Hypoxic fetoplacental vasoconstriction in humans is mediated by potassium channel inhibition

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HAMPL, Václav, Jana Bíbová, Zbynek Straňák, Xichen Wu, Evangelos D. Michelakis, Kyoko Hashimoto, and Stephen L. Archer. Hypoxic fetoplacental vasoconstriction in humans is mediated by potassium channel inhibition. Am J Physiol Heart Circ Physiol 283: H2440–H2449, 2002. First published August 22, 2002; 10.1152/ajpheart.01033.2001.—Fetal to maternal blood flow matching in the placenta, necessary for optimal fetal blood oxygenation, may occur via hypoxic fetoplacental vasodilation. HFPV was hypothesized to be mediated by K+_v (Kv) channels, increased by 10.220.32.246 on April 9, 2017 http://ajpheart.physiology.org/ Downloaded from

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toward better ventilated areas. In this manner, the systemic blood oxygenation is optimized.

In the fetus, the organ responsible for blood oxygenation is the placenta. The maternal blood flow to the placenta, and therefore the supply of O_2 to the organ, is not spatially homogeneous (33). Therefore, a mechanism for matching fetal to maternal placental blood flow would be advantageous. Although little studied, hypoxic vasoconstriction of fetal vessels in placental regions receiving poor maternal blood supply could serve this function. We hypothesize that such hypoxic fetoplacental vasoconstriction (HFPV) would redirect the flow of fetal blood from insufficiently oxygenated areas of the placenta toward regions receiving better maternal perfusion. The Po_2 in the fetal arterial blood would thus be optimized.

Despite the potential importance of HFPV, there has been minimal experimental investigation of this phenomenon (8, 15). The goal of the present study was to evaluate the mechanism of HFPV in humans. In the other known O_2-sensitive tissues, such as pulmonary vessels, carotid body glomus cells, neuroepithelial bodies, and the ductus arteriosus, a role for O_2-sensitive K^+ channels has been identified (21, 39). In general, changes in Po_2 inhibit one or more K^+ channels, thereby causing membrane depolarization, Ca^{2+} influx, and vasoconstriction. By analogy, we hypothesized that O_2-sensitive K^+ channels are involved in the mechanism of HFPV.

METHODS

All experiments were performed on tissue from healthy human placentas delivered at or near term vaginally or (less frequently) via an elective Cesarean section. The preparation of the isolated cotyledon for perfusion experiments started immediately after the delivery. For all other experiments (isolated vascular rings, patch clamp, RT-PCR, and immunoblotting), a sample of placental tissue was placed into an

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Tissue Hypoxia is a physiologically important regulator of vascular tone. In most organs, hypoxia causes local vasodilatation. This response increases blood flow to the affected organ and thus promotes restoration of tissue oxygenation. In the lung, which has low O_2 consumption and serves as an oxygenator for the organism, alveolar hypoxia causes local vasodilatation. This hypoxic pulmonary vasoconstriction reduces perfusion of poorly ventilated alveoli, diverting blood flow toward better ventilated areas. In this manner, the systemic blood oxygenation is optimized.

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ice-cold physiological saline solution and stored for <1 h before isolation of fetoplacental vessels.

All reagents were purchased from Sigma (St. Louis, MO, or Prague, Czech Republic) unless stated otherwise. Drug doses were based on preliminary experiments.

Isolated perfused human placental cotyledon. An intact cotyledon that was not damaged (as judged by visual inspection) was chosen for perfusion (14, 15, 36). The arterial and venous branches supplying the selected cotyledon were injected with heparin (2,500 IU each; Léciva, Prague, Czech Republic) and cannulated within 10 min of the end of the third stage of parturition.

The fetoplacental vasculature of the cotyledon was perfused with 4% dextran in Earle's balanced salt solution (Sigma) at 38°C. The perfusate was oxygenated by bubbling with a 40% O₂-5% CO₂-55% N₂ gas mixture. Perfusion was initiated within 10 min of the delivery of the placenta. The venous outflow was discarded until it was visibly blood free (usually ~30 min) and then it was recirculated. The initial flow rate of 2 ml/min was gradually increased to 7–8 ml/min and then kept constant. The measured changes in perfusion pressure therefore directly reflected changes in vascular resistance.

