Expression and function of potassium channels in the human placental vasculature

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POTASSIUM (K) channels have an important role in the maintenance of smooth muscle tone via their effects on membrane potential, and a variety of agonists can modify tone by alteration of K channel activity (8, 13). Several K channel subtypes have been identified in vascular smooth muscle cells (VSMCs), and altered function has been associated with cardiovascular disease (57).

It has been proposed that hypoxic fetoplacental vasoconstriction (HFPV) (47, 53) in the human placenta is a mechanism that could modulate blood flow by the diversion of blood from poorly to well-oxygenated cotyledons. In perfused human placental cotyledon, reduced partial pressure of oxygen triggered vasoconstriction; yet, large-diameter (>1 mm) arterial/venous constriction was unaltered (12, 30, 33). HFPV may occur via modification of smooth muscle K channel activity (30).

In the lung, effects of hypoxia [hypoxic pulmonary vasoconstriction (HPV)] on vessel contraction have been more thoroughly documented. HPV can be elicited in isolated pulmonary artery VSMCs without neuronal input (17) and is also observed in pulmonary veins (69); the endothelium is also thought to be a critical modulator of the process (1, 4, 26, 35). Recent studies of HPV suggest that K channels influence vascular tone directly and may also be involved in sensing the level of tissue oxygenation; they are therefore essential for the HPV response (4, 51, 67). These channels include members of the voltage-gated (Kv), calcium-activated (Kca), inward rectifying potassium channel-related acid-sensitive K channels (TASK)1 in chorionic plate arteries, veins, and placental homogenate was assessed by RT-PCR and Western blot analysis. Functional activity of K channels was assessed pharmacologically in small chorionic plate arteries and veins by wire myography using 4-aminopyridine, iberiotoxin, pinacidil, and anandamide. Experiments were performed at 20, 7, and 2% oxygen to assess the effect of oxygenation on the efficacy of K channel modulators. Kv2.1, Kv9.3, BKCa, KIR6.1, and TASK1 were all demonstrated to be expressed at the message level. Kv2.1, BKCa, KIR6.1, and TASK1 were demonstrated at the protein level. Pharmacological manipulation of voltage-gated and ATP-sensitive channels produced the most marked modifications in vascular tone, in both arteries and veins. We conclude that K channels play an important role in controlling placental vascular function.

Human potassium channels; artery; vein

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placenta; human potassium channels; artery; vein

expression of KV2.1, KV9.3, TWIK-related acid-sensitive K channels (TASK1)-1, BKCa, and KIR6.1 in arteries, veins, and placental homogenate. The rationale for choosing these channels was that they have been demonstrated previously in other tissues to directly (or indirectly in the case of KIR6.1) mediate altered vascular responsivity in relation to oxygenation. K channel function in arteries and veins was investigated pharmacologically. The influence of different levels of oxygen on K channel activity and vessel tone was also assessed [2% umbilical artery (40), 7% intervillous space (11) and 21% placental hyperoxia].

MATERIALS AND METHODS

This work was performed with the approval of the ethics committee of Central Manchester and Manchester Children’s University Hospita...
tals National Health Service Trust. Informed, written consent was obtained for all tissues used in the study. The investigation conforms to the principles outlined in the Declaration of Helsinki (65a).

**Samples**

Term (37–42 wk of gestation) placentas (n = 95) were obtained postdelivery (vaginal or after elective Caesarean section) from women with otherwise uncomplicated pregnancies (no evidence of hypertension, intrauterine growth restriction, or other medical disorders). Biopsies were taken within 20 min of delivery and placed directly into ice-cold physiological salt solution (PSS) (in mM: 119 NaCl, 25 NaHCO₃, 4.69 KCl, 2.4 MgSO₄, 1.6 CaCl₂, 1.18 KH₂PO₄, 6.05 glucose, 0.034 EDTA; pH 7.4).

