Large Conductance Ca^{2+} -Activated K^{+} Channels Modulate Uterine α_1 -Adrenergic Sensitivity in Ovine Pregnancy

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Abstract

The uteroplacental vasculature is refractory to α -adrenergic stimulation, and large conductance Ca^{2+} -activated K^{+} channels (BK_{Ca}) may contribute. We examined the effects of uterine artery (UA) BK_{Ca} inhibition with tetraethylammonium (TEA) on hemodynamic responses to phenylephrine (PE) at 101 to 117 days and 135 to 147 days of ovine gestation, obtaining dose responses for mean arterial pressure (MAP), heart rate (HR), and uteroplacental blood flow (UPBF) and vascular resistance (UPVR) before and during UA TEA infusions. The UA α_1 -adrenergic receptors (α_1 -ARs) were assessed. The PE increased MAP and UPVR and decreased HR and UPBF dose dependently at both gestations ($P < .001$, analysis of variance). The $\%\Delta\text{MAP}$ was less at 135 to 147 days before and during TEA infusions ($P \leq .008$); however, responses during TEA were greater ($P \leq .002$). The PE increased $\%\Delta\text{UPVR} \gg \%\Delta\text{MAP}$, thus $\%\Delta\text{UPBF}$ fell. The TEA enhanced PE-mediated increases in $\%\Delta\text{UPVR}$ at 135 to 147 days ($P \leq .03$). The UA α_1 -AR expression was unchanged in pregnancy. Uterine vascular responses to PE exceed systemic vascular responses throughout pregnancy and are attenuated by BK_{Ca} activation, suggesting BK_{Ca} protect UPBF.

Keywords

vascular sensitivity, pressor responses, α_1 -adrenergic receptors, uteroplacental blood flow

Introduction

Pregnancy is associated with numerous cardiovascular changes directed toward ensuring fetal growth and well-being and maternal adaptation, for example, cardiac output increases $>50\%$ and is redistributed in order to ensure the growth of the uteroplacental vascular bed and thus uterine oxygen and nutrient delivery while maintaining increases in maternal organ blood flows.¹⁻³ Pregnant women and other species also develop attenuated pressor responses to infused vasoconstrictors, including α -adrenergic agents and angiotensin II (ANG-II).⁴⁻⁷ This appears to protect against the elevated circulating levels of catecholamines and ANG-II and increased sympathetic outflow associated with normal pregnancy.^{3,8-10} The mechanisms responsible for the attenuated vascular sensitivity remain unclear but may include altered endothelial and/or vascular smooth muscle (VSM) function, receptor expression, prostaglandin or nitric oxide (NO) synthesis, or changes in receptor signaling. Notably, this refractoriness is absent in women with preeclampsia or those destined to develop gestational hypertension, resulting in enhanced pressor responses.^{7,11,12}

The uterine vascular bed in intact pregnant sheep is also refractory to α -adrenergic agents and ANG-II in pregnancy.^{13,14} However, it is more sensitive to α -adrenergic stimulation and refractory to ANG-II compared to the systemic vasculature.^{13,15-20} The attenuated uterine responses to ANG-

II are explained by predominant expression of type 2 ANG-II receptors (AT_2Rs) in uterine VSM versus type 1 (AT_1Rs) in peripheral VSM.²⁰⁻²³ The explanation for the increased uteroplacental sensitivity to α -adrenergic agents is less clear but may include differences in α -adrenergic receptors, increases in VSM Ca^{2+} sensitivity and contractile proteins, and/or remodeling.²⁴⁻²⁸ However, the mechanisms that contribute to the generalized uterine refractoriness to these agonists in pregnancy are unknown.