All visible vessels crossing the boundaries of the selected cotyledon were ligated. A piece of placental tissue containing the perfused cotyledon plus a 2-cm margin was excised and placed, maternal side down, on a wire mesh atop a funnel-shaped reservoir. The preparation was then covered with a heated (38°C) glass lid with a central opening. Through this hole, three 20-gauge needles were inserted into the lacunae of the cotyledon, thereby allowing perfusion of the maternal side of the cotyledon with a separate pool of heated and oxygenated Earle's dextran solution (38°C, equilibrated with 40% O₂-5% CO₂-55% N₂). To resemble the in vivo conditions, the flow to the maternal side of the isolated cotyledon was kept 2–3 times higher than the flow to the fetal side. The perfusate of the maternal side left the placenta through the maternal side of the isolated cotyledon was perfused with 4% dextran in Earle's balanced salt solution and stored for 1 h of perfusion, once visibly free of blood, was recirculated. The initial flow rate of 2 ml/min was gradually increased to 7–8 ml/min and then kept constant. The measured changes in perfusion pressure therefore directly reflected changes in vascular resistance.

To elicit a hypoxic vasoconstrictor response, the gas mixture bubbling both perfusates (maternal and fetal) was changed to 95% N₂-5% CO₂ until the fetoplacental perfusion pressure stabilized (10–20 min). Repeated hypoxic challenges were separated by reoxygenation (40% O₂) intervals of at least 20 min. To confirm that hypoxia rather than fluctuations in CO₂ was the stimulus for HFPV, a supplementary experiment was performed with three perfused cotyledons challenged repeatedly with moderate hypercapnia (10% CO₂) while the oxygenation was kept constant (40% O₂).

The perfused cotyledon was used in four separate experiments. The first experiment (n = 6 preparations) was designed to test the possible modulation of HFPV by endogenous prostanooids, nitric oxide (NO), and preexisting vascular tone. The role of prostaglandins was addressed by the addition of a cyclooxygenase inhibitor (20 μM sodium meclofenamate) to the perfusate after a control hypoxic response. The hypoxic responses before and after meclofenamate were compared. Similarly, the role of NO in the modulation of HFPV was tested using an inhibitor of NO biosynthesis, Nω-nitro-arginine methyl ester (L-NAME, 50 μM). To test whether the magnitude of HFPV depended on the level of preexisting vascular tone, the responses to hypoxia were compared before and after angiotensin II injection (0.2 μg bolus into fetoplacental inflow cannula). To determine the degree of active tone in the fetoplacental circuit under resting conditions, we assessed the response to sodium nitroprusside (3 doses each of 120 μg). No significant difference in perfusion pressure occurred between the second and third bolus, indicating that a maximal vasodilator dose had been reached. Perfusion pressure after the maximal dose of sodium nitroprusside compared with the value at the start of perfusion was taken as a measure of the active basal tone.

The second (n = 6) and third (n = 7) series of experiments investigated, respectively, the roles of voltage-dependent K⁺ channels (Kv) and large conductance Ca²⁺-sensitive K⁺ (BKCa) channels in the mechanism of HFPV by measuring the effects of their inhibitors on the hypoxic responses of the perfused cotyledon. 4-Aminopyridine (4-AP; 5 mM) and ibetrixitin (100 mM) were used to block Kv and BKCa channels, respectively. A supplementary experiment with four preparations tested the effect of 4-AP on nonhypoxic vasoconstriction (induced by 0.2 μg angiotensin II).

The fourth experiment was a time control with four perfused cotyledons challenged repeatedly with hypoxia without any other intervention.

Fetoplacental vascular rings in tissue bath. To assess the localization of hypoxic vasoconstriction in the placenta, we studied rings cut from the placenta along a large fetoplacental artery running on the placental surface (~1 mm resting diameter). Although we were able to isolate small arteries for measurement of mRNA and protein (see below), we could not measure tension in isolated peripheral arteries due to their small internal dimensions and fragility. However, we reasoned that if larger conduit vessels failed to constrict, the net change in vascular resistance during hypoxia in the isolated cotyledon model should reflect the contribution of the small arteries.

Placental vascular rings were studied in ring baths, as previously described for other vessels (2). Optimal resting tension was found to be ~1,100 mg. Changes in tension in response to hypoxia were recorded. Rings were studied in Krebs solution composed of (in mM) 22.6 NaHCO3, 119 NaCl, 50 sucrose, 4.7 KCl, 1.17 MgSO4, 1.18 KH2PO4, 5.5 D-glucose, 40% O₂-5% CO₂-55% N₂, pH 7.4, and 120 mmHg PO₂ or hypoxia (O₂ content of the bubbling gas mixture reduced to 2.5% for ~40 min, pH 7.4, PO₂ ~40 mmHg).

