Pregnancy Upregulates Large-Conductance Ca\(^{2+}\)-Activated K\(^{+}\) Channel Activity and Attenuates Myogenic Tone in Uterine Arteries

Xiang-Qun Hu, Daliao Xiao, Ronghui Zhu, Xiaohui Huang, Shumei Yang, Sean Wilson, Lubo Zhang

Abstract—Uterine vascular tone significantly decreases whereas uterine blood flow dramatically increases during pregnancy. However, the complete molecular mechanisms remain elusive. We hypothesized that increased Ca\(^{2+}\)-activated K\(^{+}\) (BK\(_{Ca}\)) channel activity contributes to the decreased myogenic tone of uterine arteries in pregnancy. Resistance-sized uterine arteries were isolated from nonpregnant and near-term pregnant sheep. Electrophysiological studies revealed a greater whole-cell K\(^{+}\) current density in pregnant compared with nonpregnant uterine arteries. Tetraethylammonium and iberiotoxin inhibited K\(^{+}\) currents to the same extent in uterine arterial myocytes. The BK\(_{Ca}\) channel current density was significantly increased in pregnant uterine arteries. In accordance, tetraethylammonium significantly increased pressure-induced myogenic tone in pregnant uterine arteries and abolished the difference in myogenic responses between pregnant and nonpregnant uterine arteries. Activation of protein kinase C produced a similar effect to tetraethylammonium by inhibiting BK\(_{Ca}\) channel activity and increasing myogenic tone in pregnant uterine arteries. Chronic treatment of nonpregnant uterine arteries with physiologically relevant concentrations of 17\(\beta\)-estradiol and progesterone caused a significant increase in the BK\(_{Ca}\) channel current density. Western blot analyses demonstrated a significant increase of the \(\beta1\), but not \(\alpha\), subunit of BK\(_{Ca}\) channels in pregnant uterine arteries. In accordance, steroid treatment of nonpregnant uterine arteries resulted in an upregulation of the \(\beta1\), but not \(\alpha\), subunit expression. The results indicate that the steroid hormone-mediated upregulation of the \(\beta1\) subunit and BK\(_{Ca}\) channel activity may play a key role in attenuating myogenic tone of the uterine artery in pregnancy. (Hypertension. 2011; 58:00-00.) ● Online Data Supplement

Key Words: uterine artery ■ pregnancy ■ BK\(_{Ca}\) channel ■ myogenic tone ■ steroids ■ protein kinase C

Uterine blood flow increases substantially during pregnancy, which is essential both for the growth and survival of the fetus and for cardiovascular well being of the mother. Maladaptation of the uteroplacental circulation during pregnancy is associated with high incidence of clinical complications, including preeclampsia and fetal intrauterine growth restriction. Although the mechanisms underlying the adaptation of uterine circulation to pregnancy are complex and poorly understood, recent studies have demonstrated that pressure-dependent myogenic reactivity plays a pivotal physiological role in the regulation of uterine circulation and that decreased uterine arterial myogenic tone contributes significantly to the adaptation of uterine vascular hemodynamics in pregnancy.1,2

The molecular mechanisms underlying this attenuated myogenic tone of uterine arteries in pregnancy remain elusive. Previous studies have suggested a possible role of large-conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK\(_{Ca}\)) channels in the regulation of uterine circulation during pregnancy.3–5 The BK\(_{Ca}\) channel in vascular smooth muscle contains pore-forming-\(\alpha\) subunit (encoded by KCNMA1) and \(\leq\)4 accessory identical \(\beta1\) subunits (encoded by KCNMB1).6 The \(\beta1\) subunit is predominantly expressed in smooth muscle7–9 and enhances the Ca\(^{2+}\) sensitivity of BK\(_{Ca}\) channels.10 It has been shown that BK\(_{Ca}\) channels play an important role in regulating the resting membrane potential of vascular smooth muscle, and channel blockade by iberiotoxin (IBTX) or tetraethylammonium (TEA) depolarizes the membrane resulting in vasoconstriction.11 The importance of BK\(_{Ca}\) channel in the regulation of vascular smooth muscle function was further demonstrated with the gene deletion of BK\(_{Ca}\) channel, and \(\alpha\) or \(\beta1\) subunit knockout mice displayed increased vascular tone and elevated blood pressure,8,12 although uterine vascular function was not examined.

