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Chronic Hypoxia Inhibits Pregnancy-Induced Upregulation of SK_{Ca} Channel Expression and Function in Uterine Arteries

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Summary

Small conductance Ca²⁺-activated K⁺ (SK_{Ca}) channels are crucial in regulating vascular tone and blood pressure. The present study tested the hypothesis that SK_{Ca} channels play an important role in uterine vascular adaptation in pregnancy, which is inhibited by chronic hypoxia during gestation. 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. Immunohistochemistry revealed the presence of SK_{Ca} channels type 2 (SK2) and type 3 (SK3) in both smooth muscle and endothelium of uterine arteries. The expression of SK2 and SK3 channels was significantly increased during pregnancy, which was inhibited by chronic hypoxia. In normoxic animals, both SK_{Ca} channel opener NS309 and a large-conductance (BK_{Ca}) channel opener NS1619 relaxed norepinephrine-contracted uterine arteries in pregnant but not nonpregnant sheep. These relaxations were inhibited by selective SK_{Ca} and BK_{Ca} channel blockers, respectively. NS309-induced relaxation was largely endothelium-independent. In high altitude hypoxic animals, neither NS1691 nor NS309 produced significant relaxation of uterine arteries in either nonpregnant or pregnant sheep. Similarly, the role of SK_{Ca} channels in regulating myogenic reactivity of uterine arteries in pregnant animals was abrogated by chronic hypoxia. Accordingly, the enhanced SK_{Ca} channel activity in uterine arterial myocytes of pregnant animals was ablated by chronic hypoxia. The findings suggest a novel mechanism of SK_{Ca} channels in regulating myogenic adaptation of uterine arteries in pregnancy, and in the maladaptation of uteroplacental circulation caused by chronic hypoxia during gestation.

Keywords

Hypoxia; uterine artery; pregnancy; SK_{Ca} channel; myogenic tone; relaxation

Introduction

Vascular tone constitutes the major determinant of the resistance of blood vessels, which regulates blood pressure and the distribution of blood flow among and within tissues and organs. Ca²⁺-activated K⁺ (K_{Ca}) channels contribute significantly to setting the membrane potential, and play a critical role in regulating excitability of vascular smooth muscle cells (VSMCs).^{1, 2} Based on the conductance, K_{Ca} channels are divided into large-conductance (BK_{Ca}), intermediate-conductance (IK_{Ca}), and small-conductance (SK_{Ca}) channels.³ K_{Ca}

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channels have distinct distributions in the vasculature. SK_{Ca} channels are believed to be expressed predominantly in endothelial cells,^{2, 4} and hyperpolarization produced by the activation of SK_{Ca} in endothelial cells may be transmitted to VSMCs *via* the myoendothelial gap junction.^{5, 6}

During pregnancy, uterine blood flow increases substantially to optimize the supply of oxygen and nutrients to the developing fetus *via* the placenta. Chiefly, this is achieved by adaptive changes such as remodeling of the uterine vasculature,⁷ reduced pressure-dependent myogenic reactivity,^{8–10} blunted vasoconstrictor response,^{11–13} and enhanced vasodilator response and vasodilator production.^{14–16} Chronic hypoxia during gestation has profound adverse effects on the normal adaptation of uteroplacental circulation to pregnancy,^{17–21} leading to a 2 to 4-fold increase in the incidence of preeclampsia and fetal intrauterine growth restriction.^{20–24}

