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Effect of Normoxia and Hypoxia on K^+ Current and Resting Membrane Potential of Fetal Rabbit Pulmonary Artery Smooth Muscle

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Summary

At birth, the increase in O_2 tension (pO_2) is an important cause of the decrease in pulmonary vascular resistance. In adult animals there are impressive interspecies differences in the level of hypoxia required to elicit a pulmonary vasoconstrictor response and in the amplitude of the response. Hypoxic inhibition of some potassium (K^+) channels in the membrane of pulmonary arterial smooth muscle cells (PASMCs) helps to initiate hypoxic pulmonary vasoconstriction. To determine the effect of the change in pO_2 on fetal rabbit PASMCs and to investigate possible species-dependent differences, we measured the current-voltage relationship and the resting membrane potential, in PASMCs from fetal resistance arteries using the amphotericin-perforated patch-clamp technique under hypoxic and normoxic conditions. Under hypoxic conditions, the K^+ current in PASMCs was small, and could be inhibited by 4-aminopyridine, iberiotoxin and glibenclamide, reflecting contributions by Kv, K_{Ca} and K_{ATP} channels. The average resting membrane potential was –44.3±1.3 mV (n=29) and could be depolarized by 4-AP (5 mM) and ITX (100 nM) but not by glibenclamide (10 μM). Changing from hypoxia, that mimicked fetal life, to normoxia dramatically increased the K_{Ca} and consequently hyperpolarized (–9.3±1.7 mV; n=8) fetal rabbit PASMCs. Under normoxic conditions K^+ current was reduced by 4-AP with a significant change in resting membrane potential (11.1±1.7 mV; n=8). We conclude that resting membrane potential in fetal rabbit PASMCs under both hypoxic and normoxic conditions depends on both Kv and K_{Ca} channels, in contrast to fetal lamb or porcine PASMCs. Potential species differences in the K^+ channels that control resting membrane potential must be taken into consideration in the interpretation of studies of neonatal pulmonary vascular reactivity to changes in O_2 tension.

Key words

Hypoxic pulmonary vasoconstriction • Patch-clamp • Electrophysiology • Ion channels • Oxygen • Fetal pulmonary Artery smooth muscle

Introduction

The pulmonary vascular bed is unique compared with most studied systemic vascular beds. During normoxic conditions, the adult pulmonary circulation is at low pressure – that is, vasodilated – compared with the high-pressure systemic circulation. In the systemic circulation, hypoxemia elicits vasodilatation that

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increases O_2 delivery to the tissues. In contrast, small resistance arteries in the adult pulmonary circulation constrict in response to hypoxia. However, the pulmonary hypertensive response to acute and to chronic hypoxia varies markedly among mammalian species (Tucker *et al.* 1975, Peake *et al.* 1981). There are studies demonstrating marked interspecies differences in both the level of pO_2 required to elicit a hypoxic pulmonary vasoconstrictor response and the amplitude of the response.

Knowledge of the mechanism of O_2 sensing and the signalling pathways that mediate the cellular response to changes in O_2 tension has developed rapidly in the last decade. Several studies have provided direct evidence that hypoxic vasoconstriction of pulmonary arterial smooth muscle cells (PASMCs) from adult animals is mediated, at least in part, by the inhibition of one or several potassium (K^+) channels leading to cell depolarization, opening of voltage-gated Ca^{2+} channels, and myocyte contraction (Post *et al.* 1992, Yuan 1995, Weir and Archer 1995, Archer *et al.* 1996; Osipenko *et al.* 1997). Several subtypes of voltage-sensitive K^+ channels identified in the pulmonary artery have been implicated in the response to hypoxia and the relative contribution of these subtypes to the hypoxia-sensitive whole-cell K^+ current may differ from species to species (Archer *et al.* 1996, Osipenko *et al.* 1997).

It was recently shown that O_2-induced pulmonary vasodilatation in fetal lambs is inhibited by K^+ channel blockers and that transition from a hypoxic to a normoxic environment increases calcium-activated K^+ (K_{Ca}) current, hyperpolarizes the resting membrane potential and decreases the intracellular [Ca^{2+}] recorded in fetal lamb PASMCs (Cornfield *et al.* 1996, Tristani-Firouzi *et al.* 1996, Reeve *et al.* 1998, Porter *et al.* 2001). The data involving inhibition of voltage-dependent K^+ (Kv) current in hypoxic pulmonary vasoconstriction in adult animals has been obtained primarily in rats (Yuan 1995, Archer *et al.* 1996, 1998), while that involving the effect of normoxia on K_{Ca} current or non-inactivating Kv current in fetal animals has been derived from sheep and pig (Cornfield *et al.* 1996, Tristani-Firouzi *et al.* 1996, Evans *et al.* 1998, Reeve *et al.* 1998, Porter *et al.* 2001). Hypoxic pulmonary vasoconstriction in the intact adult animal is a species-dependent phenomenon (Tucker *et al.* 1975, Peake *et al.* 1981). To investigate possible interspecies differences in fetal PASMCs we studied the basic electrophysiological properties of fetal rabbit PASMCs and the effects of normoxia and hypoxia.

