Oxygen-induced fetal pulmonary vasodilation is mediated by intracellular calcium activation of $K_{Ca}$ channels

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Am J Physiol Lung Cell Mol Physiol 281: L1379–L1385, 2001. O2 sensing in fetal pulmonary artery smooth muscle is critically important in the successful transition to air breathing at birth. However, the mechanism by which the fetal pulmonary vasculature responds to an acute increase in O2 tension is not known. Isolated fetal pulmonary artery smooth muscle cells were kept in primary culture for 5–14 days in a hypoxic environment (20–30 mmHg). These cells showed a 25.1 ± 1.7% decrease in intracellular calcium in response to an acute increase in O2 tension. Low concentrations of caffeine (0.5 mM) and diltiazem also decreased intracellular calcium. The decrease in intracellular calcium concentration in response to increasing O2 was inhibited by iberiotoxin and ryanodine. Freshly isolated fetal pulmonary artery smooth muscle cells exhibited “spontaneous transient outward currents,” indicative of intracellular calcium spark activation of calcium-sensitive potassium channels. The frequency of spontaneous transient outward currents increased when O2 tension was increased to normoxic levels. Increasing fetal pulmonary O2 tension in acutely instrumented fetal sheep increased fetal cytosolic calcium and leading to vasodilation. This study demonstrates that fetal pulmonary vasodilation is mediated by intracellular calcium activation of calcium-sensitive potassium channels.
tion, the proposed mechanism of O₂ sensing was also tested with a whole animal model. Specifically, we studied the effect of an acute increase in O₂ tension on 1) intracellular calcium in a fetal PASMC maintained in primary culture under hypoxic conditions, 2) potassium current activity in freshly dispersed hypoxic isolated fetal PASMCs, and 3) fetal pulmonary vasodilation in the presence of ryanodine in the late-gestation ovine fetus.

METHODS

Calcium imaging studies of cultured cells. Distal pulmonary arteries were quickly excised from pentobarbital sodium-anesthetized ovine fetuses ranging in gestational age from 133 to 142 days (term = 147 days) and placed in physiological saline solution (PSS) containing (in mM) 120 NaCl, 5.9 KCl, 11.5 dextrose, 25 HCO₃, 1.2 NaH₂PO₄, 1.2 MgCl₂, and 1.5 CaCl₂. PASMCs were isolated from fourth- or fifth-order resistance pulmonary arteries. Loose connective tissue and adventitia were removed, and the vessels were liberally rinsed with MEM (0.2 mM calcium). Vessel segments were carefully cut into small pieces and placed in 50-ml conical flasks containing 5.2 ml of the enzymatic dissociation mixture, which consisted of 0.125 mg/ml of elastase (Sigma, St. Louis, MO), 1 mg/ml of collagenase (Worthington Biochemical, Freehold, NJ), 2.0 mg/ml of bovine serum albumin, 0.375 mg/ml of soybean trypsin inhibitor (Sigma), and 5 ml of MEM. After incubation at 37°C for 60 min in a shaking bath, the tissue suspension was triturated 10 times every 15 min in a plastic pipette for a total incubation period of 90–120 min. The tissue suspension was then passed through a 100-μm nylon mesh (Nitex; Tetka, Elmsford, NJ) to separate dispersed cells from undigested vessel wall fragments and debris. The filtered suspension was centrifuged (200 g for 10 min), and the cell pellet was resuspended in 10 ml of MEM supplemented with 10% heat-inactivated calf serum. The dispersed cell suspension was divided into aliquots and placed on 25-mm² glass coverslips at a density of 5–10 × 10⁴ cells/cm². The cells were incubated at 37°C in a hypoxic incubation chamber containing a humidified 10% O₂-5% CO₂-85% N₂ gas mixture. After 16–18 h, the cultures were washed once with Hanks’ balanced salt solution to remove nonadherent cells and debris and were refed with fresh medium. Medium was routinely exchanged at 48- to 72-h intervals. Cells were studied between day 5 and day 14 of culture. The cell density stabilized as subconfluent monolayers after 3–5 days in culture. The cell isolation and culture conditions were optimized to select for SMCs. To verify the cell population, PASMCs were routinely stained with actin-specific antibody after 5, 10, and 14 days in culture.