Little is known about the functional role of BK\(_{Ca}\) channels in regulating pressure-dependent myogenic tone of uterine arteries and its adaptation to pregnancy. In the present study, we tested the hypothesis that upregulation of BK\(_{Ca}\) channel expression and activities accounts for attenuated myogenic tone of uterine arteries in pregnancy. This was achieved by investigating the myogenic reactivity and BK\(_{Ca}\) channel
current density and protein expression in uterine arteries obtained from nonpregnant and near-term pregnant sheep using functional, electrophysiological, and biochemical approaches. Given our recent findings that sex steroid hormones played an important role in downregulating pressure-dependent myogenic tone of the uterine artery in pregnancy,2 we further test the hypothesis that sex steroid hormones upregulate BKCa channel expression and activities in uterine arteries.

Materials and Methods
An expanded Materials and Methods section is available in the online Data Supplement at http://hyper.ahajournals.org.

Tissue Preparation and Treatment
Resistance-sized uterine arteries were isolated from nonpregnant and near-term pregnant sheep.2,13,14 For hormonal treatment, arteries were incubated in phenol red-free DMEM with 1% charcoal-stripped FBS for 48 hours at 37°C in a humidified incubator with 5% CO₂/95% air in the absence or presence of 17β-hydroxyprogesterone and/or progesterone. All of the procedures and protocols were approved by the Institutional Animal Care and Use Committee and followed the guidelines by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of Myogenic Tone
Pressure-dependent myogenic tones of resistance-sized uterine arteries were measured as described previously.2,13,14

Measurement of BKca Channel Current
Smooth muscle cells were enzymatically dissociated from resistance-sized uterine arteries, and whole-cell K⁺ currents were recorded and normalized to cell capacitance as picoampere per picofarad (pA/pF).15,16 Only relaxed and spindle-shaped myocytes were used for recording. The BKca channel current was determined as the difference between the whole-cell K⁺ current in the absence and presence of IBTX or TEA.

Western Immunoblotting Analysis
Protein abundance of BKca channel α and β1 subunits was measured in freshly isolated resistance-sized, endothelium-intact uterine arteries and after the hormonal treatment by Western blot analysis, as described previously.2,13,14

Data Analysis
Results were expressed as mean ± SEM obtained from the number of experimental animals given. Differences were evaluated for statistical significance (P<0.05) by ANOVA or t test, where appropriate.

Results
Pregnancy Upregulates BKca Channel Function in Uterine Arteries
The whole-cell K⁺ current density in uterine arterial myocytes of pregnant sheep (66.3±5.2 pA/pF) was significantly greater than that in nonpregnant animals (37.5±3.3 pA/pF) at +80 mV (P<0.05; Figure 1). TEA at concentrations ≤1.0 mmol/L selectively block BKca channels with negligible effects on voltage-gated K⁺ channels.17–19 Exposure of the myocytes to TEA (1.0 mmol/L) or IBTX (100.0 nmol/L) significantly depressed the macroscopic K⁺ currents (Figure 1), indicating the functional presence of BKca channels in uterine arteries from both nonpregnant and pregnant sheep. As shown in Figure 1, TEA and IBTX inhibited the K⁺ currents to the same extent in uterine arterial myocytes. The TEA- and IBTX-sensitive components of K⁺ currents in the myocytes at +80 mV were 38.5±2.5% and 40.0±3.1% (P>0.05) in nonpregnant sheep and 53.2±1.8% and

Figure 1. Effect of tetraethylammonium (TEA) and iberiotoxin (IBTX) on K⁺ currents in uterine arteries. Smooth muscle cells were freshly isolated from nonpregnant (A) and pregnant (B) uterine arteries, and whole-cell K⁺ currents were recorded in the absence or presence of TEA (1.0 mmol/L) or IBTX (100.0 nmol/L). Data are mean±SEM of 4 to 12 cells from 4 to 7 animals of each group. *P<0.05 vs control.
55.7±2.7% (P>0.05) in pregnant animals, respectively. The finding that TEA and IBTX inhibited the K⁺ currents to the same extent in uterine arterial myocytes validated the specificity of TEA action on the BKCa channel. The BKCa current density determined by TEA (35.1±2.9 pA/pF in pregnant myocytes versus 14.1±1.3 pA/pF in nonpregnant myocytes; P<0.05) or by IBTX (32.5±2.1 pA/pF in pregnant myocytes versus 16.8±1.9 pA/pF in nonpregnant myocytes; P<0.05) at +80 mV was significantly increased in pregnant uterine arteries (Figure 2).

