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Gestational Hypoxia Increases Reactive Oxygen Species and Inhibits Steroid Hormone–Mediated Upregulation of Ca^{2+}-Activated K^+ Channel Function in Uterine Arteries

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Abstract

Gestational hypoxia inhibits steroid hormone–induced upregulation of Ca^{2+}-activated K^+ (K_{Ca}) channel activities in uterine arteries. We tested the hypothesis that increased reactive oxygen species play an important role in hypoxia-mediated inhibition of K_{Ca} channel activities. Uterine arteries were isolated from nonpregnant (nonpregnant uterine artery) and near-term (≈142–145 day) pregnant (pregnant uterine artery) sheep maintained at either sea level or high altitude (3820 m, Pao_2: 60 mm Hg) for 110 days. In pregnant uterine arteries, hypoxia significantly decreased large conductance channel opener NS1619- and small conductance channel opener NS309-induced relaxations, which were partially restored by reactive oxygen species inhibitor N-acetylcysteine (NAC). NAC significantly increased large conductance K_{Ca} but not small conductance K_{Ca} current densities in uterine arterial smooth muscle cells in pregnant animals acclimatized to high altitude. The NAC-sensitive component of small conductance K_{Ca}–induced relaxations was diminished in endothelium-denuded arteries. In nonpregnant uterine arteries, NS1619- and NS309-induced relaxations were diminished compared with those in pregnant uterine arteries. Treatment of nonpregnant uterine arteries with 17β-estradiol and progesterone for 48 hours increased small conductance K_{Ca} type 3 protein abundance and NS1619- and NS309-induced relaxations, which were inhibited by hypoxia. This hypoxia-mediated inhibition was reversed by NAC. Consistently, steroid hormone treatment had no significant effects on large conductance K_{Ca} current density in nonpregnant uterine arteries of hypoxic animals in the absence of NAC but significantly increased it in the presence of NAC. These results suggest an important role of hypoxia-mediated reactive oxygen species in negatively regulating steroid hormone–mediated upregulation of K_{Ca} channel activity and adaptation of uterine vascular reactivity in pregnancy, which may contribute to the increased incidence of preeclampsia and fetal intrauterine growth restriction associated with gestational hypoxia.

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Disclosures

None.

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Keywords

anoxia; oxidative stress; uterine artery

Ca^{2+}-activated K^+ (K_{Ca}) channels are key regulators of vascular tone.[1,2] Based on their conductance, K_{Ca} channels are divided into large conductance (BK_{Ca}), intermediate conductance (IK_{Ca}), and small conductance (SK_{Ca}) channels. BK_{Ca} channels are expressed in vascular smooth muscle, and SK_{Ca} channels are thought to be expressed predominantly in endothelial cells.[2] Hyperpolarization produced by the activation of SK_{Ca} in endothelial cells may be transmitted to vascular smooth muscle cells via the myoendothelial gap junction.[3] Our recent studies demonstrated that both BK_{Ca} and SK_{Ca}, but not IK_{Ca} channels, contributed to the regulation of uterine vascular function.[4–6] Uterine blood flow increases dramatically during pregnancy to optimize the supply of oxygen and nutrition for the fetal development because of both vascular remodeling/growth and a decrease in uterine vascular tone.[7] Steroid hormones (estrogen and progesterone) play an important role in the hemodynamic adaption to pregnancy, in part, by upregulating K_{Ca} channel function,[4] leading to decreased uterine vascular tone. However, this adaptive change is severely compromised by gestational hypoxia, leading to increased incidence of preeclampsia and fetal intrauterine growth restriction.[8–11] This regulation of the uterine circulation involves increased vascular tone because of impaired K_{Ca} channel function.[5,6] However, the mechanism underlying the impairment of K_{Ca} channel function remains poorly understood.

Reactive oxygen species (ROS) in the cardiovascular system primarily include superoxide, hydrogen peroxide, and hydroxyl radical. Increased levels of ROS during exposure to hypoxia have been demonstrated in several arterial beds including the uterine arteries from pregnant sheep[12,13]; and oxidative stress has been implicated in the pathogenesis of various cardiovascular disorders.[14,15] Activities of K_{Ca} channels in vascular smooth muscle cells are subject to modulation by ROS.[16,17] However, the role of increased ROS in regulating K_{Ca} channel function and uterine vascular tone in response to hypoxia remains unknown. The present studies investigated whether the hypoxia-mediated heightened ROS altered K_{Ca} channel activities and their mediated relaxations of uterine arteries in pregnancy. In addition, we also investigated the effects of steroid hormones on K_{Ca} channel activities and their mediated uterine vasorelaxations and determined whether the effect of steroid hormones was changed by hypoxia-enhanced ROS.