For calcium imaging, the coverslips of subconfluent cells were placed in a custom-built hypoxic recording chamber. Dye was loaded by incubation with 100 nM fura-2 AM plus 20 ng/ml of pluronic acid (Molecular Probes, Eugene, OR) for 20 min in the hypoxic culture medium (composition given above). This was followed by a 20-min wash in calcium-containing hypoxic recording solution consisting of (in mM) 10 HEPES, 10 glucose, 135 NaCl, 5.6 KCl, 1.8 CaCl₂, and 2 MgCl₂ before the start of each recording. O₂ tension was monitored throughout with an O₂ electrode (Microelectrodes, Bedford, NH) calibrated with solutions at a range of O₂ tensions as measured with a blood gas analyzer. Hypoxic conditions were defined as those with an O₂ tension of 20–30 mmHg. O₂ tension could be rapidly controlled by changing the gas blowing over the chamber and bubbling the perfusate from 100% N₂ to room air. Normoxia was defined as an O₂ tension >140 mmHg. Ratiometric imaging was performed with excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. Images were recorded with an extended ISIS intensified charge-coupled device camera from Photonic Science (Robertsbridge, UK) with image capture and analysis software (Axon Instruments, Foster City, CA). Calcium calibration was achieved by measuring a maximum (with 1 μM ionomycin) and a minimum (with 10 mM EGTA) from each cell, assuming a dissociation constant of 224. All studies were performed on cells isolated and cultured from at least three different fetuses. All drugs, including those used for calibration, were added to the perfusate.

Electrophysiological studies of freshly isolated cells. Distal (fourth- and fifth-order, intralobal) pulmonary arteries were dissected from full-term fetal sheep (133–142 days gestation) and immediately placed in hypoxic solution. To maintain the low-O₂ state of the fetal environment, cells were prepared and stored in a hypoxic solution (O₂ tension 20–30 mmHg; composition in mM: 10 HEPES, 10 glucose, 55 NaCl, 80 sodium glutamate, 5.6 KCl, and 2 MgCl₂; pH 7.4 with NaOH). Single cells were enzymatically dispersed with a papain and collagenase digestion protocol. Briefly, arteries were incubated for 15 min at 37°C in a prewarmed solution containing 5 U/ml of papain plus 1 mg/ml of diithioerythritol plus 1 mg/ml of albumin transferred with a wide-bore pipette to a solution containing 2 U/ml of collagenase H plus 1 mg/ml of albumin and incubated at 37°C for 8 min. The arteries were then washed twice in albumin-containing solution before gentle trituration in an albumin-free solution. All cells were studied in identical conditions within 1 h of preparation.