**Inhibition of BKCa Channels Increases Pressure-Dependent Myogenic Tone in Uterine Arteries**

To determine the functional importance of heightened BKCa channel activity in regulating uterine arterial vascular tone, pressure-dependent myogenic responses of resistance-sized uterine arteries were measured in the absence or presence of the BKCa channel inhibitor TEA. Consistent with the previous studies,¹ pressure-dependent myogenic tone in uterine arteries of pregnant sheep was significantly lower than that in uterine arteries of nonpregnant sheep over the physiological range of intravascular pressures (Figure 3). TEA significantly increased the myogenic tone in uterine arteries of pregnant sheep (Figure 3). In uterine arteries of nonpregnant animals, TEA showed no significant effect on the myogenic reactivity when the data were analyzed as groups (Figure 3). However, paired analysis of data in each animal revealed that TEA appeared to increase the myogenic tone in 1 of 5 animals (animal No. 5; Figure 3). Consisting with this finding, it appeared that the uterine arteries from animal No. 5 had higher expression level of the β1 subunit of BKCa channels (Figure 3). In the presence of TEA, there were no significant differences in pressure-dependent myogenic tone of uterine arteries between nonpregnant and pregnant animals.

**Activation of Protein Kinase C Inhibits BKCa Channels and Increases Myogenic Tone in Uterine Arteries**

As shown in Figure 4, the activation of protein kinase C (PKC) by phorbol 12,13-dibutyrate (PDBu) significantly inhibited whole-cell K⁺ currents in uterine arterial myocytes. The inhibition of K⁺ currents at +80 mV by PDBu was significantly greater in the myocytes of pregnant sheep (34.0±2.6%) than that in nonpregnant animals (20.9±4.0%; Figure 4; P<0.05). However, there were no significant differences among TEA-, PDBu-, and PDBu plus TEA-produced inhibitions of K⁺ currents in either nonpregnant or pregnant uterine arterial myocytes, respectively (Figure 4), indicating that the PDBu-induced reduction of K⁺ currents is predominantly mediated by inhibiting the BKCa channel. In accordance, PDBu significantly increased pressure-dependent myogenic tone in uterine arteries of pregnant sheep (Figure 5) to the similar extent as that seen with TEA in Figure 3.

**Steroid Hormones Increase the BKCa Channel Activity in Uterine Arteries**

Previous studies demonstrated that 17β-estradiol and progesterone treatment for 48 hours resulted in a significant decrease in myogenic responses in nonpregnant uterine arteries.² The effect of steroid hormones on the BKCa channel activity was, thus, determined by the treatment of nonpregnant uterine arteries with 17β-estradiol (0.3 nmol/L) and progesterone (100.0 nmol/L) for 48 hours. As shown in

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**Figure 2.** Effect of pregnancy on Ca²⁺-activated K⁺ (BKCa) current density in uterine arteries. Smooth muscle cells were freshly isolated from nonpregnant (NPUA) and pregnant (PUA) uterine arteries. BKCa current density was determined in the presence of tetraethylammonium (TEA; A) or iberiotoxin (IBTX; B). Data are mean±SEM of 4 to 12 cells from 4 to 7 animals of each group. *P<0.05 vs NPUA.
Figure 6, the steroid hormone treatment significantly increased macroscopic whole-cell $K^+$ currents in uterine arterial myocytes. Compared with the control myocytes, the current density was increased by $2\times$ at $80\,\text{mV}$ in hormone-treated myocytes ($41.5\pm5.8$ versus $24.0\pm3.4\,\text{pA/pF}$; $P<0.05$). Whole-cell $K^+$ currents of myocytes isolated from both control and steroid hormone-treated uterine arteries were subject to the TEA inhibition. However, TEA produced a significantly greater inhibition of $K^+$ currents in hormone-treated myocytes (58.6$\pm$1.7%) than that in the control (41.3$\pm$3.5%; $P<0.05$). In accordance, the steroid hormone treatment resulted in a $2.5\times$ increase in the current density of BK$_{Ca}$ channels ($24.5\pm3.7$ versus $10.4\pm2.4\,\text{pA/pF}$; $P<0.05$; Figure 6). To determine the specific effect of