Isolation of fetoplacental vessels. Isolation of small, peripheral fetoplacental arteries for detection of Kv channel protein (by immunoblotting) and mRNA (by RT-PCR) and for electrophysiology was initiated by making a cut into the placenta along a large fetoplacental artery running on the fetal surface of the organ. This exposed small branches of the artery diving into the placental tissue (diameter ~500 μm and less). Segments (~5 mm long) of up to 10 of these arteries were dissected free from the surrounding trophoblast and connective tissue under a dissecting microscope and placed into ice-cold Hanks' balanced salt solution composed of (in mM) 140 NaCl, 4.2 KCl, 1.2 KH₂PO₄, 0.5 MgCl₂, 10 HEPES, and 0.1 EGTA, pH 7.4. The arteries were either enzymatically digested and cells dispersed for patch clamping or homogenized for RT-PCR and immunoblotting. For comparison, samples of large conduit arteries running on the surface of the placenta (~1 mm diameter) were also taken and processed identically.

Electrophysiology. After the adventitia were carefully removed under a dissecting microscope, the fetoplacental arteries were opened longitudinally, cut in small pieces, and placed in Ca²⁺-free Hanks' solution for 20 min. They were then transferred for 15 min to cold (4°C) Hanks' solution without EGTA that contained (in mg/ml) 1.0 papain, 0.75 DTT, 0.8 collagenase, and 0.8 BSA. The incubation then continued for 10 min at 37°C. The arteries were then transferred to ice-cold Hanks' solution supplemented with glucose.
Table 1. Primers for RT-PCR analysis of human potassium channels and β-actin

<table>
<thead>
<tr>
<th>Primer</th>
<th>GenBank Reference</th>
<th>Size, bp</th>
<th>Sense, nt</th>
<th>Antisense, nt</th>
<th>Tm (°C)</th>
<th>Cycles</th>
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<tbody>
<tr>
<td>Kv1.2</td>
<td>NM_004974</td>
<td>561</td>
<td>392–416</td>
<td>937–961</td>
<td>60</td>
<td>34</td>
</tr>
<tr>
<td>Kv1.5</td>
<td>M60451</td>
<td>417</td>
<td>1,530–1,554</td>
<td>1,922–1,946</td>
<td>60</td>
<td>34</td>
</tr>
<tr>
<td>Kv2.1</td>
<td>L02840</td>
<td>418</td>
<td>496–520</td>
<td>889–913</td>
<td>58</td>
<td>34</td>
</tr>
<tr>
<td>BKCa</td>
<td>U13913</td>
<td>634</td>
<td>595–619</td>
<td>1,204–1,228</td>
<td>56</td>
<td>34</td>
</tr>
<tr>
<td>β-actin</td>
<td>AB006407</td>
<td>285</td>
<td>376–397</td>
<td>639–661</td>
<td>55</td>
<td>34</td>
</tr>
</tbody>
</table>

Kv1.2–2.1, voltage-dependent K+ channels; BKCa, large conductance Ca2+-sensitive K+ channel; Tm, annealing temperature (°C).

RESULTS

Hypoxia invariably caused significant fetoplacental vasoconstriction. The time course of the hypoxic response is illustrated in Fig. 1. Vasoconstriction started within 5 min after the switch to hypoxia and reached a plateau within 10–20 min. The response was reproducible with repetition in the same preparation (not shown). The HFPV was ~80% reversible within minutes of reoxygenation. The average magnitude of control hypoxic responses from all perfusion experiments (n = 36) was 23 ± 3% of baseline perfusion pressure (from 38 ± 2 to 46 ± 2 mmHg; P < 0.0001) with a fetoplacental effluent PO2 decrease from 122 ± 8 to 61 ± 2 mmHg (P < 0.0001). The pH was 7.32 ± 0.02 before and 7.28 ± 0.02 during hypoxia (P = 0.051). PCO2 was 45.9 ± 1.4 mmHg before and 48.6 ± 1.7 mmHg during hypoxia (P = 0.07). In individual preparations, HFPV was similar regardless of whether pH and PCO2 slightly increased, decreased, or were unchanged during hypoxia.

The possible role of pH/PCO2 changes was further examined in a small supplementary experiment with hypercapnic challenges. In this particular group, eu- capnic hypoxia increased fetoplacental perfusion pressure by 29 ± 1%. Hypercapnia (CO2 increased from 5 to 10%) with unchanged oxygenation significantly increased effluent PCO2 (from 52 ± 3 to 70 ± 2 mmHg, P < 0.05, n = 3) and reduced pH (from 7.24 ± 0.03 to 7.07 ± 0.04, P < 0.01), but had no effect on perfusion pressure (27.3 ± 1.5 mmHg before and 27.9 ± 1.6 mmHg during hypercapnia, P = 0.09).

