Chronic Hypoxia Suppresses Pregnancy-Induced Upregulation of Large-Conductance Ca\(^{2+}\)-Activated K\(^{+}\) Channel Activity in Uterine Arteries

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Abstract—Our previous study demonstrated that increased Ca\(^{2+}\)-activated K\(^{+}\) (BK\(_{Ca}\)) channel activity played a key role in the normal adaptation of reduced myogenic tone of uterine arteries in pregnancy. The present study tested the hypothesis that chronic hypoxia during gestation inhibits pregnancy-induced upregulation of BK\(_{Ca}\) channel function in uterine arteries. Resistance-sized uterine arteries were isolated from nonpregnant and near-term pregnant sheep maintained at sea level (~300 m) or exposed to high-altitude (3801 m) hypoxia for 110 days. Hypoxia during gestation significantly inhibited pregnancy-induced upregulation of BK\(_{Ca}\) channel activity and suppressed BK\(_{Ca}\) channel current density in pregnant uterine arteries. This was mediated by a selective downregulation of BK\(_{Ca}\) channel β1 subunit in the uterine arteries. In accordance, hypoxia abrogated the role of the BK\(_{Ca}\) channel in regulating pressure-induced myogenic tone of uterine arteries that was significantly elevated in pregnant animals acclimatized to chronic hypoxia. In addition, hypoxia abolished the steroid hormone-mediated increase in the β1 subunit and BK\(_{Ca}\) channel current density observed in nonpregnant uterine arteries. Although the activation of protein kinase C inhibited BK\(_{Ca}\) channel current density in pregnant uterine arteries of normoxic sheep, this effect was ablated in the hypoxic animals. The results demonstrate that selectively targeting BK\(_{Ca}\) channel β1 subunit plays a critical role in the maladaptation of uteroplacental circulation caused by chronic hypoxia, which contributes to the increased incidence of preeclampsia and fetal intrauterine growth restriction associated with gestational hypoxia. (Hypertension. 2012;60:214-222.)

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

Uterine blood flow increases >30-fold in human and sheep during gestation to ensure the optimal growth and development of the fetus. Hemodynamic changes in the uterine circulation are mainly achieved through the remodeling of uterine vasculature, enhanced vasodilator response, blunted vasoconstrictor response, and reduced pressure-dependent myogenic reactivity. Hypoxia during gestation constitutes a major insult to maternal cardiovascular homeostasis, and the adaptive changes in uterine circulation are complicated by high-altitude chronic hypoxia that inhibited the pregnancy-induced reduction of myogenic tone of uterine arteries. Consequently, chronic hypoxia attenuated pregnancy-induced increase in uterine blood flow. Hypoxia-induced aberration of uterine circulation in pregnancy is believed to play an important role in the pathogenesis of many pregnancy complications. Reduced uterine blood flow and inadequate perfusion of the placenta have been attributed to the increased incidence of preeclampsia and fetal intrauterine growth restriction.

The large-conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK\(_{Ca}\)) channel is abundantly expressed in vascular smooth muscle cells. The BK\(_{Ca}\) channel is a tetramer formed by pore-forming α subunits along with accessory β1 subunits; and the channel complex is activated by membrane depolarization and/or an increase in intracellular Ca\(^{2+}\) concentrations. Opening of the channel allows K\(^{+}\) efflux across the plasma membrane leading to hyperpolarization, whereas closure of the channel causes depolarization. Therefore, the activity of the BK\(_{Ca}\) channel is critical in determining the membrane potential of vascular smooth muscle cells and, hence, vascular tone. Participation of the BK\(_{Ca}\) channel in the regulation of vascular tone is evidenced by the development of myogenic contraction and elevated blood pressure attributed to pharmacological blockade of the channel and targeted deletion of BK\(_{Ca}\) channel genes. Previous studies have suggested that the BK\(_{Ca}\) channel is involved in the regulation of uterine circulation and the increase in uterine blood flow during pregnancy. We have demonstrated recently that blockade of the BK\(_{Ca}\) channel with tetraethylammonium abolishes the pregnancy-induced attenuation of myogenic tone of uterine arteries and that upregulated expression of the BK\(_{Ca}\) channel β1 subunit and subsequently heightened BK\(_{Ca}\) channel...
activity are responsible for the attenuated myogenic tone of the uterine artery during pregnancy.\textsuperscript{12} Thus, pregnancy-mediated upregulation of BK\textsubscript{Ca} channel function plays a key role in the adaptation of uterine vascular hemodynamics during pregnancy.

