Expression and function of potassium channels in the human placental vasculature.


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Running title:
Expression of placental potassium channels

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Abstract:

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, 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 Kv2.1, Kv9.3, BKCa, Kir6.1 and TASK1 in chorionic plate arteries, veins and placental homogenate was assessed by RT-PCR and Western Blotting. 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 channels were all demonstrated to be expressed at the message level. Kv2.1, BKCa, Kir6.1 and TASK1 were all 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.

Keywords:

Placenta Human Potassium channels artery vein
Introduction.

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).

In the human placenta, it has been proposed that Hypoxic FetoPlacental Vasoconstriction (HFPV) (47, 53) 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 (pO₂) triggered vasoconstriction yet, large diameter (>1mm) arterial / venous constriction was unaltered (12, 30, 33). HFPV may occur via modification of smooth muscle K channel activity (30).

In the lung, the 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 (2, 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, two-pore domain and ATP-sensitive families. (4, 5, 10, 16, 19, 22, 23, 28, 51, 54, 56, 59).
Unlike the lung, there is little data on K channels in the fetoplacental vasculature. $K_V$, $K_{Ca}$ and $K_{ATP}$ channel activity has been demonstrated electrophysiologically in VSMCs or endothelial cells from placental allantochorial vessels (24, 25). $K_V$1.5, $K_V$2.1 and $BK_{Ca}$ have been demonstrated by RT-PCR, and variable expression of $K_V$2.1, $K_V$3.1b, $K_V$1.5 and $BK_{Ca}$ was documented in placental vessel homogenates (30). Most recently, calcitonin gene related peptide (CGRP)-induced glibenclamide-inhibitable vasodilatation of the fetoplacental vasculature has been demonstrated suggestive of a role for $K_{ATP}$ channels (20). Thus, there is limited evidence on the role of K channels in the control of fetoplacental arterial tone and no previous 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 Blotting) for $K_V$2.1, $K_V$9.3, TASK-1, $BK_{Ca}$ and $K_{IR}$6.1 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_{IR}$6.1) 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 Hospitals NHS Trust. Informed written consent was obtained for all tissue used in the study. The investigation conforms to the principles outlined in the Declaration of Helsinki (1).

Samples: Term (37-42 weeks gestation) placentas (N=95) were obtained post-delivery (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 mins of delivery and placed directly into ice-cold physiologic salt solution (PSS; in mM; 119NaCl, 25NaHCO3, 4.69KCl, 2.4MgSO4, 1.6CaCl2, 1.18KH2PO4, 6.05 glucose, 0.034 EDTA; pH 7.4).

RT-PCR: Umbilical arteries and vein were identified at the insertion of the umbilical cord in to 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 prior to dissection using a stereomicroscope. Vessels were cut into short, 2-3 mm lengths, cleaned of blood placed into cryotubes prior to snap freezing in liquid N2. Placental tissue, comprising a section through the chorionic plate and villus tree, 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 Leukema Virus Reverse Transcriptase (RT) according to the manufacturer’s instructions (Invitrogen) in a Perkin-Elmer Cetus thermal cycler (Perkin
Elmer, Beaconsfield, UK). 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 optimised for use in our samples: Kv2.1 5’-GCCTTCACCTCCATCCTCAACT-3’ (forward) with 5’-ACTCATCGAGGCCTGCTAGCTCAG-3’ (reverse), annealing temperature (Ta) 64°C (56); Kv9.3 5’-CCATGATGTGAGTACCGACTCCTC-3’ (forward) with 5’-GAACGACATGCTGTGAACG-3’ (reverse), Ta 55°C (56); BKCa α-subunit 5’-CAGACACTGACTGGCAGAGTCCTGG-3’ (forward) with 5’-GCATCGACCGTTGTACCGGTCAGG-3’ (reverse), Ta 64°C (30); Kir6.1 5’-TTGGCCAGAAAGATATCCCGAG-3’ (forward) with 5’-CATTCCACCTTTTCTCCATGTAAGC-3’ (reverse), 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 60s, annealing at Ta for 60s and extension at 72°C for 60 sec; the exception to this was the extension times for Kv2.1 and BKCa, which were 40 and 90s 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 Blotting: Placental arteries, veins and whole placenta, different to those used for RNA extraction, were collected as described above. Samples were homogenized on ice in homogenisation buffer (in M): 0.01 HEPES, 0.001 EDTA, 0.25 sucrose (pH 7.4) with an anti-protease inhibitor cocktail (104 mM 4-(2-aminoethyl)benzenesulphonyl fluoride (AEBSF), 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 post nuclear supernatant, obtained after a spin at 4,000g 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 (50-100µg as indicated) and rat brain (50-70µg) was mixed with a reducing loading buffer containing 1.25% β-mercaptoethanol (v/v), 2% SDS (w/v), 0.04% bromophenol blue (v/v) and 10% glycerol (v/v), in 0.05 mol l⁻¹ 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 PVDF membranes. The membranes were blocked for 1 hr using blocking buffer (1% dried milk powder (w/v) in 0.05% Tween 20 (v/v), Tris-buffered saline (TBS (in mol l⁻¹): 0.015 Tris, 0.150 NaCl; pH 8.0).

