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Physiology

Oxygen causes fetal pulmonary vasodilation through activation of a calcium-dependent potassium channel

(fetus/O_2-sensing/pulmonary vasculature/K^+ channels/nitric oxide/protein kinase)

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ABSTRACT **At birth, pulmonary vasodilation occurs as air-breathing life begins. The mechanism of O_2-induced pulmonary vasodilation is unknown. We proposed that O_2 causes fetal pulmonary vasodilation through activation of a calcium-dependent potassium channel (K_{Ca}) via a cyclic nucleotide-dependent kinase. We tested this hypothesis in hemodynamic studies in acutely prepared fetal lambs and in patch-clamp studies on resistance fetal pulmonary artery smooth muscle cells. Fetal O_2 tension (Pa_{O_2}) was increased by ventilating the ewe with 100% O_2, causing fetal total pulmonary resistance to decrease from 1.18 ± 0.14 to 0.41 ± 0.03 mmHg per ml per min. Tetraethylammonium and iberiotoxin, preferential K_{Ca}-channel inhibitors, attenuated O_2-induced fetal pulmonary vasodilation, while glibenclamide, an ATP-sensitive K^+-channel antagonist, had no effect. Treatment with either a guanylate cyclase antagonist (LY83583) or cyclic nucleotide-dependent kinase inhibitors (H-89 and KT 5823) significantly attenuated O_2-induced fetal pulmonary vasodilation. Under hypoxic conditions (Pa_{O_2} = 25 mmHg), whole-cell K^+-channel currents (I_K) were small and were inhibited by 1 mM tetraethylammonium or 100 nM charybdotoxin (CTX; a specific K_{Ca}-channel blocker). Normoxia (Pa_{O_2} = 120 mmHg) increased I_K by more than 300%, and this was reversed by 100 nM CTX. Nitric oxide also increased I_K. Resting membrane potential was −37.2 ± 1.9 mV and cells depolarized on exposure to CTX, while hyperpolarizing in normoxia. We conclude that O_2 causes fetal pulmonary vasodilation by stimulating a cyclic nucleotide-dependent kinase, resulting in K_{Ca}-channel activation, membrane hyperpolarization, and vasodilation.**

In the fetus, O_2 tension is low, and pulmonary arterial (PA) pressure and resistance are high. The fetal pulmonary vasculature is exquisitely sensitive to changes in O_2 tension (1, 2). Pulmonary blood flow increases 2- to 3-fold with an increase in fetal O_2 tension of only 4–6 mmHg (3). While postnatal adaptation of the pulmonary circulation is multifactorial (1, 4, 5), nitric oxide (NO) release plays a central role in both O_2-induced fetal pulmonary vasodilation and pulmonary vasodilation that occurs at birth (6–8). The mechanism whereby O_2 and/or NO regulate pulmonary vascular tone remains incompletely understood. Evidence suggests a primary role for K^+-channel activity in O_2 sensing (9) and in regulation of pulmonary vascular tone (10, 11). In adult animals, acute (11–13) and chronic (14) hypoxia both cause inactivation of K^+ channels and membrane depolarization in pulmonary artery smooth muscle cells (PA SMCs). Fetal PA SMCs may behave similarly since hypoxia causes membrane depolarization and an increase in cytosolic Ca^{2+} that is mimicked by inactivation of Ca^{2+}-dependent K^+ channels (K_{Ca}) (15).

Recent studies indicate a link between the vasodilation caused by NO and the O_2 sensing capacity of K^+ channels in vascular SMCs. Robertson *et al.* (16) demonstrated that, in cerebral artery SMCs, cGMP-dependent protein kinase acts to phosphorylate the large conductance K_{Ca} channel. In PA SMCs, NO-induced increases in intracellular levels of cGMP cause activation of a cGMP-sensitive kinase and this in turn activates a K_{Ca} channel resulting in vasodilation (17). NO has also been shown to directly activate K_{Ca} channels (18). We therefore hypothesized that increases in O_2 tension cause fetal pulmonary vasodilation through a three-step pathway involving (*i*) an increase in guanylate cyclase activity, (*ii*) cyclic nucleotide-dependent kinase activation, and (*iii*) activation of a specific K^+ channel.

To test these hypotheses, we studied the effect of an increase in O_2 tension on (*i*) fetal pulmonary hemodynamics during inhibition of guanylate cyclase, cyclic nucleotide-dependent protein kinase, or K^+-channel activity in the late-gestation ovine fetus and (*ii*) K^+-current activity and membrane potential (E_m)in freshly dispersed fetal PA SMCs.