The BK\textsubscript{Ca} channel in vascular smooth muscle cells is a major effector in response to hypoxia. Vascular responses to both acute and chronic hypoxia involve altered BK\textsubscript{Ca} channel activity and/or expression in various vascular beds.\textsuperscript{29,31} Because of the crucial role of the BK\textsubscript{Ca} channel in pregnancy-mediated adaptation of uterine arterial myogenic tone and the negative impacts on both uterine blood flow and myogenic tone exerted by chronic hypoxia and pharmacological blockade of the BK\textsubscript{Ca} channel, we hypothesized that chronic hypoxia during gestation adversely affects the uterine circulation via downregulating BK\textsubscript{Ca} channel function in uterine arterial smooth muscle cells. Given that sex steroid hormones play a pivotal role in upregulating the \(\beta_1\) subunit and increasing the BK\textsubscript{Ca} channel activity in uterine arteries during pregnancy,\textsuperscript{12} we further tested the hypothesis that chronic hypoxia inhibits steroid hormone-mediated upregulation of the \(\beta_1\) subunit and BK\textsubscript{Ca} channel activity in uterine arteries.

Materials and Methods

Tissue Preparation and Treatment

Uterine arteries were harvested from nonpregnant and near-term pregnant (\(\sim 140\) days' gestation) sheep maintained at sea level (\(\sim 300\) m) or exposed to high-altitude (3801 m) hypoxia for 110 days.\textsuperscript{32} Animals were anesthetized with thiamylal (10 mg/kg, IV) followed by inhalation of 1.5% to 2.0% halothane. An incision was made in the abdomen and the uterus exposed. For hormonal treatment, 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 CO\textsubscript{2} incubator with 20.5% O\textsubscript{2} for tissues from normoxic animals and 10.5% O\textsubscript{2} for tissues from hypoxic animals, in the absence or presence of 17\textbeta-estradiol (0.5 mmol/L; Sigma) and progesterone (100.0 nmol/L; Sigma), as reported previously.\textsuperscript{12,32,33} The concentrations of 17\textbeta-estradiol and progesterone chosen are physiologically relevant, as observed in ovine pregnancy,\textsuperscript{29} which have been shown to exhibit direct genomic effects on BK\textsubscript{Ca} channel function and pressure-dependent myogenic tone in the uterine artery.\textsuperscript{12,32,33} All of the procedures and protocols were approved by the institutional animal care and use committee and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of BK\textsubscript{Ca} Channel Current

Arterial smooth muscle cells were enzymatically dissociated from resistance-sized uterine arteries, and whole-cell K\textsuperscript{+} currents were recorded using an EPC 10 patch-clamp amplifier with Patchmaster software (HEKA, Lambrecht/Pfalz, Germany) at room temperature, as described previously.\textsuperscript{12} Briefly, cell suspension drops were placed in a recording chamber, and adherent cells were continuously superfused with HEPES-buffered physiological 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). Only relaxed and spindle-shaped cells were evoked by voltage steps from −60 mV to +80 mV by stepwise 10-mV depolarizing pulses (350-ms duration, 10-s intervals). The 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 iberiotoxin (IBTX; Sigma) or tetrodotoxin (TEA; Sigma) and that in the presence of IBTX or TEA.\textsuperscript{12}

Measurement of Myogenic Tone

Pressure-dependent myogenic tone of resistance-sized uterine arteries was measured as described previously.\textsuperscript{12,32,33} 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 PSS containing 3.0 mmol/L of EGTA to determine the maximum passive diameter. The following formula was used to calculate the percentage of myogenic tone at each pressure step: %myogenic tone = \(\left(\frac{P}{D_0}\right)\times100\), where \(P\) is the passive diameter in Ca\textsuperscript{2+}-free PSS (0Ca\textsuperscript{3+} with 3.0 mmol/L of EGTA), and \(D_0\) is the active diameter with normal PSS in the presence of extracellular Ca\textsuperscript{2+}.