**Figure 3.** Effect of tetraethylammonium (TEA) on myogenic tone in uterine arteries. Pressure-dependent myogenic tone of nonpregnant (NPUA) and pregnant (PUA) uterine arteries was determined in the absence or presence of TEA (1.0 mmol/L for 20 minutes) in each animal in a pairwise fashion. Protein abundance of Ca$^{2+}$-activated $K^+$ (BK$_{Ca}$) channel $\beta$1 subunit was determined by Western blot analysis. $^*P<0.05$, presence of TEA vs absence of TEA.

**Figure 4.** Effect of phorbol 12,13-dibutyrate (PDBu) on $K^+$ currents in uterine arteries. Smooth muscle cells were freshly isolated from nonpregnant (A) and pregnant (B) uterine arteries, and whole-cell $K^+$ currents were recorded in the absence or presence of tetraethylammonium (TEA; 1.0 mmol/L), PDBu (1.0 $\mu$mol/L), or PDBu plus TEA, respectively. Bar graphs present the effects of TEA, PDBu, or PDBu plus TEA on $K^+$ current densities obtained at $80\,\text{mV}$. Data are mean$\pm$SEM of 5 cells from 5 animals of each group. $^*P<0.05$ vs control.
17β-estradiol and progesterone, nonpregnant uterine arteries were treated with 17β-estradiol (0.3 nmol/L) or progesterone (100.0 nmol/L), respectively, for 48 hours. As shown in Figure 7, 17β-estradiol, but not progesterone, produced the similar effect seen with the combined steroids and significantly increased the BKCa channel current density by 2-fold in uterine arterial myocytes.

Pregnancy and Hormonal Treatment Increase BKCa Channel β1 Subunit Expression in Uterine Arteries

As shown in Figure 8, protein abundance of the β1 subunit detected at ~100 kDa was not significantly different between nonpregnant and pregnant uterine arteries. The β1 subunit was detected at 39 kDa, as seen in the previous findings.5,20

Figure 5. Effect of phorbol 12,13-dibutyrate (PDBu) on myogenic tone in pregnant uterine arteries. Pressure-dependent myogenic tone of pregnant uterine arteries was measured in the absence or presence of PDBu (100.0 nmol/L for 15 minutes). Data are mean±SEM of tissues from 5 to 6 animals of each group. *P<0.05 vs -PDBu.

Figure 6. Effect of steroid hormone treatment on Ca2+-activated K+ (BKCa) channel activity in nonpregnant uterine arteries. Nonpregnant uterine arteries were treated with 17β-estradiol (E2β; 0.3 nmol/L) plus progesterone (P4; 100.0 nmol/L) for 48 hours. Myocytes were then isolated and BKCa current densities were determined at +80 mV in the presence of tetraethylammonium (TEA). Data are mean±SEM of 7 cells from 4 animals of each group. *P<0.05 vs control.

Figure 7. Specific effect of 17β-estradiol and progesterone on Ca2+-activated K+ (BKCa) channel activity in nonpregnant uterine arteries. Nonpregnant uterine arteries were treated with 17β-estradiol (E2β; 0.3 nmol/L) or progesterone (P4; 100.0 nmol/L) separately for 48 hours. Myocytes were then isolated and BKCa current densities were determined at +80 mV in the presence of tetraethylammonium (TEA). Data are mean±SEM of 4 cells from 4 animals of each group. *P<0.05 vs control.

Figure 8. Effect of hormonal treatment on BKCa channel β1 subunit expression in pregnant uterine arteries.

A, K+ currents in control myocytes. *P<0.05, −TEA vs +TEA. B, K+ currents in hormone-treated myocytes. *P<0.05, −TEA vs +TEA. C, BKCa current densities in control and hormone-treated myocytes. *P<0.05, +TEA vs control.
and its expression levels were significantly greater in pregnant uterine arteries than that in nonpregnant uterine arteries. In accordance, 17β-estradiol (0.3 nmol/L) and progesterone (100.0 nmol/L) treatment for 48 hours significantly upregulated β1, but not α, subunit expression in nonpregnant uterine arteries (Figure 8).