Potassium channels regulate vascular reactivity in numerous vascular beds.^{20,29} Large conductance Ca^{2+} -activated K^{+} channels (BK_{Ca}) are expressed in VSM, can modulate vasoconstrictor and vasodilator responses, and this function varies in different vascular beds.²⁹⁻³² The BK_{Ca} consist of 4 α -subunits derived from a single gene product that form a homotetramer and undergo post-translational modification, resulting in varied function.^{30,33} They

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are associated with up to 4 regulatory β -subunits derived from separate genes, which vary in the β : α stoichiometry and channel function.^{30,34-37} The β_1 -subunit predominates in uterine artery (UA) VSM, and its C-terminal end senses increases in intracellular Ca^{2+} , resulting in BK_{Ca} activation, closure of voltage-gated Ca^{2+} channels, decreases in intracellular Ca^{2+} , and repolarization of cell membranes in order to maintain vascular tone.^{30,34,36,37} Notably, UA β_1 -subunit expression increases in the last third of ovine pregnancy,³³ increasing β_1 : α subunit which may modify UA Ca^{2+} sensitivity,³¹ contributing to the attenuated uterine vasoconstrictor responses seen in normotensive pregnancy.

In the present studies, we determined whether BK_{Ca} contribute to attenuated uteroplacental sensitivity to α_1 -adrenergic stimulation in pregnant sheep in the last third of gestation and whether α_1 -adrenergic receptor (α_1 -AR) expression is altered within the uterine vascular bed or differs from the systemic vasculature. We also examined simultaneous pressor responses to systemic phenylephrine (PE) infusions before and during UA infusions of tetraethylammonium (TEA), an inhibitor of BK_{Ca} function.^{30,38}

Methods

Animal Model

Time-dated pregnant Western sheep ($n = 8$; 6 singletons and 2 twins) were used in these studies. The surgical procedures have been reported^{6,39} and are briefly described. Animals delivered to UT Southwestern Medical Center were allowed 1 week to recover. At 95 to 104 days of gestation (term ~ 150 days), a percutaneous jugular venous catheter was placed for infusion of preoperative sedatives and intraoperative fluids, and the animals were fasted overnight. The following morning they received intramuscular (im) atropine (0.088 mg/kg), intravenous (iv) sodium pentobarbital and ketamine hydrochloride, were intubated, and the abdomen prepared for sterile surgery. During surgery, the animals received inhalation anesthesia with 1.5% isoflurane (Mallinckroft Veterinary Inc, Mundelein, Illinois) and oxygen via a rebreathing anesthesia machine. The gravid uterus was isolated through a midline abdominal incision, and electromagnetic flow probes (6.0 or 7.0 mm internal diameter; Carolina Medical, King, North Carolina) were placed on the main UA proximal to the first bifurcation in both uterine horns. Polyvinyl catheters containing heparinized saline (250 Units/mL) were inserted retrograde into a distal branch of each UA for intra-arterial infusions of tetraethylammonium chloride (TEA). The abdominal incision was closed as previously described.^{6,39} Catheters were placed in the femoral artery to monitor mean arterial pressure (MAP) and heart rate (HR) and femoral vein to infuse systemic drugs. Catheters and flow probe leads were brought to the flank via a subcutaneous tunnel, exited via a puncture wound in the skin, and stored in a canvas pouch attached to the skin with sterile stainless steel pins. Animals received 10% dextrose in 0.9% saline during surgery and postoperatively, as well as penicillin (600 000 units iv), gentamycin (150 mg im), and flunixin meglumine (100 mg iv) for 2 days after surgery. Catheters were flushed daily with heparinized saline (250 Units/mL) to maintain

patency. Studies were terminated with iv phenobarbital, 200 mg/kg. The studies described were approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center.