Western Immunoblotting

Protein abundance of the BK\textsubscript{Ca} channel \(\alpha\) subunit and \(\beta_1\) subunit was measured in freshly isolated uterine arteries and in arteries after ex vivo hormonal treatment, as described previously.\textsuperscript{12} Briefly, tissues were homogenized in a lysis buffer followed by centrifugation at 4°C for 10 minutes at 10000g, and the supernatants were collected. Samples with equal proteins were loaded onto 7.5% polyacrylamide gel with 0.1% SDS and were separated by electrophoresis at 100 V for 2 hours. Proteins were then transferred onto nitrocellulose membranes. After blocking nonspecific binding sites by dry milk, the membranes were incubated with primary antibodies against BK\textsubscript{Ca} channel \(\alpha\) and \(\beta_1\) subunits (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. The target protein abundance was normalized to the abundance of \(\beta_2\)-actin as a protein loading control.

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

Chronic Hypoxia Inhibits Pregnancy-Induced Upregulation of BK\textsubscript{Ca} Channel Activity in Uterine Arteries

In both normoxic and hypoxic animals, the whole-cell K\textsuperscript{+} current densities in uterine arterial myocytes in the voltage range of −60 mV to +80 mV were significantly higher in pregnant animals (at +80 mV: normoxia, 60.3±2.7 pA/pF; hypoxia, 46.5±3.3 pA/pF) than in nonpregnant animals (at +80 mV: normoxia, 33.9±2.7 pA/pF; hypoxia, 32.9±3.0 pA/pF; \(P<0.05\); Figure 1). In normoxic animals, pregnancy resulted in an approximate 78% increase in the whole-cell K\textsuperscript{+} current density at +80 mV in uterine arterial myocytes (Figure 1A and 1B). However, this enhancement was significantly blunted by chronic hypoxia, and pregnancy only produced an approximate 41% increase in the current density in uterine arterial
myocytes in animals acclimatized to long-term high-altitude hypoxia (Figure 1C and 1D). In accordance, chronic hypoxia caused a ~22% decrease in the whole-cell K⁺ current density in pregnant uterine arteries (P < 0.05) but had no significant effect on the current density in nonpregnant uterine arteries. Whole-cell K⁺ currents were sensitive to blockade by BKCa channel inhibitors TEA (1.0 mmol/L) or IBTX (100.0 nmol/L). Both TEA and IBTX produced similar inhibition of the K⁺ currents in uterine arterial myocytes (Figure 1). As shown in Figure 2A, BKCa current densities, determined as the differences of whole-cell K⁺ currents in the absence or presence of TEA in the voltage range of −60 mV to +80 mV, in nonpregnant uterine arterial myocytes were not altered by chronic hypoxia. In contrast, chronic hypoxia significantly suppressed BKCa current densities in pregnant uterine arterial myocytes and decreased the current density at +80 mV from

Figure 1. Chronic hypoxia decreases whole-cell K⁺ currents in uterine arteries of pregnant sheep. Arterial myocytes were freshly isolated from uterine arteries of normoxic and hypoxic sheep. Whole-cell K⁺ currents were recorded in the absence or presence of tetraethylammonium (TEA; 1.0 mmol/L) or iberotoxin (IBTX; 100.0 nmol/L). A, Normoxic nonpregnant animals. B, Normoxic pregnant animals. C, Hypoxic nonpregnant animals. D, Hypoxic pregnant animals. Data are mean ± SEM of 7 to 10 cells from 5 to 8 animals of each group. *P < 0.05 vs control (Ctr). ○, ctr; ■, TEA; □, IBTX.

