Differential oxygen sensitivity of calcium channels in rabbit smooth muscle cells of conduit and resistance pulmonary arteries

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1. Calcium currents were recorded from smooth muscle cells dispersed from conduit and resistance rabbit pulmonary arteries. We tested the hypothesis that Ca^{2+} channel activity was regulated by environmental O_2 tension.

2. Conduit (proximal) and resistance (distal) myocytes differ in their Ca^{2+} channel density and responses to low P_{O_2}. Ca^{2+} current density in distal myocytes ($20{\cdot}7 \pm 7{\cdot}4$ pA pF^{-1}, $n = 10$) is almost twice the value in proximal myocytes ($12{\cdot}6 \pm 5{\cdot}5$ pA pF^{-1}, $n = 39$). In proximal myocytes, the predominant response to reductions in P_{O_2} is inhibition of the calcium current ($n = 12$) at membrane potentials below 0 mV, whereas potentiation of current amplitude is observed in distal myocytes ($n = 24$).

3. Hypoxia also produces opposite shifts in the conductance–voltage relationships along the voltage axis. The average displacements induced by low P_{O_2} are $+5{\cdot}05 \pm 2{\cdot}98$ mV ($n = 5$) in proximal myocytes and $-6{\cdot}06 \pm 2{\cdot}45$ ($n = 10$) in distal myocytes.

4. These findings demonstrate longitudinal differences in Ca^{2+} channel density and O_2 sensitivity in myocytes along the pulmonary arterial tree. These results may help to understand the differential reactivity to hypoxia of the pulmonary vasculature: vasodilatation in conduit arteries and vasoconstriction in resistance vessels.

Blood oxygen content is known to be an important local factor in the regulation of circulation (for reviews see Sparks, 1980; Wadsworth, 1994). In systemic arteries a decrease in local O_2 tension (P_{O_2}) normally leads to vasodilatation whereas hypoxia predominantly evokes the opposite response (vasoconstriction) in the pulmonary circulation (Dawson, 1984; Harder, Madden & Dawson, 1985*a*; Madden, Vadula & Kurup, 1992). Hypoxic pulmonary vasoconstriction (HPV) is a vasomotor response of enormous physiological and pathophysiological importance (for a review see Dawson, 1984); however, the mechanisms underlying the vascular reactivity of pulmonary vessels to changes in P_{O_2} are poorly known. Although HPV can be modulated by substances released from the endothelium and the lung parenchyma, a number of studies have localized the basic O_2-sensing process to the vascular smooth muscle cell (Dawson, 1984; Hales, 1985). For example, HPV can be demonstrated in arterial rings denuded of the endothelium (Yuan, Tod, Rubin & Blaustein, 1990) and in isolated myocytes (Madden *et al.* 1992). A decade ago it was shown that hypoxia induces depolarization and spontaneous Ca^{2+}-dependent action potentials in myocytes of small pulmonary arteries, and suggested that low P_{O_2} could directly produce an increase of membrane Ca^{2+} permeability in smooth muscle cells (Harder *et al.* 1985*a*, *b*). More recently, several groups have shown that hypoxia inhibits the activity of voltage-dependent K^+ channels in pulmonary myocytes suggesting that HPV could be due, at least in part, to the inhibition of K^+ conductance, which in turn might lead to depolarization and Ca^{2+} influx (Post, Hume, Archer & Weir, 1992; Yuan, Goldman, Tod, Rubin & Blaustein, 1993), a sequence of events similar to that proposed to explain O_2 chemo-transduction in carotid body glomus cells (for a review see López-Barneo, Benot & Ureña, 1993). As it has been shown that the reduction of P_{O_2} reversibly inhibits the activity of L-type voltage-gated Ca^{2+} channels in myocytes dispersed from systemic arteries (Franco-Obregón, Ureña & López-Barneo, 1995), where hypoxaemia normally produces vasodilatation (Sparks, 1980; Marriot & Marshall, 1990), we hypothesized that an opposite modulation of Ca^{2+} channel activity by O_2 could be observed in the lung vessels. This was tested on myocytes dispersed from

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conduit and resistance pulmonary vessels. Segmental differences in the pulmonary vascular reactivity to hypoxia are well documented (Harder *et al.* 1985*a*,*b*; Marriot & Marshall, 1990; Madden *et al.* 1992; Vadula, Kleinman & Madden, 1993); thus, another objective of this work was to determine whether these differences are reflected in the electrophysiological properties of pulmonary myocytes (see also Archer *et al.* 1996). We report here that the density of L-type Ca^{2+} channels and their sensitivity to O_2 vary longitudinally along the pulmonary arterial tree. Inhibition of Ca^{2+} channel activity is the predominant response to low P_{O_2} in myocytes dispersed from the main conduit arteries whereas the opposite response is observed in cells dispersed from the distal resistance vessels. A preliminary account of these data has been published in abstract form (Ureña, Franco-Obregón & López-Barneo, 1995).