Medlofenamate, a cyclooxygenase inhibitor, had no effect on baseline perfusion pressure (29.1 ± 0.9 mmHg before and 29.6 ± 1.1 mmHg after medlofenamate) or the magnitude of HFPV (Fig. 2). L-NAME, an inhibitor of NO synthase, increased the baseline perfusion pressure from 31 ± 1 to 36 ± 2 mmHg (P = 0.02, n = 6). However, the magnitude of HFPV was not significantly affected (Fig. 2). Thus, although NO appears to contribute to maintaining low tone in the fetoplacental vasculature, it does not have a major role in HFPV. Furthermore, the data with NO synthase inhibition show that elevating vascular wall tension does not enhance HFPV. This conclusion is further supported by the observation that an additional elevation of perfusion pressure by angiotensin II to 47 ± 2 mmHg (a plateau achieved after a transient peak to 61 ± 4 mmHg) does not increase the subsequent hypoxic re-

(1 mg/ml) and dispersed by a gentle trituration with the use of a Pasteur pipette. Cells were transferred to a perfusion chamber on the stage of an inverted microscope for patch-clamp studies and left to attach to the bottom of the chamber for 10–15 min.

Whole cell patch-clamp recordings were performed as previously described (40). Micropipettes (resistance 1–5 MΩ) were filled with a solution (pH 7.2) containing (in mM) 140 KCl, 1.0 MgCl2, 10 HEPES, 5 EGTA, and 10 glucose. This solution was bubbled in its reservoir with a gas mixture of 21% O2-5% CO2-74% N2, which resulted in a PO2 of ~140 and pH of 7.4. The current-voltage relationship was measured by increasing the membrane potential in 20 mV steps (0.1 Hz, 200 ms each) from a holding potential of ~70 mV. Currents were filtered at 1 kHz and sampled at 2 or 4 kHz. Current density was expressed by dividing the whole cell K+ current (IK) by cell capacitance.

The effect of hypoxia was studied by switching the gas mixture bubbling the external solution to one containing 5% CO2-95% N2, which resulted in a PO2 of ~40 mmHg, without a change in pH. The contribution of several types of K+ channels to IK was assessed by using preferential inhibitors: ibetioxin (100 nM, a highly specific inhibitor of BKCa channels), glyburide (10 μM, an inhibitor of ATP-sensitive K channels), BaCl2 (50 μM, an inhibitor of inward rectifying K+ channels), and 4-AP (5 mM).

RT-PCR. Total RNA was isolated from homogenized peripheral fetoplacental vessels using an RNeasy mini Kit (Qiagen; Missisauga, ON, Canada). RNA (2 μg) was reverse transcribed using Qiagen Omniscript reverse transcriptase, as previously described (3). For K+ channels previously implicated in O2 sensitivity (2, 4, 9, 16, 30), PCR primers were designed based on human cloned sequences from GenBank (Table 1). The number of cycles chosen for each primer was within the linear region of the amplification curve. PCR products were sequenced and confirmed to be identical to the intended amplified sequence for each primer set. A no-RT control was used for each primer (shown only for β-actin) to ensure the absence of DNA contamination.

Immunoblotting. Immunoblotting was performed as previously described (3, 4, 22). Antibodies against putative O2-sensitive K+ channels (Alomone; Jerusalem, Israel) were detected with secondary antibodies (Pierce; Rockford, IL) and enhanced chemiluminescence (Amersham; Buckinghamshire, UK). The specificity of each primary antibody for the intended antigen was confirmed in competition experiments in which incubation with an excess of the relevant antigen neutralized the antibody.

Statistical analysis. The immunoblotting and PCR data are qualitative. Hypoxia- and pharmacological K+ channel blocker-induced changes in current-voltage curves were evaluated using ANOVA for repeated measures. All other data were analyzed using a paired t-test. P < 0.05 was considered significant. The results are reported as means ± SE. Sample sizes are noted in the figure legends.
Hypoxic fetoplacental vessels

**Fig. 1.** Hypoxia causes fetoplacental vasoconstriction in isolated, perfused human cotyledon. A composite perfusion pressure tracing from six perfused cotyledons of the first experiment is shown. Hypoxic responses were similar in all subsequent experiments, regardless of the variations in baseline perfusion pressure. Data are means ± SE. ∗P < 0.05, from the sixth minute of hypoxia until the peak of the hypoxic response, the perfusion pressure is significantly higher than at the beginning of hypoxia. †P < 0.05, from the second minute of reoxygenation, the perfusion pressure is significantly lower than at the peak of the hypoxic response (paired t-test).

Response (Fig. 2). The initial basal tone at the start of the perfusion was minimal, as judged from the negligible vasodilatation that occurred in response to a high dose of sodium nitroprusside. The baseline perfusion pressure in this series was 27 ± 2 mmHg at the beginning of the perfusion and 24 ± 3 mmHg after sodium nitroprusside (P = 0.2, n = 6).