Discussion

The present study provides direct evidence for the first time that the BKCa channel activity in uterine arteries is significantly enhanced during pregnancy. Previous studies demonstrated that intra-arterial infusion of TEA into the uterine artery circulation of late-gestation sheep caused a decrease of basal uterine blood flow from 50% to 80% in the absence of systemic effects or a change in contralateral uterine blood flow.3,4 This is consistent with the present finding that TEA inhibited K+ currents by 53% in pregnant uterine arteries. The findings that TEA significantly increased pressure-dependent myogenic tone in uterine arteries of pregnant sheep and that it abrogated the difference in the myogenic response between nonpregnant and pregnant uterine arteries indicate that the pregnancy-induced attenuation in uterine arterial myogenic tone is conferred primarily by enhanced BKCa channel activity. BKCa channels have been shown to play an important role in the regulation of resting membrane potential and control of vascular tone.21 These findings support the notion that the heightened BKCa channel activity is a predominant mechanism in maintaining uteroplacental blood flow in pregnancy. The finding that the β1 subunit was higher in the nonpregnant animal that also showed the effect of TEA is intriguing and suggests that the animal was in the follicular phase, as shown by Khan et al.20 This finding further supports the notion that elevated estrogen levels increase the β1/α stoichiometry and BKCa channel activity,20,22 which contributes to the downregulation of myogenic tone of uterine arteries. In agreement, previous studies in ovariectomized nonpregnant sheep demonstrated that TEA had no significant effects on basal uterine vascular resistance and blood flow, but it inhibited estrogen-induced rise in uterine blood flow.23 These findings suggest that the regulation of myogenic tone of uterine arteries by BKCa channels is modulated by sex steroids.

The question arises as to how pregnancy might affect BKCa channel function and its regulation of uterine arterial myogenic tone. Previous studies have demonstrated that PKC plays an important role in the regulation of pressure-dependent myogenic response of resistance arteries,1,2,24,25 and a decrease in the PKC signaling pathway accounts for the attenuated myogenic tone of the uterine artery in pregnancy.1,2,13,14,26–30 The BKCa channel is subject to modulation by PKC, and activation of PKC has been shown to inhibit BKCa channels in vascular smooth muscle cells.31–34 The present finding that activation of PKC inhibited BKCa channel activity and increased pressure-dependent myogenic tone in pregnant uterine arteries provides a functional link of the BKCa channel in pregnancy-mediated downregulation of PKC and myogenic tone of uterine arteries. The work suggests a mechanism of attenuated PKC in the heightened BKCa channel activity in pregnant uterine arteries. Although ANOVA and post hoc analysis indicated no significant differences between PDBu- and PDBu- plus TEA-produced inhibition of the total K+ currents in uterine arterial myocytes, it appeared that the combination might produce a slightly greater inhibition than the PDBu alone, which would suggest that PDBu might not completely inhibit BKCa channels under the current conditions studied.

Consistent with the previous finding of a direct genomic effect of sex steroid hormones in attenuating the PKC activity and myogenic tone of the uterine artery,2,13,14 the present study demonstrated that the enhanced BKCa channel activity seen in pregnant uterine arteries was mimicked by the treatment of nonpregnant uterine arteries with 17β-estradiol and progesterone for 48 hours in an ex vivo tissue culture system. The acute and nongenomic effects of estrogen in activating BKCa channels have been reported previously at high nanomolar-to-micromolar concentrations that are substantially greater than physiological concentrations.23,35–37 To our knowledge, the present study is the first to demonstrate that physiologically relevant concentrations of 17β-estradiol and progesterone have direct genomic effects on upregulating
the BK<sub>Ca</sub> channel activity in vascular smooth muscle cells. Although both estrogen and progesterone receptors have been identified in uterine artery vascular smooth muscle, the present study demonstrated that 17β-estradiol alone was sufficient to increase the BK<sub>Ca</sub> channel activity in uterine arterial myocytes. The conditions of the ovarian cycle of luteal or follicular phases and systemic blood steroid levels were not determined in nonpregnant animals in the present study, and, thus, we cannot exclude the possibility that previous steroid exposure in vivo may have primed the uterine arteries to respond to estrogen. In agreement with the present study, similar findings were obtained in a neuronal cell line GT1-7 in which 17β-estradiol at physiological concentrations augmented BK<sub>Ca</sub> channel currents in a genomic manner.