Figure 2. Chronic hypoxia suppresses Ca²⁺-activated K⁺ (BKCa) current density in uterine arteries of pregnant sheep. Arterial myocytes were freshly isolated from uterine arteries of normoxic and hypoxic sheep. BKCa current density was determined in the presence of tetraethylammonium (TEA; 1.0 mmol/L). A, Nonpregnant animals. B, Pregnant animals. Data are mean ± SEM of 7 to 10 cells from 5 to 8 animals of each group. *P < 0.05 vs normoxia. ○, normoxia; ■, hypoxia.
Chronic Hypoxia Abolishes Steroid Hormone-Induced Upregulation of BK$_{Ca}$ Channel Activity in Uterine Arteries

We showed recently that pregnancy-induced upregulation of BK$_{Ca}$ channel activity in uterine arteries was largely mediated by the action of 17β-estradiol and progesterone. To determine the effects of chronic hypoxia on the steroid hormone-mediated enhancement of BK$_{Ca}$ channel activity, uterine arteries isolated from normoxic and hypoxic nonpregnant sheep were treated ex vivo with 17β-estradiol (0.3 nmol/L) and progesterone (100.0 nmol/L) under 20.5% O$_2$ and 10.5% O$_2$, respectively, for 48 hours. The regulation of BK$_{Ca}$ channel activity by steroid hormones is illustrated in Figure 4. In a way similar to the effect of pregnancy, the ex vivo hormonal treatment of uterine arteries from normoxic nonpregnant sheep significantly increased whole-cell K$^+$ current density at +80 mV in uterine arterial myocytes from 26.2±1.8 pA/pF (Figure 4A) to 44.7±4.0 pA/pF (Figure 4B; P<0.05). Accordingly, the hormonal treatment resulted in significant increases in BK$_{Ca}$ current densities (at +80 mV: 25.5±2.5 pA/pF versus 10.7±1.5 pA/pF; P<0.05; Figure 4C). In contrast, the hormonal treatment of uterine arteries from nonpregnant animals acclimatized to long-term high-altitude hypoxia had no significant effect on whole-cell K$^+$ current densities (at +80 mV: 28.9±1.4 pA/pF in control myocytes versus 32.3±0.8 pA/pF in hormone-treated myocytes; P>0.05; Figure 4D and 4E) or BK$_{Ca}$ current densities (at +80 mV: 12.5±0.8 pA/pF in control myocytes versus 13.9±0.6 pA/pF in hormone-treated myocytes; P>0.05; Figure 4F) in uterine arterial myocytes.

Chronic Hypoxia Inhibits Pregnancy- and Hormone-Induced Upregulation of the BK$_{Ca}$ Channel β1 Subunit in Uterine Arteries

The impacts of chronic hypoxia on molecular expression of BK$_{Ca}$ channels in uterine arteries were determined with Western immunoblotting analysis. Chronic hypoxia had no significant effects on protein abundance of either BK$_{β1}$ channel α or β subunits in uterine arteries of nonpregnant sheep (data not shown). In pregnant sheep exposed to long-term high altitude hypoxia, uterine arterial BK$_{Ca}$ channel α subunit protein abundance was not changed, but BK$_{β1}$ channel β1 subunit protein abundance was significantly decreased (Figure 5A). In contrast to the previous finding in normoxic sheep in which pregnancy upregulated the β1 subunit in uterine arteries, pregnancy had no significant effects on either α or β subunit protein abundance in animals acclimatized to long-term high-altitude hypoxia (Figure 5B). Similarly, ex vivo hormonal treatment of uterine arteries from nonpregnant sheep exposed to long-term high-altitude hypoxia failed to modify protein abundance of the BK$_{Ca}$ channel α and β1 subunits (Figure 5C).