Previous studies have demonstrated that BK_{Ca} channels participate in the regulation of vascular tone and uterine blood flow during pregnancy.^{25–27} Upregulated expression of the BK_{Ca} channel $\alpha 1$ subunit and enhanced BK_{Ca} channel activity contribute to the attenuated myogenic tone of uterine arteries during pregnancy.¹⁰ Chronic hypoxia during gestation inhibited pregnancy-induced upregulation of BK_{Ca} channel function in uterine arteries by selectively targeting the $\alpha 1$ subunit.¹⁹ Although SK_{Ca} channels are predominantly expressed in endothelial cells,² apamin-sensitive K^+ currents and positive staining of SK_{Ca} channels have been detected in VSMCs of various vascular beds.^{28–31} Functional roles of SK_{Ca} channels in VSMCs remain elusive. SK_{Ca} channels are also expressed in the myometrium and are regulated by estrogen during pregnancy.³² Of interest, SK_{Ca} channels are subject to regulation by oxygen.^{33, 34} However, the role of SK_{Ca} channels in the regulation of uterine circulation under physiological and pathophysiological conditions such as pregnancy and chronic hypoxia is unclear. In the present study, we tested hypotheses that SK_{Ca} channels play an important role in regulating the contractility of uterine arteries during pregnancy; and that chronic hypoxia during gestation impairs this regulation. To test these hypotheses, we first determined whether SK_{Ca} channels were expressed in the uterine vasculature and how pregnancy and chronic hypoxia regulated their expression. We then determined SK_{Ca} -mediated relaxations of uterine arteries and their regulation by pregnancy and chronic hypoxia. Furthermore, we measured the SK_{Ca} channel activity in uterine vascular smooth muscle cells using patch-clamp analysis to see whether pregnancy and chronic hypoxia altered their activities. In addition, we determined the role of SK_{Ca} channels in pressure-dependent myogenic tone of uterine arteries and its regulation by pregnancy and chronic hypoxia.

Materials and Methods

An expanded Methods section is available in the online data supplement at <http://hyper.ahajournals.org>.

Tissue preparation and treatment

Uterine arteries were harvested from nonpregnant and near-term (142–145 days of gestation, the term is about 150 days) pregnant sheep maintained at sea level (~300 m) or exposed to high-altitude (3801 m) hypoxia for 110 days.¹⁸ All of the procedures and protocols were approved by the Institutional Animal Care and Use Committee guidelines.

²Disclosures: None.

Western immunoblotting

Protein abundance of SK_{Ca} type 2 (SK2) and type 3 (SK3) channels was measured in freshly isolated uterine arteries, as described previously.¹⁰

Immunohistochemistry

Uterine arteries were fixed in 10% neutral buffered formalin and embedded in paraffin. Immunohistochemical detection of SK2, SK3, and eNOS was performed using the Anti-Ig HRP Detection Kit (BD Biosciences PharMingen, San Diego, CA) as described previously.^{35, 36}

Contraction studies

Fourth-generation branches of the main uterine arteries from nonpregnant and pregnant sheep were separated from the surrounding tissue, and cut into 2-mm ring segments. Isometric tension was measured in the Krebs solution in a tissue bath at 37°C, as described previously.^{37, 38}

Measurement of SK_{Ca} channel current

Arterial smooth muscle cells were dissociated enzymatically 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.¹⁰

Measurement of myogenic tone

Pressure-dependent myogenic tone of resistance-sized uterine arteries was measured as described previously.^{10, 18, 39}

Data analysis

Data are 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.

Results

Effect of pregnancy and chronic hypoxia on SK_{Ca} channel expression

Protein abundance of both SK2 and SK3 channels was significantly greater in uterine arteries of pregnant sheep than that in nonpregnant animals (Figure 1A). Chronic hypoxia during gestation significantly decreased the expression of SK2 and SK3 channels in uterine arteries of pregnant animals (Figure 1B).

Effect of pregnancy and chronic hypoxia on SK_{Ca} channel-mediated relaxation

The effect of SK_{Ca}/IK_{Ca} channels on contractility of uterine arteries was examined by exposing norepinephrine-contracted arteries to NS309 (Figure 2). In normoxic animals, NS309 had no effect on uterine artery relaxation in nonpregnant sheep, but produced concentration-dependent relaxations of uterine arteries in pregnant animals, with a maximal relaxation of $64.3 \pm 7.5\%$ (Figure 2A). As shown in Figure 2B, blocking of BK_{Ca} channels with IBTX or blocking of BK_{Ca}/IK_{Ca} channels with CTX had no significant effect on NS309-induced relaxation, but the SK_{Ca} channel blocker apamin significantly inhibited NS309-mediated relaxation, suggesting that NS 309-induced relaxation was conferred chiefly by SK_{Ca} channel activation. Long-term, high altitude hypoxia did not change NS309's effect in nonpregnant sheep, but abrogated NS309-mediated relaxations of uterine

arteries in pregnant animals (Figure 2C). Similarly, the BK_{Ca} channel activator NS1619-induced relaxations were significantly increased in uterine arteries of pregnant sheep, which was inhibited by chronic hypoxia (Online Figure S1).