Experimental Protocol

Animals were maintained in large holding pens within the laboratory. Studies were not performed until 5 days postoperative and scheduled to start at 104 days ($n = 7$) and 136 days ($n = 7$) of gestation, requiring 10 days to complete each time period. In all, 6 ewes were studied at both gestations, while 1 animal in each age group was studied at only 1 gestational age. This allowed us to determine whether age-dependent changes occurred in vascular responsiveness to PE and/or the BK_{Ca} channel inhibitor TEA (Sigma, St Louis, Missouri). On the day of study, baseline MAP, HR, and uteroplacental blood flow (UPBF) were recorded for 1 hour, after which a control cumulative dose-response curve for iv PE was generated at each gestational age using 1.29, 2.58, 12.9, 55.7 and 129 $\mu\text{g}/\text{min}$ (Figure 1; Neosynephrine; Winthrop Laboratories, New York, New York). Each PE dose was infused 12 minutes to obtain steady-state responses, which occurred by 8 to 12 minutes, and was used for analyses. After completing the control PE dose responses, animals were monitored until all hemodynamic parameters returned to baseline and remained stable. At this time, TEA, a relatively selective BK_{Ca} inhibitor at concentrations <1.0 mmol/L,^{30,38} was continuously infused for 72 minutes via a UA catheter in the gravid horn to achieve the calculated concentrations of 0.005, 0.01, 0.02, 0.035, or 0.04 mmol/L. These concentrations are less than previously used^{32,39} and were chosen to minimize the effects on basal UPBF while providing partial BK_{Ca} blockade (Figure 1). These TEA levels are more specific for BK_{Ca} inhibition and are less likely to block most voltage-gated K^+ channels (K_V).^{29,30} Rates of TEA infusion were determined using the following equation and the measurement of UPBF in the infused uterine horn: Rate infusion ($\mu\text{g}/\text{min}$) = TEA level desired ($\mu\text{g}/\text{mL}$) $\times$ UPBF (mL/min). A cumulative PE dose response was repeated during the continuous TEA infusion. At completion, animals were monitored until hemodynamic parameters returned to baseline; 48 hours were allowed between doses of TEA. No more than 2 TEA doses were studied each week in the same order to prevent any cumulative effects on BK_{Ca} function. Measurements of MAP and HR were monitored via femoral arterial catheters using transducers (Gould, Houston, Texas); UPBF was monitored with flow meters (Carolina Medical Instruments Inc, East Bend, North Carolina). Hemodynamic parameters were collected and stored using "Pohnema" Data Acquisition software (Data Sciences International, Valley View, Ohio).

Immunoblot Analysis

The α_1 -AR is the predominant adrenergic receptor in UA VSM⁴⁰⁻⁴²; but it is not known whether changes in UA sensitivity to α -adrenergic agonists in pregnancy or differences in uterine and systemic responses reflect alterations in receptor expression. We, therefore, collected proximal third- and fifth-generation

Figure 1. Representative recording of the effects of a unilateral uterine arterial infusion of TEA (0.04 mmol/L) on mean arterial pressure, heart rate, and left and right uterine blood flow in a term pregnant ewe before and during the cumulative PE dose response. Responses shown are at 10 minutes of PE infusion $\pm$ 2 minutes. TEA indicates tetraethylammonium; PE, phenylephrine.

Table 1. Baseline Hemodynamic Measurements in Mid- and Late-Gestation Sheep.

	MAP, mm Hg	HR, bpm	UPBF _{TX} , mL/min	UPBF _C , mL/min	UPVR _{TX} , mm Hg·min/mL	UPVR _C , mm Hg·min/mL
Early (n = 7)	80 $\pm$ 2.8	89 $\pm$ 5.9	611 $\pm$ 78	340 $\pm$ 49	0.15 $\pm$ 0.02	0.24 $\pm$ 0.03
Late (n = 7)	77 $\pm$ 5.4	94 $\pm$ 3.9	780 $\pm$ 95	476 $\pm$ 71	0.11 $\pm$ 0.01 ^a	0.20 $\pm$ 0.05

Abbreviations: TX, TEA-treated uterine horn; C, control uterine horn.
^a $P = .047$.

UA from 6 nonpregnant ewes and third-generation (n = 6), pre-cotyledonary or placental (n = 6) and mesenteric (n = 5) arteries from 6 near-term pregnant sheep. We examined precotyledonary arteries since placental blood flow accounts for >80% of total UPBF at term.¹⁻³ Arteries were homogenized and 20 μ g of soluble protein were loaded on 10% polyacrylamide gels, subjected to electrophoresis, and transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Billerica, Maryland). Immunoblots were blocked in buffer containing powdered milk (5% wt/vol) and incubated overnight at 4°C with antiserum against α_1 -ARs (1:500; ab54730; Abcam Inc, Cambridge, Massachusetts). Membranes were incubated with anti-mouse immunoglobulin G (IgG) at 1:1000. Regions containing receptor proteins were visualized by enhanced chemiluminescence. Densitometry was performed, and values expressed as arbitrary units. The loading protein was α -smooth muscle actin.