Chronic Hypoxia Suppresses Protein Kinase C–Mediated Inhibition of BK$_{Ca}$ Channel Activity in Uterine Arteries of Pregnant Animals

Given that the BK$_{Ca}$ channel presents a functional link in pregnancy-mediated downregulation of protein kinase C (PKC) and myogenic tone of uterine arteries, the effect of chronic hypoxia on PKC-mediated modulation of BK$_{Ca}$ channel activity...
in uterine arteries of pregnant sheep was assessed. To determine the effect of PKC on BKCa channel activity, we selectively blocked the K+ current mediated by voltage-gated K+ channels (Kv) with 4-aminopyridine (4-AP) and examined the effect of phorbol 12,13-dibutyrate (PDBu) on the 4-AP-insensitive component of whole-cell K+ currents (ie, BKCa component). As predicted, the application of 4-AP inhibited whole-cell K+ currents in uterine arterial myocytes. However, the resultant Kv current densities determined as the differences of whole-cell K+ currents were not significantly different between normoxic and hypoxic animals (Figure 6). As shown in Figure 7A, PKC activation by PDBu in the presence of 4-AP decreased the current density at +80 mV from 40.7 ± 2.9 to 26.8 ± 1.5 pA/pF (P < 0.05) in uterine arterial myocytes from normoxic sheep. In pregnant sheep acclimatized to long-term high-altitude hypoxia, the BKCa component was significantly decreased from 40.7 ± 2.9 to 22.6 ± 1.8 pA/pF at +80 mV (P < 0.05; Figure 7A and 7B). In contrast to the finding in normoxic sheep, PDBu did not reduce BKCa currents in hypoxic animals at +80 mV (22.6 ± 1.8 versus 19.0 ± 1.7 pA/pF; P > 0.05; Figure 7B).

Discussion
In the present study we present evidence to support the hypothesis that chronic hypoxia increases myogenic tone of uterine arteries of pregnant sheep via inhibiting BKCa channel function in uterine arterial vascular smooth muscle cells. It has been demonstrated previously that decreased myogenic tone in the uterine artery plays a key role in increased uterine blood flow during pregnancy.9–12,33 The attenuated myogenic tone is largely demonstrated previously that decreased myogenic tone in the uterine artery plays a key role in increased uterine blood flow during pregnancy.9–12,33 The attenuated myogenic tone is largely

Figure 4. Chronic hypoxia inhibits steroid hormone-induced upregulation of Ca2+-activated K+ (BKCa) channel activity in uterine arteries. Uterine arteries were isolated from nonpregnant sheep and were treated ex vivo with 17β-estradiol (E2; 0.3 nmol/L) plus progesterone (P4; 100.0 nmol/L) under 20.5% O2 and 10.5% O2, respectively, for 48 hours. Arterial myocytes were then isolated and whole-cell K+ currents were recorded in the absence or presence of tetraethylammonium (TEA; 1.0 mmol/L). A, Whole-cell K+ currents in myocytes of normoxic animals without hormonal treatment. *P < 0.05 vs control (Ctr). B, Whole-cell K+ currents in myocytes of normoxic animals with hormonal treatment. *P < 0.05 vs control (Ctr). C, BKCa current density in myocytes of normoxic animals without and with hormonal treatment. Data are mean ± SEM of 8 to 10 cells from 6 animals of each group. A, B, D, and E, ○, ctr; ●, TEA. C and F, ○, E2; ●, E2 + P4; ○, E2 + P4.

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Figure 5. Chronic hypoxia inhibits pregnancy- and steroid hormone-induced upregulation of Ca^{2+}-activated K^- (BK_{Ca}) channel β1 subunit in uterine arteries. Protein abundance of BK_{Ca} channel α (αBK_{Ca}) and β1 (β1BK_{Ca}) subunits was determined by Western blot analyses. A, Freshly isolated uterine arteries of pregnant sheep in normoxic and hypoxic animals. *P<0.05 vs normoxia. B, Freshly isolated uterine arteries from nonpregnant (NPUA) and pregnant (PUA) sheep of hypoxic animals. C, Uterine arteries from nonpregnant sheep of hypoxic animals were treated ex vivo with 17β-estradiol (E2; 0.3 nmol/L) plus progesterone (P4; 100.0 nmol/L) under 10.5% O2 for 48 hours. Data are mean±SEM of tissues from 5 to 6 animals of each group. A, □, normoxia; ■, hypoxia. B, □, PUA; ■, NPUA. C, □, -E2β&P4; ■, +E2β&P4.