Suspensions of single PASMCs were transferred to a perfusion chamber (250-μl volume) on the stage of an inverted microscope (Nikon Eclipse TE300) for amphotericin B-perfused patch-clamp studies (14). After a brief period to allow partial adherence to the bottom of the recording chamber, the cells were superfused with hypoxic recording solution (composition given in Calcium imaging studies of cultured cells). Fire-polished microelectrodes with resistances between 3 and 4 MΩ and containing a solution of 140 mM KCl, 1.0 mM MgCl₂, 10 mM HEPES, 0.1 mM EGTA, and 100 μg/ml of amphotericin B (pH 7.2 with KOH) were positioned above single cells with both coarse and micromanipulators. The electrodes were lowered onto the surface of a cell, at which point slight negative pressure, applied by mouth, formed a high-resistance seal with the membrane, electrically isolating the small area of membrane under the electrode. Electronic compensation of whole cell capacitance provided an estimate of the cell size (in pF). To reduce voltage errors, recordings were not made until access resistance was <5 MΩ. Changes in current amplitude were recorded while holding the cell at a fixed membrane potential of ~30 mV. This potential was chosen because it is close to the average resting membrane potential of these cells. All patch-clamp studies were done at room temperature. O₂ tension was kept constant and was adjusted by changing the perfusate from one bubbled with 100% N₂ to one exposed to room air. With the use of a small recording chamber and short dead space, complete bath exchange could be achieved in <30 s. Preliminary measurements with an O₂ electrode established the time frame for consistent bath exchange and reestablishment of stable O₂ levels. However, it was not possible to simultaneously measure O₂ in the bath during patch clamping due to the small volume of the bath. For presentation and analysis, traces were selected from periods of the record in which we
were confident of the O₂ levels in the bath, allowing a full 60 s for bath exchange and reestablishment of stable O₂ levels.

Whole animal studies. All procedures were reviewed and approved by the Animal Care and Use Committee at the University of Minnesota. Mixed-breed (Dorset-Hampshire) pregnant ewes between 133 and 142 days of gestation (term = 147 days) were fasted for 24 h before surgery. Ewes were sedated (pentobarbital sodium at 2–4 g iv over the operative and study periods) and anesthetized (tetracaine at 30 mg intratracheally) and breathed spontaneously throughout the surgery and study periods. The left forelimb of the fetal lamb was delivered through a midline uterine incision, and a skin incision was made after local infiltration with lidocaine (2–3 ml of a 1% solution). Polyvinyl catheters were advanced into the ascending aorta and the superior vena cava after insertion into the axillary artery and vein. Catheters were inserted into the left pulmonary artery (LPA) and main pulmonary artery by direct puncture and secured. 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 the exposed surfaces bathed in warm towels. Fetal skin temperature was monitored. After a minimum recovery time from surgery of 1 h, serial arterial blood gas tensions and pH were recorded at 15-min intervals throughout. Hemodynamic parameters including mean LPA blood flow, heart rate, and pressures in the main pulmonary artery and aorta were monitored continuously. Each animal was studied only once; n refers to the number of animals. Left pulmonary vascular resistance was calculated as mean PA pressure divided by LPA flow.

The experimental protocol used was comparable to that used previously (4, 10). These previous studies have consistently shown that with a 1-h recovery period, repeat exposure to O₂, and this was confirmed in the current study with control animals that did not receive the drug. In this study, after baseline measurements, the fraction of inspired O₂ to O₂ levels did not have any effect on calculated PA pressure divided by LPA flow.

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All salts and enzymes were obtained from Sigma. Fluorescent dyes and ryanodine were obtained from Molecular Probes. Iberiotoxin was obtained from Alomone Labs (Jerusalem, Israel). Throughout, results are presented as means ± SE. Statistical significance was tested with Student’s t-test (paired or unpaired as appropriate). P ≤ 0.05 was taken as the threshold level for statistical significance. Experiments were designed to have a statistical power of at least 90% at a probability level of P ≤ 0.05.

RESULTS

Calcium imaging studies with cultured cells. Hypoxic cultured cells had a calculated resting intracellular calcium level of 111 ± 10 nM (n = 117 cells) at the start of the experiment in a low-O₂ environment (O₂ tension 20–30 mmHg). Increasing the O₂ tension to >140 mmHg led to a decrease in intracellular calcium (e.g., Fig. 1, A and C). In normoxia, the mean intracellular calcium concentration was calculated at 83 ± 7 nM (n = 117 cells). This was a mean decrease of 25.1 ± 1.7%. The decrease in intracellular calcium in response to an increase in O₂ tension (as indicated by the change in fura 2 ratio) was blocked by prior application of either ryanodine (5 × 10⁻⁵ M) or the K Ca channel blocker diltiazem (10 μM; n = 29 cells). *P < 0.05 by paired t-test vs. hypoxic control period.