Membranes were probed for 2h at room temperature with either anti-KV2.1 at 1:1000 (Upstate Biotech, Lake Placid, NY, USA), anti-BKCa (Alomone Labs, Jerusalem, Israel) at 1:500, anti-KIR6.1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) 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. KIR6.1 rabbit polyclonal antibody was raised against residues 345-424 (C-terminus) of human KIR6.1. TASK1 rabbit polyclonal antibody was raised against the peptide (C-terminus) corresponding to residues 252-269 of the human TASK1 channel. Three 10min washes in TBS/0.05% Tween 20 were followed by incubation
with a horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody at a 1:2000 dilution (DAKO, Ely, UK) for 1h. After three 10min 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±7µm; n=186) and veins (294±10µm; n=164) were cut into 2-3 mm lengths and mounted onto 40µm steel wires on a M610 wire myograph (Danish Myotech, Aarhus, Denmark), bathed in 6ml of PSS and warmed to 37°C. Vessels were normalised as described previously (62, 64) to 0.9 of L_{5.1kPa} to mimic a physiological resting tension of approximately 25mmHg (39). Post-normalisation, vessels were equilibrated for 20min. Functional studies were performed in vessels normalised and equilibrated in 5% CO₂ in air (termed 20% oxygen) to mimic placental hyperoxia, 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 WPI oxygen meter (WPI Inc., USA; measurement accuracy +/-1%). Following equilibration, concentration-response curves were constructed to the thromboxane mimetic U46619 (0.1-2000nM in 2min increments / 5min plateau (62, 64)). Placental vessel viability was assessed using 120mM KCl in PSS (equimolar substitution of KCl for NaCl). Vessels greater than 500µm in diameter were excluded from the study.

**Role of K channels in the 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 un-stimulated chorionic plate arteries and veins as follows:
• Voltage-gated (K\textsubscript{V}) channels were inhibited with 4-aminopyridine (4-AP; 1mM)

• Large conductance calcium activated K channels (BK\textsubscript{Ca}) were inhibited with iberiotoxin (IBTX; 100nM) TWIK-related acid-sensitive K channels (TASK1) were inhibited with anandamide (AEA; 20\textmu M)

• ATP sensitive K channel (K\textsubscript{ATP}) were opened with pinacidil (PIN; 50\textmu M).

Basal tone was assessed pre- and 5min post-addition of the pharmacological agent.

**Role of K channels in the control of placental chorionic plate arterial and venous constriction and relaxation:**

Following incubation of arteries and veins with K channel modulators for 5min, vessels were constricted with U46619 (0.1-2000nM). To assess the vasodilator effect of pinacidil, arteries were constricted with an EC\textsubscript{80} dose of U46619. Once a stable constriction was achieved, relaxation was assessed with incremental doses of pinacidil (0.01-100\textmu M). Time control vessels were performed in parallel (constricted with EC\textsubscript{80} dose of U46619 only).

**General chemicals:** General chemicals and pharmacological agents were obtained from Sigma-Aldrich (Poole, Dorset, UK) or BDH (Poole, Dorset, UK). U46619 was obtained from Calbiochem (CN Biosciences (UK) Ltd., Nottingham, UK).