Statistical Analyses

Data were analyzed with SAS V9.01 using 3-way repeated measures analysis of variance (ANOVA) to examine interactions between PE dose, TEA, and gestational group. To determine differences in PE responses between treatment and within

age groups at specific PE doses, we used 2-way ANOVA and all pairwise multiple comparison procedures using the Holm-Sidak method. Responses were examined to determine TEA dose effects, PE dose effects, differences between studies at 101 to 117 days and 135 to 147 days of gestation, and differences in simultaneous responses in MAP, HR, UPBF, and UPVR. Responses in the infused and noninfused uterine horns were compared. Uteroplacental vascular resistance (UPVR) was calculated using MAP (mm Hg) $\div$ UBF (mL/min) during steady-state responses. Student independent samples *t* test was used where noted. Data are presented as means $\pm$ 1 standard error of the mean (SEM).

Results

Baseline Hemodynamic Data

Baseline hemodynamic variables did not differ before infusions of PE or different doses of TEA at either gestational age ($P > 0.1$); thus, baseline values were averaged for each animal and used to calculate baseline mean hemodynamic parameters at each gestational age. Although MAP and UPVR in each uterine horn decreased between study periods while HR and UPBF increased

Figure 2. Comparison of PE-induced increases in mean arterial pressure in pregnant sheep at 101 to 117 days (Δ , $\blacktriangle$) and 135 to 147 days ($\circ$, $\bullet$) gestation in the absence (A, open symbols) and presence (B, solid symbols) of uterine artery TEA infusion. Data are the percentage change, analyzed by 2-way ANOVA and are shown as means $\pm$ SEM. The PE responses are dose dependent ($P < .001$, ANOVA), modified by gestational age ($P \leq .008$, ANOVA), and increased in the presence of TEA. TEA indicates tetraethylammonium; PE, phenylephrine; SEM, standard error of mean; ANOVA, analysis of variance.

(Table 1), only the fall in TEA-treated uterine horn (UVR_{Tx}), that is, the gravid horn, achieved statistical significance. Similarly, total UPBF rose 28% from 943 ± 105 to 1207 ± 114 ($P = .1$), while the total UPVR fell 32% from 0.09 ± 0.01 to 0.06 ± 0.01 ($P < .001$); however, the rise in UPBF varied between animals, explaining the inability to detect a significant rise.

Blood Pressure Responses to PE

Since each hemodynamic parameter changed during the study period (Table 1), we used the relative responses or percentage change ($\% \Delta$) from baseline to compare time periods and assess the responses to PE and TEA + PE. This also permits a comparison of changes in vascular sensitivity to PE in the absence and presence of UA BK_{Ca} blockade by each hemodynamic variable, which have different units of measurement.^{13,18} The PE alone increased MAP dose dependently at both gestational ages (Figure 2A; $P < .0001$, ANOVA); but responses were significantly less at 135 to 147 days ($P \leq .05$, 2-way ANOVA), reflecting an interaction between gestational age and PE dose ($P \leq .008$). The HR fell dose dependently ($P < .0001$, ANOVA), decreasing $\sim 30\%$ at the highest PE dose (data not shown); but unlike MAP, there was no gestational age effect, $P > .1$.