The BK<sub>Ca</sub> channel in vascular smooth muscle consists of a pore-forming α subunit and ≤4 accessory β subunits. In ovine uterine arteries, both α and β1 subunits were detected exclusively in vascular smooth muscle cells with no evidence of their existence in the endothelium. In the present study, we found that the α subunit was not significantly different in uterine arteries between nonpregnant and pregnant sheep, suggesting no changes in the BK<sub>Ca</sub> channel density by pregnancy. This is somewhat different from the previous finding showing an ∼60% increase in protein abundance of the α subunit in pregnant uterine arteries, although the mRNA was not significantly changed. One possible reason for this apparent difference may be because the previous study used larger diameter first- and second-generation uterine arteries, whereas the present study used resistance-sized uterine arteries. In agreement with the present finding, it has been shown in sheep that increased endogenous estrogen in the follicular phase or infusion of exogenous estrogen in ovariectomized nonpregnant ewes has no significant effect on α subunit levels in uterine arteries. The present finding of an increased β1 subunit in pregnant uterine arteries as compared with nonpregnant uterine arteries is consistent with the previous study in sheep. Although the β2 subunit was present in uterine artery smooth muscle, the expression was minimal and unchanged by pregnancy. Unlike the effect of pregnancy, both β1 and β2 subunits were found elevated in the follicular as compared with the luteal phase of the ovarian cycle in nonpregnant sheep, probably because of relatively high estrogen and low progesterone that was produced endogenously by the ovaries. Given that pregnancy is a state with substantially higher levels of estrogen and progesterone as compared with the nonpregnant state, it is possible that high progesterone inhibits the expression of the β2 subunit. Similar findings were obtained in the ovariectomized estrogen-treated sheep showing an increase in the β1 subunit, although the β2 subunit was not examined.

The increase in β1 subunit expression of the BK<sub>Ca</sub> channel would have functional consequences. It has been shown that β1 subunits of BK<sub>Ca</sub> channels play a pivotal role in regulating vascular tone and blood pressure. The association of β1 subunits with α subunits is critical in regulating the Ca<sup>2+</sup> sensitivity of BK<sub>Ca</sub> channels. Downregulation of the β1 subunit and altered α:β1 stoichiometry decrease the Ca<sup>2+</sup> sensitivity of the channel, resulting in decreased BK<sub>Ca</sub> channel activity. In contrast, upregulation of the β1 subunit increases the Ca<sup>2+</sup> sensitivity of the BK<sub>Ca</sub> channel and channel activity in vascular smooth muscle. In the present study, the increased β1 subunit abundance of the BK<sub>Ca</sub> channel and facilitate the activation of the channel and the consequent reduction in myogenic tone of the uterine arteries in pregnancy. The previous findings of the increased β1 subunit mRNA and protein levels in uterine arteries and myometrial smooth muscle after prolonged exposure of ovariectomized sheep or mice to estrogen suggested a possible role of the steroid hormone in modulating BK<sub>Ca</sub> channel expression. The present study provides clear evidence of the direct effect of steroid hormones on upregulating the β1 subunit in the uterine artery. Although the molecular mechanisms remain elusive, the estrogen responsive elements have been identified at the promoter of the ovine β1 subunit gene.