**RT-PCR**

Umbilical arteries and veins were identified at the insertion of the umbilical cord into the chorionic plate of the placenta. Chorionic plate small arteries and veins, which traverse the surface of the placenta, can be easily identified by tracing their origin from this insertion point before dissection using a stereomicroscope. Vessels were cut into short, 2- to 3-mm lengths, cleaned of blood, and placed into cryotubes before dissection using a stereomicroscope. Vessels were cut into small arteries and veins, which traverse the surface of the placenta, to the principles outlined in the Declaration of Helsinki (65a). Obtained for all tissues used in the study. The investigation conforms to the principles outlined in the Declaration of Helsinki (65a).

**Expression of Placental Potassium Channels**

R438 EXPRESSION OF PLACENTAL POTASSIUM CHANNELS

**RT-PCR**

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**Western Blot Analysis**

Placental arteries, veins, and whole placenta, different to those used for RNA extraction, were collected as described in Samples. Samples were homogenized on ice in homogenization buffer consisting of 0.01 M HEPES, 0.001 M EDTA, 0.25 M sucrose (pH 7.4) with an anti-protease inhibitor cocktail consisting of 104 mM 4-(2-aminoethyl)benzenesulphonyl fluoride, 1.5 mM pepstatin A, 1.4 mM E-64, 3.6 mM bestatin, 2.1 mM leupeptin, and 80 µM aprotinin (Sigma-Aldrich, Poole, UK). Rat brain (animals killed by cervical dislocation according to UK Home Office guidelines) was used as a positive control for K⁺ channel protein expression. We routinely used the postnuclear supernatant, obtained after a spin at 4,000 g for 10 min, for our blotting experiments. All sample protein concentrations were determined using a commercial protein assay kit (Bio-Rad, Hemel Hempstead, UK). Samples were stored at −80°C until used.

**Arterial and Venous Basal Tone**

For microvessel studies were performed in vessels normalized and equilibrated in 5% CO₂ in nitrogen (final dissolved oxygen content of 0.8–1.0%; termed 7% oxygen) to mimic intervillus space oxygenation, or 5% CO₂ in nitrogen (final dissolved oxygen content of 8.5–10%; termed 2% oxygen) to mimic placental hypoxia. Oxyhemoglobin was measured in the myograph chamber using a oxygen meter (World Precision Instruments, Sarasota, FL; measurement accuracy ±1%). Following equilibration, concentration-response curves were constructed to the thromboxane mimetic U-46619 [0.1–2,000 nM in 2-min increments/5-min plateau (62, 64)]. Placental vessel viability was assessed using 120 mM KCl in PSS (equimolar substitution of KCl for NaCl). Vessels greater than 500 mm in diameter were excluded from the study.

**Role of K Channels in Control of Placental Chorionic Plate Arterial and Venous Basal Tone**

The role of K channels in the control of placental vascular tone was assessed in unstimulated chorionic plate arteries and veins as follows:

Chorionic plate small arteries (274 ± 7 µm; n = 186) and veins (294 ± 10 µm; n = 164) were cut into 2- to 3-mm lengths and mounted onto 40-µm steel wires on a M610-wire myograph (Danish Myotech, Aarhus, Denmark), bathed in 6 ml of PSS, and warmed to 37°C. Vessels were normalized as described previously (62, 64) to 0.9 mm Hg for the vessel diameter in vivo if subjected to a transmural pressure of 5.1 KPa to mimic a physiological resting tension of ~25 mmHg (39). Postnormalization, vessels were equilibrated for 20 min. Functional studies were performed in vessels normalized and equilibrated in 5% CO₂ in air (termed 20% oxygen) to mimic placental hypoxia, 5% CO₂ in 5% oxygen (final dissolved oxygen content of 4.8–6.0%; termed 7% oxygen) to mimic intervillus space oxygenation, or 5% CO₂ in nitrogen (final dissolved oxygen content of 0.8–1.0%; termed 2% oxygen) to mimic placental hypoxia. Oxygenation was measured in the myograph chamber using a oxygen meter (World Precision Instruments, Sarasota, FL; measurement accuracy ±1%). Following equilibration, concentration-response curves were constructed to the thromboxane mimetic U-46619 [0.1–2,000 nM in 2-min increments/5-min plateau (62, 64)]. Placental vessel viability was assessed using 120 mM KCl in PSS (equimolar substitution of KCl for NaCl). Vessels greater than 500 µm in diameter were excluded from the study.
Role of K Channels in Control of Placental Chorionic Plate Arterial and Venous Constriction and Relaxation