To assess the localization of HFPV, the response to hypoxia was measured in isolated fetoplacental vessels mounted in an organ bath. Large conduit fetoplacental arteries responded to hypoxia by relaxation: their tension decreased from 1,065 ± 35 mg in normoxia to 789 ± 106 mg in hypoxia (P < 0.05, n = 9). Large veins exhibited a similar tendency. In seven venous rings, tension was 1,101 ± 68 mg in normoxia and 960 ± 39 mg in hypoxia (P = 0.17). Although we could not measure tone in the small peripheral vessels, the existence of hypoxic vasoconstriction in the whole cotyledon and its absence in the large fetoplacental vessels implicate fetoplacental microvessels as the site of HFPV.

RT-PCR analysis of K+ channels implicated in O2 sensing (2, 4, 16, 30) showed that homogenates from peripheral human fetoplacental arteries contain mRNA for Kv1.5, Kv2.1, and BKCa, but not Kv1.2 (Fig. 3). All primers (except Kv3.1b) detected the relevant channel mRNA in human brain homogenate (Fig. 3), which was used as a standard because of its known abundant expression of virtually all K+ channel types. Immunoblotting confirmed the presence of proteins for the putative O2-sensitive K+ channels, where the mRNA was detected by RT-PCR. Kv3.1b protein was also detected, although we did not have a primer that detected the relevant mRNA under our experimental conditions (Fig. 4).

Initial patch-clamp measurements using smooth muscle cells isolated from a mixture of large and small fetoplacental vessels showed that within minutes of being switched to hypoxia (Po2 ~ 40 mmHg), I\textsubscript{K} dropped by ~30%, and this response was fully reversible on reoxygenation (Fig. 5). Because the results with isolated vascular rings suggested a differential involvement of small and large fetoplacental vessels (see above), cells from these two sources were separated in subsequent patch-clamp experiments.

Smooth muscle cells isolated from small peripheral arteries had significantly higher I\textsubscript{K} density than cells from large conduit arteries (Fig. 6). The combination of iberiotoxin, glyburide, and barium (to inhibit K+ channels other than K\textsubscript{ATP} diminished I\textsubscript{K} to a similar degree in both cell populations, whereas 4-AP caused significant I\textsubscript{K} reduction only in cells from small arteries (Fig. 6). Iberiotoxin alone had similar effects as it had in combination with glyburide and barium, and glyburide alone did not affect I\textsubscript{K} in these cells (data not shown). The conclusion that the two smooth muscle cell populations have similar non-K\textsubscript{ATP} current and cells from small arteries differ from those from large vessels by also having a significant K\textsubscript{ATP} current was confirmed by subtraction analysis (Fig. 7).

In the presence of iberiotoxin, glyburide, and barium, hypoxia significantly reduced I\textsubscript{K} in cells from small, but not large, arteries (Fig. 6, E and F). In the presence of 4-AP, hypoxia did not cause any further I\textsubscript{K} inhibition in any cell population (Fig. 6, A and D). Subtraction analysis revealed that the hypoxia-sensitive portion of I\textsubscript{K} was considerably greater in cells from peripheral than from conduit arteries, where it was minimal (Fig. 7). Importantly, subtraction current...
voltage relationships were almost superimposable for hypoxia and 4-AP (Fig. 7), implying that both hypoxia and 4-AP act on the same channel(s).

The functional relevance of the hypoxic reduction of $I_K$ in fetoplacental vascular smooth muscle was studied in isolated perfused cotyledon. The $K_v$ channel blocker 4-AP (5 mM) mimicked the vasoconstrictor effect of hypoxia. In this series of six experiments, adding 4-AP (5 mM) into the perfusate caused a rise in perfusion pressure from 41 ± 6 to 52 ± 6 mmHg (Fig. 8, A and B). This increase was similar in magnitude to that elicited by hypoxia (Fig. 8, A and B). When the preparation was challenged with hypoxia in the presence of 4-AP, the fetoplacental perfusion pressure did not rise above the level to which it had already been elevated by 4-AP (Fig. 8, A and B).

To confirm that the inhibitory effect of 4-AP was selective for the hypoxic response, a small supplementary experiment was performed with a similar protocol except that after reaching the plateau of the 4-AP vasoconstriction (from 35 ± 4 to 44 ± 5 mmHg, $P < 0.05$), angiotensin II was given instead of the hypoxic challenge. Unlike hypoxia, angiotensin II caused a large vasoconstriction in the fetoplacental vascular bed already constricted by 4-AP (to a peak of 66 ± 6 mmHg, $P < 0.01$, $n = 4$). This shows that the abolition of HFPV by 4-AP was not due to achieving maximal vasoconstriction with 4-AP.