MATERIALS AND METHODS

Whole Animal Studies. All procedures were reviewed and approved by the Animal Care and Use Committee at the Veteran's Administration Medical Center. Mixed-breed (Dorset–Hampshire) pregnant ewes betweeen 133 and 142 days of gestation (term = 147 days) were fasted for 24 h prior to surgery. Ewes were sedated (pentobarbital at 2–4 g i.v. over the the operative and study periods), anesthetized (tetracaine at 30 mg intrathecally), and mechanically ventilated (Siemans 900A; tidal volume, 600 ml; 20 breaths per min) throughout the surgery and study periods. The fetal lamb's left forelimb was delivered through a midline uterine incision and a skin incision was made after local infiltration with lidocaine (2–3 ml, 1% solution). Polyvinyl catheters were advanced into the ascending aorta and the superior vena cava after insertion in the axillary artery and vein. Catheters were inserted into the left pulmonary artery (LPA) and main pulmonary artery by direct puncture and secured as described (6, 7). An ultrasonic flow transducer, size 4.0 (Transonic Systems, Ithaca, NY), was placed around the LPA to measure blood flow. The uteroplacental circulation was kept intact and the fetuses were gently replaced in the uterus, with exposed surfaces bathed in warm towels. Fetal skin temperature was monitored and maintained at 39°C. After a minimum recovery time from surgery of 1 h, serial arterial blood gas tensions and pH were recorded at

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Abbreviations: K_{Ca} channel, calcium-dependent potassium channel; PA, pulmonary arterial; SMC, smooth muscle cell; IBX, iberiotoxin; TEA, tetraethylammonium; CTX, charybdotoxin; LPA, left pulmonary artery; STOC, spontaneously transient outward current; $[cGMP]_i$, intracellular cGMP concentration; I_K, K^+ current; TPR, total pulmonary vascular resistance.

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15-min intervals throughout baseline and study periods. Hemodynamic parameters, including mean LPA blood flow, heart rate, and pressures in the main pulmonary artery and aorta, were monitored continuously. For each protocol *n* refers to the number of animals studied. Each animal was studied only once. Total pulmonary vascular resistance (TPR) was calculated as mean PA pressure divided by LPA flow.

After baseline measurements, the fraction of inspired O_2 (FiO_2) provided to the ewe was increased to 1.00 for 30 min. Fetal hemodynamics and blood gas parameters were measured. This represents the control period for each animal. After a 1-h recovery period (maternal $FiO_2 = 0.21$), the effect of K^+-channel inhibition, guanylate cyclase inhibition, or cyclic nucleotide kinase inhibition on O_2-induced vasodilation was measured. All study drugs were administered via the LPA at a rate of 0.1 ml/min.

Protocol 1. To investigate the role of K^+-channel activity, we studied the hemodynamic effects of three K^+-channel blockers, tetraethylammonium [TEA, a preferential K_{Ca}-channel antagonist at low millimolar concentrations (19)], glibenclamide [an ATP-sensitive K^+-channel blocker (19)], and iberiotoxin (IBX) [a specific K_{Ca} blocker (20)], on the response of the fetal pulmonary vasculature to increased O_2 tension. After the control period of increased fetal Pa_{O_2} and recovery, TEA, glibenclamide, or IBX, was infused into the LPA over a 10-min period (protocol shown in Fig. 1). Maternal O_2 concentration was again increased to 1.00. After 30 min of increased O_2 concentration, lemakalim at 10 mg, an ATP-sensitive K^+-channel agonist (19), was infused into the LPA over a 10-min period.

Protocol 2. To examine the role of guanylate cyclase activity in the vasodilation caused by O_2, a guanylate cyclase antagonist, LY83583 (6-anilino,5,8-quinolinedione), was used (21). To prove the dose of LY83583 was sufficient to block guanylate cyclase activity (protocol shown in Fig. 2), the ability to attenuate the pulmonary vasodilation caused by acetylcholine infusion in the LPA at 1.5 μg/min was measured.

Protocol 3 (n = 5). The role of cAMP- and cGMP-dependent protein kinases were investigated using antagonists of adenylyl kinase, H-89, and guanylyl kinase, KT 5823 (22–24). To prove the efficacy of kinase blockade with the doses of H-89 and KT 5823 (120 μg of H-89, followed by 120 μg of KT 5823 administered over consecutive 10-min periods via the LPA), 8-Br-cGMP was infused via the LPA before and after kinase inhibition (Fig. 2 *Inset*).