Basal MAP, HR, and UPBF in both uterine horns were unchanged during the initial 10 minutes of the unilateral UA infusion of TEA (Figure 1). During the TEA infusions, PE dose dependently increased MAP at both gestational ages and the relative rise in MAP remained less at 135 to 147 days (Figure 2B; $P \leq .004$, 2-way ANOVA). The HR also decreased dose dependently ($P = .05$, data not shown). Notably, PE-mediated increases in $\% \Delta$ MAP were greater during UA TEA infusions at both gestational ages ($P \leq .002$, 2-way ANOVA), especially with the highest PE doses, which were always studied last. This suggests TEA may have entered and accumulated in the systemic circulation during the 72-minute infusion. This

was also associated with a greater fall in HR ($P = .05$, data not shown), likely reflecting an enhanced baroreponse due to the increased pressor response. There was no TEA dose effect on PE-induced responses in MAP or HR at 101 to 117 days ($P > .1$, ANOVA); however, a modest dose effect occurred at term, $P = .05$, suggesting increased sensitivity.

Uterine Vascular Responses

The best way to assess uterine responses to systemic infusions of vasoactive agents is to compare the relative changes ($\% \Delta$) in UPVR, MAP, and UPBF, which takes into account simultaneous changes in perfusion pressure and vascular resistance.^{13,18} The PE alone increased UPVR and decreased UPBF dose dependently at both gestational ages (Figures 3A and 4A; $P < .0001$, ANOVA); however, the relative rise in UPVR was modestly less at 135 to 147 days, $P = .08$, while the fall in $\% \Delta$ UPBF did not differ, $P > .1$. Notably, the rise in $\% \Delta$ UPVR exceeded the rise in $\% \Delta$ MAP ($P \leq .003$) at both gestational ages, thus $\% \Delta$ UPBF fell, demonstrating greater uterine versus systemic vascular sensitivity to PE throughout pregnancy (Figures 3A and 4A). The fall in $\% \Delta$ UPBF was not proportional to increases in $\% \Delta$ UPVR due to the simultaneous rise in perfusion pressure, that is, $\% \Delta$ MAP.

At 101 to 117 days, there was no TEA dose effect. However, PE in the presence of UA TEA increased $\% \Delta$ UPVR dose dependently (Figure 3B; $P < .0001$, ANOVA), which exceeded the simultaneous rise in $\% \Delta$ MAP ($P < .01$) and $\% \Delta$ UPBF fell dose dependently. Notably, the uterine responses to PE were greater in the presence of UA TEA, thus the fall in $\% \Delta$ UPBF increased ($P = .018$, 2-way ANOVA). Responses to PE in the untreated or contralateral uterine horn were not significantly affected ($P > .1$, ANOVA).

At 135 to 147 days, TEA had a modest dose effect on uterine responses to PE (data not shown, $P = .03$). In the presence of UA

Figure 3. Comparison of the simultaneous relative changes in uteroplacental vascular resistance (UPVR, ○), mean arterial pressure (MAP, Δ), and uteroplacental blood flow (UPBF, □) during systemic PE infusions in the absence (A) and presence (B) of UA infusions of TEA at 101 to 117 days of gestation. Data were analyzed by 2-way ANOVA and are shown as means $\pm$ SEM. The PE increased UPVR and MAP and decreased UPBF dose dependently without and with TEA ($P < .0001$, ANOVA), and $\% \Delta$ UPVR exceeded $\% \Delta$ MAP at the doses noted ($*P \leq .008$, $**P \leq .01$; Holm-Sidak method). TEA indicates tetraethylammonium; PE, phenylephrine; SEM, standard error of mean; ANOVA, analysis of variance; UA, uterine artery.

Figure 4. Comparison of the simultaneous relative changes in uteroplacental vascular resistance (UPVR, ●), mean arterial pressure (MAP, ▲), and uteroplacental blood flow (UPBF, ■) during systemic PE infusions in the absence (A) and presence (B) of UA infusions of TEA at 135 to 147 days of gestation. Data were analyzed by 2-way ANOVA and are shown as means $\pm$ SEM. The PE increased UPVR and MAP and decreased UPBF dose dependently without and with TEA ($P < .0001$, ANOVA), and $\% \Delta$ UPVR exceeded $\% \Delta$ MAP at the doses noted ($*P < .001$; $**P \leq .002$; Holm-Sidak method). TEA indicates tetraethylammonium; PE, phenylephrine; SEM, standard error of mean; ANOVA, analysis of variance; UA, uterine artery.