Following incubation of arteries and veins with K channel modulators for 5 min, vessels were constricted with U-46619 (0.1–2,000 nM). To assess the vasodilator effect of pinacidil, arteries were constricted with an EC_{50} dose of U-46619. Once a stable constriction was achieved, relaxation was assessed with incremental doses of pinacidil (0.01–100 μM). Time control vessels were performed in parallel (constricted with EC_{50} dose of U-46619 only).

General Chemicals

General chemicals and pharmacological agents were obtained from Sigma-Aldrich (Poole, Dorset, UK) or BDH Laboratory Supplies (Poole, Dorset, UK). U-46619 was obtained from Calbiochem (CN Biosciences, Nottingham, UK).

Statistical Analysis

Vessel tension production was calculated as follows. To standardize for the length of the vessel segment, tension production (in mN) was divided by the length of the vessel segment (in mm) to give active wall tension ΔT (mN/mm). Active effective pressure (P, in kPa), was calculated by dividing ΔT by the normalized internal radius (in mm) of the vessel. An assessment of whether data was normally distributed was performed using the Kolmogorov-Smirnov normality test. Data for the effect of K channel inhibitors and openers on basal tone were compared by repeated-measures (RM)-ANOVA. Relaxation for the effect of K channel inhibitors and openers on basal tone were compared by using the Wilcoxon signed-rank (WSR) test. Statistical significance was calculated as a percentage of the contraction achieved with an EC_{50} dose of U-46619. Concentration-response curves for contraction and relaxation were compared by repeated-measures (RM)-ANOVA. The Bonferroni post hoc test was used to assess statistical significance at individual concentrations of the agonist. Data are expressed as means ± SE with the number of vessels (n) from the number of placentas (N). P < 0.05 was taken to indicate statistical significance.

RESULTS

K Channel Gene Expression

Figure 1 shows two representative examples of PCR products amplified from chorionic arterial (PA) and venous (PV) samples and whole placenta (PL). PA1, PV1, PL1, PA2, PV2, and PL2 blots are matched products amplified from chorionic arterial (PA) and venous (PV) samples and whole placenta (PL). PA1, PV1, PL1, PA2, PV2, and PL2 blots are matched products from individual term placentas. cDNA integrity was confirmed with β-actin. −ve, dH2O negative control; +ve, human brain cDNA positive control; BK_{Ca}, large-conductance Ca^{2+} K channel; K_{IR}, voltage-gated K^{+} channel; K_{IR}6.1, inward-rectified K^{+} channel; TASK, TWIK-related acid-sensitive K channel.

K Channel Protein Expression

Using anti-K_{V}2.1 (representative blot of a minimum of three different placentas; Fig. 2A), we detected bands of ~115 kDa in both placental arteries and veins. Control tissue (rat brain) also gave a single band of 115 kDa. When arterial and venous vessel homogenates and rat brain were probed with anti-BK_{Ca} antibody, a strong signal was observed at 125 kDa (Fig. 2B). Smaller, less intense bands were observed at similar sizes in all three tissues. Signals of ~51 kDa in rat brain and 55 kDa in arterial and venous samples were observed when using the antibody raised to K_{IR}6.1 (Fig. 2C). Anti-TASK1 gave rise to a signal of ~122 kDa (Fig. 2D). For anti-K_{V}2.1, anti-BK_{Ca} and anti-TASK-1, exposure of the primary antibody to its antigenic peptide resulted in ablation of the observed signals (Fig. 2, A–C). This maneuver was not performed for anti-K_{IR}6.1 because a competing peptide was not commercially available. However, primary antibody omission resulted in loss of signal (Fig. 2D). Similar negative control experiments were also performed for all of the other antibodies; the results demonstrated a loss of signal (not shown). Expression of K_{V}9.3 in placental tissues was not assessed because of the lack of a commercially available antibody.