**Statistical analysis:** Vessel tension production was calculated as follows. To standardise 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\textsubscript{i} in kPa), was calculated by dividing ΔT by the normalised internal radius (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 using Wilcoxon-signed rank (WSR) test. Relaxation was calculated as a percentage of the contraction achieved with EC$_{80}$ dose of U46619. Concentration-response curves for contraction and relaxation were compared by repeated measures (RM-) ANOVA. Bonferroni post hoc test was used to assess statistical significance at individual concentrations of agonist. Data are expressed as mean ± standard error of the mean (SE) with $n$ vessels from $N$ placentas. $P<0.05$ was taken to indicate statistical significance.
Results:

K channel gene expression.

Figure 1 shows two representative examples of PCR products obtained from matched arterial, venous and placental RNA using the gene-specific primers for Kv2.1, Kv9.3, BKCa, Kir6.1 and TASK1. All the observed signals co-migrated with those amplified in the positive controls (human brain library) and appeared as single amplicons at the predicted molecular size (compared to 1kb pair DNA ladder; not shown).

Kv2.1 signals were qualitatively weak and observed in 2/5 arterial samples, 4/5 venous samples and 2/5 placental samples. Unlike Kv2.1, Kv9.3 gave signals in all samples (5/5 artery, veins and placenta). BKCa was detected in all 5 arterial samples, 4/5 venous samples (which were qualitatively always weaker than the arterial samples from the same placenta) and 3/5 placental samples. Kir6.1 was almost identical to Kv9.3, in that strong signals were seen in all but 1/5 placental samples. TASK-1 signal intensity was weak but detectable in 4/5 arterial and 3/5 venous samples, and readily detected in 4/5 placental samples.

K channel protein expression.

Using anti-Kv2.1 (representative blot of a minimum of 3 different placentas; Figure 2A) we detected bands of approximately 115kD in both placental arteries and veins. Control tissue, rat brain, also gave a single band of 115kD. When arterial and venous vessel homogenates and rat brain were probed with anti-BKCa antibody, a strong signal was observed at 125kD (Figure 2B). Smaller less intense bands were observed at similar sizes in all three tissues. Signals of approximately 51 kD in rat brain and 55 kD in arterial and venous samples were observed using the antibody raised to Kir6.1 (Figure 2C). Anti-TASK1 gave rise to a signal of approximately 122kD (Figure 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
(Figure 2A-C). This manoeuvre was not performed for anti-Kir6.1 as a competing peptide
was not commercially available. However, primary antibody omission resulted in loss of
signal (Figure 2D). Similar negative control experiments were also performed for all the other
antibodies, the results demonstrated a loss of signal (not shown). Expression of Kv9.3 in
placental tissues was not assessed due to the lack of a commercially available antibody.

**Functional assessment of K channels in fetoplacental arteries and veins.**

1. **General vessel characteristics:**

   We utilized 95 normal term placentas (Table 1). Baseline active effective pressure
   maintained by chorionic plate arteries was 2.71±0.13kPa (20.3±1.0mmHg; n = 186) and
   2.75±0.15kPa (20.7±1.1mmHg; 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)
   normalisation at the 3 different levels of oxygenation did not significantly affect baseline
   active effective pressure (data not shown).

2. **Pharmacological investigations:**

   **Voltage-gated (Kv) channels:** 1mM 4-AP significantly increased basal tone in unstimulated
   chorionic plate arteries and veins at 7% oxygenation (P<0.05; WSR-test; Figure 3A,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 U46619 concentration-response curve in arteries and veins at 7%
oxygenation (P<0.05; RM-ANOVA; Figure 3C,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 arteries and veins at 2% and 20% oxygenation (data not
shown).
Large conductance calcium-activated (BKCa) channels: 100nM 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 U46619 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 U46619 concentration-response relationship at 2% or 20% oxygenation (P>0.05; RM-ANOVA; Figure 4A,C). However, at 7% oxygenation maximal contraction with U46619 increased (P<0.05; RM-ANOVA & WSR-test) but EC50 was unaffected (P>0.05; WSR test; Figure 4B) by IBTX.

ATP-sensitive (KATP) channels: 50µM pinacidil significantly decreased basal tone in unstimulated chorionic plate arteries and veins at 2% oxygenation (P<0.05; WSR-test; Figure 5A,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 pre-contracted (EC80 dose of U46619) at 2% oxygenation (P<0.05; RM-ANOVA; Figure 5C,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 U46619 in 2% oxygenation (P<0.05; RM-ANOVA; Figure 5E, 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). U46619-induced arterial and venous contraction were unaffected by pinacidil at 7% oxygenation (P>0.05; RM-ANOVA; data not shown).