METHODS

Smooth muscle cells were enzymatically dispersed from arteries of male rabbits following the general methodology described previously (Franco-Obregón *et al.* 1995). Rabbits weighing 1–2 kg were killed by a sodium pentobarbitone overdose. Lungs were quickly removed from rabbits and immediately placed into cold (4 °C) Hanks' solution where the pulmonary arteries were dissected carefully from the surrounding tissue. Pieces of the vessels (1 mm^2) were placed into 5 ml cold (4 °C) Hanks' solution to which 7 mg papain (Sigma), 5 mg collagenase (type IA; Sigma) and 3·5 mg bovine serum albumin (fraction V; Sigma) had been added. The tissue was stored for 1–6 h at 6 °C and afterwards transferred to fresh Hanks' solution containing bovine serum albumin (10 mg in 50 ml). Cells were mechanically dissociated and plated on pieces of glass coverslips coated with poly-L-lysine. During the experiments a coverslip was placed in a chamber of approximately 0·2 ml with continuous flow of solution. Most of our recordings were done on large (> 150 μm in length) fibre-like cells with a birefringent appearance. The external solution was equilibrated with either air (P_{O_2} ~150 mmHg) or mixtures of N_2 and air to obtain the desired O_2 levels. P_{O_2} in the chamber was monitored with an O_2-sensing electrode. The mean interval of time between the switching of bath perfusion to a drop in P_{O_2} was ~3 s and saturation of the bath at a low P_{O_2} level of ~20 mmHg was achieved within ~50 s. Calcium currents were recorded using the whole-cell configuration of the patch-clamp technique after blockade of the voltage-dependent K^+ channels. Membrane currents were recorded using an EPC-7 patch-clamp amplifier (List Electronics) and on-line subtraction of linear capacity and leakage currents. The speed at which clamping was achieved was improved by the use of low resistance electrodes (1–3 MΩ) and ballistic charge of membrane capacitance. Given the relatively small size of the currents studied we did not systematically use series resistance compensation. Data acquisition and analysis were done as previously described (Franco-Obregón *et al.* 1995). The external recording solution contained (mM): 140 NaCl, 2·7 KCl, 10 $BaCl_2$, 10 Hepes; pH 7·4. The internal solution (the solution in the patch pipette and inside the cell) contained (mM): 100 CsCl, 25 CsF, 2 $MgCl_2$, 10 Hepes, 10 EGTA, 5 BAPTA, 4 MgATP; pH 7·3. The pH of the extracellular recording solution changed less than 0·05 pH units while equilibrating with N_2. Ba^{2+} was used as charge carrier instead of Ca^{2+} to favour the flow of current through the Ca^{2+} channels. ATP was used to prevent the wash-out of the channels. All experiments were conducted at room temperature (22–25 °C). Unless otherwise noted, all the data are given as means ± S.D.

RESULTS

The dual effect of P_{O_2} on Ca^{2+} channel activity in pulmonary vascular smooth muscle is illustrated in Fig. 1*A* with recordings of calcium currents obtained from isolated, voltage-clamped myocytes during depolarizing steps to −10 mV. In cells dispersed from the main arterial trunk (proximal myocyte) hypoxia produced inhibition of current amplitude. This response to low P_{O_2} is qualitatively similar to the one reported previously in systemic myocytes (Franco-Obregón *et al.* 1995). By contrast, in cells obtained from finer, tertiary, arterial branches (distal myocyte) exposure to hypoxia produced potentiation of current amplitude, a response rarely seen in proximal myocytes and never observed in over fifty recordings of myocytes dispersed from various systemic arteries. Reversibility of the responses to hypoxia in the two types of myocytes is shown by the superposition of the control and recovery traces obtained in normoxic solutions. In spite of the differential sensitivity to oxygen, calcium channels of proximal and distal myocytes share a common pharmacology and voltage dependence. Both currents are blocked by nifedipine (not shown) and, as demonstrated in Fig. 1*B* and *C* (top panel), exhibit similar current–voltage relationships. However, peak current amplitude at +10 mV in proximal myocytes (423 ± 214 pA, $n = 41$) was significantly smaller than in distal myocytes (801 ± 360 pA, $n = 21$; $P < 0·01$). With the estimated whole-cell capacitance values of 31·2 ± 7 and 40·6 ± 10 pF for proximal and distal myocytes, respectively, current density (the ratio of membrane current to membrane capacitance in picoamps per picofarad) was also smaller in proximal (12·6 ± 5·5 pA pF^{-1}, $n = 39$) than in distal (20·7 ± 7·4 pA pF^{-1}, $n = 10$) pulmonary myocytes ($P < 0·01$) (Fig. 1*C*, bottom panel). Thus, the effect of hypoxia (either inhibition or facilitation) on Ca^{2+} channels in pulmonary myocytes, as well as calcium current density, vary longitudinally along the pulmonary arterial tree. Hypoxic potentiation of current elicited with moderate depolarizations is the most frequent response (see below) observed in distal myocytes, these cells having the highest density of Ca^{2+} channels.