Involvement of smooth muscle cells in SK_{Ca} channel-mediated relaxations

Given that SK_{Ca} channels are expressed in endothelial cells, we determined NS309-induced relaxation in endothelium-intact and -denuded uterine arteries. The validity of endothelium removal was confirmed by the absence of eNOS in immunohistochemical staining (Figure 3A). As shown in Figure 3B, endothelial removal did not significantly alter NS309-induced relaxation of uterine arteries (pD₂: endothelium-intact: 6.5 ± 0.3 ; endothelium-denuded: 6.6 ± 0.2 ; Emax: endothelium-intact: $64.3 \pm 7.5\%$; endothelium-denuded: $51.3 \pm 11.4\%$, $P > 0.05$). This suggests that NS309-induced relaxation of uterine arteries was mediated mainly by SK_{Ca} channels in vascular smooth muscle cells. Immunohistochemical staining revealed that both SK2 and SK3 channels were expressed in endothelial as well as smooth muscle cells in the uterine artery (Figure 3C).

Chronic hypoxia inhibited SK_{Ca} channel activity in uterine arteries

To determine the effect of pregnancy and chronic hypoxia on SK_{Ca} channel activity in uterine arterial smooth muscle cells, whole-cell K⁺ currents were recorded in the absence or presence of apamin or NS309 in myocytes freshly isolated from uterine arteries of normoxic control and hypoxic animals. As shown in normoxic pregnant sheep, apamin significantly reduced whole-cell K⁺ currents (from 60.7 ± 2.7 pA/pF to 49.6 ± 2.3 pA/pF at +80 mV, $P < 0.05$) in uterine arterial myocytes (Figure 4A). In contrast, in myocytes of hypoxic animals, apamin was without effect on whole-cell K⁺ currents (Figure 4B). Similarly, NS309 significantly enhanced whole-cell K⁺ currents in myocytes of normoxic pregnant animals (from 57.5 ± 1.3 pA/pF to 72.4 ± 2.9 pA/pF at +80 mV, $P < 0.05$) (Figure 4C) but not in hypoxic animals (Figure 4D). Neither apamin nor NS 309 altered whole-cell K⁺ currents in uterine arterial myocytes of nonpregnant sheep in either normoxic or hypoxic animals (data not shown).

Effect of pregnancy and chronic hypoxia on uterine artery SK_{Ca} channel-mediated myogenic tone

As reported previously, pressure-dependent myogenic tone of uterine arteries was significantly reduced in pregnant sheep in normoxic control animals (Figure 5A and 5B). Blockade of SK_{Ca} channels with apamin had no significant effect on pressure-dependent myogenic reactivity in uterine arteries of nonpregnant animals (Figure 5A), but resulted in a significant increase in myogenic tone in uterine arteries of pregnant animals (Figure 5B). In the presence of apamin, there was no significant difference in myogenic tone of uterine arteries between nonpregnant and pregnant animals (Figure 5A and 5B). In hypoxic animals, apamin had no significant effect on pressure-dependent myogenic tone of uterine arteries in either nonpregnant or pregnant animals (Figure 5C and 5D).