**TWIK-related acid-sensitive (TASK1) channels:** In 20% oxygenation, 20µM AEA significantly increased basal tone unstimulated in chorionic plate arteries and veins (P<0.05; WSR-test; Figure 6 A, 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 (P>0.05; WSR-test; data not shown).

AEA significantly modified the U46619 concentration-response relationship in arteries and veins at 20% oxygenation (P<0.05; RM-ANOVA; Figure 6C, D). In arteries, AEA significantly increased maximal contraction (P<0.05; WSR-test) but EC₅₀ was unaffected (P>0.05; WSR test; Figure 6C). In veins, AEA significantly increased contraction at 1µM U46619 (P<0.05; WSR-test) but EC₅₀ was unaffected (P>0.05; WSR test; Figure 6E). Similar results were observed in arteries at 2% oxygenation. AEA significantly modified U46619-induced contraction (P<0.05; RM-ANOVA; Figure 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 U46619 (P>0.05; RM-ANOVA; data not shown). At 7% oxygenation AEA did not affect the U46619 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 level. We also demonstrated using wire myography, that pharmacological manipulation of these channels with known modulators leads to altered vascular function.

**K<sub>V</sub> channels:** K<sub>V</sub>9.3 is an electrically silent K channel that modifies the activity of other channels, including K<sub>V</sub>2.1, when co-expressed as heteromeric channels (56). Furthermore, K<sub>V</sub>9.3 / K<sub>V</sub>2.1 and K<sub>V</sub>1.2 / K<sub>V</sub>1.5 heteromeric channels are hypoxia inhibitable and may be an important HPV initiators in mouse pulmonary VSMCs (35). In this study we have assessed the expression of pore-forming α-subunits only. K<sub>V</sub> 1.1 and 1.2 β-subunits have been suggested to have a role in oxygen sensing in pulmonary artery VSMC’s (67), however as the K<sub>V</sub> 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 K<sub>V</sub>2.1 and K<sub>V</sub>9.3 α-subunits. This confirms (30) and extends previous studies to now include novel data regarding channel expression and function in placental veins for not only K<sub>V</sub> channels but other channel subtypes (see below).

Western blotting indicated K<sub>V</sub>2.1 protein expression in placental arteries and veins, at a size comparable to that seen previously (4, 7, 58). Smaller bands were observed with anti-K<sub>V</sub>2.1, however these bands remained after addition of the antigenic peptide suggesting that they are a result of non-specific binding of the primary antibody. K<sub>V</sub>9.3 protein expression was not determined due to a lack of commercially available antibody.

Our and previous expression data (30) suggests the presence of fetoplacental vascular K<sub>V</sub> channels. Increased basal tone with 4-AP indicates K<sub>V</sub>2.1 and K<sub>V</sub>9.3, as well as other
members of the \( K_v \) channel family, are open at rest, adapting \( E_m \), and are therefore important determinants of fetoplacental vascular activity. The upward-shift in the U46619 concentration-response curve, without altered agonist sensitivity, further implies an effect of altered baseline channel activity rather than a mechanistic change in U46619-induced contraction of the smooth muscle.

With 4-AP, parallel upward shifts in the U46619 concentration-response curves at all oxygenations without modification in agonist sensitivity, implies a \( K_v \) channel independent effect. Furthermore vasoconstriction at 2% and 7% oxygenation were comparable indicative of a non-HPV-like response, which may also explain the lack of effect oxygenation on the 4-AP-induced alterations in vascular function. A role for \( K_v \) channels in hypoxic fetoplacental responses requires detailed studies using oxygen tensions below 1% in pressurised vessels in the presence of luminal flow.

**\( K_{Ca} \) channels:** \( BK_{Ca} \) \( \alpha \)-subunit mRNA was strongly detected in arteries with qualitatively weaker signals in matched venous and placental homogenates. Western blotting detected \( BK_{Ca} \) protein at a similar size (~125kD) 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 U46619-induced contraction without altering agonist sensitivity. Upon vasoconstriction, raised intracellular \( Ca^{2+} \) opens \( BK_{Ca} \) channels induces smooth muscle cell membrane hyperpolarisation which results in a reduction in \( Ca^{2+} \) 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 U46619-induced contraction in modified oxygenation. At low oxygenation, animal pulmonary VSMCs data suggests BK$_{Ca}$ inhibition (16, 68). Why IBTX is ineffective at increased oxygenation is unclear but may partly result from actions of reactive oxygen species which 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 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 which requires further functional study.