The modifications of Ca^{2+} channel activity induced by changes of low P_{O_2} are dose dependent and during ramp-like exposures to hypoxia current amplitude decreases in parallel with O_2 tension in the chamber. This is illustrated in Fig. 2*A* and *B* with recordings from a myocyte exposed to low P_{O_2}. Peak current amplitude is plotted as a function of the P_{O_2} value achieved in the bath, which was extrapolated from the signal of the oxygen-sensing electrode placed in the vicinity of the cell. It is clear that

the amplitude of the current decreases within seconds of lowering P_{O_2} with a time course roughly similar to bath exchange. Also note that the current fully recovers its initial amplitude promptly following the re-establishment of normoxia to the bath.

The results shown in Fig. 1*A* represent an extreme manifestation of the opposite voltage dependence of the interaction of O_2 with the Ca^{2+} channels of proximal and distal myocytes shown in Fig. 3. In more than 80% of proximal myocytes exhibiting stable recordings, the inhibitory effect of low P_{O_2} on Ca^{2+} channels was more potent at negative membrane potentials and diminished as the membrane was depolarized to progressively more positive voltages (set of traces at the left in Fig. 3*A*). This voltage-dependent inhibition of Ca^{2+} channel activity is similar to the effect of hypoxia described in systemic arteries (Franco-Obregón *et al.* 1995). An opposite effect was seen in most distal pulmonary myocytes (87%, $n = 24$), where

