Expression and function of potassium channels in the human placental vasculature

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Expression and function of potassium channels in the human placental vasculature. Am J Physiol Regul Integr Comp Physiol 291: R437–R446, 2006. First published March 2, 2006; doi:10.1152/ajpregu.00040.2006.—In the placental vasculature, where oxygenation may be an important regulator of vascular reactivity, there is a paucity of data on the expression of potassium (K) channels, which are important mediators of vascular smooth muscle tone. We therefore addressed the expression and function of several K channel subtypes in human placentas. The expression of voltage-gated (Kv)2.1, Kv9.3, large-conductance Ca2+-activated K channel (BKCa), inward-rectified K+ channel (KIR)6.1, and two-pore domain inwardly 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-amino-pyridine, iberiotoxin, pinacidil, and anandamide. Experiments were performed at 20, 7, and 2% oxygen to assess the effect of oxygenation on K channel activity and vessel tone. K channel function in arteries and veins was assessed by RT-PCR, and variable expression of Kv2.1, Kv3.1b, Kv1.5, and BKCa was demonstrated electrophysiologically in VSMCs or endothelial cells from placental allantochorial vessels (24, 25, 28, 51, 54, 59). Most recently, calcitonin gene-related peptide-induced glibenclamide-inhibited vasodilatation of the fetoplacental vasculature has been demonstrated, suggestive of a role for KATP 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.

Unlike in the lung, there are few data on K channels in the fetoplacental vasculature. Kv, KCa, and KATP channel activity have been demonstrated electrophysiologically in VSMCs or endothelial cells from placental allantochorial vessels (24, 25), Kv1.5, Kv2.1, and large-conductance Ca2+-activated potassium channels (BKCa) have been demonstrated by RT-PCR, and variable expression of Kv2.1, Kv3.1b, Kv1.5, and BKCa 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 KATP 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 Kv2.1, Kv9.3, TWIK-related acid-sensitive K channels (TASK)1, BKCa, and KIR6.1 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 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 Hospitat;
the exception to this was the extension times for KV2.1 and BKCa. homology with any other known gene products. The number of cycles

\[ Ta = 59^\circ C (6) \]

BLAST searches were performed to ensure primers had no contamination with genomic DNA. Subsequent RT-PCR, in which the RT was omitted, was performed to ensure that all RNA samples were not contaminated with genomic DNA. Subsequent RT-PCR, using standard techniques with a hot start, was employed using primer pairs previously described in other studies of human K channel expression, and optimized for use in our samples: KV2.1 5'-GCTCTGATGTGAGTACCGACTCCTC-3' (forward) with 5'-GAACTCCGACATGCTGTGAACG-3' (reverse), annealing temperature (Ta) 64°C (56); BVa1.3 5'-GCTCTGATGTGAGTACCGACTCCTC-3' (forward) with 5'-GAACTCCGACATGCTGTGAACG-3' (reverse), Ta 55°C (56); BKCa subunit 5'-CAGACACTGACTGGCAGAGTCCTGG-3' (forward) with 5'-GATCACGTGGTTGAGCACTGGCTCTC-3' (reverse), Ta 55°C (18); TASK1 5'-CTCCTTCTTACTTCCACCATCAT-3' (reverse) with 5'-CATTCCACTTTTCTCATGTAAGGC-3' (forward), Ta 59°C (6). BLAST 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 50 s, annealing at Ta for 60 s, and extension at 72°C for 20 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.

**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 snap freezing in liquid N2. Placental tissue, comprising a small arteries and veins, which traverse the surface of the placenta, was also excised, rinsed in PSS, and snap frozen.

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, in which the RT was omitted, was performed to ensure that all RNA samples were not contaminated with genomic DNA. Subsequent RT-PCR, using standard techniques with a hot start, was employed using primer pairs previously described in other studies of human K channel expression, and optimized for use in our samples: KV2.1 5'-GCTCTGATGTGAGTACCGACTCCTC-3' (forward) with 5'-GAACTCCGACATGCTGTGAACG-3' (reverse), annealing temperature (Ta) 64°C (56); BVa1.3 5'-GCTCTGATGTGAGTACCGACTCCTC-3' (forward) with 5'-GAACTCCGACATGCTGTGAACG-3' (reverse), Ta 55°C (56); BKCa subunit 5'-CAGACACTGACTGGCAGAGTCCTGG-3' (forward) with 5'-GATCACGTGGTTGAGCACTGGCTCTC-3' (reverse), Ta 55°C (18); TASK1 5'-CTCCTTCTTACTTCCACCATCAT-3' (reverse) with 5'-CATTCCACTTTTCTCATGTAAGGC-3' (forward), Ta 59°C (6). BLAST 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 50 s, annealing at Ta for 60 s, and extension at 72°C for 20 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. β-actin was routinely amplified from all samples, confirming sample integrity and amplification capacity.

**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 0.1 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.

**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).

**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 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 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:

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1) K_v channels were inhibited with 4-aminopyridine (4-AP; 1 mM); 2) BK_{Ca} channels were inhibited with iberiotoxin (IBTX; 100 nM); 3) TASK1 channels were inhibited with anandamide (AEA; 20 µM); 4) K_ATP 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 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 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 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 placenta (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) using the gene-specific primers for K_v2.1, K_v9.3, BK_{Ca}, K_{IR}6.1, and TASK1. All of the observed signals comigrated with those amplified in the controls (human brain library) and appeared as single amplicons at the predicted molecular size (compared to 1-kb pair DNA ladder; not shown).

K_v2.1 signals were qualitatively weak and observed in two of five arterial samples, four of five venous samples, and two of five placental samples. Unlike K_v2.1, K_v9.3 gave signals in all samples (5 of 5 artery, veins, and placenta, respectively). BK_{Ca} was detected in all five arterial samples, four of five venous samples (which were qualitatively always weaker than the arterial samples from the same placenta), and three of five placental samples. K_{IR}6.1 was almost identical to K_v9.3, in that strong signals were seen in all but one of five placental samples. TASK-1 signal intensity was weak but detectable in 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-K_v2.1 (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_v2.1, 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_v9.3 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;
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Fig. 2. K⁺ channel protein expression. Membranes were probed with anti-K₂.1 (1:1,000; A), anti-BKCa (1:500; B), anti-K₉.6.1 (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.

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 2% oxygenation (P < 0.05; RM-ANOVA; data not shown). 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 K₅₅₆ 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 at 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 2% 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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<tbody>
<tr>
<td>95</td>
<td>28 (16–45)</td>
<td>2 (1–8)</td>
<td>1 (0–6)</td>
<td>110 (80–135)</td>
<td>68 (50–90)</td>
<td>39/1 (36/0–42/0)</td>
<td>3300 (2410–4520)</td>
</tr>
</tbody>
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Data are medians with range in parenthesis; No. is number of subjects. IBR, individualized birth ratio.

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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 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 alteration in 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.

Kv 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). 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_ATP channel function, as previously suggested in other tissues (29, 61). The K_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_ATP6.1/K_ATP may thus play an important role in the control of fetoplacental vascular tone. Therapeutically, specific K_ATP channel activation may reverse hypercontracture in disease states, such as intrauterine growth restriction (IUGR), where chiorionic plate arteries demonstrate increased agonist-induced contraction (45). In support of this notion, ET1 inhibits K_ATP channels in rabbit (50) and guinea pig (65). ET1 has also been suggested to promote an IUGR phenotype (48). Thus K_ATP channel openers may offer a pharmacological tool to combat such an effect.

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 chiorionic 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 rest 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) 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 Kv or K_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_ATP 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 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_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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