Materials and Methods

Tissue Preparation and Treatment

Uterine arteries were harvested from both nonpregnant and near-term (≈142–145 days of gestation) pregnant sheep maintained at sea level (≈300 m) or exposed to high-altitude (3801 m) hypoxia (Pao_2: 60 mm Hg) for 110 days (from 30 days of gestation for pregnant animals).[18] Animals were anesthetized with intravenous injection of propofol (2 mg/kg) followed by intubation, and anesthesia was maintained on 1.5% to 3.0% isoflurane balanced in O_2 throughout the surgery. An incision was made in the abdomen and the uterus exposed. The uterine arteries were isolated and removed without stretching and placed into a Krebs

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solution containing (in mmol/L) 130.0 NaCl, 10.0 HEPES, 6.0 glucose, 4.0 KCl, 4.0 $NaHCO_3$, 1.8 $CaCl_2$, 1.2 $MgSO_4$, 1.18 KH_2PO_4, and 0.025 EDTA (pH 7.4) in room air with Po_2 of ≈100 mm Hg. To determine the ex vivo hypoxic effect, uterine arteries were treated under normoxic or hypoxic conditions for 48 hours. Given an ≈50% decrease in arterial Po_2 observed in high-altitude hypoxic sheep,[5,18,19] uterine arteries were incubated in a given culture dish with 5 mL of phenol red–free DMEM with 1% charcoal-stripped fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and treated at 37°C in humidified incubators for 48 hours with oxygen levels at either 21% O_2 for normoxic or 10.5% O_2 for hypoxic conditions as described previously.[5,18,19] In some tissues, the endothelium was removed by gentle rotation of arterial rings on an approximately sized blunt hypodermic needle, and the validity of endothelium removal was confirmed by the absence of endothelial nitric oxide synthase in immunohistochemical staining as shown previously.[6] Endothelium removal had no significant effect on KCl- or norepinephrine-induced contractions. Each experimental group had 4 to 6 animals. All 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.

Contraction Studies

The fourth generation branches of main uterine arteries were separated from surrounding tissues and cut into 2-mm ring segments. In uterine arteries of pregnant sheep, isometric tension was measured in the Krebs solution in a tissue bath at 37°C as described previously.[17–20] Briefly, each ring segment was equilibrated for 60 minutes and then gradually stretched to the optimal resting tension, as determined by the tension that developed in response to 3× 120 mmol/L KCl, each added at a stretch level. In the studies of arterial contractility, vessels are commonly stimulated with repeated (normally 3×) maximal concentrations of KCl to stabilize the vascular reactivity. Without the KCl stimulation, norepinephrine-induced contractions and subsequent NS1619 compound–mediated relaxations were unstable and were not comparable between vessels. After stable responses to KCl were obtained, arteries were treated with 0 or 1 mmol/L N-acetylcysteine (NAC) for 20 minutes. Tissues were then precontracted with submaximal concentrations of norepinephrine that produced ≈70% to 80% of the maximal contraction, followed by additions of NS1619 or NS309 in a cumulative manner.

In uterine arteries of nonpregnant sheep, tissues were treated with 17β-estradiol (0.3 nmol/L, Sigma) and progesterone (P_4, 100 nmol/L, Sigma) for 48 hours in the absence or presence of 1 mmol/L NAC. For hormonal treatments, arteries from nonpregnant sheep were incubated under normoxia of 21% O_2 for tissues from normoxic animal or hypoxia of 10.5% O_2 for tissues from hypoxic animals in the absence or presence of 17β-estradiol and P_4 as reported previously.[4,5,18–20] The concentrations of 17β-estradiol and P_4 were chosen based on the previous study of their concentration-dependent effects on uterine arteries,[20] and they are physiologically relevant as observed in ovine pregnancy.[21] Our previous studies have shown that the hormonal treatment regulates K_{Ca} channel activity and pressure-dependent myogenic tone in the uterine artery.[4,5,18–20] After the treatments, NS1619- or NS309-induced relaxations were determined as described above.

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Western Immunoblotting

Our previous study demonstrated that pregnancy increased protein abundance of type 2 (SK2) and type 3 (SK3) SK_{Ca} channels in ovine uterine arteries.[6] In the present study, the effect of steroid hormones on SK2 and SK3 expression was measured in uterine arteries of nonpregnant sheep. Briefly, fourth order uterine arteries were treated with 17β-estradiol (0.3 nmol/L) and progesterone (P_4, 100 nmol/L) or vehicle control for 48 hours. Tissues from each animal provided enough protein for Western blotting. After the treatments, tissues were homogenized in a lysis buffer followed by centrifugation at 4°C for 10 minutes at 10 000*g*, and 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. After blocking nonspecific binding sites by dry milk, membranes were incubated with primary antibodies against SK2 channel (Alomone Ltd, Jerusalem, Israel) and SK3 channel (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.

Measurement of K_{Ca} Channel Current

Arterial smooth muscle cells were enzymatically dissociated from resistance-sized uterine arteries, and whole-cell K^+ currents were recorded using an EPC 10 patch-clamp amplifier with Patchmaster software (HEKA, Lambrecht/Pfalz, Germany) at room temperature as previously described.[4–6] 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 $CaCl_2$, 1.2 $MgCl_2$, 10.0 HEPES, and 10.0 glucose (pH 7.4). Only relaxed and spindle-shaped myocytes were used for recording. Micropipettes were pulled from borosilicate glass and had resistances of 2 to 5 mol/LΩ when filled with the pipette solution containing (in mmol/L) 140.0 KCl, 1.0 $MgCl_2$, 5.0 Na_2ATP, 5.0 EGTA, 10.0 HEPES (pH 7.2). $CaCl_2$ was added to bring free Ca^{2+} concentrations to 200 nmol/L as determined using WinMAXC software (Chris Patton, Stanford University). Cells were held at −50 mV, and whole-cell K^+ currents were evoked by voltage steps from −60 mV to +80 mV by stepwise 10-mV depolarizing pulses (350-ms duration, 10-second intervals) in the absence and presence of 1 mmol/L BK_{Ca} channel blocker tetraethylammonium[4,5] or 1 μmol/L SK_{Ca} channel blocker apamin.[6] The K^+ currents were normalized to cell capacitance and were expressed as picoampere per picofarad (pA/pF).