Pharmacological blockade of voltage-dependent calcium channels with diltiazem (10 μM) decreased intracellular calcium in hypoxic cultured fetal PASMCs...
when studied in a hypoxic environment (Fig. 1C). The application of low concentrations of caffeine (0.5 mM) also produced a decrease in intracellular calcium in the hypoxic cultured fetal PASMCs when studied in a hypoxic environment (Fig. 1, B and C).

Electrophysiological studies with freshly isolated cells. Cells isolated and maintained in hypoxic conditions up to and including the time of recording showed a low frequency of STOC activity. Currents recorded showed small (<5 pA) single-channel openings and large STOCs (e.g., Fig. 2A). In the hypoxic environment, STOC frequency was low (0.14 ± 0.07 STOCs/s; n = 5 cells), with an amplitude of 27.8 ± 7.6 pA. When the O₂ level of the perfusate was increased to normoxic levels, STOC frequency increased to 0.74 ± 0.32 STOCs/s (n = 5 cells; Fig. 2B), with an amplitude of 34.9 ± 5.0 pA (n = 5 cells; Fig. 2C).

Whole animal studies. Administration of 100% O₂ to the pregnant ewe led to an elevation of fetal PA O₂ tension from a baseline of 18.2 ± 2.1 to 28.6 ± 2.7 Torr (Table 1); fetal LPA flow increased from 27.9 ± 8.9 to 241.5 ± 71.4 ml/min (n = 5 sheep; Fig. 3). Baseline and test fetal blood gas values did not differ between the control and study periods of maternal O₂ exposure (Table 1). Preliminary control experiments confirmed the observations made in previous studies (4, 10). After a 1-h recovery period, repeat exposure of the pregnant ewe to 100% O₂ caused an increase in fetal LPA flow that was not significantly different from the first exposure to O₂ (paired t-test; n = 3 sheep). Ryanodine had no effect on fetal baseline mean arterial pressure, aortic pressure, LPA flow, or heart rate. In the presence of ryanodine, administration of 100% O₂ to the ewe in-

Table 1. Fetal blood gases and hemodynamics in response to increasing maternal inhaled O₂

<table>
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<tr>
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<th>Control</th>
<th>With Ryanodine</th>
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<tr>
<td></td>
<td>Baseline</td>
<td>100% O₂</td>
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<tr>
<td>Arterial pressure</td>
<td>63.5 ± 5.2</td>
<td>46.4 ± 10.0</td>
</tr>
<tr>
<td>Aortic pressure</td>
<td>57.4 ± 8.2</td>
<td>41.1 ± 12.8</td>
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<tr>
<td>pH</td>
<td>7.26 ± 0.06</td>
<td>7.27 ± 0.04</td>
</tr>
<tr>
<td>Heart rate</td>
<td>150 ± 1.6</td>
<td>164.2 ± 5.9</td>
</tr>
<tr>
<td>PO₂, Torr</td>
<td>18.2 ± 2.1</td>
<td>28.6 ± 2.7*</td>
</tr>
<tr>
<td>PCO₂, Torr</td>
<td>47.9 ± 4.5</td>
<td>45.9 ± 3.98</td>
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Values are means ± SE. O₂ (100%) inhaled by the ewe in control and test (with ryanodine) periods caused similar changes in blood gas values (*P < 0.05 vs. baseline). There was no change in mean arterial pressure, aortic pressure, pH, or heart rate between the control and test periods or from baseline.
increased fetal LPA flow to only 118.4 ± 42.6 ml/min (P < 0.05 vs. control; n = 5 sheep; Fig. 3).

