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 cotyledons, 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), two-pore domain, and ATP-sensitive families (K\textsubscript{ATP}). (4, 5, 10, 16, 19, 22, 23, 28, 51, 54, 56, 59).

Unlike in the lung, there are few data on K channels in the fetoplacental vasculature. Kv, KCa, and K\textsubscript{ATP} channel activity have been demonstrated electrophysiologically in VSMCs or endothelial cells from placental allantochorial vessels (24, 25), Kv\textsubscript{1.5}, Kv\textsubscript{2.1}, and large-conductance Ca\textsuperscript{2+}-activated potassium channels (BK\textsubscript{Ca}) have been demonstrated by RT-PCR, and variable expression of Kv\textsubscript{2.1}, Kv\textsubscript{3.1b}, Kv\textsubscript{1.5}, and BK\textsubscript{Ca} was documented in placental vessel homogenates (30). Most recently, calcitonin gene-related peptide-induced glibenclamide-inhibitable vasodilatation of the fetoplacental vasculature has been demonstrated, suggestive of a role for K\textsubscript{ATP} channels (20).

Thus there is limited evidence on the role of K channels in the control of fetoplacental arterial tone; there are no previously published studies of fetoplacental venous tone.

Our hypothesis is that K channels have a role in the control of small vessel function in the human chorionic plate. We determined expression of mRNA (RT-PCR) and protein (Western blot analysis) for Kv\textsubscript{2.1}, Kv\textsubscript{9.3}, TWIK-related acid-sensitive K channels (TASK1)-1, BK\textsubscript{Ca}, and K\textsubscript{IR6.1} in arteries, veins, and placental villous 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 K\textsubscript{IR6.1}) mediate altered vascular reactivity 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 Hospital.
EXPRESSION OF PLACENTAL POTASSIUM CHANNELS


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. Vessels were cut into short, 2- to 3-mm lengths, cleaned of blood, and placed into cryotubes until used.

After thawing on ice, total RNA was isolated from arterial, venous, and whole placental tissue by homogenization in Trizol reagent (Invitrogen, Paisley, UK). RNA was reverse-transcribed using Moloney murine leukemia virus RT according to the manufacturer’s instructions (Invitrogen) in a PerkinElmer (Beaconsfield, UK) Cetus thermal cycler. An initial RT-PCR, using β-actin primers, was carried out to verify RNA integrity before proceeding. Subsequent RT-PCR, using standard techniques with a hot start, was performed using primer pairs previously described in other studies of human K channel expression, and optimized for use in our samples: Kv2.1 5'-GCC-TACCCCTCATCTCCAATC-3' (forward) with 5'-ACTCATCGAG-GCTCTGTAGCTCAG-3' (reverse), annealing temperature (Ta) 64°C (56); Kv3.3 5'-CCATGATGTGAGGACCCCTCCT-3' (forward) with 5'-GAACCTCCGACATGCTGTAAGC-3' (reverse), Ta 55°C (56); BKCa α-subunit 5'-CAGACACTGATGCGAGACTCCTG-3' (forward) with 5'-GAGTGGCTGCTATCCGACG-3' (reverse), Ta 53°C (18); TASK1 5'-CTCCTTTCATCACTTCCTC-3' (forward) with 5'-CATCCACTTTTCTCAGAGC-3' (reverse), Ta 59°C (18). Blot searches were performed to ensure primers had no homology with any other known gene products. The number of cycles was 35 for each primer pair, with one cycle consisting of denaturation at 95°C for 60 s, annealing at Ta for 60 s, and extension at 72°C for 60 s; the exception to this was the extension times for Kv2.1 and BKCa, which were 40 and 90 s, respectively. Appropriate positive (human brain RNA; Becton Dickinson Bioscience, Oxford, UK) and negative controls (water replacing template) were used at all times.

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 stunning, followed 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.

Protein from arteries, veins, placenta (50–100 μg as indicated), and rat brain (50–70 μg) was mixed with a reducing loading buffer containing 1.25% β-mercaptoethanol (vol/vol), 2% SDS (wt/vol), 0.04% bromophenol blue (vol/vol), and 10% glycerol (vol/vol), in 0.05 mol/l Tris-HCl (pH 6.8) and heated at 95°C for 5 min. Proteins were then subsequently electrophoretically separated in 8–10% polyacrylamide gels and transferred to polyvinylidene difluoride membranes. The membranes were blocked for 1 h using blocking buffer [1% dried milk powder (wt/vol) in 0.05% Tween 20 (vol/vol), Tris-buffered saline (TBS; 0.015 in mol/l Tris, 0.150 in mol/l NaCl; pH 8.0)].