Drugs. TEA (Sigma), glibenclamide (Upjohn), and IBX (Peptide Institute, Osaka), lemakalim (Beecham Pharmaceuticals), acetylcholine (Sigma), and 8-Br-cGMP (Sigma) were dissolved in saline. LY83583 (Calbiochem), and H-89 (LC Services, Woburn, MA) were dissolved in less than 0.15% ethanol/balance saline; KT 5823 (LC Services) was dissolved in less than 0.1% dimethyl sulfoxide/balance saline; glibenclamide was dissolved as described (25). Vehicle controls were performed for each drug.

Electrophysiology. To obtain enzymatically dispersed ovine fetal or newborn (<24 h) SMCs, arterial segments were dissected daily from fourth and fifth divisions of the PA and placed in Ca^{2+}-free Hanks' for 20 min. The Hanks' solution contained 140 mM NaCl, 4.2 mM KCl, 1.2 mM KH_2PO_4, 0.5 mM $MgCl_2$, 10 mM Hepes, and 0.1 mM EGTA (pH 7.4). Arteries were then transferred to Hanks' solution without EGTA for 30 min. Arteries were digested for 45 min at 4°C in a solutiion of collagenase (1 mg/ml), protease (0.5 mg/ml), elastase (0.067 mg/ml), and bovine albumin (0.67 μg/ml) and for an additional 10 min in the same solution at 37°C. Arteries were washed thoroughly in Hanks' solution for at least 10 min and and kept at 4°C. Gentle trituration produced a suspension of single cells that was then pipetted into a perfusion chamber on the stage of an inverted microscope for patch-clamp studies (26). After a brief period to allow partial adherence to the bottom of the recording chamber, cells were perfused with an hypoxic solution of 115 mM NaCl, 25 mM $NaHCO_3$, 4.2 mM KCl, 1.2 mM KH_2PO_4, 0.5 mM $MgCl_2$, 1.5 mM $CaCl_2$, and 10 mM Hepes (pH 7.4; Pa_{O_2} = 25 mmHg by bubbling with 3.5% CO_2/balance N_2). For perforated patch-clamp recordings (27), electrodes were filled with a solution of 140 mM KCl, 1.0 mM $MgCl_2$, 10 mM Hepes, 5 mM EGTA, and 2 mM phosphocreatinine (pH 7.2). Amphotericin B was included in the pipette at a final concentration of 120 μg/ml. Normoxic solutions (Pa_{O_2} = 120 mmHg) were bubbled with 3.5%CO_2/