Data Analysis

Concentration–response curves were analyzed by computer-assisted nonlinear regression to fit the data using GraphPad Prism (GraphPad Software, San Diego, CA). 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.

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Results

NAC Inhibited the Hypoxic Effect in Suppressing K_{Ca} Channel–Mediated Relaxations of Uterine Arteries in Pregnant Sheep

In normoxic animals, both BK_{Ca} channel opener NS1619 and SK_{Ca} channel opener NS309 induced concentration-dependent relaxations of uterine arteries from pregnant sheep, which were not significantly affected by NAC (Figure 1A and 1B). In pregnant animals acclimatized to high-altitude hypoxia, both NS1619- and NS309-induced relaxations were significantly attenuated (Figure 1C and 1D). Compared with the relaxations seen in normoxic animals, chronic hypoxia caused a significant decrease in the maximal response of NS1619 from 39.3±3.8% to 13.7±3.8% (P<0.05) and NS309 from 58.0±11.2% to 17.7±3.3% (P<0.05). As shown in Figure 1C and 1D, treatment of the vessels with NAC inhibited the hypoxic effect and significantly increased the maximal response of NS1619-induced relaxations from 13.7±3.8% to 46.2±9.5% (P<0.05) and NS306-induced relaxations from 17.7±3.3% to 34.9±4.6% (P<0.05). In the presence of NAC, there was no significant difference in NS1619-induced relaxations between normoxic and hypoxic animals (43.9±5.0% versus 46.2±9.5%; P>0.05). Unlike NS1619, NAC partially blocked the effect of hypoxia on NS309-mediated relaxations.

NAC Increased BK_{Ca} Channel Activities in Uterine Arteries of Hypoxic Animals

To determine the role of ROS in the hypoxia-mediated effect on K_{Ca} channel activities in uterine arteries, whole-cell K^+ and K_{Ca} currents were determined in the absence or presence of NAC in smooth muscle cells freshly isolated from uterine arteries of normoxic and hypoxic pregnant animals. Consistent with the previous finding,[5] chronic hypoxia significantly decreased both whole-cell K^+ currents (from 52.8±1.7 to 42.4±0.8 pA/pF at +80 mV; P<0.05) and BK_{Ca} current density (from 29.1±2.0 to 18.5±1.4 pA/pF; P<0.05). NAC was without effect on whole-cell K^+ currents (50.8±2.0 versus 52.8±1.7 pA/pF; P>0.05), BK_{Ca} current density (27.8±1.8 versus 29.1±2.0 pA/pF; P>0.05), or SK_{Ca} current density (10.8±1.1 versus 12.5±2.2 pA/pF; P>0.05) in myocytes of normoxic animals. However, NAC significantly increased whole-cell K^+ currents from 42.4±0.8 to 50.4±2.1 pA/pF (P<0.05; Figure 2A) and BK_{Ca} currents from 18.5±1.4 to 26.6±2.4 pA/pF (P<0.05; Figure 2B) in uterine arterial myocytes of hypoxic animals. Consistent with the previous finding,[6] no apamin-sensitive SK_{Ca} current was detected in uterine arterial myocytes of hypoxic animals, and NAC treatment failed to upregulate SK_{Ca} currents (Figure S1 in the online-only Data Supplement).

Endothelium Mediated the NAC-Sensitive Component of SK_{Ca}-Induced Relaxations of Uterine Arteries in Pregnant Sheep

The finding that NAC increased NS309-induced relaxations of uterine arteries from hypoxic animals but had no effect on SK_{Ca} channel current density in uterine arterial smooth muscle cells is intriguing and suggests an endothelium-dependent mechanism in the NAC-sensitive component of SK_{Ca}-mediated relaxation under hypoxic conditions. To test this hypothesis, intact and endothelium-denuded uterine arteries of pregnant sheep were treated ex vivo under normoxic (21% O_2) or hypoxic (10.5% O_2) conditions for 48 hours. After the treatment, NS309-induced relaxations were determined in the absence or presence of NAC

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pretreatment for 20 minutes. Our previous study showed that endothelium removal caused a modest 20% decrease in the maximal response of NS309-induced relaxations in normoxic vessels.[6] As shown in Figure 3A, NAC had no effect on NS309-mediated relaxations in the normoxic condition. Hypoxia significantly decreased the maximal response of NS309-induced relaxations (22.7±2.6% versus 56.0±2.6%; $P<0.05$), and NAC significantly increased NS306-induced relaxations from 22.7±2.6% to 44.8±1.9% ($P<0.05$) in endothelium intact vessels (Figure 3B). In contrast, NAC showed no effect on NS306-induced relaxations in endothelium-denuded uterine arteries (Figure 3C).