ity, therefore, provides a mechanism that increases vascular contraction. The present finding that TEA failed to alter myogenic tone of uterine arteries from pregnant animals exposed to chronic hypoxia suggests a loss of the regulatory role of the BK_{Ca} channel in myogenic reactivity. Consistent with this finding, the BK_{Ca} current density in uterine arteries was decreased by ≈44% in pregnant animals, and the normal pregnancy-induced upregulation of BK_{Ca} channel activity was diminished in animals acclimatized to long-term high altitude hypoxia. Overall, these findings suggest that the regulation of uterine arterial myogenic tone by the BK_{Ca} channel in pregnant animals is inhibited by chronic hypoxia during gestation.

Our recent study in sheep revealed that the increased BK_{Ca} channel activity in uterine arteries during pregnancy was primarily mediated by the actions of sex steroid hormones 17β-estradiol and progesterone. Specifically, molecular expression of the BK_{Ca} channel β1 subunit and BK_{Ca} channel activity were upregulated in uterine arteries of pregnant sheep, which was mimicked by ex vivo steroid hormonal treatment of uterine arteries from nonpregnant animals. In contrast to these findings, the present study demonstrated that chronic hypoxia impeded pregnancy-associated and steroid-induced upregulation of BK_{Ca} channel β1 subunit expression and BK_{Ca} channel activity in the uterine artery. Accordingly, the abundance of the BK_{Ca} channel β1 subunit was significantly decreased in uterine arteries of pregnant animals acclimatized to long-term high-altitude hypoxia as compared with normoxic animals. Thus, chronic hypoxia during gestation selectively downregulates the BK_{Ca} channel β1 subunit in uterine arteries of pregnant animals. Not surprisingly, chronic hypoxia also selectively downregulates the BK_{Ca} channel β1 subunit in other vascular beds, such as pulmonary arteries and aortas, as well as cultured vascular smooth muscle cells. Hence, the BK_{Ca} channel in vascular smooth muscle cells constitutes a major effector of decreasing Po2, and chronic hypoxia selectively targets the BK_{Ca} channel β1 subunit. In agreement with our finding that the regulation of uterine arterial myogenic tone by the BK_{Ca} channel was ablated in pregnant sheep exposed to chronic hypoxia, Navarro-Antolin et al. also observed the attenuated vasodilator response mediated by the BK_{Ca} channel in the aorta from hypoxic rats. These observations support the notion that blunted molecular and functional expressions of the BK_{Ca} channel in vascular smooth muscle cells from hypoxic animals contribute to enhanced vascular tone.

The BK_{Ca} channel β1 subunit is ubiquitously expressed in vascular smooth muscle cells. The association of the β1 subunit with the pore-forming α subunit significantly increases the Ca^{2+} sensitivity of the channel complex. In accordance, deletion of the BK_{Ca} channel β1 subunit gene dramatically decreased BK_{Ca} channel Ca^{2+} sensitivity in
Figure 7. Chronic hypoxia diminishes protein kinase C (PKC)-mediated modulation of Ca\(^{2+}\)-activated K\(^+\) (BK\(_{Ca}\)) channel in uterine arteries. Arterial myocytes were freshly isolated from uterine arteries of pregnant sheep in normoxic (A) and hypoxic (B) animals. Whole-cell K\(^+\) currents were recorded in the absence or presence of 4-aminopyridine (4-AP; 5.0 mmol/L) and 4-AP plus phorbol 12,13-dibutyrate (PDBu; 1.0 \(\mu\)mol/L), respectively. Data are mean±SEM of 5 cells from 5 animals of each group. a, P<0.05 vs control (Ctr); b, P<0.05 vs 4-AP. ○, ctr; ●, 4-AP; ■, 4-AP+PDBu.