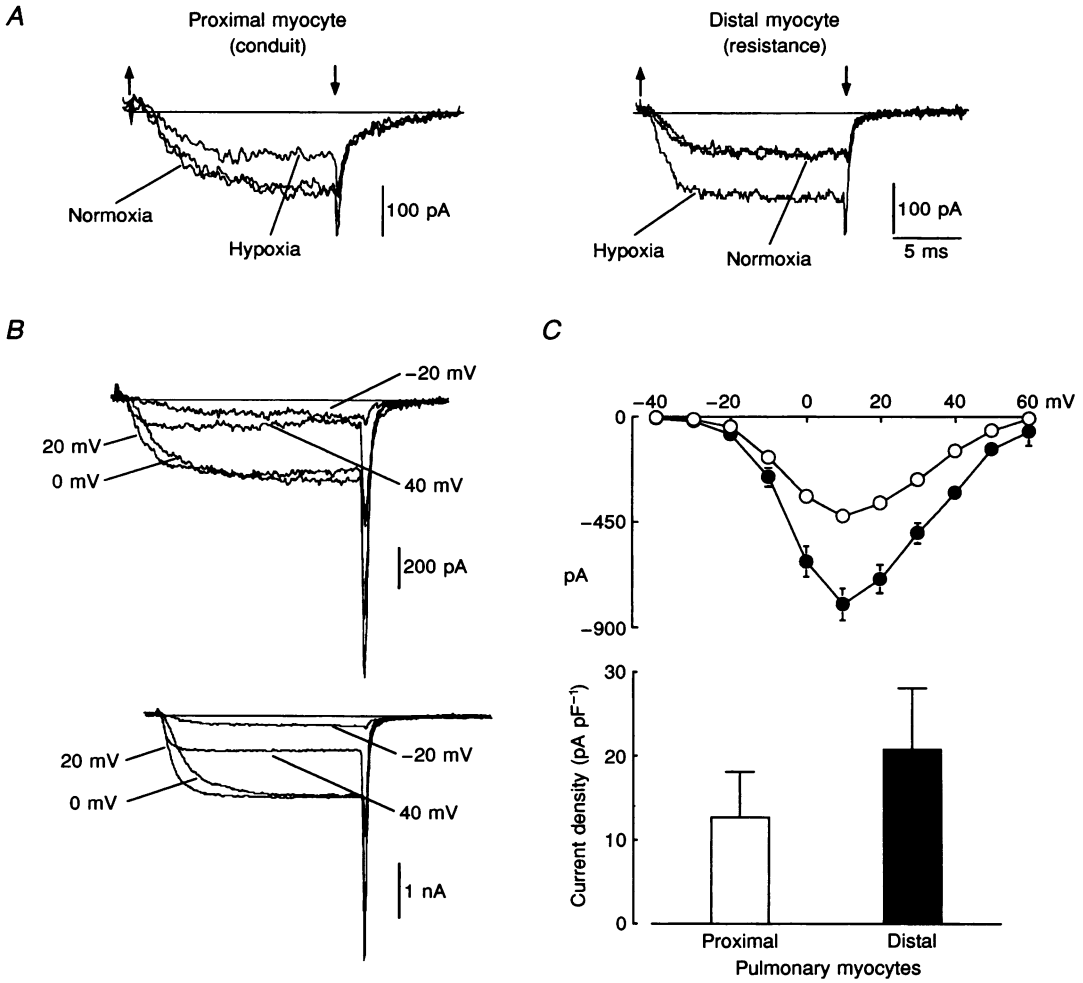

Figure 1. Dual modulation of Ca^{2+} channel activity by low P_{O_2} in pulmonary arterial myocytes

A, currents recorded from patch-clamped smooth muscle cells dispersed from proximal (left panel) and distal (right panel) pulmonary arteries during depolarizations to −10 mV from a holding potential of −80 mV. The onset and end of the depolarizing pulses are indicated by the arrows. The control and recovery traces recorded in the normoxic solution are shown superimposed. *B*, families of Ca^{2+} currents recorded from representative proximal (upper panel) and distal (lower panel) pulmonary myocytes during depolarizations to the indicated membrane potentials from a holding potential of −80 mV. *C*: top panel, mean calcium current–voltage relationships in proximal (○; $n = 41$) and distal (●; $n = 21$) myocytes of the pulmonary arterial tree. The plot shows the average peak inward current (ordinate, mean ± S.E.M.) elicited with voltage pulses to the indicated membrane potentials (abscissa) from the holding potential of −80 mV. Despite the differences in magnitude, the shapes of the current–voltage curves are the same in the two classes of smooth muscle cells. *C*: bottom panel, current density in proximal and distal myocytes.

we observed an inverse voltage dependence. Here, hypoxia produced a facilitatory effect on current amplitude generated during moderate depolarizations but elicited inhibition at positive membrane potentials (set of traces at the right in Fig. 3*A*). A summary of the modulation by P_{O_2} of Ca^{2+} currents recorded over a broad range of membrane potentials is shown in Fig. 3*B*. At −30 mV, which is a membrane potential value near the threshold for action potential in vascular smooth muscle (Harder *et al.* 1985*a*, *b*), low P_{O_2} inhibits current amplitude by approximately 90% in most proximal myocytes whereas potentiation of current amplitude (~80%) was observed in distal myocytes. As evidenced by the size of the standard deviation bars, the facilitatory response to hypoxia at negative membrane potentials varied from almost no effect in some cells to more than a 100% increase in current amplitude in other cases. Low P_{O_2} will surely have an even larger effect on Ca^{2+} channel open probability at more negative membrane potentials; however, this could not be studied due to the small size of the currents with pulses below −30 mV.

The inverse voltage-dependent interaction of O_2 with the Ca^{2+} channels in proximal and distal myocytes can be explained by the data shown in Fig. 4. The currrent–voltage relationship in Fig. 4*A* (left panel) illustrates the typical voltage-dependent hypoxic reduction of current amplitude in proximal myocytes. Note also that on exposure to hypoxia the peak of the current–voltage curve is displaced to positive voltages. This is also manifested as a ~5 mV shift toward more positive membrane potentials of the conductance–voltage relationship while in the presence of low P_{O_2}. The average, reversible shift measured in several proximal myocytes ($n = 5$) was 5·05 ± 2·98 mV (see Fig. 4 legend). By contrast, hypoxia produced an opposite displacement (towards negative membrane potentials) of the current–voltage relationship in distal myocytes (Fig. 4*B*). The shaded areas in Fig. 4*B* (left panel) show how, as a result of the shifts, hypoxia would produce both current potentiation (at voltages below 0 mV) and current inhibition at positive membrane potentials. In distal myocytes, the displacement of the current–voltage curve