Steroid Hormones Enhanced K_{Ca} Channel–Mediated Relaxations of Uterine Arteries From Nonpregnant Sheep

Compared with uterine arteries of pregnant animals (Figure 1A and 1B), both NS1619- and NS309-induced relaxations were significantly attenuated in uterine arteries of nonpregnant animals (Figure 4). Our recent study demonstrated that steroid hormone treatment upregulated BK_{Ca} channel expression and current density in uterine arteries.[4] In the present study, we further examined the functional significance of steroid hormone–mediated effect on K_{Ca} channel–induced relaxations of uterine arteries. As shown in Figure 4A, the hormonal treatment significantly enhanced the potency (pD_2, $-\log EC_{50}$; 5.4±0.3 versus 7.4±0.4; $P<0.05$) and had a tendency to increase the maximal response (12.6±2.1% versus 18.8±2.4%; $P=0.08$) of NS1619-induced relaxations. Both the potency (5.6±0.2 versus 6.4±0.2; $P<0.05$) and the maximal response (10.4±1.0% versus 24.2±2.1%; $P<0.05$) of NS309-induced relaxations were significantly increased by the hormonal treatment (Figure 4B). Consistent with the increased NS309-induced relaxations, the hormonal treatment significantly upregulated the protein abundance of SK3 but not SK2 channels in uterine arteries (Figure 5).

NAC Rescued the Effect of Steroid Hormones on K_{Ca} Channel–Induced Relaxations of Uterine Arteries in Hypoxic Nonpregnant Sheep

Steroid hormones increased K_{Ca} channel–mediated relaxations of uterine arteries in normoxic nonpregnant animals (Figure 4). However, the hormonal treatment had no effect on either NS1619-induced relaxations (E_{max}: 11.2±1.0% versus 12.9±1.1%; $P>0.05$; Figure 6A) or NS309-induced relaxations (E_{max}: 11.0±0.5% versus 10.3±1.4%; $P>0.05$; Figure 6B) of uterine arteries in hypoxic nonpregnant sheep. Inhibition of ROS by NAC rescued the effect of steroid hormones on uterine arteries of hypoxic animals, and both NS1619- and NS309-induced relaxations were significantly increased by the hormonal treatment in the presence of NAC (Figure 6).

NAC Restored the Effect of Steroid Hormones on Upregulation of BK_{Ca} Channel Activities in Uterine Arteries of Hypoxic Nonpregnant Sheep

We further examined K_{Ca} channel activities in uterine arteries from hypoxic nonpregnant sheep treated with steroid hormones in the presence of NAC for 48 hours. Our recent study demonstrated that chronic hypoxia abolished steroid hormone–induced upregulation of BK_{Ca} channel activity in uterine arteries of nonpregnant sheep.[5] The present study showed that in the presence of NAC, the hormonal treatment of uterine arteries from nonpregnant

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animals acclimatized to long-term high-altitude hypoxia was able to increase whole-cell K^+ densities (Figure 7A) and BK_{Ca} current densities significantly (Figure 7B), similar to those seen in normoxic animals in the previous study.[5] In contrast, whole-cell K^+ currents in uterine arteries treated with NAC were not sensitive to SK_{Ca} blocker apamin, and enhanced whole-cell K^+ currents by the hormonal treatment in the presence of NAC were not inhibited by apamin (Figure S2).

Discussion

In the present study, we have demonstrated that the function of both BK_{Ca} and SK_{Ca} channels in uterine arteries of pregnant animals is suppressed by heightened oxidative stress during chronic hypoxia. Our results suggest that chronic hypoxia–induced oxidative stress exerts its adverse effect on K_{Ca} channel–mediated relaxations of uterine arteries through suppressing steroid hormone–induced upregulation of K_{Ca} channel activities. These findings provide strong evidence that heightened ROS is a common mechanism to impair BK_{Ca} and SK_{Ca} channel function in uterine arteries and contributes to the dysfunction of uterine circulation caused by chronic hypoxia during gestation.

Consistent with our previous studies,[6] the present finding that both NS1619- and NS309-induced relaxations of uterine arteries were significantly attenuated by chronic hypoxia in pregnant animals, further suggesting that chronic hypoxia downregulates both BK_{Ca} and SK_{Ca} channel activities. However, the molecular mechanisms underlying chronic hypoxia–mediated downregulation of BK_{Ca} channel– and SK_{Ca} channel–mediated uterine arterial relaxation remain incompletely understood. The present finding that treatment with NAC, an antioxidant to scavenge free radicals,[22] significantly enhanced both NS1619- and NS309-induced relaxations in hypoxic but not normoxic animal suggests that hypoxia-mediated increase in ROS may be one of the key mechanisms in attenuating both BK_{Ca} channel– and SK_{Ca} channel–mediated relaxations of uterine arteries during gestation. ROS play an important role in pathogenesis of cardiovascular dysfunctions.[14,15] Increased ROS have been shown to inhibit BK_{Ca} channel activity.[16,23–25] We recently demonstrated that gestational hypoxia increased ROS production in uterine arteries.[17] Thus, heightened ROS likely attribute to the suppressed function of BK_{Ca} and SK_{Ca} channels in uterine arteries from pregnant sheep by chronic hypoxia. In the present study, our finding that NAC partially reversed the impairment of BK_{Ca} channel activity suggests that ROS may directly alter BK_{Ca} channel activity, resulting in the regulation of BK_{Ca}-mediated uterine vascular contractility in response to hypoxia exposure. These observations are consistent with previous findings that the nicotinamide adenine dinucleotide phosphate oxidase inhibitor apocynin partially reversed the suppression of BK_{Ca} channel function by chronic hypoxia.[17] Other studies in insulin resistant rats showed that impaired BK_{Ca} channel–mediated relaxations of cerebral arteries were restored by superoxide dismutase plus catalase that catalyze the breakdown of free radicals.[26]