Discussion

In the present study, we have demonstrated for the first time the expression and function of SK_{Ca} channels in uterine arteries. The capacity of activation of SK_{Ca} channels to relax uterine arteries was markedly increased during pregnancy. Additionally, the SK_{Ca} channel blocker apamin significantly increased pressure-dependent myogenic tone in uterine arteries of pregnant sheep and blunted the difference in the myogenic response of uterine arteries between nonpregnant and pregnant animals. These pregnancy-induced changes were accompanied by increased expression of both SK2 and SK3 channels. Consistently, we detected increased activities of SK_{Ca} channels in uterine artery smooth muscle cells of

pregnant sheep. The concurrence of those findings suggests that pregnancy-induced upregulation of expression and activity of SK_{Ca} channels contributes to the reduced myogenic reactivity and vascular contractility of uterine arteries during gestation. Decreased myogenic tone and increased vasorelaxing responses of uterine arteries have been implicated in the increase in uterine blood flow during pregnancy.^{8–10, 14–16} Hence, our observations provide a novel mechanism of upregulation and heightened activity of SK_{Ca} channels in the adaptation of uteroplacental circulation during pregnancy. Furthermore, the up-regulation of SK3 channels appears to have a role in remodeling of the uterine vasculature. A recent study demonstrated that uterine arteries from nonpregnant transgenic SK3^{T/T} mice that overexpress SK3 channels had larger basal diameters and blunted vasoconstrictor response compared to those from wild-type animals,⁴⁰ although the expression of SK3 channels in uterine arteries was not determined.

At present, the mechanisms responsible for upregulating expression and function of SK_{Ca} channels in uterine arteries during pregnancy are not clear. It is conceivable that sex steroid hormones may contribute to this regulation. Activation of estrogen receptors may alter gene transcription, which has a profound impact on cardiovascular function.⁴¹ Pregnancy up-regulates the expression of estrogen receptor and in uterine arteries.^{18,42} Moreover, we recently demonstrated the 17 β -estradiol-mediated increase in expression and heightened activity of BK_{Ca} channels in uterine arteries of pregnant sheep.¹⁰ Similarly, SK3 channel expression also was regulated by 17 β -estradiol in recombinant expression system,⁴³ hypothalamus,⁴⁴ and myometrium.³²

Although SK_{Ca} channels were expressed in uterine arteries of nonpregnant animals, the channel activity was not detected with an electrophysiological approach. Moreover, it appeared that these channels did not participate in regulating myogenic tone and contractility of uterine arteries in nonpregnant animals. One possibility is that in nonpregnant animals SK_{Ca} channels are not expressed in the cell membrane of uterine arteries smooth muscle, but rather are retained inside cells. The incapability of those channels to insert into membrane would prevent them from being activated. It is also possible that in nonpregnant animals despite being present in the myocyte membrane, the efficacy of these channels may be too low to be functional. Similar findings have been reported for both IK_{Ca} and BK_{Ca} channels. Although IK_{Ca} channels were stained at both the plasma membrane and within the cytoplasm,³⁰ the selective IK_{Ca} channel blocker TRAM-34 was unable to alter vascular tone of cerebral arteries.⁴⁵ Similarly, BK_{Ca} channels in uterine arteries did not participate in the regulation of relaxation (the present study), myogenic tone, vascular resistance and blood flow in nonpregnant animals.^{10, 46}

Initially, SK_{Ca} channels were detected in endothelial cells, but not in VSMCs of SK3^{T/T} mice.⁴⁷ The regulatory role of SK_{Ca} channels on vascular function is thought to be mediated exclusively by the endothelium. This notion was supported by the finding that a genetic deficit of SK3 and IK1 channels caused hypertension by abolishing endothelium-derived hyperpolarizing factor-mediated vasodilation.⁴⁸ In the present study, immunostaining demonstrated the expression of SK2 and SK3 channels in both vascular smooth muscle and endothelial cells in uterine arteries. The functional presence of SK_{Ca} channels in uterine arterial smooth muscle cells was confirmed with electrophysiological technique; and a selective SK_{Ca} channel blocker apamin decreased whole-cell K⁺ currents by ~20%. This is in agreement with the previous findings in myocytes of rabbit aorta²⁹ and rat myometrium³⁶ that apamin reduced whole-cell K⁺ currents by about 20%. Furthermore, previous studies also have shown the presence of SK_{Ca} channels in other visceral and vascular smooth muscle cells by immunohistochemistry,^{31, 49, 50} although the functional roles of these channels are not known. In uterine arteries, NS309-induced relaxation was largely endothelium-independent, suggesting that SK_{Ca} channels in vascular smooth muscle