**DISCUSSION**

This study shows that isolated ovine fetal PASMCs are capable of directly sensing an acute increase in O2 tension. We studied the effect of an acute increase in O2 tension on freshly isolated PASMCs and, in separate experiments, PASMCs maintained in primary culture. Whole animal physiology was used to study the mechanism of action of O2 in the intact animal. Specifically, the present study demonstrates that 1) SMCs maintained in hypoxic primary culture for 7–14 days respond to an increase in O2 tension with a decrease in intracellular calcium that can be inhibited by either ryanodine or by the KCa channel blocker iberiotoxin; 2) intracellular calcium decreases in the presence of low concentrations of caffeine or voltage-dependent calcium channel blocker; 3) freshly isolated SMCs respond to an increase in O2 tension with an increase in STOC frequency; and 4) in the whole animal, ryanodine attenuates O2-induced fetal pulmonary vasodilation. Our interpretation of these findings is that O2 can cause fetal pulmonary vasodilation through a ryanodine-sensitive activation of smooth muscle KCa channels.

These results indicate that fetal PASMCs can respond directly to an acute increase in O2 tension via a mechanism that does not necessarily require an intact endothelium or release of other vasoactive mediators. This study is unique in that the cells were prepared, cultured, and maintained under hypoxic conditions (to mimic the fetal environment) up to and during the start of the experiment. The experimental design allowed analysis of the effects of the first exposure to normoxic levels of O2. The decrease in fetal PASMC intracellular calcium concentration in response to increasing O2 tension was blocked by either the KCa channel blocker iberiotoxin or by ryanodine, which blocks calcium release from intracellular stores. This indicates that the O2 response is contingent on both a functional sarcoplasmic reticulum ryanodine-sensitive store and functional KCa channels. We hypothesized that the most likely way in which intracellular calcium stores and KCa channels could be acting in a coordinated fashion to produce a decrease in intracellular calcium would be that previously outlined (9, 12) for cerebral vascular smooth muscle and recently identified as present in rabbit fetal PASMCs (13). Specifically, KCa channels are opened by the quantal, localized release of intracellular calcium from a ryanodine-sensitive store close to the cell membrane. These small bursts of calcium have been termed calcium sparks. Calcium sparks are localized events that do not contribute to the global intracellular calcium concentration and do not reach a high enough concentration to cause contraction. Calcium spark activation of KCa channel STOCs hyperpolarizes the cell, closing voltage-dependent calcium channels and causing a decrease in cytosolic calcium and, ultimately, vasodilation (9). Blocking the intracellular release channel with ryanodine inhibits calcium sparks and therefore also inhibits any resulting STOCs (12). Iberiotoxin does not block sparks but does prevent the opening of KCa channels in response to an intracellular calcium spark. Inhibition of the O2-induced decrease in PASMC intracellular calcium concentration by either ryanodine or iberiotoxin provides evidence that this spark-STOC activation pathway mediates O2-induced perinatal pulmonary vasodilation.

Inherent in this hypothesis is the assumption that voltage-dependent calcium channels are at least partially open at the resting membrane potential of hypoxic fetal PASMCs. This assumption is supported by the evidence that diltiazem (a voltage-dependent calcium channel blocker) caused a decrease in cytosolic calcium when applied to cells in a hypoxic environment. We hypothesize that O2 leads to an activation of KCa channels (by release of calcium from intracellular stores). This increased opening of KCa channels would hyperpolarize the membrane and tend to close the voltage-dependent calcium channels. Low concentrations of caffeine caused a decrease in cytosolic calcium. Caffeine at concentrations less than those that would
release all the stored calcium at once, causing the frequently observed global increase in cytosolic calcium (6), should increase the frequency of calcium sparks (9). This provides indirect evidence that the hypoxic K_{Ca} channels are capable of responding to an increase in spark frequency. However, to fully address this question, it will be necessary to measure calcium sparks directly.