**Perspectives**

The present study suggests a possible mechanism of 17β-estradiol–mediated upregulation of the β1 subunit in the heightened BK<sub>Ca</sub> channel activity in uterine arteries during pregnancy, resulting in the reduced myogenic tone of uterine artery in pregnancy. Given that the BK<sub>Ca</sub> channel plays a pivotal role in regulating vascular tone and, thus, blood flow and pressure, dysregulation of the stoichiometric composition of BK<sub>Ca</sub> channels and the channel activity in the uterine artery is likely to contribute significantly to the maladaptation of uterine vascular hemodynamics in pregnancies complicated by preeclampsia. Indeed, reductions in uteroplacental blood flow and chronic uteroplacental ischemia in a variety of animal models lead to a hypertension state that closely resembles preeclampsia in women. The present findings provide an understanding of the mechanisms of the BK<sub>Ca</sub> channel in uterine vascular adaptation to pregnancy and may suggest new insights of therapeutic strategies by enhancing the BK<sub>Ca</sub> channel activity in vascular smooth muscle that may be beneficial for pregnant women with preeclampsia. In addition, the present study offers insights into the mechanisms in the hormonal regulation of the BK<sub>Ca</sub> channel activity and myogenic tone of resistance arteries in general and improves our understanding of vascular benefits of hormone replacement therapy in postmenopausal women, given the well-established finding that premenopausal women are at lower risk of developing hypertension and coronary heart disease than men of the same age and that the cardiovascular risk increases only after the cessation of ovarian function.

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**Disclosures**

None.

**References**


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By

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Materials and Methods

Tissue Preparation and Treatment
Uterine arteries were harvested from nonpregnant and near-term pregnant (~140 days’ gestation with the term at about 145 days) sheep at the same time point in the Spring season. As described previously,\textsuperscript{1-3} sheep were anesthetized with thiamylal (10 mg/kg) administered \textit{via} the external left jugular vein. The ewes were then intubated, and anesthesia was maintained on 1.5% to 2.0% halothane in oxygen throughout surgery. An incision was made in the abdomen and the uterus exposed. The resistance-sized uterine arteries (~150 μm in diameter) were isolated and removed without stretching and placed into a modified Krebs solution. For the steroid hormone treatment, uterine arteries from nonpregnant sheep were incubated in phenol red-free DMEM with 1% charcoal-stripped FBS for 48 hours at 37°C in a humidified incubator with 5% CO2/95% air in the absence or presence of 17\(\beta\)-estradiol (0.3 nmol/L, Sigma) and/or progesterone (100.0 nmol/L, Sigma), as reported previously.\textsuperscript{1-3} The concentrations of 17\(\beta\)-estradiol and progesterone chosen are physiologically relevant as observed in ovine pregnancy,\textsuperscript{4} which have been shown to exhibit direct genomic effects on pressure-dependent myogenic tone in the uterine artery.\textsuperscript{1} All of the procedures and protocols were approved by the Institutional Animal Care and Use Committee and followed the guidelines by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of Myogenic Tone
Pressure-dependent myogenic tone of resistance-sized uterine arteries was measured as described previously.\textsuperscript{1-3} Briefly, the arterial segments were mounted and pressurized in an organ chamber (Living Systems Instruments, Burlington VT). The intraluminal pressure was controlled by a servo-system to set transmural pressures and arterial diameter was recorded using the SoftEdge Acquisition Subsystem (IonOptix LLC, Milton MA). After the equilibration period, the intraluminal pressure was increased in a stepwise manner from 10 to 100 mmHg in 10-mmHg increments, and each pressure was maintained for 5 minutes to allow vessel diameter to stabilize before the measurement. The passive pressure-diameter relationship was conducted in Ca\textsuperscript{2+}-free physiologic saline solution (PSS) containing 3.0 mmol/L of EGTA to determine the maximum passive diameter. The following formula was used to calculate percent myogenic tone at each pressure step: \%myogenic tone = \((D_1 - D_2)/D_1 \times 100\), where \(D_1\) is the passive diameter in Ca\textsuperscript{2+}-free physiologic saline solution (0 Ca\textsuperscript{2+} with 3.0 mmol/L of EGTA) and \(D_2\) is the active diameter with normal physiologic saline solution in the presence of extracellular Ca\textsuperscript{2+}.