vascular smooth muscle cells. Changes in the stoichiometry of \(\alpha\) and \(\beta\) subunits have been implicated in a variety of physiological and pathophysiological conditions. BK\(_{Ca}\) channel activity was enhanced because of the upregulation of the \(\beta\) subunit in the mesenteric artery after hemorrhagic shock\(^{41}\) and in the uterine artery during pregnancy.\(^{12}\) On the other hand, depressed BK\(_{Ca}\) channel activity that resulted from the downregulation of the \(\beta\) subunit was demonstrated in the cerebral artery of hypertensive animals.\(^{42,43}\) In the present study, we demonstrated that chronic hypoxia during gestation selectively ablated the pregnancy-induced upregulation of the BK\(_{Ca}\) channel \(\beta\) subunit without affecting the \(\alpha\) subunit protein abundance in uterine arteries of pregnant animals. The decreased \(\beta:\alpha\) subunit stoichiometry in uterine arteries of pregnant animals exposed to chronic hypoxia thus impedes BK\(_{Ca}\) channel activation, leading to membrane depolarization and increased vascular tone. These findings of molecular and electrophysiological impacts of chronic hypoxia are consistent with the results of functional studies showing increased vascular tone of uterine arteries from pregnant sheep acclimatized to long-term high-altitude hypoxia. Thus, the present study provides a novel mechanism of aberrant BK\(_{Ca}\) channel activity in reduced uterine blood flow caused by chronic hypoxia during gestation. In contrast to the BK\(_{Ca}\) channel, the K\(_V\) channel current density in the uterine artery of pregnant sheep was not significantly different between hypoxic and normoxic animals, indicating minimal role of K\(_V\) channels in the chronic hypoxia-induced alteration of uterine vascular tone. However, this lack of an effect of chronic hypoxia on K\(_V\) responses in uterine arteries is not ubiquitous to all vascular smooth muscle. Unlike the uterine artery, chronic hypoxia caused a decrease in the activity of the K\(_V\) channel in pulmonary arteries,\(^{44}\) suggesting the heterogeneity of tissue response to chronic hypoxia.

Our previous study demonstrated that 17\(\beta\)-estradiol alone was sufficient to upregulate the expression of the BK\(_{Ca}\) channel \(\beta\) subunit in the uterine artery.\(^{12}\) Hence, the downregulation of the BK\(_{Ca}\) channel \(\beta\) subunit in uterine arteries of pregnant animals exposed to high-altitude chronic hypoxia could be a result of decreased 17\(\beta\)-estradiol in the maternal circulation. However, this is unlikely, because maternal plasma 17\(\beta\)-estradiol levels in sheep were not altered by chronic hypoxia.\(^{32}\) Estrogen receptor-\(\alpha\) is the predominant estrogen receptor in the uterine artery.\(^{32}\) Thus, the genomic effect of the steroid hormone in upregulating the BK\(_{Ca}\) channel is likely mediated through the interaction between 17\(\beta\)-estradiol and estrogen receptor-\(\alpha\). Hypoxia has profound impacts on gene expression and protein synthesis.\(^{45,46}\) Chronic hypoxia during gestation downregulates estrogen receptor-\(\alpha\) expression in uterine arteries.\(^{32}\) Consequently, suppressed BK\(_{Ca}\) channel \(\beta\) subunit expression in uterine arteries could be attributed to reduced expression of estrogen receptor-\(\alpha\). Accordingly, chronic hypoxia suppressed pregnancy- and steroid hormone-mediated attenuation of uterine arterial myogenic tone.\(^{32}\)