Membranes were probed for 2 h at room temperature with either anti-Kv2.1 at 1:1,000 (Upstate Biotech, Lake Placid, NY), anti-BKCa (Alomone Labs, Jerusalem, Israel) at 1:500, anti-Kv6.1 (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:500, or anti-TASK1 (Alomone Labs) at 1:100 in blocking buffer. Kv2.1 rabbit polyclonal antibody was raised against residues 837–853 of rat Kv2.1. BKCa rabbit polyclonal antibody was raised against residues 1184–1200 of mouse BKCa α-subunit. Kv6.1 rabbit polyclonal antibody was raised against residues 345–424 (COOH terminus) of human Kv6.1. TASK1 rabbit polyclonal antibody was raised against the peptide (COOH terminus) corresponding to residues 252–269 of the human TASK1 channel. Three 10-min washes in TBS/0.05%/Tween 20 were followed by incubation with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody at a 1:2,000 dilution (Dako, Ely, UK) for 1 h. After three 10-min washes in TBS/0.05%/Tween 20, membranes were developed by enhanced chemiluminescence (Amersham Pharmacia Biotech, Little Chalfont, UK). Appropriate negative controls without primary antibody or in the presence of competing peptide were also performed.

Myography

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 of 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 hyperoxia, 5% CO2 in 5% oxygen (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 nM KC1 in PSS (equimolar substitution of KC1 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 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:
Kv channels were inhibited with 4-aminopyridine (4-AP; 1 nM); 2) BKCa channels were inhibited with iberiotoxin (IBTX; 100 nM); 3) TASK1 channels were inhibited with anandamide (AEA; 20 μM); 4) KATP channels were opened with pinacidil (50 μM); and 5) basal tone was assessed pre- and 5-min postaddition of the pharmacological agent.

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 EC50 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 EC50 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 Kolomogorov-Smirnov normality test. Data for the effect of K channel inhibitors and openers on basal tone were compared by using the Wilcoxon signed-rank (WSR) test. Relaxation was calculated as a percentage of the contraction achieved with an EC50 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 placentas (PL). PA1, PV1, PL1, PA2, PV2, and PL2 blots are matched pairs obtained from matched arterial, venous, and placental samples from individual term placentas. cDNA integrity was confirmed with β-actin. −ve, ΔH2O negative control; +ve, human brain cDNA positive control; BKCa, large-conductance Ca2+-activated K+ channel; Kv1, voltage-gated K+ channel; KIR, inward-rectified K+ channel; TASK, TWIK-related acid-sensitive K channel.

Four of five arterial and three of five venous samples and were readily detected in four of five placental samples.

K Channel Protein Expression

Using anti-KV2.1 (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-BKCa 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 KIR6.1. Anti-TASK1 gave rise to a signal of ~122 kDa (Fig. 2D). For anti-KV2.1, anti-BKCa 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-KIR6.1 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 KV9.3 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;
n = 164) in chorionic plate veins [i.e., similar to that suggested to be present in vivo (39)]. In agreement with our previous observations (14, 15), normalization at the three different levels of oxygenation did not significantly affect baseline active effective pressure (data not shown).

Pharmacological investigations. In Kv channels, 1-mM 4-AP significantly increased basal tone in unstimulated chorionic plate arteries and veins at 7% oxygenation (P < 0.05; WSR test; Fig. 3, A and B). Basal tone was unaltered in parallel time controls (P > 0.05, WSR test; data not shown). The effects of 4-AP were independent of oxygenation (data not shown), 4-AP induced a significant upward shift in the U-46619 concentration-response curve in arteries and veins at 7% oxygenation (P < 0.05, RM-ANOVA; Fig. 3, C and D). Maximal contraction increased significantly (P < 0.05, WSR test), but EC50 was unaffected (P > 0.05, WSR test). Comparable significant effects of 4-AP were seen in arteries and veins at 2 and 20% oxygenation (data not shown).

In BKCa channels, 100-nM IBTX did not significantly affect basal tone in unstimulated chorionic plate arteries and veins at 2, 7, or 20% oxygenation (P > 0.05; WSR test; data not shown). IBTX did not modify the U-46619 concentration-response relationships in veins at 2, 7, or 20% oxygenation (P > 0.05; RM-ANOVA; data not shown). In arteries, IBTX did not affect the U-46619 concentration-response relationship at 2 or 20% oxygenation (P > 0.05; RM-ANOVA; Fig. 4, A and C). However, at 7% oxygenation, maximal contraction with U-46619 increased (P < 0.05; RM-ANOVA and WSR test) but EC50 was unaffected (P > 0.05; WSR test; Fig. 4B) by IBTX.