TEA, PE continued to dose dependently affect uterine hemodynamic parameters (Figure 4B; $P < .0001$, ANOVA), and the relative rise in UPVR exceeded simultaneous increases in $\% \Delta$ MAP ($P < .002$), thus $\% \Delta$ UPBF fell. Importantly, UA TEA was associated with greater PE-mediated increases in $\% \Delta$ UPVR and decreases in $\% \Delta$ UPBF at the highest doses of PE ($P \leq .03$, 2-way ANOVA); that is, $\% \Delta$ UPVR increased 2-fold greater with TEA + PE at 128.9 μ g/min. Additionally, the rise in $\% \Delta$ UPVR during UA BK_{Ca} blockade was $\sim$ 2-fold greater at 135 to 147 days versus 101 to 117 days of gestation, that is, 501% versus 250%, respectively. The PE-mediated responses in the contralateral uterine horn were unaffected by TEA infusions.

Adrenergic Receptors

There was no difference in α_1 -AR expression in proximal third-generation UA VSM from nonpregnant and term pregnant ewes

(Figure 5; $P > .1$) or in UA and mesenteric arteries from the pregnant sheep ($P > .1$; data not shown). Since $>80\%$ of total UPBF represents placental blood flow at term pregnancy,¹⁻³ we also compared third-generation UA with precotyledonary or placental artery in pregnant ewes. There also was no difference within the uterine vascular bed (Figure 6; $P > .1$). Finally, α_1 -AR expression was not different between precotyledonary arteries in pregnant sheep and fifth-generation UA from nonpregnant ewes ($P > .1$; data not shown).

Discussion

Pregnancy is associated with the development of refractoriness to several pressor agents,⁴⁻⁷ and this is absent in women with and destined to develop gestational hypertension or preeclampsia.^{7,11,12} The uteroplacental vascular bed also becomes refractory to α -adrenergic agonists, and this also is absent in pregnant

Figure 5. Immunoblot analysis of α_1 -adrenergic receptor expression in proximal third generation uterine artery smooth muscle from nonpregnant and term pregnant ewes. α -smooth muscle actin was the loading protein. $P = .5$, independent samples t test.

Figure 6. Immunoblot analysis of α_1 -adrenergic receptor expression in proximal third-generation uterine and cotyledon/placental arterial smooth muscle from term pregnant ewes. α -smooth muscle actin was the loading protein. $P = .4$, independent samples t test.

women with hypertension.^{13,15-18} Thus, the mechanisms modulating vascular sensitivity within the uterine and peripheral vascular beds may be similar, and understanding them might provide clues to the pathophysiology of gestational hypertension and related complications, for example, decreases in UPBF and fetal growth restriction. We determined whether VSM BK_{Ca}, which contribute to uterine vasodilation in pregnancy^{32,39} but are Ca²⁺ sensitive, also modulate uterine vascular responses to α_1 -adrenergic stimulation. The uteroplacental vascular responses to PE were greater than systemic during the last third of ovine pregnancy. However, uterine responses were

attenuated at term compared to the earlier stage of gestation, and importantly, this was not due to changes in UA α_1 -AR expression. Notably, PE-mediated increases in % Δ UPVR were enhanced during UA TEA infusions and greater at 135 to 147 days versus 101 to 117 days of gestation. Pressor responses to PE were also attenuated at term and were greater at both gestational ages during the continuous UA infusions of TEA. Thus, BK_{Ca} modulate α_1 -adrenergic sensitivity in the uteroplacental circulation and may also contribute to the attenuated systemic pressor responses in pregnancy. Thus, abnormal BK_{Ca} function or expression may contribute to the increased vascular