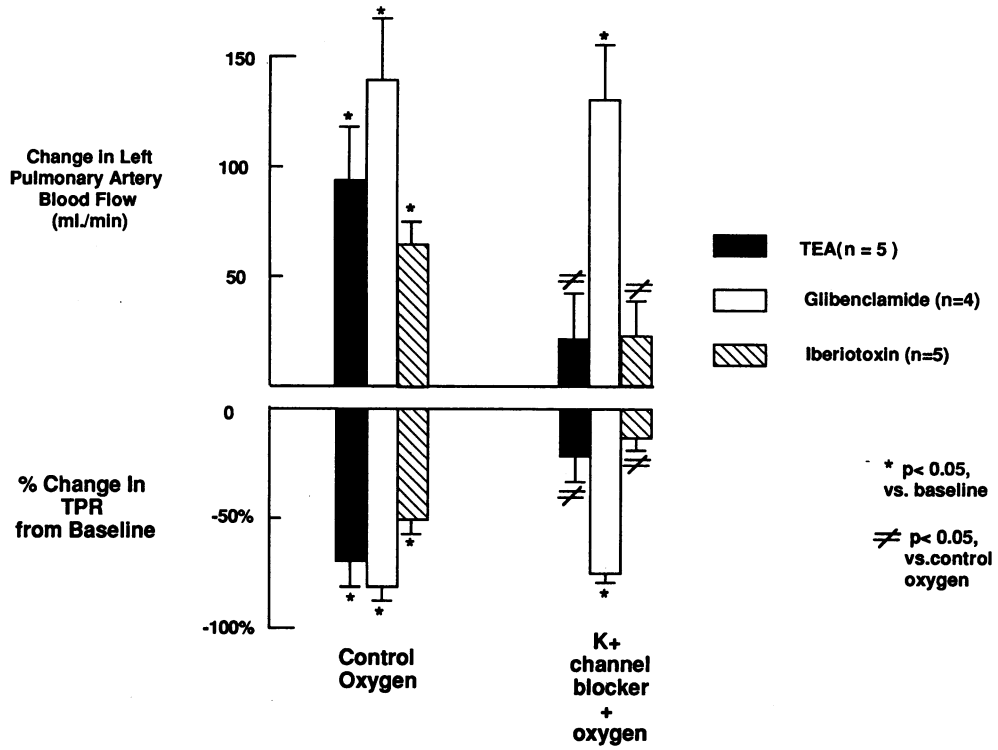

FIG. 1. Effect of K^+-channel inhibition on O_2-induced fetal pulmonary vasodilation. In each of the control periods, O_2 caused a decrease in TPR ($P < 0.05$) and a commensurate in increase in LPA blood flow ($P < 0.05$). TEA blocked the O_2-induced fetal pulmonary vasodilation ($P < 0.05$). Glibenclamide (GLI), an ATP-sensitive K^+-channel blocker, had no effect on O_2-induced fetal pulmonary vasodilation. IBX (IBER), a specific K_{Ca}-channel blocker, attenuated O_2-induced fetal pulmonary vasodilation ($P < 0.05$).

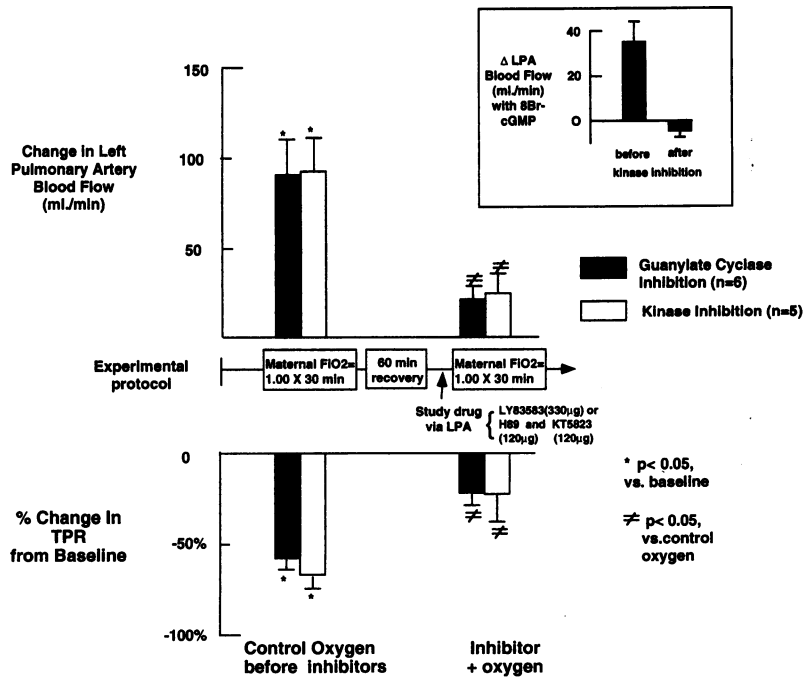

FIG. 2. Effect of LY83583, a guanylate cyclase inhibitor, and protein kinase inhibition with H-89, a cAMP-dependent kinase inhibitor, and KT 5823, a cGMP-dependent kinase inhibitor, on O_2-induced fetal pulmonary vasodilation. In each of the control periods, O_2 caused a decrease in TPR ($P < 0.05$) and a commensurate in increase in LPA blood flow ($P < 0.05$). Guanylate cyclase inhibition alone did not change pulmonary vascular tone. With guanylate cyclase inhibition, O_2-induced fetal pulmonary vasodilation was attenuated ($P < 0.05$). Protein kinase inhibition alone did not change pulmonary vascular tone. Compared with the control period, the O_2-induced increase in fetal pulmonary blood flow was attenuated after protein kinase inhibition ($P < 0.05$). (*Inset*) Effect of kinase inhibition on pulmonary vasodilation caused by 8-Br-cGMP.

20% O_2/balance N_2. All drugs were applied to the cells dissolved in the extracellular perfusate via gravity perfusion at a rate of 2 ml/min. Electrode resistances ranged from 1 to 5 MΩ after fire polishing. For most studies, cells were voltage-clamped at a holding potential of −70 mV and currents were evoked by 10 mV steps to more positive potentials by using test pulses of 200-ms duration at a rate of 0.033–0.1 Hz. Currents were filtered at 1 kHz and sampled at 2 or 4 kHz. Single-step time-series plots were recorded continuously during drug application at a frequency of 0.05 Hz, in order that the time course of onset of any effect may be determined. Steady-state spontaneous outward currrents were recorded at −30 mV. For E_m experiments, cells were held in current clamp at their resting E_m. Control recordings of E_m were made for at least 1 min before application of drug to ensure stability. All data were recorded and analyzed using PCLAMP 6.01 software (Axon Instruments, Foster City, CA). All experiments were performed at 32°C.

