7 kPa, 5th trace). In accordance with the delayed depolarization (fig.1B), the change in P_o is slow in onset and outlasts the period of hypoxia, i.e. it undergoes maximal decrease 90 s after the onset of hypoxia (5th trace). Such a finding stresses the interconnection between channel closing and depolarization and allows one to suggest that a second messenger could mediate the effect of hypoxia. From this, the single K^+ channel should be mediated by some metabolic agent [8].

In conclusion, the behaviour of this P_{O_2}-sensitive K^+ channel indicates a possible mechanism to explain the effect of hypoxia on type-I cells. This channel could also be responsible for instability of the resting potential (fig.1), as it appears to be active at negative potentials (fig.4), i.e. continuous flow of inward current. However, further experiments are necessary in order to clarify whether other mechanism could also be involved in O_2-chemotransduction, as in aggregated type-I cells the depolarization is preceded by a transient hyperpolarization (fig.1A).

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