sensitivity to vasoconstrictors seen in pregnant women with hypertension.^{8,9}

The UPBF rises ~30-fold in ovine pregnancy, optimizing fetal nutrient and oxygen delivery and the maintenance of fetal growth and well-being.¹⁻³ This is paralleled by maternal systemic vasodilation and compensatory increases in the renin-angiotensin and sympathetic systems.^{2,8-10} Thus, maintenance of UPBF requires a balance between vasoconstrictors and vasodilators. The uterine vasculature is always refractory to ANG-II in women and sheep due to AT₂R predominance in UA VSM throughout reproduction.^{18,20-22,43} In contrast, it is sensitive to α -adrenergic agonists and this increases in pregnancy^{20,23,24,27,28,44}; thus, infusion of α -adrenergic agents increases % Δ UPVR more than simultaneous increases in % Δ MAP and % Δ UPBF falls.^{13,19,45} This was seen at both gestational ages and was not due to differences in α_1 -AR expression in UA VSM during pregnancy. Annibale et al^{24,25} reported that this reflected UA remodeling in pregnant sheep with VSM hypertrophy and increased VSM actin-myosin contents and myosin light chain phosphorylation. This has been confirmed in several species and women^{20,23,26,27} and is associated with increased VSM Ca²⁺ sensitivity.²⁸

The attenuated UA responses could reflect downregulation of α_1 -AR, which is predominant in UA VSM from several species.⁴⁰⁻⁴² However, no one has compared nonpregnant and pregnant animals. Farley et al⁴⁰ observed increased α_1 -AR binding in nonpregnant porcine UA versus mesenteric, which might explain in part the differences in uterine and systemic responses. However, pregnant UA were not examined. We²³ recently reported that UA α_1 -AR expression did not differ in nonpregnant and pregnant women. In the present study, α_1 -AR expression in proximal UA VSM was also unchanged during ovine pregnancy. Furthermore, α_1 -AR expression did not differ in proximal third-generation and preplacental UA, which account for >85% of total UPBF at term.³ This parallels the enhanced α_1 -adrenergic responses seen throughout the ovine uteroplacental vascular bed.²⁰ Additionally, there was no difference in UA and mesenteric artery α_1 -AR expression, as noted earlier, or between nonpregnant fifth and pregnant preplacental VSM. Thus, α_1 -AR expression is unchanged within the uterine vascular bed throughout reproduction. The enhanced UA sensitivity to adrenergic agonists could explain the flight or fight physiology that protects the mother by redirecting ~25% of cardiac output from the uterus to essential organs and tissues in pregnancy.

Although UA responses to PE increase during pregnancy,^{24,25} the uterine vasculature as a whole is refractory, protecting uterine oxygen and nutrient delivery.¹³ This was observed to increase in the last third of ovine gestation. Although UA synthesis of prostacyclin and/or nitric oxide may contribute, this is unclear.^{44,46-48} Alternatively, UA K⁺ channels may be involved. BK_{Ca} are expressed in human and ovine UA VSM and contribute to NO-induced relaxation, maintenance of basal UPBF and UA tone, estrogen-mediated increases in UPBF during pregnancy, and importantly, modulation of PE-induced contractions.^{32,38,39,49,50} The β_1 -regulatory subunit senses increases in intracellular Ca²⁺,

predominates in UA VSM, and β_1 : α -subunit stoichiometry increases in the last third of ovine pregnancy.^{31,33,38} Thus, α_1 -AR-induced increases in intracellular Ca²⁺ would enhance BK_{Ca} open state, close voltage-gated Ca²⁺ channels, and attenuate vasoconstriction via a negative-feedback mechanism.^{30,34,36,37} We infused TEA doses at 1 order of magnitude less than prior studies to prevent a fall in basal UPBF while obtaining partial BK_{Ca} inhibition.^{32,39} Importantly, these doses also are less likely to inhibit K_V within the uterine circulation.^{29,30} This is the first in vivo evidence that BK_{Ca} regulate UA sensitivity to α_1 -AR stimulation and this increases in late gestation, paralleling increases in β_1 : α stoichiometry and thus, enhanced sensitivity to changes in intracellular Ca²⁺.^{35,36} BK_{Ca}, therefore, modulate basal UPBF via membrane hyperpolarization and vasodilation via nitric oxide (NO)-cyclic guanosine monophosphate and estrogens and attenuate UA responses to increased circulating levels of catecholamines and increased sympathetic outflow in normal pregnancy via a negative-feedback mechanism. Abnormal UA BK_{Ca} expression and/or function may result in the loss of refractoriness to α -agonists and a fall in basal UPBF in hypertensive women.^{8,9} This is supported by studies showing increased channel activity in ovine UA in pregnancy.³⁸