Methods

The animal study was approved by the Institutional Animal Care and Use Committee of the Minneapolis VA Medical Center and conforms to current National Institutes of Health and American Physiological Society guidelines for the use and care of laboratory animals.

Isolation of fetal PASMCs

Fetal rabbit PASMCs were freshly dissociated for electrophysiological studies every day. Pregnant New Zealand White rabbits at 28 days gestation (normal gestation period is from 28 to 32 days) were anaesthetized with 75 mg ketamine and 20 mg xylazine intramuscularly and then 50 mg sodium pentobarbital intravenously. Fetal pups were delivered by caesarean section and decapitated before initiation of respiration. A midline sternotomy was performed on each pup and the heart, lungs and great vessels removed en block and placed in chilled hypoxic, Ca^{2+}-free Hanks' solution (see "Solutions and drugs"). Resistance pulmonary arteries (4th-divisions) were dissected. The arteries were then transferred to Hanks' solution containing 0.5 mg/ml of papain, 1 mg/ml of albumin and 1 mg/ml of dithiothreitol, without EGTA, and kept at 4 °C for 30 min. After this time the arteries were incubated at 37 °C for 8-10 min. The arteries were washed thoroughly in enzyme-free Hanks' solution for at least 10 min and then maintained at 4 °C. To maintain the low oxygen state of the fetal environment, cells were prepared and stored in hypoxic Hanks' solution. Several digestions were done each day to ensure cell viability. Gentle trituration produced a suspension of single cells, which were then aliquoted into a perfusion chamber on the stage of the inverted microscope (Diaphot 200, Nikon). After a brief period to allow partial adherence to the bottom of the recording chamber, cells were superfused *via* gravity with an extracellular solution (see "Solutions and drugs") at a rate of 2 ml/min.

Numbers presented (n) indicate the actual number of PASMCs used, which were harvested from fetuses of at least three different maternal rabbits to allow for animal variation. Thirteen maternal rabbits were used for the study.

Current recording and analysis

Whole-cell recordings were performed using the amphotericin-perforated patch-clamp technique (Rae *et*

al. 1991). Patch pipettes were pulled from glass tubes (PG 150T, Warner Instruments Corp). The pipettes were fire-polished directly before the experiments and had a resistance of 2-3 MΩ when filled with a pipette solution. Conventional whole-cell patch-clamp recording (Hamill *et al.* 1981) was performed using a modified pipette solution (potassium ions replaced for cesium ions) to determine whether the current was carried solely by K^+ ions. The patch-clamp amplifiers were Axopatch 200A and B (Axon Instruments, Foster City, CA, USA) in all voltage- and current-clamp experiments. Offset potentials were nulled directly before formation of the seal. Capacitance was corrected for, and perforation was monitored by changes in series resistance. Compensation of whole-cell capacitance allowed an estimation of cell size (8.3±0.4 pF, n=30). The small currents recorded in the fetal cells made series resistance compensation unnecessary. PASMCs were voltage clamped at a holding potential of –70 mV, and currents were evoked by +20 mV steps to more positive potentials with test pulses of 300-ms duration. For K_{Ca} studies, cells were also voltage-clamped at a holding potential of –20 mV to inactivate Kv channels (Osipenko *et al.* 1997, Patel *et al.* 1997, Reeve *et al.* 1998) and then depolarized to more positive potentials in +10 mV steps. Steady-state current-voltage relationships were obtained by measuring the current at the end of the voltage-clamp pulse. The effective corner frequency of the low-pass filter was 1 kHz. The frequency of digitization was at least twice that of the filter. For resting membrane potential (E_m) experiments, cells were held in current-clamp at their resting E_m (without current injection). The data were stored and analysed with commercially available pCLAMP software (Axon Instruments, Foster City, CA, USA). All experiments were performed at 30 °C and in low light intensity because of the light-sensitivity of amphotericin B.

Solutions and drugs

The Hanks' solution contained (in mM): NaCl 145, KCl 4.2, $MgCl_2$ 1, KH_2PO_4 1.2, HEPES 10, glucose 10, EGTA 0.1 (pH was adjusted to 7.4 by KOH). The extracellular or bath solution contained (in mM): NaCl 115, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1.8, $NaHCO_3$ 25 HEPES 10, glucose 10, (pH 7.4 with NaOH). The standard intracellular pipette solution contained (in mM): KCl 145, $MgCl_2$ 1, EGTA 0.1, HEPES 10, and 120 µg/ml amphotericin B (pH was adjusted to 7.2 by KOH). In the modified pipette solution CsCl was used instead of KCl.