To ensure that K^+ was the main ion carrying outward current, the reversal potentials of tail currents recorded in solutions of various extracellular K^+ at 30°C was calculated using the Nernst equation. Reversal potential were recorded using three different external K^+ concentrations and a two-step protocol. Cells were first depolarized by steping to +80 mV from −70 mV for 40 ms and then tail currents measured at various test potentials (−100 through −20 mV) over 200 ms.

K^+-channel characterization was performed using pharmacologic probes, including 1 mM TEA and 100 nM charybdotoxin (CTX; a specific K_{Ca}-channel blocker). To determine the effect of increasing O_2 tension on PA SMCs, K^+ current (I_K) was recorded under hypoxic (Pa_{O_2} = 25 mmHg) and normoxic (Pa_{O_2} = 120 mmHg) conditions. KT 5823 was used to evaluate the effect of kinase inhibition on I_K under hypoxic and normoxic conditions. To determine the effect of NO on I_K, a 10-μl bolus dose of 2 mM saturated NO was administered to PA SMCs under hypoxic conditions. E_m was recorded under hypoxic and normoxic conditions and in the presence of CTX.

Table 1. Effect of increasing maternal FiO_2 (fraction of inspired O_2) on fetal blood gas values before and after treatment with study drugs

Study drug	Maternal FiO_2	Control pH	Control pCO_2	Control pO_2	After study drug pH	After study drug pCO_2	After study drug pO_2
TEA	0.21	7.36 ± 0.04	39.2 ± 6.0	17.6 ± 1.2	7.38 ± 0.01	41.5 ± 3.8	18.7 ± 1.2
	1.00	7.33 ± 0.02	41.0 ± 2.9	27.5 ± 2.4*	7.37 ± 0.03	43.1 ± 5.1	26.4 ± 2.2*
Glibenclamide	0.21	7.30 ± 0.03	55.7 ± 3.0	17.7 ± 1.6	7.27 ± 0.03	59.0 ± 4.6	17.7 ± 1.6
	1.00	7.28 ± 0.03	59.0 ± 4.6	26.0 ± 2.3*	7.27 ± 0.02	54.8 ± 1.0	24.7 ± 2.3*
IBX	0.21	7.29 ± 0.04	46.9 ± 5.3	18.7 ± 1.0	7.32 ± 0.04	48.8 ± 6.0	18.7 ± 1.4
	1.00	7.29 ± 0.04	47.1 ± 5.6	24.7 ± 0.5*	7.30 ± 0.03	49.9 ± 5.0	25.5 ± 0.9*
LY83583	0.21	7.30 ± 0.02	49.4 ± 3.1	20.3 ± 0.8	7.25 ± 0.03	56.7 ± 2.3	17.8 ± 0.5
	1.00	7.28 ± 0.02	54.8 ± 1.8	26.4 ± 1.1*	7.25 ± 0.02	60.2 ± 3.0	24.3 ± 1.0*
H-89, KT 5823	0.21	7.25 ± 0.03	52.0 ± 4.1	18.0 ± 0.5	7.25 ± 0.03	57.1 ± 3.6	17.4 ± 1.0
	1.00	7.25 ± 0.03	57.0 ± 3.6	27.5 ± 1.5*	7.25 ± 0.03	57.0 ± 5.5	26.0 ± 1.9*

Ventilation of the ewe with FiO_2 = 1.00 caused similar changes in fetal blood gas values before and after treatment with study drugs. There were no differences in fetal blood gas values between experimental protocols. *, $P < 0.05$, vs. maternal FiO_2 = 0.21.