In a separate series of seven experiments, iberiotoxin, a selective BKCa inhibitor, had no significant effect on normoxic perfusion pressure (48 ± 4 mmHg before and 53 ± 5 mmHg after iberiotoxin, $P = 0.126$), although there was a small tendency for an increase in three of the preparations (Fig. 8, C and D). In the presence of iberiotoxin, HFPV was reduced by ~30% (Fig. 8, C and D).

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**Fig. 3.** Small fetoplacental vessels contain mRNA for $K_v$ channels implicated in O$_2$ sensing. The panels show electrophoresis of the RT-PCR products. Arrows show 600-bp mark on a standard DNA ladder in the left lanes. Second right lane, RNA from human brain homogenate (B). The brain, a rich source of most types of $K_v$ channels, was used to positively confirm the effectiveness of each primer. The three lanes at right contain RNA from peripheral fetoplacental vessels (P), with each lane coming from a separate placenta. $\beta$-Actin is shown for standardization. The absence of a signal in the lane depicting $\beta$-actin PCR without reverse transcription confirms the absence of DNA contamination in the RNA isolates. $K_v$1.2, $K_v$1.5, and $K_v$2.1, types of voltage-dependent $K_v$ (Kv) channels; BKCa, large conductance Ca$^{2+}$-sensitive $K_v$ channel.

**Fig. 4.** Small fetoplacental vessels contain proteins of putative O$_2$-sensitive $K_v$ channels. Immunoblots from a homogenate of peripheral vessels isolated from one placenta (P) compared with human brain homogenate (B) are shown. For each channel, immunoblots were prepared from three more placentas, with similar results. The brain, as a rich source of most types of $K_v$ channels, was used to positively confirm the effectiveness of each primary antibody. The intact immunoblotting assay on the left of each panel is complemented with a competition assay on the right, confirming the specificity of the primary antibodies. Note that the competed antibodies cause much less intense signals confirming their specificity for the target antigen. The approximate expected location of each immunoblot is marked on the left of each panel (arrows). The bar at right is a molecular mass marker. Right, the absence of any spurious signal from the secondary antibody is shown.
We hypothesized that HFPV might be in-
ulated by endothelial production of vasodilatory pros-
testosterone, and we demonstrated that this was the case (Fig. 2), suggesting that endogenous production of prostaglandins does not modulate HFPV in humans. Our experiments do not distinguish whether the reason for this finding is that cyclooxygen-
ase activity is not altered by hypoxia or that the human HFPV is insensitive to changes in prostaglandin levels. Our data are consistent with those of King et al. (19), who showed no effect of cyclooxygenase inhibition on baseline perfusion pressure and on vasoconstrictor re-
activity to the thromboxane analog U-46619 in per-
fused lobules of human placenta.

Another potent modulator of reactivity in many vas-
cular beds is endothelial NO. NO synthase is richly expressed in the fetoplacental vasculature (25, 26). Therefore, we addressed the possibility that endoge-
ous NO modulates HFPV in a similar manner as it alters reactivity to numerous pharmacological vaso-
constrictors (19). In agreement with several previous studies (8, 11, 19, 24), we found NO synthase inhibition by L-NAME to cause vasoconstriction during normoxia (confirming effectiveness of the dose). The hypoxic va-
soconstriction, however, was unaltered (Fig. 2). Our interpretation of this finding is that in the human placent a there is a basal, “tonic” production of NO (the inhibition of which increases vascular tone), which is not altered during acute exposure to hypoxia.

Our finding of HFPV unaltered by NO synthase inhibition contradicts the study of Byrne et al. (8), where L-NAME abolished hypoxic reactivity in per-
fused placental cotyledon. This discrepancy may be related to a considerably higher L-NAME dose (0.3 mM) or to the lower Po2 (~40 mmHg) achieved during the hypoxic challenges in the study of Byrne et al. (8). High doses of NO synthase blockers may be associated with nonspecific effects (7, 31, 38), possibly contribut-
ating to unexpected results. The L-NAME dose used in
our study has been shown to be sufficient to inhibit NO synthase (12).

In general, lowering Po2 is expected to inhibit NO biosynthesis, because O2 is a substrate for NO syn-
that. However, because of various cellular compen-
satory mechanisms, the drop in Po2 needed to cause a significant inhibition of NO synthase appears quite large (13, 35). It is thus possible that the Po2 achieved by Byrne et al. (8) was low enough to inhibit NO synthesis, whereas Po2 in our experiments was not. Although the degree of hypoxia reported by Byrne et al. (8) is closer to that expected in vivo, our finding of a normal HFPV during NO synthase inhibition refutes their conclusion that reduction of NO synthesis is the basis of HFPV. This shows that other mechanisms must be in action, and we turned to the possible role of K+ channels.