The effect of hypoxia was studied by switching between normoxic and hypoxic perfusate reservoirs. Normoxic extracellular solutions were equilibrated with 20 % O_2-5 % CO_2–balance N_2. Hypoxic extracellular solutions were achieved by bubbling with 0 % O_2-5 % CO_2–balance N_2, for at least 20 min before cell perfusion. These procedures produced pO_2 values in the cell chamber of 140 to 160 mm Hg (21 % O_2), 35-44 mm Hg (0 % O_2), measured with a Rapidlab Chiron blood gas analyzer in samples taken from the experimental chamber during perfusion. PCO_2 was 36-42 mm Hg, and pH was 7.37-7.42 under these conditions.

Iberiotoxin was obtained from Alomone Laboratories (Jerusalem, Israel). All other compounds were purchased from Sigma Chemical Company (St. Louis, MO). Iberiotoxin was solubilized in distilled water. All other drugs were dissolved in Hanks' solution, with the exception of glibenclamide, which was dissolved in ethanol as a stock solution. pH of solutions containing drugs was tested and corrected to eliminate potential pH-induced effects. Stock solutions in ethanol were diluted at least 1:10 000 in the bath solution. The diluted ethanol vehicle was tested and found to have no effect on potassium channel activity.

Statistical analysis

Numerical values are given as mean ± S.E.M. of n cells. Currents at different potentials were compared between experimental conditions by paired Student's *t*-test with Bonferroni correction. $P<0.05$ value was considered statistically significant. When changes of membrane potential in individual cells were analyzed, the Student's paired *t*-test was also used. For statistical comparison of membrane potential between the normoxic and hypoxic group, the unpaired *t*-test was used. In all figures the SEM is indicated when it exceeds the symbol size.

Results

Whole-cell outward potassium currents in fetal PASMCs under hypoxia and normoxia recorded from different holding potentials

Basal whole-cell outward potassium currents (I_k) in fetal pulmonary artery smooth muscle cells from resistance vessels were recorded in hypoxia from holding potentials of –70 mV and –20 mV (the latter to inactivate Kv current), using a perforated patch-clamp technique. I_k under hypoxic conditions was small, 391.6±9.4 pA

recorded from a holding potential of –70 mV (at +50 mV; n=8), and 215.9±20.7 pA recorded from a holding potential of –20 mV (at +50 mV; n=7). Initially the cells were superfused with a hypoxic bath solution to mimic fetal conditions, and then I_k was recorded during a 4-min exposure to normoxia (Fig. 1). Under these conditions normoxia rapidly enhanced I_k, as shown in Figure 1A, during a step depolarization to +50 mV from a holding potential of –20 mV. The current-voltage relationships for I_k illustrating the change from normoxia to hypoxia are shown in Figure 1B. The activation of I_k was increased 139.1±15.0 % (holding potential of –70 mV; n=8) and 429.9±132.6 % (holding potential of –20 mV, n=7) under normoxic conditions. When the PASMCs were kept at –20 mV the normoxia-activated I_k could be inhibited by 100 nM ITX, a K_{Ca} antagonist, to 27.6±11.7 % (at +50 mV; n=4; Fig 3B), suggesting that at least part of the effect of normoxia is to increase the K_{Ca} current.