mediated mainly NS309-induced vasorelaxation. To our knowledge, the present study is the first to demonstrate that SK_{Ca} channels in vascular smooth muscle significantly contribute to the regulation of vascular contractility and tone in this vascular bed. Lines of evidence have also implicated SK_{Ca} channels in regulating excitability and contraction of smooth muscle cells from the uterus and urinary bladder, which are highly responsive to sex steroids and in particular estrogen.^{51–53} Our findings thus provide a novel mechanism of SK_{Ca} channels in regulating vascular tone and cardiovascular function.

Previously, chronic hypoxia has been found to abrogate the capacity of BK_{Ca} in regulating myogenic reactivity of uterine arteries in pregnant sheep.¹⁹ The present findings of diminishment of vasodilator response to NS309 and failure of apamin to alter myogenic tone of uterine arteries in pregnant animals exposed to long-term high altitude hypoxia suggest that chronic hypoxia resulted in a loss of the regulatory role of SK_{Ca} channels in vascular smooth muscle excitability and contractility. Hence, the nullification of the regulatory role of K_{Ca} channels may attribute to chronic hypoxia-induced reduction in uterine blood flow in pregnancy.^{20, 21} Our data also suggest that the loss of regulatory role of SK_{Ca} channels in uterine arteries of pregnant animals resulted chiefly from reduced channel activities due to suppressed expression of these channels. Similar findings were obtained for BK_{Ca} channels in uterine arteries of pregnant sheep¹⁹ and IK_{Ca} channels in pulmonary arteries from animals exposed to chronic hypoxia.³⁴ The effect of chronic hypoxia seems to be specific for K_{Ca} channels, as voltage-gated K⁺ (K_V) channels were largely unaffected.¹⁹ Taken together, experimental evidence suggests that targeted suppression of K_{Ca} channels is a major mechanism to alter uterine vascular function by chronic hypoxia and the uterine arteries from chronic hypoxic animals are losing their adaptation to pregnancy. This may account for the increased incidence of preeclampsia and fetal intrauterine growth restriction associated with chronic hypoxia exposure during gestation. Estrogens have been shown to regulate expression of BK_{Ca}^{10, 54} and SK_{Ca}^{32, 43} channels. Ablation of pregnancy-induced upregulation of SK_{Ca} and BK_{Ca} channels in uterine arteries by chronic hypoxia during gestation likely occurred at the genomic level. Expression of estrogen receptor in uterine arteries during gestation, but not plasma estrogen levels, was significantly depressed by chronic hypoxia¹⁸ due to heightened promoter methylation.⁵⁵ It is possible that chronic hypoxia-mediated suppression of estrogen receptor expression led to abrogation of upregulation of K_{Ca} channels in uterine arteries during pregnancy. However, Jobe et al has shown there are numerous types of estrogens and estrogen metabolites that are decreased in preeclampsia.⁵⁶ Therefore, the regulation of K_{Ca} channels by estrogens in VSMCs likely has a significant role in physiological and pathophysiological conditions.

Perspectives

The striking increase of uterine blood flow during pregnancy is essential both for optimal growth and survival of the fetus and for cardiovascular wellbeing of the mother. Maladaptation of the uteroplacental circulation during pregnancy is associated with a high incidence of clinical complications including preeclampsia and fetal intrauterine growth restriction. Thus, a comprehensive understanding of regulatory mechanisms of uterine vascular adaptation in pregnancy has long been sought, but has not been achieved. The present study demonstrates a novel mechanism of SK_{Ca} channels in uterine arterial smooth muscle, and thus has a major impact in advancing our knowledge in the molecular mechanisms of uterine vascular adaptation to pregnancy. This will help to improve our understanding of the pathophysiological mechanisms underlying maladaptation of uteroplacental circulation and pregnancy complications including preeclampsia and fetal growth restriction associated with chronic hypoxia during gestation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

What Is New?