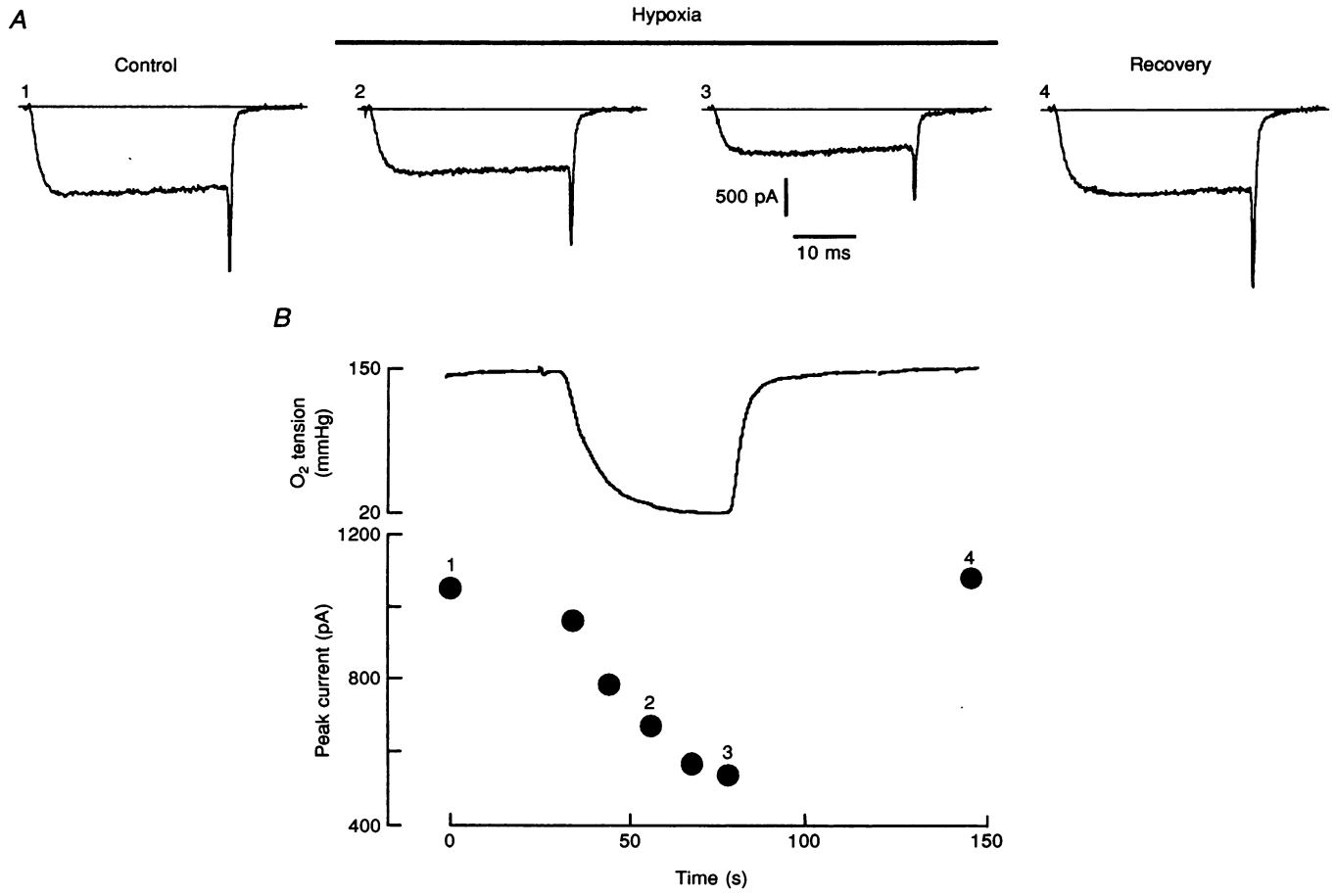

Figure 2. Time course of the low P_{O_2}-induced modifications in current amplitude

A, traces demonstrating the reversible hypoxic inhibition of the current recorded in a distal myocyte during the application of voltage pulses to +10 mV from a holding potential of −80 mV. *B*, relationship between O_2 tension in the bath and inhibition of the peak inward current. The numbers indicate the times during which the current traces in panel *A* were taken.

was due to a reversible shift toward negative potentials of the conductance–voltage curve of 6·06 ± 2·45 mV ($n = 10$) (Fig. 4*B*, right panel). The effects of low P_{O_2} on the biophysical parameters of the Ca^{2+} current appear to be selective for activation since no modifications are observed in the time courses of inactivation or deactivation. Thus, proportional hypoxic reduction of peak and steady-state current amplitudes were observed with long (> 500 ms) depolarizing pulses (data not shown; see also Franco-Obregón *et al.* 1995).