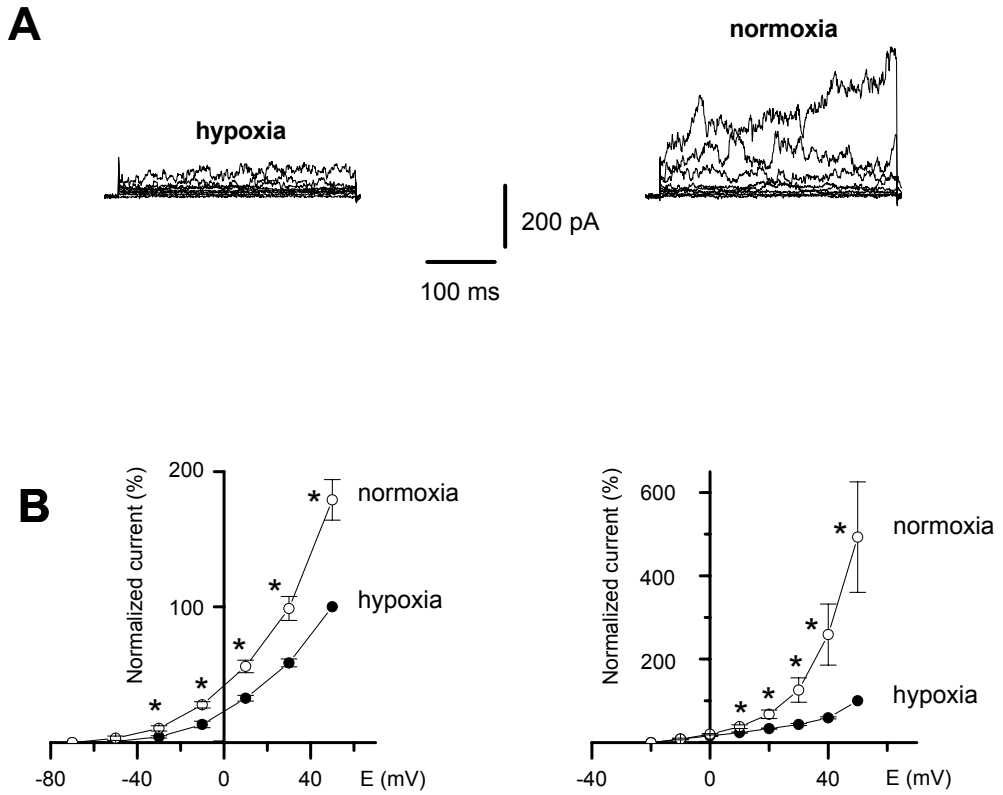

Fig. 1. Activation of outward potassium current recorded from fetal rabbit PASMCs under normoxia. **(A)** Representative traces demonstrate whole-cell potassium currents from fetal rabbit PASMC under hypoxic (left) or normoxic conditions (right). Currents were evoked from a holding potential of –20 mV to +50 mV in 10 mV steps. **(B)** Averaged whole-cell I-V plots of outward potassium currents recorded in fetal rabbit PASMCs. Steady-state currents were recorded in hypoxia and in normoxia, and normalized to maximum current under hypoxia at +50 mV. Cells were clamped at –70 mV (left; n = 8) and at –20 mV (right; n = 7). Values are mean ± S.E.M. * $p<0.05$ difference from control (hypoxia).

Pharmacological properties of I_k in fetal PASMCs under hypoxia

The whole cell current in fetal rabbit PASMCs under hypoxic circumstances was sensitive to inhibition by a number of pharmacological agents known to block K^+ channels. This is illustrated in Figure 2A by voltage-clamp recordings obtained during 300 ms steps to +50 mV applied from a holding potential of –70 mV in a control hypoxic experimental solution and after application of 5 mM 4-AP (Kv antagonist), 100 nM ITX, or 10 μM glibenclamide (ATP-sensitive potassium channel antagonist). These drugs were tested because they have been helpful in distinguishing between different components of I_k in adult and fetal PASMCs of other species. At the concentrations used, all three drugs caused pronounced inhibition of the compound current activated by depolarizing steps over a wide range of test potentials. When potassium ions were replaced by cesium ions in the pipette solution, using conventional whole-cell patch-clamp recording, the whole-cell current

disappeared, indicating that the current was entirely carried through K^+ channels (results not shown). At +50 mV 5 mM 4-AP reduced the current to 62.4±4.7 % (n=4), 100 nM ITX inhibited the current to 85.7±4.8 % (n = 4), and 10 μM glibenclamide to 81.6±2.3 % (n = 5; Fig. 2B).