Expression and activity of SK_{Ca} channels in uterine arteries are upregulated during pregnancy.

Chronic hypoxia during gestation inhibits pregnancy-induced upregulation of SK_{Ca} channels in uterine arteries.

Blunted SK_{Ca} channel function results in an increase in uterine arterial contractility and myogenic reactivity.

What Is Relevant?

The present study identifies a novel mechanism of SK_{Ca} in regulating myogenic adaptation of uterine arteries in pregnancy and in the maladaptation of uteroplacental circulation caused by chronic hypoxia during gestation.

Summary

The present study demonstrates a novel mechanism of SK_{Ca} channels in uterine arterial smooth muscle, and thus has a major impact in advancing our knowledge in molecular mechanisms of uterine vascular adaptation to pregnancy and in improving our understanding of pathophysiological mechanisms underlying maladaptation of uteroplacental circulation and pregnancy complications including preeclampsia and fetal growth restriction associated with chronic hypoxia during gestation.

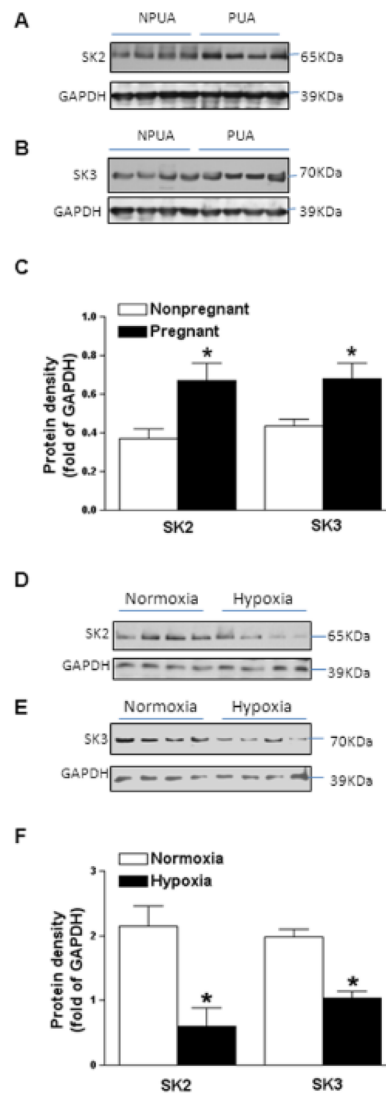


Figure 1. Effect of pregnancy and chronic hypoxia on SK_{Ca} channel expression

Protein abundance of SK2 and SK3 channels were determined by Western blot analyses in uterine arteries of normoxic nonpregnant (NPUA) and pregnant (PUA) animals (Panels A, B, C), and uterine arteries of normoxic and high altitude hypoxic pregnant animals (Panels D, E, F). Data are means \pm SEM of tissues from 4–6 animals of each group, * $P < 0.05$.