Pregnant women are also refractory to pressor agents, and those destined to develop gestational hypertension or preeclampsia have increased sensitivity to infused α -adrenergic agents and ANG-II.^{7,11,12} Defining the mechanisms involved would aid in delineating the pathophysiology of these hypertensive disorders and their consequences. Pregnant sheep also are refractory to these agents,^{6,10,13} which was confirmed in the present study, paralleling changes in women.¹¹ Although vascular NO and/or prostaglandin synthesis may contribute to the attenuated pressor responses, this remains unclear.^{44,46,48,51} Animal models of hypertension in pregnancy may not recapitulate preeclampsia, which is unique to women. An alternative would be to define the mechanisms that regulate the development of refractoriness in pregnancy.

As noted, BK_{Ca} regulate basal tone and sensitivity to vasoconstrictors.²⁹⁻³¹ When activated by increased intracellular Ca²⁺ and voltage, they attenuate VSM contractions by repolarizing VSM membranes, closing voltage-gated Ca²⁺ channels, and decreasing intracellular Ca²⁺.^{29,30,34,37} Decreases in either VSM α - or β_1 -regulatory subunits are associated with enhanced Ca²⁺ sensitivity, contractility, and hypertension.⁵²⁻⁵⁵ The role of BK_{Ca} in blood pressure regulation in pregnancy is unknown. Although the doses of intra-arterial TEA did not affect baseline MAP, pressor responses to systemic PE infusions were enhanced at the 2 highest PE doses infused. Thus, TEA appears to have entered the systemic circulation and altered peripheral VSM BK_{Ca} function. This was unanticipated and may reflect TEA accumulation in the systemic circulation due to a low TEA clearance rate during the 72-minute infusion. Unfortunately, we were unable to measure TEA levels. This, however, is consistent with the enhanced responses to PE observed in human UA rings after incubation with TEA.⁵⁰ Alternatively, TEA may have evoked a central nervous system response; but this is unlikely since changes in MAP and HR were not seen

with the higher doses previously infused into pregnant ewes.^{34,39} It is also possible that the BK_{Ca} sensitivity to TEA inhibition is greater than previously considered.³² Finally, it is unlikely this is due to TEA-induced release of a placental factor since the shorter infusions at higher concentrations had no effect on MAP, HR, or contralateral UPBF.^{34,39} Thus, BK_{Ca} may contribute to the regulation of pressor responses to α -adrenergic agents and maintenance of blood pressure in pregnancy. Future studies will need to characterize peripheral artery BK_{Ca} expression, activation, and function in pregnancy.

We have shown that uterine refractoriness to α_1 -adrenergic agonists occurs throughout ovine pregnancy and the uteroplacental vasculature is more sensitive to these agents than the peripheral vasculature at all times. We report for the first time that BK_{Ca} contribute to the attenuated uterine responses to α_1 -adrenergic agents seen in ovine pregnancy, suggesting a role in regulating UPBF in normal pregnancy. Surprisingly, pressor responses to PE were augmented during TEA infusions. It is unclear whether the attenuated pressor responses reflect changes in α -subunit density, β : α -subunit stoichiometry, and/or other mechanisms that modify BK_{Ca} activity. It also is unclear whether other TEA-sensitive K⁺ channels, for example, voltage-gated K_V, are involved.⁵⁶ Nonetheless, changes in α_1 -AR expression do not explain changes in UA sensitivity in pregnancy or difference between the uterine and systemic vasculature. Future studies should clarify the role of BK_{Ca} in blood pressure regulation and determine whether they contribute to increases in vascular sensitivity in the presence of hypertensive diseases, an area not yet explored.

Declaration of Conflicting Interests

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