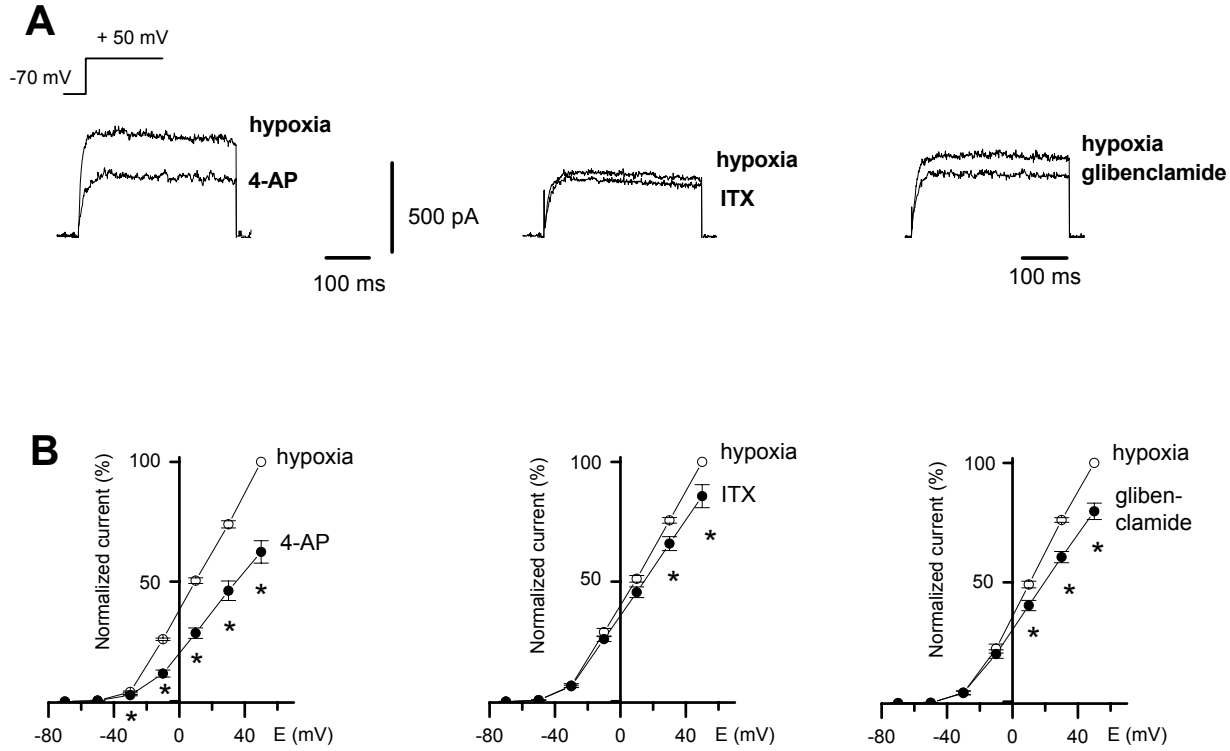

Fig. 2. Pharmacology of whole-cell potassium current in fetal rabbit PASMCs under hypoxia. **(A)** Representative traces recorded from fetal rabbit PASMC during control (hypoxia) and after exposure to 5 mM 4-AP (left), or 100 nM ITX (middle), or 10 μM glibenclamide (right) during the voltage step from –70 mV to +50 mV. **(B)** Relationship between test potential and current measured under hypoxic control and after application of 5 mM 4-AP (left; n = 4), or 100 nM ITX (middle; n = 4), or 10 μM glibenclamide (right; n = 5), and normalized to maximum current under hypoxia at +50 mV. Values are mean ± S.E.M. * $p<0.05$ difference from control (hypoxia).

Pharmacology of I_k in fetal PASMCs under normoxia

Two types of K^+ current could be identified in fetal rabbit PASMCs under normoxic circumstances using the perforated patch-clamp technique. The extracellular application of 4-AP, ITX, or tetraethyl-ammonium chloride (TEA, non-specific inhibitor of K_{Ca}) markedly decreased I_k, while glibenclamide had no significant effect on I_k. The effects of 4-AP and ITX on I_k are illustrated in Figure 3. At +50 mV, 5 mM 4-AP reduced the current to 41.2±7.4 % (n=4; Fig. 3A), 100 nM ITX to 68.0±8.0 % (n=4; Fig. 3B), 5 mM TEA to 54.5±6.0 % (n=4; not shown), and 10 μM glibenclamide to 96.1±3.9 % (n=4; not shown).

Resting membrane potential (E_m) in fetal PASMCs

Average resting membrane potential from fetal PASMCs in in hypoxia was –44.3±1.3 mV (n=29) and after 10 min exposure to normoxia –49.5±0.8 mV (n=27; $p<0.005$). The switching of the perfusion solution from hypoxia to normoxia hyperpolarized the cell membrane from –41.0±2.1 mV to –50.3±2.1 mV (n=8; $p<0.005$). Membrane hyperpolarization occurred rapidly (within 4 min). When the cells were kept for 10 min in a normoxic bath solution (E_m –49.4±1.5 mV) superfusion of the cells with a hypoxic experimental solution caused significant depolarization to –42.6±2.6 mV (n=6; $p<0.05$). Figure 4 shows averaged resting membrane potentials of PASMCs before and after application of 5 mM 4-AP, 100 nM ITX and 10 μM glibenclamide. In a hypoxic environment, the cell membrane could be depolarized by 4-AP (9.7±2.1 mV; n=10; $p<0.005$) and ITX (7.1±1.3 mV; n=9; $p<0.005$), but was not affected by exposing the cells to glibenclamide (n=5). When cells were kept for 10 min under normoxic conditions, the 4-AP- and the ITX-induced depolarization was 11.1±1.2 mV (n=5; $p<0.005$) and 11.0±1.0 mV (n=8; $p<0.005$), respectively.