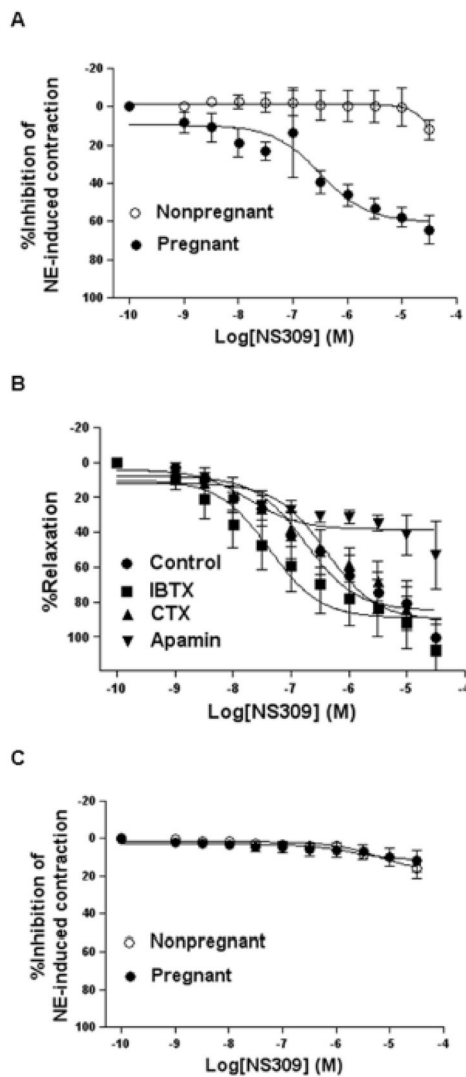


Figure 2. Concentration-response curves of NS309-induced relaxation

Uterine arteries were contracted with norepinephrine (NE, 3 $\mu\text{mol/L}$) and followed by additions of NS309. **A.** Normoxic animals. **B.** Normoxic pregnant animals in the absence (control) or presence of iberitoxin (IBTX, 100 nmol/L), charybdotoxin (CTX, 70 nmol/L), or apamin (500 nmol/L). **C.** High altitude hypoxic animals. Data are means \pm SEM of tissues from 5–6 animals in each group.

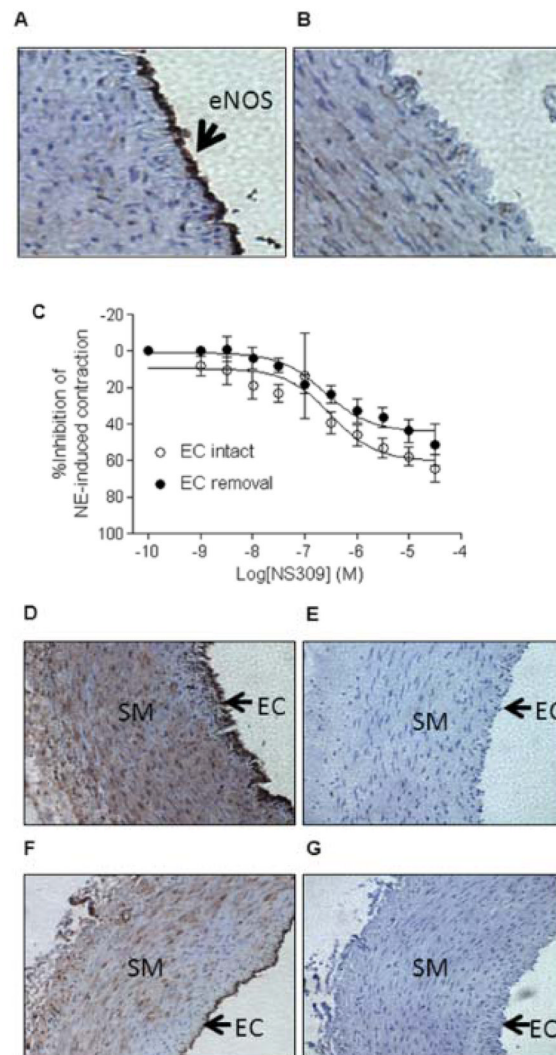


Figure 3. Effect of endothelium on NS309-induced relaxation

Immunoreactivity of eNOS was present in endothelium-intact arteries (Panel A) but absent in endothelium-denuded arteries (Panel B), demonstrating the effectiveness of endothelium removal. C. NS309-induced relaxation of norepinephrine (NE, 3 $\mu\text{mol/L}$)-contracted pregnant uterine arteries with (EC intact) or without (EC removal) endothelium. Data are means \pm SEM of tissues from 4–6 animals in each group. Immunoreactivity of SK2 (Panel D) and SK3 (Panel F) channels in endothelium (EC) and vascular smooth muscle (SM) of pregnant uterine arteries. Panels E and G show negative controls of SK2 and SK3 staining.