In several O2-sensing tissues, hypoxic inhibition of K+ channels (particularly homo- or heterotetramers containing Kv1.2, Kv1.5, and Kv2.1 α-subunits, and possibly Kv3.1b) is thought to contribute to the mecha-

This study clearly demonstrates a marked vasocon-
striction of the human fetoplacental vessels in re-
response to acute hypoxia. We show that hypoxic inhibi-
tion of K+ channels present in the fetoplacental vascular smooth muscle underlies this phenomenon. HFPV is most likely localized in small fetoplacental vessels, as implicated by the absence of hypoxic con-
striction in the larger fetoplacental vessels and because the hypoxic response of K+ channels in smooth

Although the existence of HFPV has been frequently assumed (23, 32), it has not previously been well experimen-
tally documented. Its existence has been in-
ferred from the fetoplacental vasoconstriction elicited by a mechanical restriction of the perfusion of the uterus (37). However, factors other than hypoxia could act as stimuli in this situation, e.g., reduced supply of nutrients or impaired removal of fetal metabolites. The first direct indication that hypoxia per se causes fetoplacental vasoconstriction was in a study by Howard et al. (15). In their experiments on perfused human cotel-
ledons, the increases in perfusion pressure with hyp-
oxia were small (~10% above baseline). In our study, the hypoxic responses were more than twice as large. This demonstration of a functionally relevant magni-
itude of HFPV is in agreement with a recent study by
Byrne et al. (8).

Because vascular reactivity in many organs is mod-
ulated by endothelial production of vasodilatory pros-
taglandins, we hypothesized that HFPV might be in-
creased by cyclooxygenase inhibition. That proved not to be the case (Fig. 2), suggesting that endogenous production of prostaglandins does not modulate HFPV in humans.
vessels (9). We found that these K^+ channels, except for Kv1.2, are expressed in peripheral fetoplacental vessels (Figs. 3 and 4). The presence of a 4-AP and voltage-sensitive Kv current in the patch-clamp studies is consistent with a contribution of Kv1.5 and Kv2.1 to the I_K ensemble. The ability of iberiotoxin to reduce I_K shows that BKCa channels also contribute to I_K. However, the portion of the current that is inhibited by hypoxia is sensitive to 4-AP but not iberiotoxin. Importantly, we demonstrated the specificity of all RT-PCR products and antibodies and furthermore showed that these probes effectively detected channel mRNA and protein in the human brain, a rich source of K^+ channels.

The fetoplacental vascular smooth muscle cells display rapid and reversible hypoxic inhibition of I_K (Figs. 5 and 7). Because K^+ channels are central to the regulation of membrane potential in vascular smooth muscle (28), their inhibition is expected to cause membrane depolarization that, in turn, activates the voltage-gated Ca^{2+} channels and thereby causes Ca^{2+} influx. The resulting increase in intracellular Ca^{2+} concentration is a known stimulus for activation of the contractile apparatus. This proposed chain of events remains to be tested in human fetoplacental arterial smooth muscle cells. The mechanism whereby hypoxia inhibits K^+ channels also has yet to be found. This may be a direct effect of hypoxia on the channel or alternatively act through an O_2 sensor mechanism (for review, see Ref. 5).

Our data with K^+ channel inhibitors in perfused placenta show that the electrophysiologically documented inhibition of fetoplacental vascular smooth muscle K^+ channels by low P_O2 does indeed underlie the vasoconstrictor response of the placenta to hypoxia. Importantly, Kv channels are much more important in this respect than BKCa channels. A dose of 4-AP that is selective for Kv channel inhibition (27, 28) mimics and blocks HFPV, suggesting that 4-AP and hypoxia act by the same mechanism. By contrast, iberiotoxin at a dose highly selective for BKCa channel inhibition (27, 28) has a minimal effect on fetoplacental perfusion pressure and only partially reduces HFPV. In light of our electrophysiological finding of small hypoxic sensitivity of BKCa-dominated I_K in smooth muscle cells from large arteries, contrasted with large hypoxic reactivity of Kv-dominated I_K of cells from small arteries, these data are consistent with an interpretation that a major portion of HFPV is mediated by Kv closure in small...
vessels. Hypoxic inhibition of BKCa channels (also in large vessels) plays a minor role.