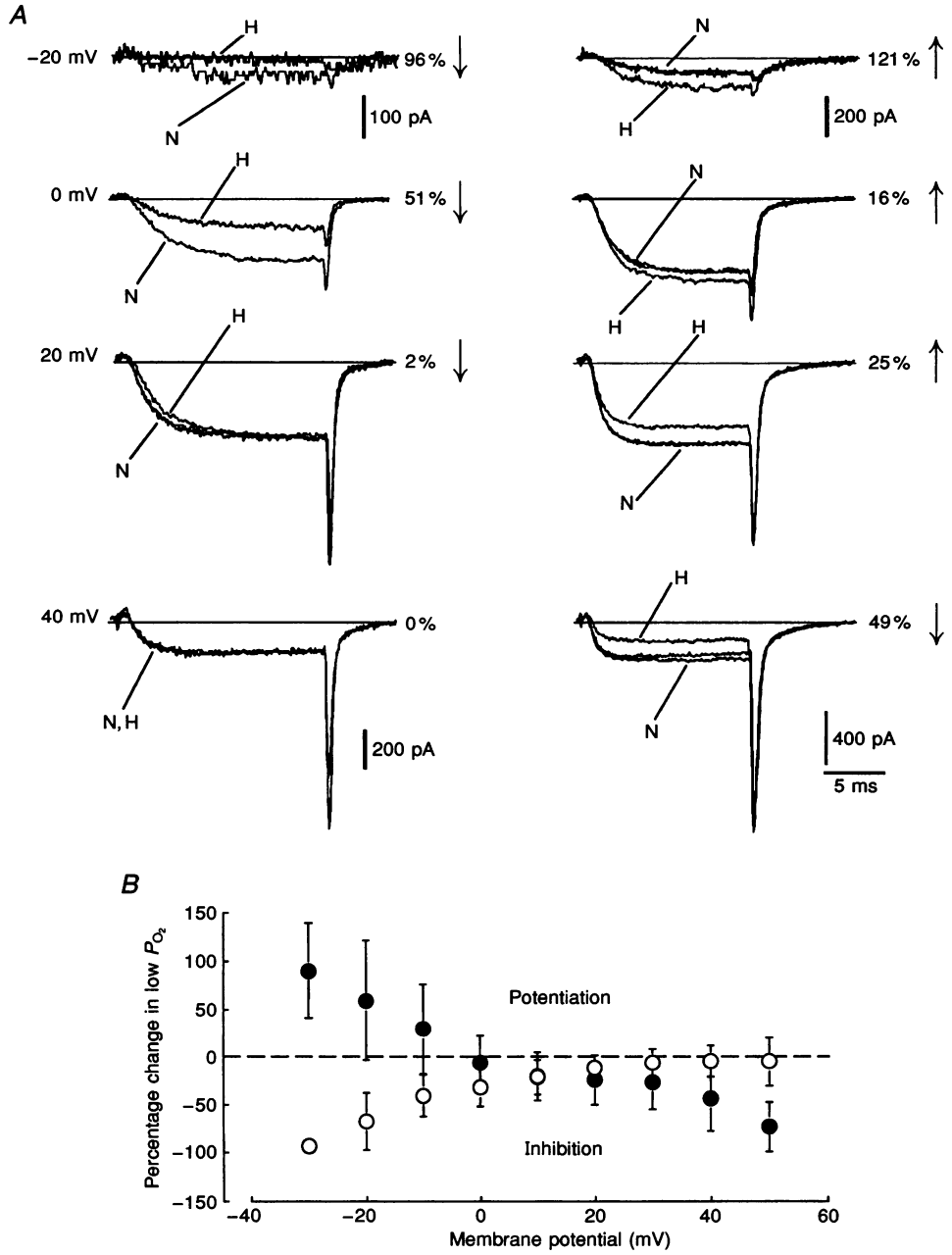

Figure 3. Opposed O_2 sensitivity of Ca^{2+} channels in the pulmonary vasculature

A, current traces demonstrating the effect of hypoxia (H) on the calcium currents elicited by voltage pulses to −20, 0, +20 and +40 mV in proximal (left panel) and distal (right panel) pulmonary myocytes. In most cases, control and recovery traces obtained in normoxic conditions (N) are shown superimposed. The changes in peak current amplitude, expressed as a percentage of the control values, are given in each case to the right of the traces. Upward arrows indicate potentiation and downward arrows indicate inhibition. Note that the recordings at −20 mV have a higher magnification than the rest. *B*, summary of the results, illustrating the inverse voltage dependence of the interaction of O_2 with the Ca^{2+} channels in proximal (○) and distal (●) pulmonary myocytes. The data points are the means ± S.D. of measurements from 24 distal and 12 proximal myocytes.