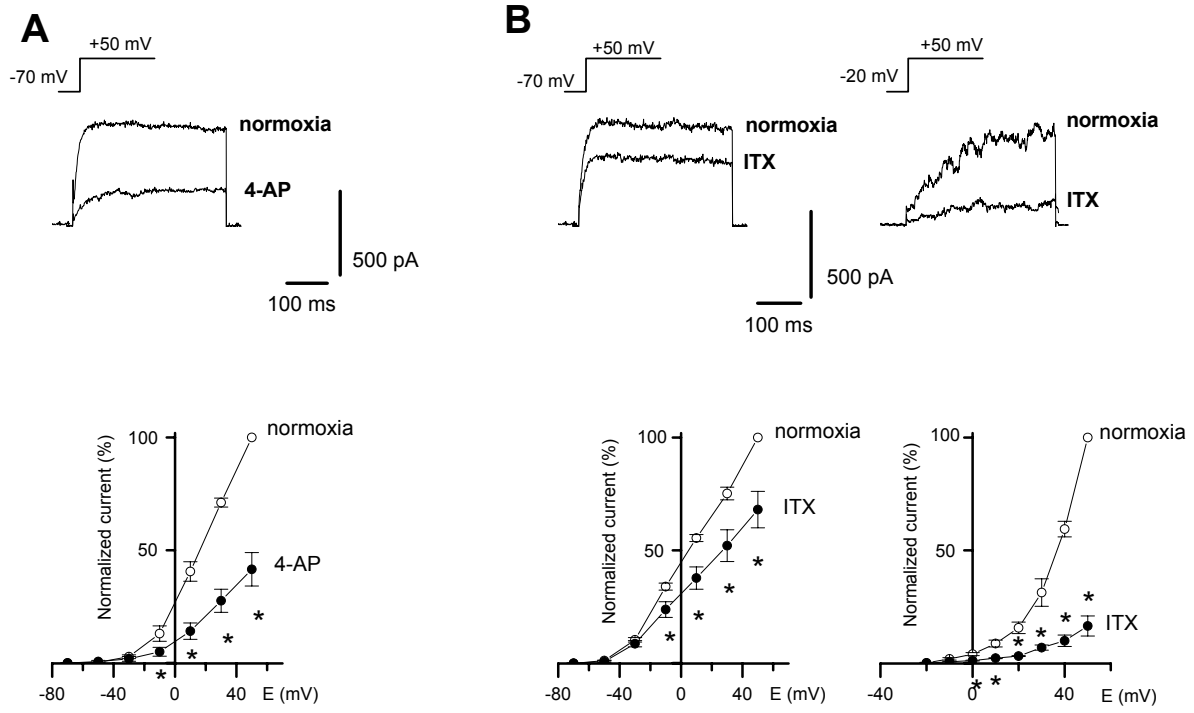

Fig. 3. Effect of 4-AP and ITX on whole-cell potassium current of fetal rabbit PASMCs under normoxia. **(A)** Representative traces recorded from fetal rabbit PASMC during control (normoxia) and after addition of 5 mM 4-AP during a voltage step from –70 mV to +50 mV (upper panel). Averaged whole-cell I-V relationship of outward potassium currents normalized to maximum current under normoxia and after application of 5 mM 4-AP (left; n = 4; lower panel). Values are mean ± S.E.M. **(B)** Actual traces recorded from fetal PASMCs under normoxia and after exposure to 100 nM ITX, during a voltage step from –70 mV to +50 mV (left) and from –20 mV to +50 mV (right). The relationship is shown between test potential and current measured at the end of the voltage step, constructed from records above, in absence or presence of 100 nM ITX (n = 4) and normalized to maximum current under normoxia at +50 mV. Values are mean ± S.E.M. * $p<0.05$ difference from control (normoxia).

Discussion

In this study we have described the basic electrical properties of fetal rabbit PASMCs under hypoxic and normoxic conditions. This characterization is of interest since it is well known that pulmonary vascular resistance at birth rapidly falls from the high fetal level. This allows pulmonary blood flow to increase 8- to 10-fold and enables the lung to assume its postnatal role in gas exchange (Rudolph 1985, Ghanayem and Gordon 2001). The mechanisms that contribute to the normal decrease in pulmonary vascular resistance include increased oxygen tension, mechanical distension of the lung by ventilation, shear stress and enhanced production of vasodilator substances (Abman *et al.* 1990, Accurso *et al.* 1986, Cassin *et al.* 1964a,b, Cornfield *et al.* 1992, Fineman *et al.* 1994, Morin *et al.* 1988).

The ability to sense the change in O_2-tension is of fundamental biological importance. While the effector mechanism of the change in vascular tone in response to an alteration in O_2 tension is fairly well characterized, the exact identity of the O_2 sensor and how the variations of O_2 tension are translated into modifications of vascular contractility has not been definitively established. There is increasing evidence that K^+ channels play a central role in mediating responses to changes in O_2 tension. O_2-regulated K^+ channels, initially observed in type I cell of the carotid body (Lopez-Barneo *et al.* 2001, Lopez-Lopez *et al.* 1989, 1997), are present in neuroepithelial cells (Youngson *et al.* 1993, 1994), pheochromocytoma cells (Zhu *et al.* 1996), PASMCs (Post *et al.* 1992, Yuan *et al.* 1993, 1995, Weir and Archer 1995, Archer *et al.* 1996, Osipenko *et al.* 1997) and central neurons (Jiang and Haddad 1994a,b).