A novel and interesting finding in the present study is that heightened ROS regulate SK_{Ca} channel–mediated uterine vascular relaxations but without alteration of SK_{Ca} channel activity. Our data indicated that hypoxia-mediated impairment of SK_{Ca} channel–mediated relaxations of uterine arteries were alleviated by NAC. However, NAC treatment failed to

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restore SK_{Ca} channel current density in vascular smooth muscle cells of uterine arteries. It is likely that the NAC enhanced SK_{Ca}-mediated relaxations of uterine arteries via an endothelium-dependent mechanism. SK_{Ca} channels are present in both vascular smooth muscle cells and endothelial cells of uterine arteries.[6] The finding that the effect of NAC on NS309-mediated relaxations was lost in endothelium-denuded uterine arteries under the hypoxic condition suggests an endothelium-dependent mechanism in the NAC-sensitive component of SK_{Ca}-mediated relaxations, possibly by releasing vasodilators (eg, nitric oxide or prostacyclin) and transmitting hyperpolarization into vascular smooth muscle through myoendothelial gap junctions.[3] Given the finding that the increased blood pressure in the rat model with reduced uterine perfusion pressure was alleviated by NAC,[27] the upregulation of K_{Ca} function by NAC may have therapeutic implications.

Whereas 48 hours of estrogen plus progesterone treatment is a short duration compared with the long duration of estrogen and progesterone being exposed to the blood vessels in vivo during gestation, our previous studies demonstrated that this ex vivo hormonal treatment increased BK_{Ca} channel activity and decreased uterine arterial myogenic tone.[4,5,18–20] We have demonstrated that BK_{Ca} channel function is directly regulated by estrogen and progesterone through a genomic effect.[4] Similarly, 17β-estradiol and progesterone also selectively upregulated the expression of SK_{Ca} type 3 channels in uterine arteries. This finding mimicked enhanced SK_{Ca} channel activity seen in pregnant uterine arteries form normoxic animals.[6] The lack of hormonal effect on SK2 channels is intriguing, but the mechanisms remain unknown at present. Both estrogen receptors α (ER-α) and β (ER-β) are present in the uterine artery.[18,28] Estrogen has been shown to control SK_{Ca} channel expression in human myometrial cells via the specificity protein family of transcription factors.[29] Although this significant, albeit small, change in SK3 channel expression may contribute to the channel function, the finding of the acute effect of NAC in the present study suggests that chronic hypoxia (either in vivo or ex vivo for 48 hours)–mediated ROS persist and predominately affect K_{Ca} channel activities in the uterine arteries.

In contrast to the upregulation of K_{Ca} channel activities and K_{Ca} channel–mediated relaxations in uterine arteries of normoxic animals by estrogen and progesterone, the effect of steroid hormones on the regulation of K_{Ca} channel activities and their function of inducing relaxations was diminished in hypoxic animals. These findings suggest that hypoxia-mediated downregulation of K_{Ca} channel activities and function may be regulated through steroid hormone–mediated signaling. These observations are not surprising because ER-α receptor is downregulated during gestational hypoxia via increased methylation of the receptor gene.[18,19] Thus, it is possible that downregulation of ER-α may play a role in reduced K_{Ca} channel activities.

Of interest, the present study demonstrates that the hormonal treatment in the presence of NAC restored K_{Ca} channel–mediated relaxations of uterine arteries from hypoxic animals, suggesting that increased oxidative stress causing by chronic hypoxia diminishes the effect of steroid hormones to regulate of K_{Ca} channel activities. ROS has been shown to induce post-translational modifications of ER-α, leading to ER-α downregulation in human breast cancer cells.[30] Scavenging free radicals by NAC removed inhibitory effects of ROS, allowing steroid hormones to upregulate K_{Ca} channel function. Correspondingly, BK_{Ca}

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channel activity in uterine arterial smooth muscle cells was also restored by NAC with the hormonal treatment. Although cotreatment of uterine arteries from hypoxic animals with steroid hormones and NAC failed to upregulate SK_{Ca} channel activity in vascular smooth muscle cells, it remains possible to upregulate SK_{Ca} channel function in endothelial cells, which in turn results in enhanced SK_{Ca} channel–mediated relaxations via the interaction of endothelial and smooth muscle cells of uterine arteries as aforementioned. It has been shown that NAC supplement improves human coronary and peripheral endothelium-dependent vasodilation.[31]

In conclusion, the results suggest that heightened ROS production by chronic hypoxia attenuates steroid hormone–mediated signaling, which leads to downregulation of K_{Ca} channel activities and results in decreased relaxations of uterine arteries during gestation. The attenuation of K_{Ca} channel–mediated relaxations may contribute to enhanced uterine vascular tone and increased incidence of preeclampsia and fetal intrauterine growth restriction associated with maternal hypoxia.