The electrophysiological measurements from freshly isolated PASMCs provide further support for this hypothesis. STOC activity in fetal PASMCs has been reported previously (4, 5, 13, 16). However, the increase in fetal PA STOC frequency with increasing O_2 tension to normoxia has not been previously studied. STOCs are a result of the coordinated opening of a number of K_{Ca} channels and can be completely inhibited with potassium channel blockers such as tetraethylammonium, charybdotoxin, and the more specific K_{Ca} channel blocker iberiotoxin (9, 12, 16). An increase in STOC frequency, as demonstrated in the present study by increasing O_2 tension, could, in a non-voltage-clamped cell, result in hyperpolarization of the membrane, leading to closure of voltage-dependent calcium channels and a decrease in PASMC intracellular calcium concentration. Thus the electrophysiological data in freshly isolated PASMCs are consistent with the calcium imaging data in cultured PASMCs.

Whether the increase in STOC frequency is due to an increase in spark frequency, an increase in the calcium sensitivity and/or open probability of the K_{Ca} channel, or a combination of these factors remains undefined. Nitric oxide (NO), another important mediator of perinatal pulmonary vasodilation (1) released from the endothelium or possibly produced in the SMCs (8), can also activate K_{Ca} channels (2). Recent data (17) confirm that NO causes perinatal pulmonary vasodilation through ryanodine-sensitive K_{Ca} channel activation. Thus the same mechanism by which the smooth muscle directly responds to an increase in O_2 may mediate the response of the perinatal circulation to NO. O_2 and/or O_2-induced release of NO may lead to an increase in the localized release of calcium and K_{Ca} channel activation.

Single-cell studies were performed with both freshly isolated and cultured PASMCs. This combination of techniques was used to avoid introducing a systematic bias to the data analysis; cells in culture can undergo a range of phenotypic changes leading to complications in interpretation, whereas freshly isolated cells can suffer temporary cell damage from the isolation procedure. The consistency of the observations in both freshly dispersed and cultured cells with our hypothesis is compelling and mitigates the possibility that our findings result from the use of a single-cell preparation method. However, to determine the physiological significance of the observed response to O_2, we also used a whole animal model. In these studies, O_2-induced fetal pulmonary vasodilation was studied in the presence and absence of ryanodine. We found that blocking the release of intracellular calcium with ryanodine attenuated O_2-induced fetal pulmonary vasodilation. The present data fit well with previously published observations (4) in the fetal pulmonary vasculature that show that iberiotoxin attenuates O_2-induced fetal pulmonary vasodilation to a similar degree. This indicates that calcium release from intracellular stores (probably calcium sparks) leading to K_{Ca} channel activation is, at least in part, responsible for the O_2-induced fetal pulmonary vasodilation in the whole animal. The contribution of this sparks-STOCs mechanism in the response to O_2 was different in the whole animal compared with that in the isolated cells in which ryanodine completely blocked the response. This is to be expected because there are many other factors contributing to the tone of the intact vascular bed. The presence of the intact endothelium, differences in temperature and antioxidant defense mechanisms, and the presence of O_2-carrying molecules in the whole animal often lead to difficulties in making direct comparisons between single-cell studies and whole animal responses. Given these differences, the fidelity in the sensitivity of the O_2 response to ryanodine between the single-cell experiments and the response in the whole animal studies is interesting.

In conclusion, this study identifies a powerful O_2-sensing mechanism in fetal PASMCs. The ryanodine sensitivity of the response to an initial increase in O_2 tension was seen to be consistent between the whole animal and cells maintained in hypoxic culture for several days before study. It is concluded that an increase in O_2 to normoxic levels may lead to the release of calcium from ryanodine-sensitive stores, leading to an increase in STOC frequency, which, in turn, leads to a decrease in cytosolic calcium and ultimately contributes to a reduction in fetal pulmonary resistance. This is an important step in understanding the complicated cascade of events that work together to enable the postnatal adaptation of the newborn animal to air-breathing life. Furthermore, abnormalities in the subcellular response to critical birth-related physiological stimuli such as an acute increase in O_2 tension may play a role in perinatal pulmonary vascular disease.

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REFERENCES