DISCUSSION

The electrophysiological observations reported here strongly suggest the existence of two major types of pulmonary arterial myocytes regarding the modulation by P_{O_2} of their voltage-gated Ca^{2+} channels. The main arterial trunk predominantly contains 'proximal myocytes' where Ca^{2+} currents are inhibited by low P_{O_2}. 'Distal myocytes', with an opposite response to hypoxia (potentiation of the Ca^{2+} current), are rarely seen in the main trunk or proximal branches but are highly concentrated in the smaller arterial ramifications. These results are in accordance with recent data showing that pulmonary vascular smooth muscle cells are heterogeneous in terms of their morphology and responses to vasoactive agonists (see Archer *et al.* 1996 for references). Moreover, it has been recently reported that pulmonary myocytes dispersed from resistance vessels possess a higher density of O_2-sensitive, voltage-dependent K^+ channels than conduit myocytes (Archer *et al.* 1996). Together, these observations help to explain the differential responses to hypoxia of the proximal and distal segments of the lung vasculature. As in systemic vessels, hypoxia-induced vasodilatation is the

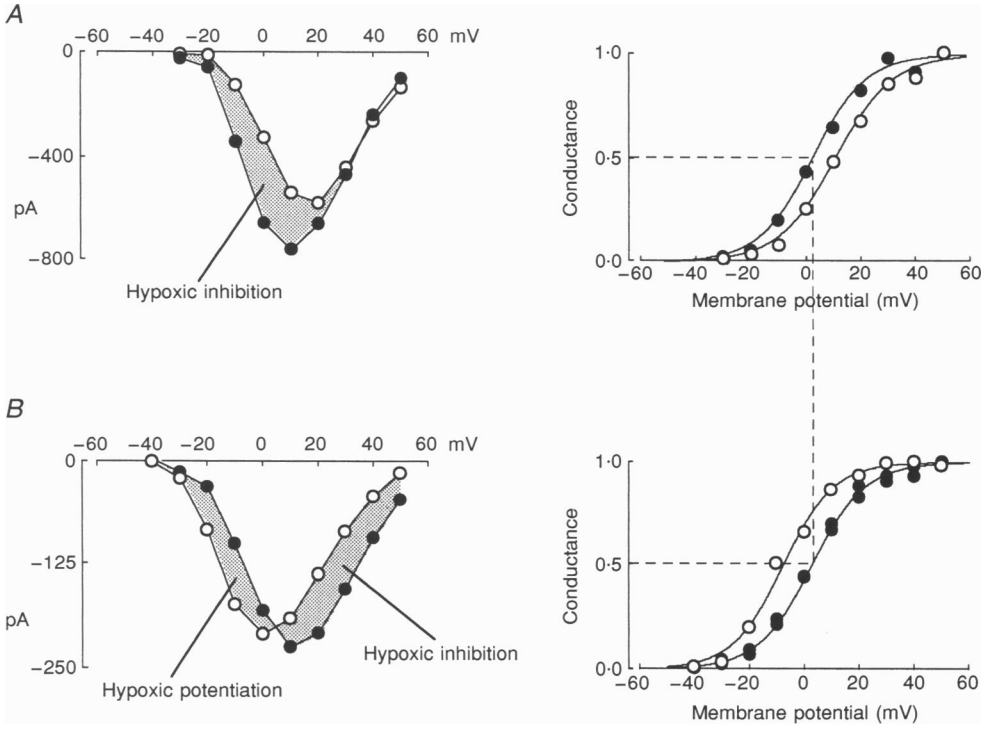

Figure 4. Differential effects of low P_{O_2} on the Ca^{2+} conductance–voltage relationship in distal and proximal pulmonary smooth muscle cells