Functional Assessment of K Channels in Fetoplacental Arteries and Veins

General vessel characteristics. We used 95 normal-term placentas (Table 1). Baseline active effective pressure maintained by chorionic plate arteries was 2.71 ± 0.13 kPa (20.3 ± 1.0 mmHg; n = 186) and 2.75 ± 0.15 kPa (20.7 ± 1.1 mmHg;
In K^+ channel protein expression, membranes were probed with anti-K-2.1 (1:1,000; A), anti-BK_Ca (1:500; B), anti-Kg6.1 (1:500; C), or anti-TASK1 (1:100; D). Primary antibody exposed to paired samples from a minimum of three term placentas. Exposure of primary antibody to its antigenic peptide or its omission resulted in signal ablation. Molecular mass standards (in kDa) are indicated. RB, rat brain.

Pinacidil induced significant relaxation of arteries and veins precontracted (EC_80 dose of U-46619) at 2% oxygenation (P < 0.05; RM-ANOVA; Fig. 5, C and D). Significant relaxation was also achieved with 7 and 20% oxygenation (P < 0.05; RM-ANOVA; data not shown). Oxygenation did not significantly alter the sensitivity (P > 0.05 EC_50 data; WSR test) or the maximal relaxation achieved with pinacidil (P > 0.05; WSR test; data not shown).

Pinacidil significantly modified the response of arteries and veins at 2% oxygenation by 10.22 ± 3.4 on November 8, 2017 http://ajpregu.physiology.org/ Downloaded from

Table 1. Subject details

<table>
<thead>
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<th>No.</th>
<th>Age, yr</th>
<th>Gravidity</th>
<th>Parity</th>
<th>Booking Blood Pressure, mmHg</th>
<th>Gestation, wk/day</th>
<th>Birth weight, g</th>
<th>IBR, centile</th>
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<td>95</td>
<td>28 (16–45)</td>
<td>2 (1–8)</td>
<td>1 (0–6)</td>
<td>110 (80–135)</td>
<td>39/1 (36/0–42/0)</td>
<td>3300 (2410–4520)</td>
<td>53 (5–99)</td>
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Data are medians with range in parenthesis; No. is number of subjects. IBR, individualized birth ratio.
In veins, AEA significantly increased contraction at 1 μM U-46619 (*P < 0.05; WSR test), but EC50 was unaffected (*P > 0.05; WSR test; Fig. 6E). Similar results were observed in arteries at 2% oxygenation. AEA significantly modified U-46619-induced contraction (*P < 0.05; RM-ANOVA; Fig. 6D); maximal contraction was significantly increased in the presence of AEA (*P < 0.05; WSR test), but EC50 was unaffected (*P > 0.05; WSR test). However, in veins at 2% oxygenation, AEA did not affect the concentration-response relationship to U-46619 (*P > 0.05; RM-ANOVA; data not shown). At 7% oxygenation, AEA did not affect the U-46619 concentration-response curve in both arteries and veins (*P > 0.05; RM-ANOVA; data not shown).

DISCUSSION

We demonstrated the presence of a number of K channels at both mRNA and protein levels. We also demonstrated using wire myography, showing that pharmacological manipulation of these channels with known modulators leads to altered vascular function.