Perspectives

The present study demonstrates a novel mechanism that hypoxia-mediated heightened ROS attenuates K_{Ca} channel activities and their mediated uterine vascular relaxation in pregnancy. Given that K_{Ca} channels play an important role in regulating vascular tone and thus blood flow and pressure, dysregulation of K_{Ca} channel function in the uterine artery may contribute significantly to maladaptation of uterine vascular hemodynamics in pregnancy, leading to an increased risk of preeclampsia. Reductions in uteroplacental blood flow and chronic uteroplacental ischemia in a variety of animal models lead to a hypertensive state that closely resembles preeclampsia in women. The present findings provide a mechanistic understanding of dysfunction of K_{Ca} channels in uterine vascular adaptation to pregnancy in chronic hypoxia and may suggest new insights of therapeutic strategies by enhancing the K_{Ca} channel activities in vascular smooth muscle, which may be beneficial for pregnant women with preeclampsia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What Is New?

- Chronic hypoxia during gestation attenuates both large conductance Ca^{2+}-activated K^+ (K_{Ca}) and small conductance K_{Ca} channel mediated relaxations of uterine arteries.
- Increased reactive oxygen species production contributes to hypoxia-induced attenuation of K_{Ca} channel function in uterine arteries during pregnancy.
- Gestational hypoxia inhibits steroid hormonal regulation of K_{Ca} channel activities and their mediated uterine vascular relaxations via reactive oxygen species signaling.

What Is Relevant?

- Chronic hypoxia during pregnancy is one of the most common factors in increasing uterine vascular tone and decreasing uterine blood flow.
- K_{Ca} channels play an important role in maintaining vascular tone. Chronic hypoxia–mediated maladaptation of K_{Ca} channel function may be a key mechanism in aberrant uteroplacental circulation and fetal growth restriction.

Summary

The present study demonstrates a novel role of heighted oxidative stress on sex steroid hormone–mediated K_{Ca} channel function in uterine arterial smooth muscle in pregnancy acclimated to high-altitude hypoxia. Understanding of the interaction of reactive oxygen species and steroid hormones in the regulation of K_{Ca} channel function will help improve our understanding of pathophysiological mechanisms underlying maladaptation of the uteroplacental circulation and pregnancy complications including preeclampsia and fetal growth restriction associated with chronic hypoxia during gestation.

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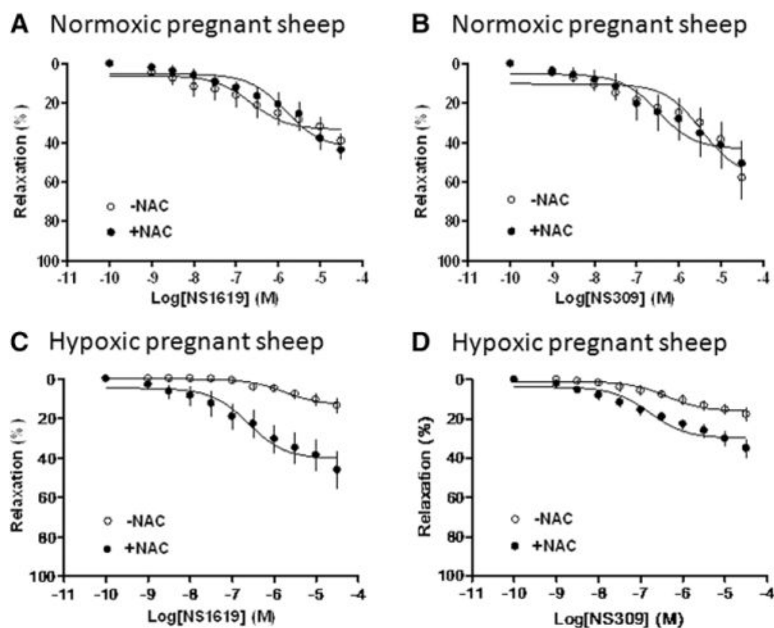

Figure 1.

Effect of N-acetylcysteine (NAC) on Ca^{2+}-activated K^+ channel–induced relaxations of uterine arteries from pregnant sheep. Uterine arteries were isolated from normoxic (**A** and **B**) and hypoxic (**C** and **D**) pregnant sheep. Concentration–response curves were determined in NS1619-induced relaxations and NS309-induced relaxations in the absence or presence of NAC (1 mmol/L). The *x* axis indicates cumulative increases of the agonists in approximately one-half log increments. Data are means±SEM from 4 to 5 animals in each group. The E_{max} values are presented in the text.

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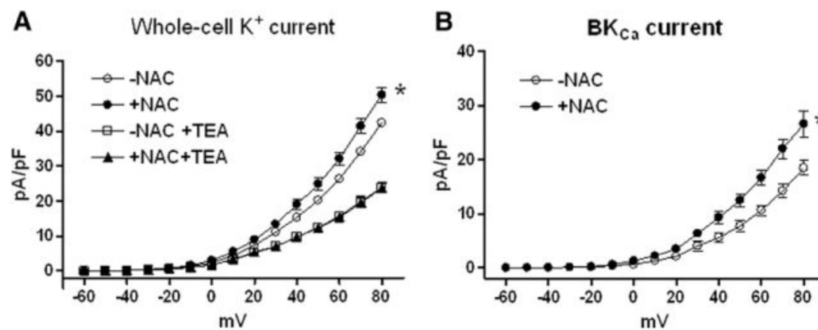

Figure 2.