Though K^+ channels have been shown to play a significant part in the regulation of pulmonary vascular tone in the adult, the role of K^+ channels in the perinatal circulation is less well understood. In PASMCs obtained from the adult rat, dog and rabbit a decrease in O_2 tension inhibits K^+ channels, depolarizes the cell membrane, opens voltage-activated Ca^{2+} channels and increases intracellular [Ca^{2+}], thus causing vasoconstriction (Post *et*

al. 1992, Yuan 1995, Weir and Archer 1995, Archer *et al.* 1996, Osipenko *et al.* 1997). Pulmonary vasocon-striction diverts mixed venous blood away from hypoxic alveoli, thus optimizing the matching of perfusion and ventilation and preventing intrapulmonary shunting and arterial hypoxemia. The vasoconstrictor effect of hypoxia on the pulmonary circulation in the adult could be initiated in part by closing of these O_2-sensitive K^+ channels. Conversely, opening of O_2-sensitive K^+ channels with subsequent hyperpolarization of PASMCs could also account for the drop in pulmonary vascular resistance associated with an increase of oxygen at the time of birth.

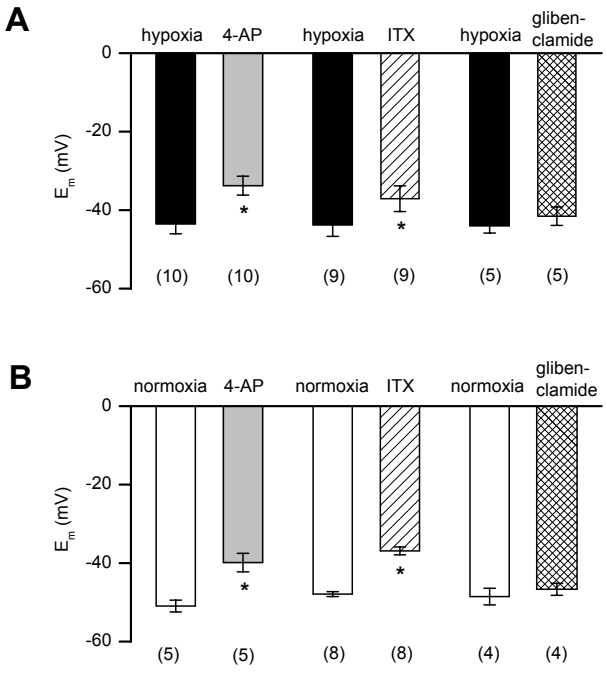

Fig. 4. Modulation of resting membrane potential of fetal PASMCs by 4-AP, ITX and glibenclamide in hypoxic and normoxic environment. **(A)** Average membrane potentials recorded from fetal PASMC under hypoxic (black bars) conditions and after exposure to either 4-AP (gray bars), or ITX (hatched bars) or glibenclamide (crossed bars). **(B)** Average membrane potentials recorded from fetal PASMC under normoxic (white bars) conditions and after exposure to either 4-AP (gray bars), or ITX (hatched bars) or glibenclamide (hatching). Values are mean ± S.E.M. Numbers of cells shown in parentheses. * $p<0.05$ difference from control.

Several groups have investigated the role of a variety of K^+ channels in oxygen-induced vasodilatation and pulmonary vascular tone in the fetal and perinatal pulmonary circulations and some of these investigations have yielded different results, in some cases in different species. Cornfield *et al.* (1996) reported that TEA and ITX attenuated, while glibenclamide had no effect on normoxia-induced pulmonary vasodilatation in the fetal lamb. This suggests a role for K_{Ca} channels in normoxic vasodilatation. Consistent with this observation, Tristani-Firouzi *et al*. (1996) showed that the perinatal pulmonary vasodilatation of fetal lambs is mediated through TEA-sensitive channels, but not through K_{ATP} channel activation and neither TEA nor glibenclamide had any effect on basal pulmonary tone. On the contrary, Mital and Konduri (2000) reported that oxygen-induced pulmonary vasodilatation was inhibited by both TEA and glibenclamide, suggesting that K_{ATP} channels, in addition to K_{Ca} channels, may mediate the pulmonary vasodilator response to oxygen in the fetal lamb. Finally, it has been recently reported that 4-AP but not charybdotoxin, TEA, or glibenclamide, significantly attenuate normoxic vasodilatation in the fetal rat lung indicating a potential role for Kv channels in the rat (Gosche 2001).