**Kᵥ Channels**

Kᵥ9.3 is an electrically silent K channel that modifies the activity of other channels, including Kᵥ2.1, when coexpressed as heteromeric channels (56). Furthermore, Kᵥ9.3/Kᵥ2.1 and Kᵥ1.2/Kᵥ1.5 heteromeric channels are hypoxia inhibitable and may be an important HPV initiator in mouse pulmonary VSMCs (35). In this study, we have assessed the expression of pore-forming α-subunits only. Kᵥ1.1 and Kᵥ1.2 β-subunits have been suggested to have a role in oxygen sensing in pulmonary artery VSMCs (67); however, because the Kᵥ α-subunit modifies contractile function (via the passage of K ions through the channel pore), we focused on these units initially. Here, we detected mRNA expression for Kᵥ2.1 and Kᵥ9.3 α-subunits. This confirms and extends previous studies (30) to now include novel data regarding channel expression and function in placental veins for not only Kᵥ channels but other channel subtypes, such as K_ATP, K_CA, and TASK (see below).

Western blot analysis indicated Kᵥ2.1 protein expression in placental arteries and veins at a size comparable to that seen previously (4, 7, 58). Smaller bands were observed with anti-Kᵥ2.1; however, these bands remained after the addition of the antigenic peptide, suggesting that they are a result of nonspecific binding of the primary antibody. Kᵥ9.3 protein expression was not determined because of a lack of commercially available antibody.

Our and previous expression data (30) suggest the presence of fetoplacental vascular Kᵥ channels. Increased basal tone with 4-AP indicates Kᵥ2.1 and Kᵥ9.3, as well as other members of the Kᵥ channel family, are open at rest and adapting membrane potential and are therefore important determinants of fetoplacental vascular activity. The upward shift in the U-46619 concentration-response curve, without altered agonist sensitivity, further implies an effect of altered baseline channel activity, rather than a mechanistic change in U-46619-induced contraction of the smooth muscle.

With 4-AP, parallel upward shifts in the U-46619 concentration-response curves at all oxygenations without modification in agonist sensitivity imply an effect independent of Kᵥ channels. Furthermore, vasoconstriction at 2 and 7% oxygenation was comparable and indicative of a non-HPV-like response, which may also explain the lack of effect oxygenation had on the 4-AP-induced alterations in vascular function. A
role for KV channels in hypoxic fetoplacental responses requires detailed studies using oxygen tensions below 1% in pressurized vessels in the presence of luminal flow.

**KCa Channels**

BKCa α-subunit mRNA was strongly detected in arteries with qualitatively weaker signals in matched venous and placental homogenates. Western blot analysis detected BKCa protein at a similar size (~125 kDa) to that in human myometrium (44) and rat brain (42). Smaller signals were observed with anti-BKCa that remained after competition with the antigenic peptide, suggesting that they contained the epitope of interest; these bands may reflect immature or partially degraded forms of the protein.

IBTX did not alter basal tone. At 7% oxygenation, IBTX increased U-46619-induced contraction without altering agonist sensitivity. On vasoconstriction, raised intracellular Ca²⁺ opens BKCa channels and induces smooth muscle cell membrane hyperpolarization, which results in a reduction in Ca²⁺ entry, and relaxation ensues. IBTX prevents the induction of this feedback, and increased smooth muscle contraction results. This was not seen in veins at 7% oxygenation. These data suggest a difference in the control of arterial and venous contraction; we previously documented similar differences in the responses of fetoplacental arteries and veins to oxygenation (63).

IBTX did not affect arterial or venous U-46619-induced contraction in modified oxygenation. At low oxygenation, animal pulmonary VSMC data suggest BKCa inhibition (16, 68). Why IBTX is ineffective at increased oxygenation is unclear but may partly result from actions of reactive oxygen species that can inhibit (9) or activate (60) BKCa in vascular preparations.