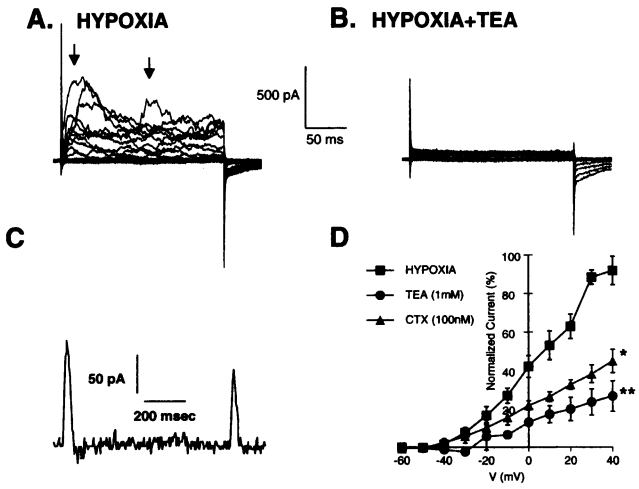

FIG. 3. Pharmocologic characterization of K^+ currents in fetal PA SMCs. (*A* and *B*) Actual current traces recorded from a fetal PA SMC under hypoxic conditions before (*A*) and during (*B*) 1 mM TEA application. Cell was held at −70 mV and stepped to +40 mV using 10-mV steps. Note presence of spontaneous transient outward currents (STOCs) prior to TEA (indicated by arrow). (*C*) Recordings of steady-state activity in a different fetal cell showing two spontaneous outward currents. Cell was held at −30 mV and activity was recorded continously under hypoxic conditions. (*D*) Average current–voltage relationships showing inhibitory effects of 1 mM TEA (●) and 100 nM CTX (▲) during hypoxia. Currents have been normalized and plotted as percentages of the peak current recorded at each test potential under hypoxic conditions (each point plotted as the mean ± SEM; n = 3–6). Levels of significance different from control: *, $P < 0.05$; **, $P < 0.005$ (repeated ANOVA).

Statistics. Results are presented as the mean ± SEM. Intergroup differences and dose–response data were assessed by repeated measures analysis of variance using STATVIEW II (4.0, Abacus Concepts, Berkeley, CA). Student's paired t test was used for E_m data. A P value of <0.05 was considered statistically significant.

RESULTS

Whole Animal Studies. In the control period elevation of fetal Pa_{O_2} from 18.5 ± 0.5 to 26.4 ± 0.7 mmHg (Table 1), caused PA pressure to decrease from 50.6 ± 1.89 to 48.7 ± 1.94 ($P < 0.01$) and TPR to decrease from 1.18 ± 0.14 to 0.41 ± 0.03 mmHg per ml per min ($n = 25$; $P < 0.01$). Fetal blood gas values did not differ between the control and study periods in any experimental protocol. TEA and IBX attenuated, while glibenclamide, had no effect on O_2-induced pulmonary vasodilation (Fig. 1). LY83583 attenuated fetal O_2-induced pulmonary vasodilation (Fig. 2). KT 5823 and H-89, at doses that blocked the vasodilatory effect of 8-Br-cGMP (Fig. 2 *Inset*), inhibited O_2-dependent fetal pulmonary vasodilation (Fig. 2).

Electrophysiology. Initial studies were performed to confirm that the observed currents were mainly carried by K^+. In solutions of external K^+ concentration of 4.2 mM, 8.4 mM, and 16.8 mM, reversal potentials were calculated as −83.1 ± 1.5 mV ($n = 4$), −67.1 ± 1.7 mV ($n = 3$), and −50.5 ± 1.1 mV ($n = 3$), respectively. These values approximate to those calculated by the Nernst equation (at 30°C) of −91.6 mV, −73.5 mV, and −55.4 mV, confirming these were K^+ currents.

Under hypoxic conditions, outward currents recorded from PA SMCs were small (400 ± 47 pA at +40 mV; $n = 19$) and showed brief bursts of superimposed outward current characteristic of spontaneously transient outward currents (STOCs; Fig. 3 *A* and *C*). STOCs, defined as a transient rapid increase in outward current carried by a K_{Ca} channel (28), were also observed when the cells were held at −30 mV (Fig. 3*C*). TEA and CTX inhibited I_k (Fig. 3*B*). Normoxia increased I_k (to 376 ± 80% at +40 mV; $n = 6$; $P < 0.05$), as shown in Fig. 4*A*, and this increase was inhibited by 100 nM CTX. Maximal increase in I_K was always observed within 5 min of switching solutions. KT 5823 had no effect on basal hypoxic I_K but prevented the normoxia-induced increase in I_k ($n = 3$; data not shown). NO increased I_k to 253 ± 28% at +40 mV ($n = 4$; Fig. 4*B*). Fetal PA SMCs had a E_m of −37.2 ± 1.9 mV during hypoxia ($n = 14$). Normoxia hyperpolarized the membrane by 11.9 ± 2.6 mV (Fig. 5). CTX depolarized the membrane by 17.6 ± 3.1 mV.