**K$_{IR6.1}$ channels:** K$_{IR6.1}$ mRNA was readily detectable in all tissues. Western blotting yielded a signals at ~55 kD in vessels, which compares favourably to that seen here in the control tissue (51 kD, rat brain) (41) and 44kD 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 U46619 pre-contracted 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 $K_{\text{ATP}}$ channels (63). Here, the effects of pinacidil were independent of oxygenation. U46619-induced contractions were attenuated by pinacidil but only in hyper- and hypoxia. The lack of oxygenation effect was unexpected. One would expect decreased oxygenation to inhibit ATP production. This would promote $K_{\text{ATP}}$ 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 down-regulation of ATP production. Alternatively, hypoxia may also affect levels of vasodilators such as prostacyclin, which has previously been demonstrated to alter $K_{\text{ATP}}$ channel activity (37), or influence relaxation by other non-ATP channel mechanisms. Furthermore, plasmalemmal $K_{\text{ATP}}$ channels of VSMCs may also have different sensitivities to K channel openers compared to mitochondrial or endothelial cell subtypes (8, 23). Consequently it is less surprising that pinacidil-induced relaxation was oxygen-independent.

U46619-induced contraction was unaltered 7% oxygenation but 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 $K_{\text{ATP}}$ channel function, as previously suggested in other tissues (29, 61). $K_{\text{ATP}}$ channels opening would be expected to blunt U46619-induced contraction, as seen at 20 and 2% oxygenation but at 7% oxygenation, pinacidil alone may be insufficient to produce such an effect.

$K_{\text{IR6.1}}/K_{\text{ATP}}$ may thus play an important role in the control of fetoplacental vascular tone. Therapeutically, specific $K_{\text{ATP}}$ channel activation may reverse hypercontraction 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 $K_{\text{ATP}}$ channels in rabbit (50) and guinea pig (65). ET1 has also been suggested to promote an IUGR phenotype (48). Thus $K_{\text{ATP}}$ channel openers may offer a pharmacological tool to combat such an effect.

**TASK1:** TASK1 mRNA was present in vessels but 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 to other channels, at a size consistent with our previous study in placental trophoblast (6).

We attempted to address if 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 $E_m$ as a background current. AEA’s small affect on U46619-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 affect of AEA is not via TASK1 inhibition. The similarity of the U46619 concentration-response curve with AEA to that with 4-AP, coupled with the observation that AEA can inhibit the activity of $K_V1.2$ (52) and $K_V1.5$ (32), may explain this alteration in arterial contractility. However, AEA did not cause a similar affect 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 U46619-induced constriction or mediating contractile responses at oxygen tensions prevalent in situ.
This is an important observation as 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 >50µM (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 to 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 (Figure 3). However modified oxygenation which is also apparent in IUGR, did not alter 1) affects of 4-AP on basal tone 2) U46619-induced contraction or 3) vessel sensitivity to U46619. Hypersensitivity to U46619(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 ischaemia-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.

**Summary**

We demonstrated the presence of a number of K channels in chorionic plate arteries and veins using RT-PCR and Western blotting. Furthermore pharmacological manipulation 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 characterisation of vascular responses using pressure myography in the presence and absence of the endothelium.
Acknowledgements:

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References:


Figure legends:

**Figure 1. K⁺ channel gene expression.** Two representative examples of PCR products amplified from chorionic arterial (PA), venous (PV) samples and whole placenta (PL). PA1, PV1 and PL1, and PA2, PV2 and PL2 are matched samples from individual term placentas. –ve: dH2O negative control. +ve: human brain cDNA positive control, cDNA integrity confirmed with β-actin.

**Figure 2. K⁺ channel protein expression.** Membranes were probed with (A) anti-Kv2.1 (1:1000), (B) anti-BKCa (1:500), (C) anti-KIR6.1 (1:500) or (D) anti-TASK1 (1:100). Primary antibody exposed to paired samples from a minimum of 3 term placentas. Exposure of primary antibody to its antigenic peptide or its omission resulted in signal ablation. Molecular weight standards (in kD) as indicated. RB-rat brain, PA- artery, PV-vein.