A, relationships between peak current amplitude (left panel) and conductance (right panel) with membrane potential in a typical proximal pulmonary myocyte exposed to normoxic (●) and hypoxic (○) solutions. Conductance was estimated from the amplitude of tail currents recorded upon repolarization to −80 mV of voltage pulses delivered to variable membrane potentials. Control and recovery values in the normoxic solution have been pooled together for the plot. The values were normalized to the amplitude of the tails at +50 mV. Curves on the right are least-square fits to a Boltzmann function of the form: $G = G_{max}/(1 + \exp(V - V_{½})/k)$, where G_{max} is maximal conductance, V the membrane potential during the pulse, $V_{½}$ the potential at which 50% of G_{max} is obtained, and k a slope factor that indicates the steepness of the curve. The estimated values of k were affected little by low P_{O_2} (10 mV in normoxia and 9 mV in hypoxia). By contrast, $V_{½}$ in the normoxic solution was shifted by ~5 mV to positive potentials during exposure to low P_{O_2}. *B*, similar analysis as in *A* performed with the currents recorded from a distal myocyte. Hypoxia (○) produces a displacement of ~8 mV toward negative membrane potentials of both the peak current–voltage (left) and the conductance–voltage (right) relationships. Note that under normoxic conditions (●) the positions of the current–voltage curves and the conductance–voltage relationships (dotted line) were almost identical in the two types of myocytes. In the text we give the averages of individual shifts calculated for each of the 15 proximal and distal myocytes exposed to normoxia and hypoxia. In addition, $V_{½}$ values measured in normoxia and hypoxia in the population of cells studied were also significantly different in the two types of myocytes ($P < 0·05$ for proximal and $P < 0·001$ for distal myocytes; Student's paired t test).

most frequent response observed in the large, conduit pulmonary arteries (Marriot & Marshall, 1990; Vadula *et al.* 1993), whereas hypoxic vasoconstriction is known to occur in the finer resistance vessels with diameters between 200 and 600 μm (Harder *et al.* 1985*a*, *b*; Madden *et al.* 1992; Vadula *et al.* 1993). These vasomotor responses to low P_{O_2} may serve different physiological roles. Vasodilatation of the conduit pulmonary arteries in response to hypoxaemia favours the perfusion of the lung and the uptake of O_2; by contrast, vasoconstriction of resistance vessels in response to local decreases of P_{O_2} contributes to the proper distribution of blood in the lung by favouring the irrigation of the better ventilated alveoli. Our results also give support to the notion that HPV is due, at least in part, to a direct interaction of O_2 with arterial smooth muscle cells. They are also in accord with previous accounts showing that Ca^{2+} channel antagonists, such as verapamil and dihydropyridines, prevent the appearance of acute HPV (McMurtry, Davidson, Reeves & Grover, 1976), and that hypoxia increases cytosolic [Ca^{2+}] in myocytes of the small pulmonary vessels (Salvaterra & Goldman, 1993). Thus it is highly likely that the hypoxic potentiation of the calcium current observed in distal myocytes is one of the cellular mechanisms responsible for HPV.

It has been shown that the biophysical properties of Ca^{2+} channels suit them well for a major participation in the regulation of the contractile force near the resting membrane potential both in vascular (Nelson, Standen, Brayden & Worley, 1988) and tracheal (Fleischmann, Murray & Kotlikoff, 1994) myocytes. At the resting potential of vascular smooth muscle cells (−50 to −45 mV; see Harder *et al.* 1985*a*, *b*) only a few Ca^{2+} channels will be active. However, channel open probability would increase dramatically if the voltage dependence of activation is shifted toward negative membrane potentials, which is the situation in distal pulmonary myocytes upon exposure to hypoxia. Given the high Ca^{2+} channel density in distal pulmonary myocytes, this would lead to sufficient Ca^{2+} entry as to produce membrane depolarization and contraction (see Fleischmann *et al.* 1994). These phenomena explain the abolishment of the hypoxia-induced depolarizations of distal pulmonary myocytes by the Ca^{2+} channel blocker verapamil (Harder *et al.* 1985*a*, *b*), as well as the external Ca^{2+} dependence of the hypoxia-induced increase in 'resting' tension (Yuan *et al.* 1990). Besides the potentiation of Ca^{2+} channel activity described here, the inhibition of voltage-gated K^+ currents by hypoxia in peripheral pulmonary myocytes is another mechanism proposed to participate in HPV (Post *et al.* 1992; Yuan *et al.* 1993; Archer *et al.* 1995). In this respect, concomitant reduction of the K^+ conductance and the potentiation of Ca^{2+} channel activity in distal myocytes would enhance the pressor response of the pulmonary arteries to low P_{O_2}.

In conclusion, we have observed longitudinal differences in the modulation of Ca^{2+} channel activity by hypoxia in pulmonary arterial myocytes, which may contribute to the differential reactivity of pulmonary vessels to hypoxia. The molecular mechanisms underlying the dual and opposite modulation of Ca^{2+} channels by P_{O_2} are for the moment unknown and open avenues for future experimental work.

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