DISCUSSION

The primary finding in these studies is that O_2 causes fetal pulmonary vasodilation through activation of a K_{Ca} channel and membrane hyperpolarization. K_{Ca} channel inhibition attenuated O_2-induced pulmonary vasodilation, while K_{ATP} channel inhibition had no effect. Patch-clamp studies confirm a central role for the K_{Ca} channel in fetal O_2 sensing since O_2 increased a CTX-sensitive K^+ current. Studies of E_m are consistent with these findings. Under hypoxic conditions, PA SMCs had an E_m of −37.2 ± 1.9 mV and were depolarized by CTX (data not shown). O_2 caused membrane hyperpolarization that was reversed by CTX.

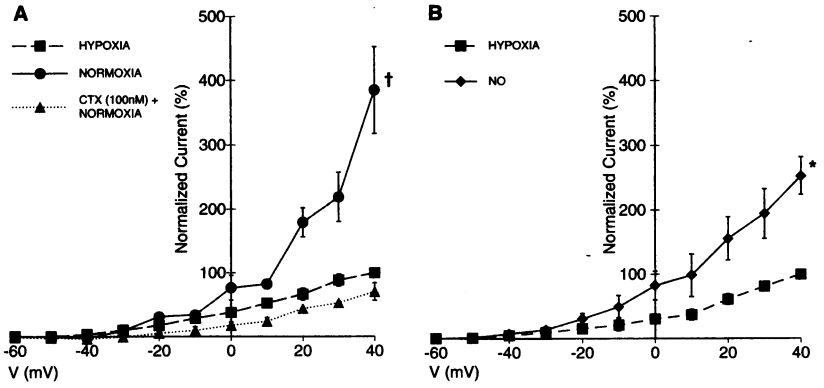

FIG. 4. Effect of normoxia and NO on K^+ currents. (*A*) Average currect–voltage relationships recorded from fetal PA SMCs under hypoxic conditions (■) and after exposure to normoxia (●). Also shown is the effect of 100 nM CTX on currents in the continued presence of normoxia (▲). (*B*) Average currect–voltage relationships recorded from cells under hypoxic conditions and after a 10-μl bolus dose of 2 mM saturated NO (◆). For *A* and *B*, currents have been normalized and plotted as percentages of the peak current recorded at each test potential under hypoxic conditions (each point plotted as the mean ± SEM; n = 4–6). Levels of significance different from control: †, $P < 0.01$ (repeated ANOVA); *, $P < 0.05$ (Student's paired t test for +40 mV).

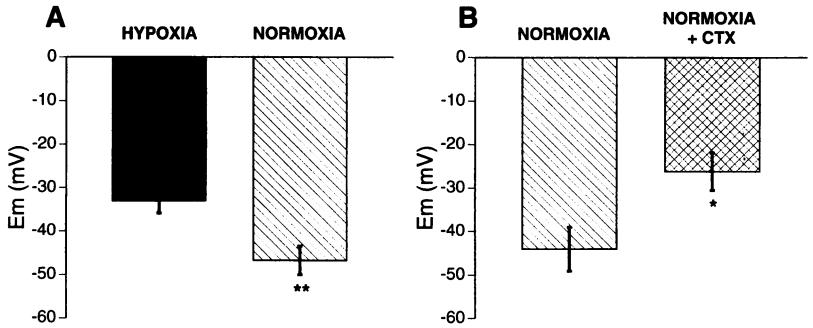

FIG. 5. Modulation of E_m by normoxia and K_{Ca} blockade. (*A*) Average membrane potentials recorded from fetal PA SMCs under hypoxic and normoxic conditions ($n = 5$ for both groups). (*B*) Average membrane potentials recorded from fetal PA SMCs during normoxic conditions and after application of 100 nM CTX ($n = 3$). Significance between groups: *, $P < 0.05$; **, $P < 0.005$ (Student's paired t test)

Further studies were performed to address the mechanism whereby increases in O_2 tension activate K^+ channels. Previous studies have demonstrated that O_2 causes fetal pulmonary vasodilation through increased NO activity (29–31) and elevation of intracellular cGMP concentration ($[cGMP]_i$) (32). Inhibition of guanylate cyclase activity attenuated O_2-induced pulmonary vasodilation. While patch-clamp studies did not address a link between $[cGMP]_i$ and I_k, both O_2 and NO caused a similar increase in I_k. Thus, in fetal PA SMCs, the possibility that NO and O_2 may act through a common mechanism cannot be excluded.