We found minimal effects of BKCa on chorionic plate vessel function, yet NO-mediated relaxation of human umbilical arteries occurs via activation of Kv and BKCa channels (43). A similar mechanism has been suggested in endothelin-1 (ET1)-contracted placental arteries; NO produces cGMP-dependent and independent relaxation, which may be via an action on BKCa (55). This suggests that BKCa may indirectly promote fetoplacental vascular relaxation to different stimuli, an area that requires further functional study.

**KIR6.1 Channels**

KIR6.1 mRNA was readily detectable in all tissues. Western blot analysis yielded signals at ~55 kDa in vessels, which compares favorably to that seen here in the control tissue (51 kDa, rat brain) (41) and 44 kDa in primary human coronary artery endothelial and smooth muscle cells (66).

KATP channel activation with pinacidil induced arterial and venous relaxation at rest and in U-46619 precontracted vessels. Pinacidil was used in the current experiments since we have previously demonstrated that glibenclamide, the best described blocker of KATP channels, produces effects in the placental vasculature that cannot be wholly attributed purely to inhibition of KATP channels (63). Here, the effects of pinacidil were independent of oxygenation. U-46619-induced contractions were attenuated by pinacidil but only in hyperoxia and hypoxia. The lack of oxygenation effect was unexpected. One would expect decreased oxygenation to inhibit ATP production. This would promote KATP channel opening and perhaps modify the sensitivity of the tissue to pinacidil. However, in this system, we may not have achieved the level of prolonged and severe hypoxia required to achieve a downregulation of ATP production. Alternatively, hypoxia may also affect levels of vasodilators, such as prostacyclin, which has previously been demonstrated to alter KATP channel activity (37) or influence relaxation by other non-ATP channel mechanisms. Furthermore, plasmalemmal KATP channels of VSMCs may also have different sensitivities to K channel openers compared...
Fig. 5. A and B: functional responses to pinacidil. All data in 2% oxygenation. Effect of pinacidil on basal tone in arteries (A) and veins (B). Prepinacidil (solid bar); 5 min of postpinacidil (50 μM; hatched bar). All data are given as means ± SE; P values from Wilcoxon signed rank test. C and D: relaxation of U-46619 precontracted arteries (C) and veins (D). P values in lower left corner (C and D) from repeated-measures ANOVA; *P < 0.05 from Bonferroni post hoc test (C and D). Effect of pinacidil on U-46619-induced contraction in arteries (E) and veins (F). P values from repeated-measures ANOVA; *P < 0.05 from Bonferroni post hoc test (E and F).

Fig. 6. Functional responses to anandamide. A and B: effect of 20 μM anandamide on basal tone in arteries (A) and veins (B) at 20% oxygenation. Preanandamide (solid bar); 5 min postanandamide (20 μM; hatched bar). All data are means ± SE; P values from Wilcoxon signed-rank test. C–E: effect of anandamide on U-46619-induced contraction in arteries (C and D) and veins (E). Tissue was exposed to 20% oxygenation (C and E) or 2% oxygenation (D). P values by repeated-measures ANOVA (bottom right; C–E); *P < 0.05 by Bonferroni post hoc test (C and D).
with mitochondrial or endothelial cell subtypes (8, 23). Consequently, it is less surprising that pinacidil-induced relaxation was oxygen independent.

U-46619-induced contraction was unaltered by 7% oxygenation but was inhibited by pinacidil in raised/lowered oxygenation. The reason(s) for this are unclear. One possible explanation is that pinacidil coupled with reactive oxygen species modulates K<sub>ATP</sub> channel function, as previously suggested in other tissues (29, 61). The K<sub>ATP</sub> channel opening would be expected to blunt U-46619-induced contraction, as seen at 20 and 2% oxygenation, but at 7% oxygenation, pinacidil alone may be insufficient to produce such an effect.