Consistent with the previous studies showing that BK\(_{Ca}\) channel activity was inhibited by PKC in vascular smooth muscle cells,\(^{47-50}\) the present study demonstrated that PDBu significantly decreased the 4-AP–insensitive component of whole-cell K\(^+\) currents, that is, the BK\(_{Ca}\) current in uterine arteries of pregnant animals. This finding, combined with our previous results that the activation of PKC by PDBu produced the same increase in pressure-dependent myogenic tone of uterine arteries in pregnant animals as the inhibition of BK\(_{Ca}\) channel by TEA,\(^{12}\) provides evidence that PKC-mediated inhibition of BK\(_{Ca}\) channel activity is functionally coupled to myogenic tone of uterine arteries. Given that the PKC activity in uterine arteries of pregnant sheep was enhanced by chronic hypoxia,\(^{13}\) we reasoned that activation of PKC may exert greater inhibition on BK\(_{Ca}\) channel activity in the uterine arteries of pregnant animals exposed to high-altitude hypoxia. Surprisingly, our data suggest that PKC-mediated inhibition of BK\(_{Ca}\) activity in uterine arteries of pregnant animals was diminished by...
chronic hypoxia. Although this may be because of a loss of the regulatory role of PKC on the BKCa channel, the significantly decreased BKCa channel activity in the uterine arteries caused by chronic hypoxia during gestation may also contribute to the diminished effect of PDBu. Similar findings have been reported in pulmonary arteries, showing that chronic hypoxia inhibits the Kᵥ channel activity and diminishes the PKC-mediated inhibition of the Kᵥ channel.⁴⁴,⁵¹,⁵² It should be noted, however, that the Kᵥ channel activity in uterine arteries does not appear to be modulated by PKC, because PDBu had no effect on TEA-insensitive K⁺ currents, that is, Kᵥ current density in uterine arterial myocytes from both nonpregnant and pregnant animals.¹²

**Perspectives**

Chronic hypoxia during gestation has profound adverse effects on the normal adaptation of uteroplacental circulation to pregnancy and increases the incidence of preeclampsia and fetal intrauterine growth restriction.¹⁴–¹⁷,³⁶,⁵³,⁵⁴ Studies in a variety of animal models have demonstrated a critical link between reductions in uteroplacental blood flow with prolonged uteroplacental ischemia and a hypertension state that closely resembles preeclampsia in women.⁵⁵,⁵⁶ BKCa channels play a key role in regulating the uterine circulation during pregnancy.¹²,²⁶–²⁸ The present study provides evidence of a novel mechanism of aberrant BKCa channel function in abnormal uteroplacental circulation caused by chronic hypoxia during gestation and, hence, improves our understanding of the pathophysiology of preeclampsia and intrauterine growth restriction. Further studies on the regulation of BKCa channel transcription, translation, and trafficking should provide more insights into mechanisms at the molecular level.

**Sources of Funding**

This work was supported by National Institutes of Health grants HD031226 (to L.Z.), HL089012 (to L.Z.), HL110125 (to L.Z.), and DA025319 (to S.Y.) and by National Science Foundation grant MRI-DBI 0923559 (to S.M.W.).

**Disclosures**

None.

**References**


Diminished BK Ca channel function accounts for heightened uterine vasoreactivity in shock.

Chronic hypoxia during gestation selectively inhibits the BKCa channel activity in uterine arteries.

The inhibition of BKCa channel activity by hypoxia is attributed to the loss of steroid hormone-mediated upregulation of the BKCa channel β1 subunit.

Diminished BKCa channel function accounts for heightened uterine arterial myogenic reactivity.

What Is Relevant?

Heightened uterine arterial myogenic tone leads to reductions in uteroplacental blood flow observed in pregnancy with hypoxia.

Gestational hypoxia and reduced uteroplacental perfusion are major risk factors of preeclampsia.
Chronic Hypoxia Suppresses Pregnancy-Induced Upregulation of Large-Conductance Ca
2+-Activated K+ Channel Activity in Uterine Arteries
Xiang-Qun Hu, Daliao Xiao, Ronghui Zhu, Xiaohui Huang, Shumei Yang, Sean M. Wilson and
Lubo Zhang

Hypertension. published online June 4, 2012;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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