**Figure 3. Functional responses to 4-AP.** All data in 7% oxygenation.

Effect of 4-AP on basal tone in arteries (A) and veins (B). Key: pre-4-AP (solid bar); 5 mins post-4-AP (1mM; hatched bar); All data mean +/- SE; P = Wilcoxon signed rank test.

Effect of 4-AP on U46619-induced contraction in arteries (C) and veins (D). Key: P = Repeated measures ANOVA; *P<0.05 Bonferroni post hoc test; n vessels from N placentas.

**Figure 4. Functional responses to iberiotoxin.** All data from arteries exposed to U46619 (0.1-2000nM) in 20% (A), 7% (B) and 2% (C) oxygenation. Key: All data mean +/- SE, N placentas; P = Repeated measures ANOVA.

**Figure 5. Functional responses to pinacidil.** All data in 2% oxygenation.
Effect of pinacidil on basal tone in arteries (A) and veins (B). Key: pre-pinacidil (solid bar); 5min post-pinacidil (50µM; hatched bar); All data mean +/- SE; P = Wilcoxon signed rank test.

Relaxation of U46619 pre-contracted arteries (C) and veins (D). N placentas; P = Repeated measures ANOVA; * P<0.05 Bonferroni post hoc test.

Effect of pinacidil on U46619-induced contraction in arteries (E) and veins (F). Key: N placentas; P = Repeated measures ANOVA; * P<0.05 Bonferroni post hoc test.

Figure 6. Functional responses to anandamide.

Effect of 20µM anandamide on basal tone in arteries (A) and veins (B) at 20% oxygenation. Key: pre-anandamide (solid bar); 5min post-anandamide (20µM; hatched bar); All data mean +/- SE; P = Wilcoxon signed rank test; n vessels.

Effect of anandamide on U46619-induced contraction in arteries (C, D) and veins (E). Tissue exposed to 20% oxygenation (C, E) or 2% oxygenation (D). Key: P = Repeated measures ANOVA; * P<0.05 Bonferroni post hoc test; n vessels from N placentas.
Table 1. Subject details.

<table>
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<th>N</th>
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<th>Gestation</th>
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<td>mmHg</td>
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<td>3300</td>
<td>53</td>
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<td></td>
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<td>(80-135)</td>
<td>(50-90)</td>
<td>(36w 0d –42w 0d)</td>
<td>(2410-4520)</td>
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All data are medians with range in parenthesis (IBR; individualised birth ratio).
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- **Kv2.1**
- **Kv9.3**
- **BK$_{Ca}$**
- **K$_{IR}$6.1**
- **TASK1**
- **β-actin**
A) Anti-K_{\text{v}2.1}  
- 115 kD
- 95 kD
- 54 kD

B) Anti-BK_{Ca}  
- 125 kD
- 82 kD
- 40 kD

C) Anti-K_{\text{ir}6.1}  
- 51 kD
- 36 kD
- 29 kD

D) Anti-TASK1  
- 115 kD
- 95 kD
- 54 kD

+epitope
Active effective pressure (kPa)

Control (n=14, N=5)
Post 4-AP (n=14, N=5)

P<0.05

Passive tension (kPa)

Control (n=17, N=6)
Post 4-AP (n=17, N=6)

P<0.05

Active effective pressure (kPa)

Control (n=14, N=5)
Post 4-AP (n=14, N=5)

P<0.05

Passive tension (kPa)

Control (n=17, N=6)
Post 4-AP (n=17, N=6)

P<0.05

Active effective pressure (kPa)

Control (n=14, N=5)
Post 4-AP (n=14, N=5)

P<0.05

Passive tension (kPa)

Control (n=17, N=6)
Post 4-AP (n=17, N=6)

P<0.05
Pre Post
0 1 2 3 4 5 P<0.05
(14) (5)
Passive tension (kPa)

Pre Post
0 1 2 3 4 5 P<0.05
(12) (4)
Passive tension (kPa)

Control (n=14, N=5) Post AEA (n=14, N=5)

Active effective pressure (kPa)
P<0.05

Control (n=12, N=4) Post AEA (n=12, N=4)

Active effective pressure (kPa)
P<0.05

A; artery 20%
B; artery 2%
C; artery 20%
D; artery 2%
E; vein 20%

A
B
C
D
E