K<sub>IR6.1/KATP</sub> may thus play an important role in the control of fetoplacental vascular tone. Therapeutically, specific K<sub>ATP</sub> channel activation may reverse hypercontraction in disease states, such as intrauterine growth restriction (IUGR), where choriionic plate arteries demonstrate increased agonist-induced contraction (45). In support of this notion, ET1 inhibits K<sub>ATP</sub> channels in rabbit (50) and guinea pig (65). ET1 has also been suggested to promote an IUGR phenotype (48). Thus K<sub>ATP</sub> channel openers may offer a pharmacological tool to combat such an effect.

**TASK1**

TASK1 mRNA was present in vessels, but it was more readily detectable in whole placental homogenate, consistent with our previous study in cytotrophoblast cells (6). Similarly, TASK-1 protein was expressed, at reduced levels compared with other channels, at a size consistent with our previous study in placental trophoblast (6).

We attempted to address whether TASK1 channels were functional within choriionic plate vessels using AEA, one of the more selective blockers of TASK1. AEA increased basal tone in arteries and veins at 20% oxygenation but not at lower oxygenation where the channels would be expected to be closed (10, 27). Thus TASK1 may maintain basal tone, perhaps contributing to resting E<sub>ma</sub> as a background current. AEA’s small effect on U-46619-induced contraction at 20% oxygenation fits with this role. However, altered contraction at 2% oxygenation is inconsistent with such a hypothesis. The lack of effect of AEA on basal tone and data, suggesting that reduced oxygenation closes TASK1 (10, 27) implies that the latter effect of AEA is not via TASK1 inhibition. The similarity of the U-46619 concentration-response curve with AEA to that with 4-AP, coupled with the observation that AEA can inhibit the activity of Kv1.2 (52) and Kv1.5 (32), may explain this alteration in arterial contractility. However, AEA did not cause a similar effect in veins or either vessel type at 7% oxygenation where 4-AP enhanced constriction.

Overall, these data do not suggest an obligatory role for TASK1 in mediating U-46619-induced constriction or mediating contractile responses at oxygen tensions prevalent in situ. This is an important observation because it implies that the control of vascular tone in the fetoplacental vasculature contrasts markedly with the data from pulmonary artery VSMCs, where TASK1 has a key role in the mediation of oxygen-sensitive contractile function (28). However, AEA may also inhibit gap junctions, although this is only thought to be significant at >50 μM (34). Thus a clarification of the role of TASK1 K channels in vascular tissues may await the development of more specific pharmacological tools.

**IUGR**

In IUGR, umbilical artery Doppler waveforms indicate increased fetoplacental resistance compared with normal pregnancies (3, 38). Increased tone could be a consequence of aberrant Kv channel function, as Kv channel inhibition elicits increased tone in fetoplacental arteries and veins (Fig. 3). However, modified oxygenation, which is also apparent in IUGR, did not alter 1) effects of 4-AP on basal tone, 2) U-46619-induced contraction, or 3) vessel sensitivity to U-46619. Hypersensitivity to U-46619 (46) and ET1 (48) has previously been demonstrated in IUGR. These effects may be via actions on Kv or K<sub>ATP</sub> channels (50, 65), but changes in oxygenation per se did not modify the actions of 4-AP or pinacidil on fetoplacental vessels. Conversely, K<sub>ATP</sub> and Kv channel function may be modified during ischemia-reperfusion injury (31, 49) and by free radicals (21, 36), respectively, and perhaps these influences, in addition to hypoxia, are required to produce the IUGR phenotype.

In summary, we demonstrated the presence of a number of K<sub>ATP</sub> channels in chorionic plate arteries and veins using RT-PCR and Western blot analysis. Furthermore, pharmacological manipulation of K<sub>ATP</sub> channels modified fetoplacental vascular function. In particular, administration of K<sub>ATP</sub> channel openers may be a strategy to promote relaxation of the fetoplacental vasculature in pathological states of inappropriately increased vascular tone. Further elucidation of the role for these channels in the control of fetoplacental vascular tone necessitates characterization of vascular responses using pressure myography in the presence and absence of the endothelium.

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**REFERENCES**