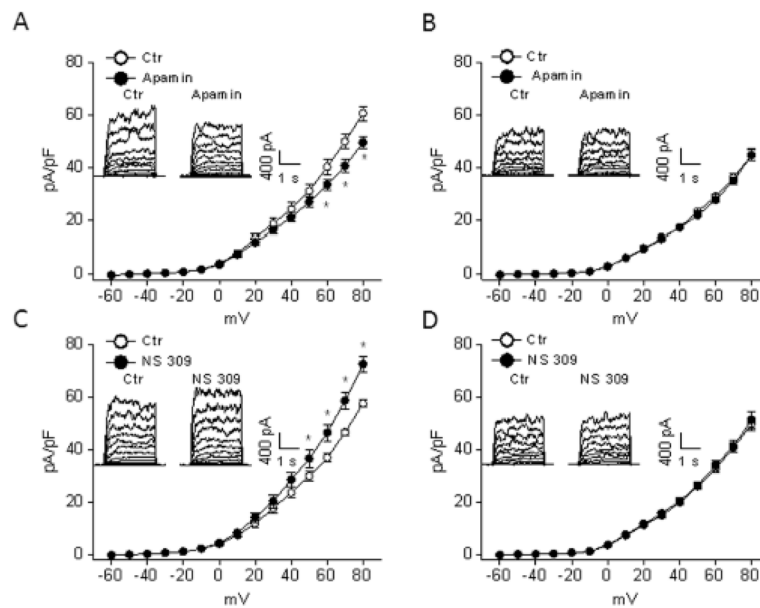


Figure 4. Effect of chronic hypoxia on SK_{Ca} channel currents in uterine arteries of pregnant sheep

Arterial myocytes were freshly isolated from uterine arteries of pregnant sheep in normoxic and high altitude hypoxic animals. Whole-cell K⁺ currents were recorded in the absence or presence of apamin (1 μmol/L) or NS309 (1 μmol/L). **A, C.** Normoxic animals. **B, D.** High altitude hypoxic animals. Data are means ± SEM of cells from 5–6 animals of each group. * P < 0.05, versus control (Ctr).

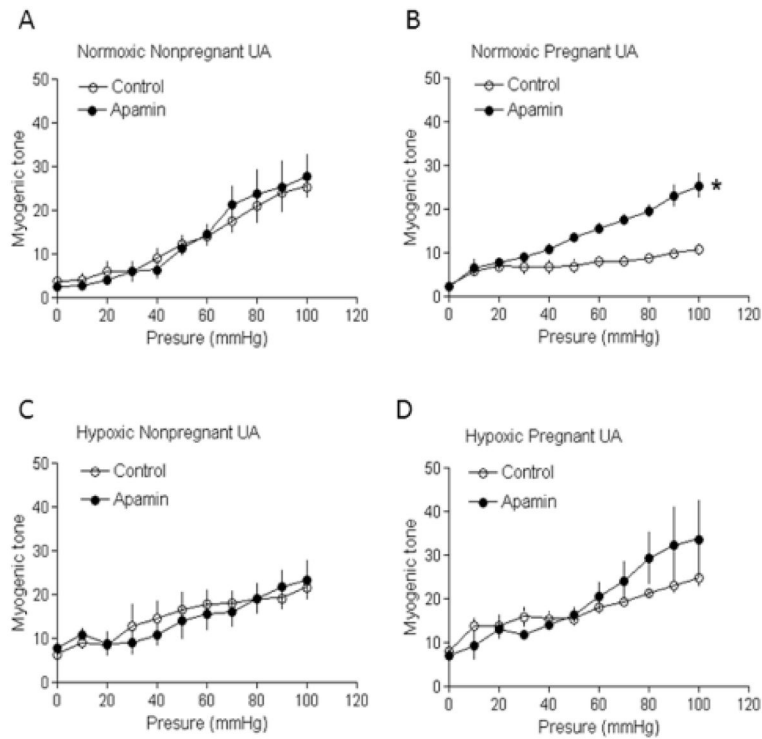


Figure 5. Effect of pregnancy and chronic hypoxia on SK_{Ca} channel-mediated myogenic tone Pressure-dependent myogenic tone was determined in the absence or presence of apamin (500 nmol/L). Data are means \pm SEM of tissues from 5–6 animals of each group. * $P < 0.05$, versus control.