To determine the effect of the change in pO_2 on fetal rabbit PASMCs, we measured the current-voltage relationship, as well as the resting membrane potential, in PASMCs from fetal resistance arteries using the amphotericin-perforated patch-clamp technique under hypoxic and normoxic conditions. Normoxia dramatically increased the whole cell outward K^+ current and consequently hyperpolarized fetal rabbit PASMCs, in comparison to the hypoxic condition that mimicked fetal life. The response to normoxia was almost completely reversed upon return to hypoxia. It was noteworthy that the percent normoxic increase in K^+ current was markedly enhanced when the cells were kept at a more depolarized holding potential (–20 mV) closer to the resting membrane potential of these cells, where the Kv channel activity is inhibited and only K_{Ca} channel activity is recorded. This conclusion is strengthened by the fact that ITX reversibly inhibited the normoxia-induced increase in whole-cell K^+ current, indicating that K_{Ca} channels may serve as O_2 sensors in fetal rabbit PASMCs. These results are consistent with those of Reeve *et al.* (1998) and Cornfield *et al.* (1996) who showed that in fetal lamb PASMCs the exposure to normoxia markedly increased the whole-cell, CTX-sensitive K^+ current. Evans *et al.* (1998) have also reported an approximately 40 % block of the whole-cell K^+ current by TEA, a non-specific blocker of K_{Ca} channels, under normoxic conditions in fetal porcine PASMCs. In PASMCs isolated from fetal lambs, the effect of hypoxia on fetal PASMCs intracellular Ca^{2+} was mimicked by CTX (Cornfield *et al.* 1994). In addition, ITX prevented the effect of normoxia (Porter *et al.* 2001),

providing support for the hypothesis that the K_{Ca} current is involved in keeping levels of cytosolic Ca^{2+} low under normoxic conditions in the fetal lamb and rabbit.

As described earlier, there is an emerging consensus that in adult PASMCs the inhibition of voltage-gated K^+ channels by hypoxia leads to cell depolarization and, hence to Ca^{2+} influx and consequent vasoconstriction. The potassium channels which control E_m in these cells, conduct an outward current which is slowly inactivating and blocked by the Kv channel blocker 4-AP but not by inhibitors of K_{Ca} or K_{ATP}, at least in the rat (Yuan 1995, Archer *et al.* 1996, 1998). Consequently, we looked for the evidence of Kv channels in fetal rabbit PASMCs. 4-AP inhibited the whole-cell K^+ current under hypoxic as well as under normoxic conditions and led to depolarization in these cells. The existence of Kv channels in fetal PASMCs is also supported by Evans *et al.* (1998) who observed a marked (approximately 60 %) block of whole-cell K^+ current by 4-AP in the pig. They had previously shown a hypoxia-sensitive non-inactivating component of Kv current in adult rabbit PASMCs, which they could not clearly identify in the fetal porcine PASMCs (Evans *et al.* 1996, 1998). Observations in fetal ovine PASMCs under hypoxic conditions by Reeve *et al.* (1998) showed that a small component of the residual whole-cell K^+ current, after dialysis of the cells with the rapid Ca^{2+} chelator BAPTA, was due to 4-AP-sensitive Kv channels. Although 4-AP (1 mM) caused slight inhibition in whole-cell K^+ current in fetal lamb PASMCs, the resting membrane potential was not affected by this concentration of the Kv channel blocker (Reeve *et al.* 1998). These data indicate that some Kv channels in fetal rabbit and porcine PASMCs are O_2-sensitive, while the role of Kv channels may be less prominent in the fetal lamb.

Further studies have been performed to examine the role of ATP-dependent K^+ channels in fetal PASMCs. Glibenclamide-sensitive K^+ current develops progressively in fetal porcine PASMCs under normoxic conditions after dialysis with ATP-free pipette solution, suggesting the presence of K_{ATP} channels (Evans *et al.* 1998). However, no effect of glibenclamide on resting membrane potential of fetal ovine PASMCs was found by Reeve *et al.* (1998). Our results suggest the presence of an ATP-sensitive K^+ current under hypoxia, when ATP levels may be low. However, glibenclamide had no effect on the resting membrane potential of fetal rabbit PASMCs, neither under normoxic nor hypoxic conditions, suggesting that K_{ATP} channels are unlikely to be involved in O_2-sensing in these cells.

In summary, this work offers the first evidence that the K^+ channels controlling the resting membrane potential of fetal rabbit PASMCs appears to be a combination of K_{Ca} and Kv channels, as resting membrane potential was depolarized by ITX and 4-AP but not by glibenclamide, under both hypoxic and normoxic conditions. The role of Kv, K_{Ca}, and K_{ATP} channels in the fetal pulmonary circulation has been investigated by several groups and these studies have yielded varied results, indicating species-dependent differences in the potassium channels responsible for regulation of the membrane potential of the fetal PASMCs and for their response to changes in O_2 tension.

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Reprint requests

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