The absence of hypoxic contraction in isolated rings of large conduit fetoplacental arteries suggests that HFPV is localized in small vessels. An alternative explanation could be that placental parenchyma responds to hypoxia by releasing constrictor factor(s) that subsequently increase placental tone. HFPV thus would be lost once the arteries are isolated, regardless of their size. Certain, although probably not crucial, involvement of surrounding tissue in O2 sensing related to vascular regulation has been documented in the lung (1, 10, 18), which is analogous to the placenta by also responding to hypoxia with vasoconstriction. However, our patch-clamp data show that hypoxia-sensitive K/H11001 current was much higher in the cells from small arteries, whereas it was minimal in cells from large vessels (Figs. 6 and 7). Because of the demonstra-

Fig. 7. Subtraction analysis of whole cell K+ current responses to K+ channel blockers and hypoxia. Note that the portion of whole cell K+ current sensitive to the combination of IbTX, Ba, and glyburide (A) is similar in cells isolated from small (closed symbols, n = 10) arteries and large fetoplacental arteries (open symbols, n = 10), whereas the 4-AP-sensitive portion (B) is much larger in cells from small arteries. Hypoxia-sensitive currents (n = 7) (C) are virtually identical to the 4-AP-sensitive currents in cells from both sizes of vessels.

Fig. 8. K+ channel blockers inhibit hypoxic vasoconstriction in perfused human cotyledon. A: representative original recording of perfusion pressure in isolated cotyledon under constant flow conditions, showing hypoxic vasoconstriction, followed by vasoconstriction elicited by the K+ blocker 4-AP (5 mM) and inhibition of a response to subsequent hypoxic challenge. B: means ± SE data from 6 experiments performed according to the protocol shown in A. Note that 4-AP elicits a similar vasoconstriction as hypoxia and obliterates the subsequent hypoxic response, suggesting the stimuli act via a common mechanism. C: representative pressure recording from an experiment with a similar protocol as in A except that the BKCa inhibitor IbTX (100 nM) was used instead of 4-AP. D: means ± SE data from 7 experiments shown in C. Note that the tendency toward IbTX-induced vasoconstriction is not significant for the whole group. Unlike 4-AP, IbTX reduces hypoxic fetoplacental vasoconstriction only partially. *P < 0.05, the response is significantly different from zero. †P < 0.05, the response is significantly different from the control response to hypoxia (i.e., from the first bar).
tion that the hypoxic K\(^+\) channel participates in HFPV (see above), these data strongly support the idea that small arteries are much more important in HFPV than the large ones.

A methodological issue that requires a brief comment is that of baseline perfusion pressures in isolated cotyledons. Because the preparations differed considerably in the size of the perfused placental tissue while the perfusion flow rate was always similar, the values of baseline perfusion pressure varied greatly (range 20–61 mmHg). In many vascular beds, the vasoconstrictor reactivity is affected by the level of perfusion, typically in the sense of greater vasoconstriction if the baseline tension is higher. In the present study, however, we found no correlation between the baseline perfusion pressure and HFPV magnitude within our range of pressures (analysis not shown). The average baseline perfusion pressure (38 ± 2 mmHg, \(n = 36\)) was close to the value reported in vivo (41).

One methodological limitation of this study that has not been yet mentioned is that of \(P_{O_2}\) during the hypoxic challenges. Because of the \(O_2\) permeability of tubing in perfusion systems and of the placental tissue itself, we were not able to lower effluent \(P_{O_2}\) to <33 mmHg. In fact, the average \(P_{O_2}\) during the hypoxic challenges was \(~60\) mmHg (effluent) in perfusion experiments and \(~40\) mmHg (bath) in patch-clamp studies. Although the drop in \(P_{O_2}\) from baseline was considerable, these values are still higher than the normal \(P_{O_2}\) in the fetoplacental circulation in vivo (\(~30\) mmHg) (29). On the basis of an analogy with other \(O_2\)-sensing tissues, it is unlikely that HFPV is fundamentally different at lower \(P_{O_2}\); however, the exact characteristics of HFPV in vivo remain to be determined.

We speculate that HFPV is pathophysiologically important in humans. In fact, despite the limited published information on HFPV to date, it is often cited as a principal factor in the pathogenesis of intrauterine growth retardation (IUGR) (23, 32), a major cause of neonatal mortality and morbidity affecting \(3\%\) of newborns (17, 20, 34). Newborns with IUGR, especially when combined with prematurity, are at increased risk for extreme respiratory distress syndrome (20), severe bronchopulmonary dysplasia (17), and adverse neurodevelopmental outcome (34). Because of that risk, management of premature newborns with IUGR remains one of the main challenges in neonatology. Of those who survive the neonatal period, 8–10% do not catch up postnatally in growth. In addition, the long-term consequences of IUGR include cardiovascular disease, obesity, and non-insulin-dependent diabetes mellitus (6).

How does HFPV relate to IUGR? We speculate that if HFPV affects most of the placenta (due to maternal hypoxia or uteroplacental dysfunction), it increases the total fetoplacental hemodynamic resistance and consequently impairs the perfusion of the fetal side of the cotyleda (23, 32). Reduced fetoplacental blood flow, in turn, is thought to impair fetal growth (23, 32). Understanding the mechanism of HFPV thus might ultimately facilitate new treatments to prevent or minimize IUGR.

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