In KATP channels, 50-μM pinacidil significantly decreased basal tone in unstimulated chorionic plate arteries and veins at 2% oxygenation (P < 0.05; WSR test; Fig. 5, A and B). Basal tone was unaltered in parallel time controls (P > 0.05; WSR test; data not shown). The effects of pinacidil were independent of oxygenation (P < 0.05; WSR test; data not shown).

Pinacidil induced significant relaxation of arteries and veins precontracted (EC80 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 EC50 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 to U-46619 in 2% oxygenation (P < 0.05; RM-ANOVA; Fig. 5, E and F); maximal contraction was significantly reduced (P < 0.05; WSR test), but EC50 was unaffected (P > 0.05; WSR test). Similar results were observed at 20% oxygenation in arteries and veins (P < 0.05; RM-ANOVA; data not shown); however, maximal contraction was significantly reduced (P < 0.05; WSR test) and EC50 was significantly increased (P > 0.05; WSR test; data not shown). U-46619-induced arterial and venous contraction were unaffected by pinacidil at 7% oxygenation (P > 0.05; RM-ANOVA; data not shown).

In 20% oxygenation, 20 μM AEA significantly increased basal tone unstimulated in chorionic plate arteries and veins (P < 0.05; WSR test; Fig. 6 A and B). Basal tone was unchanged in parallel time controls (P > 0.05; WSR test; data not shown). AEA did not significantly modify basal tone of arteries or veins at 7 or 20% oxygenation, respectively (P > 0.05; WSR test; data not shown).

AEA significantly modified the U-46619 concentration-response relationship in arteries and veins at 20% oxygenation (P < 0.05; RM-ANOVA; Fig. 6, C and D). In arteries, AEA significantly increased maximal contraction (P < 0.05; WSR test), but EC50 was unaffected (P > 0.05; WSR test; Fig. 6C).

Table 1. Subject details

<table>
<thead>
<tr>
<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>Systolic</td>
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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 KATP, KCa, 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.

**K_Ca Channels**

BK_Ca α-subunit mRNA was strongly detected in arteries with qualitatively weaker signals in matched venous and placental homogenates. Western blot analysis detected BK_Ca protein at a similar size (~125 kDa) to that in human myometrium (44) and rat brain (42). Smaller signals were observed with anti-BK_Ca 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 BK_Ca 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 BK_Ca 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) BK_Ca in vascular preparations.

We found minimal effects of BK_Ca on chorionic plate vessel function, yet NO-mediated relaxation of human umbilical arteries occurs via activation of KV and BK_Ca 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 BK_Ca (55). This suggests that BK_Ca may indirectly promote fetoplacental vascular relaxation to different stimuli, an area that requires further functional study.

**K_IR6.1 Channels**

K_IR6.1 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).

K_ATP 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 K_ATP channels, produces effects in the placental vasculature that cannot be wholly attributed purely to inhibition of K_ATP 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 K_ATP 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 K_ATP channel activity (37) or influence relaxation by other non-ATP channel mechanisms. Furthermore, plasmalemmal K_ATP 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\textsubscript{ATP} channel function, as previously suggested in other tissues (29, 61). The K\textsubscript{ATP} 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\textsubscript{ATP} \textsubscript{6.1} channels may thus play an important role in the control of fetoplacental vascular tone. Therapeutically, specific K\textsubscript{ATP} channel activators may reverse hypercontractility in disease states, such as intrauterine growth restriction (IUGR), where chorionic plate arteries demonstrate increased agonist-induced contraction (45). In support of this notion, ET1 inhibits K\textsubscript{ATP} channels in rabbit (50) and guinea pig (65). ET1 has also been suggested to promote an IUGR phenotype (48). Thus K\textsubscript{ATP} 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 chorionic 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\textsubscript{m} 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 \textmu M (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 K\textsubscript{v} channel function, as K\textsubscript{v} 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) affects 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 K\textsubscript{v} or K\textsubscript{ATP} channels (50, 65), but changes in oxygenation per se did not modify the actions of 4-AP or pinacidil on fetoplacental vessels. Conversely, K\textsubscript{ATP} and K\textsubscript{v} 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 channels in chorionic plate arteries and veins using RT-PCR and Western blot analysis. Furthermore, pharmacological manipulation of K channels modified fetoplacental vascular function. In particular, administration of K\textsubscript{ATP} 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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GRANTS

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