Effect of N-acetylcysteine (NAC) on large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel current density in myocytes of uterine arteries from hypoxic pregnant sheep. **A**, Whole-cell K^+ currents were recorded in the absence or presence of NAC (1 mmol/L) and tetraethylammonium (TEA, 1 mmol/L). **B**, BK_{Ca} currents were determined as the difference between the whole-cell K^+ current in the absence of TEA and that in the presence of TEA. The K^+ currents were normalized to cell capacitance and were expressed as picoampere per picofarad (pA/pF; *y* axis), as a function of stepwise 10-mV depolarizing pulses (*x* axis). Data are means±SEM of cells from 5 animals of each group (*P<0.05; +NAC vs −NAC).

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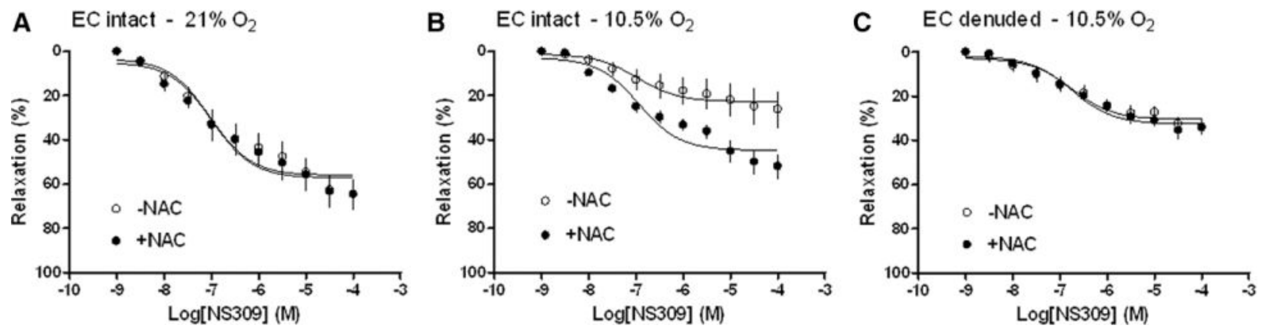

Figure 3.

Effect of endothelium on N-acetylcysteine (NAC)–mediated response in NS309-induced relaxations. Uterine arteries were isolated from pregnant sheep. Endothelium (EC) intact and denuded vessels were treated ex vivo under 21% O_2 (**A**) or 10.5% O_2 (**B** and **C**) for 48 hours. Concentration–response curves were determined in NS309-induced relaxations in the absence or presence of NAC (1 mmol/L). The *x* axis indicates cumulative increases of the agonist in approximately one-half log increments. Data are means±SEM from 5 animals in each group. The E_{max} values are presented in the text.

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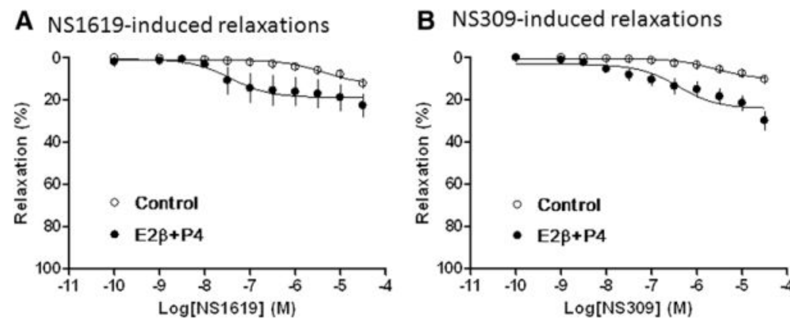

Figure 4.

Effect of hormonal treatment on Ca^{2+}-activated K^+ channel–induced relaxations of uterine arteries from normoxic nonpregnant sheep. Uterine arteries were isolated from normoxic nonpregnant sheep and treated ex vivo with 17β-estradiol ($E_2β$; 0.3 nmol/L) plus progesterone (P4; 100 nmol/L) under 21% O_2 for 48 hours. Concentration–response curves were determined in NS1619-induced relaxations (**A**) and NS309-induced relaxations (**B**) in uterine arteries treated without (control) or with steroid hormones ($E_2β$+P4). The *x* axis indicates cumulative increases of the agonists in approximately one-half log increments. Data are means±SEM from 4 to 6 animals in each group. The E_{max} and pD_2 values are presented in the text.

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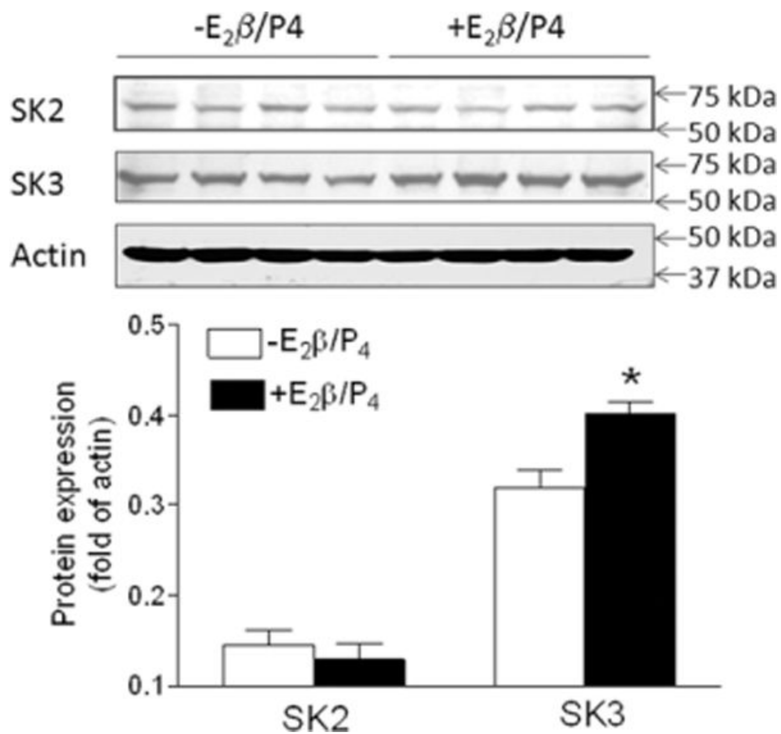