cAMP- and cGMP-dependent protein kinase antagonists were used to test the hypothesis that K_{Ca} activation is contingent upon cyclic nucleotide-dependent protein kinase activity that is sensitive to $[cGMP]_i$. We demonstated that inhibitors of cAMP- and cGMP-protein kinase attenuate O_2-induced fetal pulmonary vasodilation. Since elevation of $[cGMP]_i$ with the membrane-permeable analogue of cGMP 8-Br-cGMP does not cause vasodilation in the presence of cAMP- and cGMP-protein kinase antagonists, it appears cGMP causes vasodilation through activation of a protein kinase (17). In single-cell experiments, the cGMP-dependent protein kinase inhibitor KT 5823 blocked the normoxia-induced increase in I_k. This suggests that $[cGMP]_i$ may modulate I_k through cGMP-sensitive kinase effects on the K_{Ca} channel. A similar relationship between $[cGMP]_i$ and K_{Ca}-channel activation has been described (16, 17, 33). Both patch-clamp and whole animal studies in this paper suggest that O_2 causes pulmonary vasodilation through K_{Ca}-channel activation and membrane hyperpolarization. The K_{Ca} channel may be more important in fetal than in adult PA SMCs. K_{Ca} channels contribute to resting E_m in fetal PA SMCs, since CTX causes membrane depolarization, whereas in PA SMCs from adult rats, E_m is controlled by the delayed rectifier K^+ channel (34, 35). Maturational changes in the expression of subtypes of K^+ channels may relate to developmental differences in vascular reactivity of the pulmonary circulation.

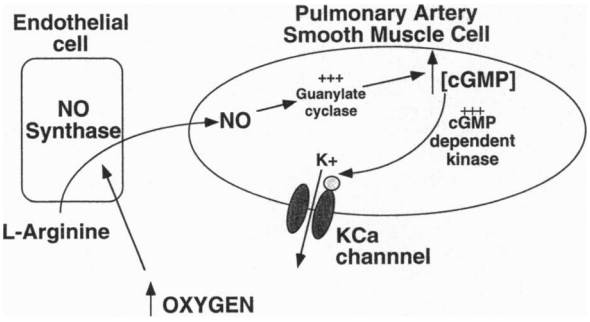

FIG. 6. Schematic diagram of the proposed mechanism for O_2-induced fetal pulmonary vasodilation. O_2 acts to increase NO production. NO diffuses into the PA SMC to cause an increase in guanylate cyclase activity and subsequently an increase in cytosolic cGMP concentration. Elevated cytosolic concentrations of cGMP activate a cGMP-sensitive kinase that phosphorylates a K_{Ca} channel. Activation of the K_{Ca}-channel membrane hyperpolarization and vasodilation then occurs.

The presence of STOCs in fetal PA SMCs may be evidence of such maturation-related changes in the pulmonary circulation. These currents were not present in PA SMCs from 2-week-old sheep (data not shown) and adult rats (34, 35). STOCs may result from transient increases in PA SMC cytosolic Ca^{2+} (36). It has previously been shown (15) that acute hypoxia causes membrane depolarization, entry of Ca^{2+} via dihydropyridine-sensitive Ca^{2+} channels, release of Ca^{2+} from intracellular stores, and an increase in fetal PA SMC cytosolic Ca^{2+}. STOCs may exert a counterregulatory effect in the high-pressure, low-flow, low-O_2-tension environment of the fetal circulation and prevent excessive vasoconstriction, thereby enabling normal pulmonary vascular development.

The observation that K_{Ca}-channel inhibition blocks both the vasodilation and the increase in I_k in single PA SMCs caused by O_2 implies that O_2 causes vasodilation through activation of a large-conductance K_{Ca} channel. Recent work showing that NO has both direct (18) and indirect (16, 17, 32) effects on K_{Ca}-channel activity in vascular smooth muscle cells supports this finding since the vasodilation caused by increasing O_2 tension has been shown to be mediated, in part, by NO (29, 30). Since both guanylate cyclase and cyclic nucleotide-dependent protein kinase inhibition attenuate O_2-induced fetal pulmonary vasodilation, it is unlikely that O_2 causes vasodilation through a direct effect on K_{Ca} channels.

In summary, this work offers the first direct evidence that increases in K_{Ca}-channel activity mediate the pulmonary vasodilation caused by elevation of O_2 tension in the late-gestation ovine fetus. Moreover, the increase in K^+-channel activity is mediated by a cGMP-sensitive kinase (Fig. 6). In these studies, O_2 appears to act via intracellular second messengers rather than directly on the K_{Ca} channel.

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