Measurement of BK_{Ca} Channel Current
Smooth muscle cells were enzymatically dissociated from resistance-sized uterine arteries. Briefly, uterine arteries were minced and incubated in low-Ca\textsuperscript{2+} (0.1 mmol/L CaCl\textsubscript{2}) HEPES-buffered PSS containing 0.6 mg/ml papain (Worthington Biochemical; Lakewood, NJ), 1 mg/ml bovine serum albumin and 1 mg/ml dithioerythritol for 35 minutes at 37°C. The tissues were then transferred to a new low-Ca\textsuperscript{2+} HEPES-buffered PSS containing 0.75 mg/ml collagenase type IV (Worthington) and 1 mg/ml bovine serum albumin and incubated for 30 minutes at 37°C. Following the enzyme treatment, tissues were washed with low Ca\textsuperscript{2+} PSS. Single smooth muscle cells were released by gentle trituration with a fire-polished glass Pasteur pipette. The cells were kept at 4°C and experiments were conducted within 6 hours of cell isolation. Whole-cell currents were recorded from freshly isolated arterial myocytes using an EPC 10 patch-clamp.
amplifier with Patchmaster software (HEKA, Lambrecht/Pfalz, Germany) at room temperature, as previously described.\textsuperscript{5,6} The cell membrane is much more fluid at physiological temperature of 37°C, which presents a technical difficulty in maintaining a patch with a good seal for patch-clamp recording. Therefore, most patch-clamp recordings were conducted at room temperature (22–24°C) for the better stability of the patch membrane. Several drops of cell suspension were placed in a recording chamber and the adherent cells were continuously superfused with HEPES-buffered physiologic salt solution containing (in mmol/L): 140.0 NaCl, 5.0 KCl, 1.8 CaCl\textsubscript{2}, 1.2 MgCl\textsubscript{2}, 10.0 HEPES, and 10.0 glucose (pH 7.4). Relaxed and spindle-shaped myocytes were used for recording. Micropipettes were pulled from borosilicate glass and had resistances of 2 to 5 MΩ when filled with the pipette solution containing (in mmol/L) 140.0 KCl, 1.0 MgCl\textsubscript{2}, 5.0 Na\textsubscript{2}ATP, 5.0 EGTA, 10.0 HEPES (pH 7.2). CaCl\textsubscript{2} was added to bring free Ca\textsuperscript{2+} concentrations to 100.0 nmol/L, as determined using WinMAXC software (Chris Patton, Stanford University). Cells were held at −50 mV and whole-cell K\textsuperscript{+} currents were evoked by voltage steps from −60 mV to +80 mV by stepwise 10-mV depolarizing pulses (350-ms duration, 10-second intervals). Whole-cell K\textsuperscript{+} currents were normalized to cell capacitance and were expressed as picoampere per picofarad (pA/pF). The BK\textsubscript{Ca} channel current was determined as the difference between the whole-cell K\textsuperscript{+} current in the absence of IBTX or TEA and that in the presence of IBTX or TEA.

Western Immunoblotting Analysis
Protein abundance of BK\textsubscript{Ca} channel α subunit and β1 subunit was measured in freshly isolated resistance-sized, endothelium-intact uterine arteries and after the hormonal treatment by Western blot analysis, as described previously.\textsuperscript{1–3} Briefly, tissues were homogenized in a lysis buffer followed by centrifugation at 4°C for 10 minutes at 10,000g, and the supernatants were collected. Samples with equal proteins were loaded onto 7.5% polyacrylamide gel with 0.1% sodium dodecyl sulfate, and were separated by electrophoresis at 100 V for 2 hours. Proteins were then transferred onto nitrocellulose membranes. Nonspecific binding sites in the membranes were blocked by an overnight incubation at 4°C in Tris-buffered saline solution containing 5% dry milk. The membranes were incubated with primary antibodies against BK\textsubscript{Ca} channel α and β1 subunit (Santa Cruz Biotechnology, Santa Cruz CA). After washing, membranes were incubated with secondary horseradish peroxidase-conjugated antibodies. Proteins were visualized with enhanced chemiluminescence reagents, and blots were exposed to Hyperfilm. Results were quantified with the Kodak electrophoresis documentation and analysis system and Kodak ID image analysis software. Preliminary studies showed that protein abundance of both alpha smooth muscle actin and GAPDH in uterine arteries was altered by pregnancy. Given the uncertainty of proteins that were not changed by pregnancy in the uterine artery, loading controls were not used and the samples were directly compared by scanning densitometry using arbitrary units.

Data Analysis
Results were expressed as means ± SEM obtained from the number of experimental animals given. Differences were evaluated for statistical significance (P<0.05) by ANOVA or t test, where appropriate.

References