Figure 5.

Effect of steroid hormones on small conductance calcium–activated K+ channel (SK)2 and SK3 protein abundance in uterine arteries from normoxic nonpregnant sheep. Uterine arteries were isolated from normoxic nonpregnant sheep and treated ex vivo with 17β-estradiol ($E_2β$; 0.3 nmol/L) plus progesterone (P_4; 100 nmol/L) under 21% O_2 for 48 hours. Protein abundance of SK2 and SK3 was determined by Western blot analyses. Data are means±SEM of tissues from 4 animals of each group (*P<0.05; +$E_2β/P_4$ vs −$E_2β/P_4$).

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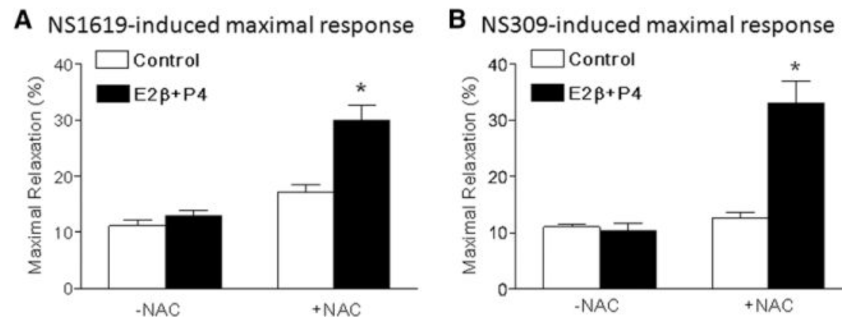

Figure 6.

Effect of N-acetylcysteine (NAC) on steroid hormone–mediated response in K_{Ca} channel–induced relaxations of uterine arteries from hypoxic nonpregnant sheep. Uterine arteries were isolated from hypoxic nonpregnant sheep and treated ex vivo with 17β-estradiol ($E_2\beta$; 0.3 nmol/L) plus progesterone (P_4; 100 nmol/L) under 10.5% O_2 for 48 hours in the absence and presence of NAC (1 mmol/L). NS1619-and NS309-induced relaxations were determined after the treatment. **A**, NS1619-induced maximal response. **B**, NS309-induced maximal response. Data are means±SEM from 4 to 6 animals in each group (*P<0.05; $E_2\beta$+P_4 vs control).

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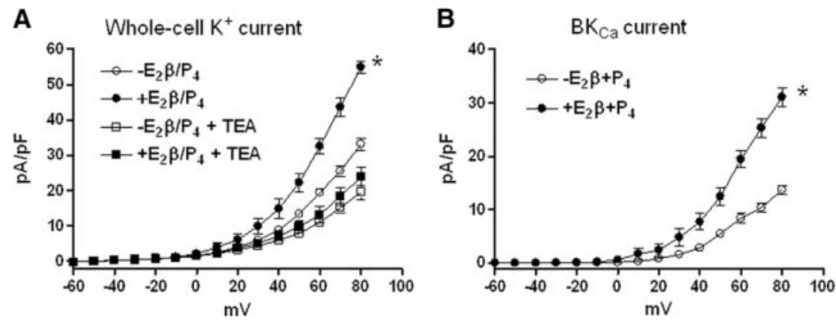

Figure 7.

Effect of N-acetylcysteine (NAC) on steroid hormone–mediated response in large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel current density in uterine arteries from hypoxic nonpregnant sheep. Uterine arteries were isolated from hypoxic nonpregnant sheep and treated ex vivo with 17β-estradiol ($E_2β$; 0.3 nmol/L) plus progesterone (P_4; 100 nmol/L) under 10.5% O_2 for 48 hours in the presence of NAC (1 mmol/L). After the treatment, whole-cell K^+ currents were recorded in the absence or presence of tetraethylammonium (TEA, 1 mmol/L) in myocytes isolated from uterine arteries. The K^+ currents were normalized to cell capacitance and were expressed as picoampere per picofarad (pA/Pf; *y* axis), as a function of stepwise 10-mV depolarizing pulses (*x* axis). **A**, Whole-cell K^+ currents. **B**, BK_{Ca} currents were determined as the difference between the whole-cell K^+ current in the absence of TEA and that in the presence of TEA. Data are means±SEM of cells from 5 animals of each group (*$P<0.05$; $+E_2β/P_4$ vs $-E_2β/P_4$).