Role of K_{ATP} and L-type Ca²⁺ channel activities in regulation of ovine uterine vascular contractility: effect of pregnancy and chronic hypoxia

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Abstract

OBJECTIVE—Our objective was to determine whether the pregnancy and high altitude long-term hypoxia (LTH)-mediated changes in uterine artery (UA) contractility were regulated by KATP and L-type Ca^{2+} channel activities.

STUDY DESIGN—UAs were isolated from nonpregnant (NPUA) and near-term pregnant (PUA) ewes that had been maintained at sea level (~300 m) or exposed to high altitude (3801 m) for 110 days. Isometric tension was measured in a tissue bath.

RESULTS—Pregnancy increased diazoxide- but not verapamil-induced relaxations. LTH attenuated diazoxide-induced relaxations in PUA, but enhanced verapamil-induced relaxations in NPUA. Diazoxide decreased the maximal response (E_{max}) of phenylephrine (PE)-induced contractions in PUA but not NPUA in normoxic sheep. In contrast, diazoxide had no effect on PE-induced E_{max} in PUA but decreased it in NPUA in LTH animals. Verapamil decreased the E_{max} and pD2 (−logEC50) of PE-induced contractions in both NPUA and PUA in normoxic and LTH animals, except NPUA of normoxic animals in which verapamil showed no effect on the pD_2.

CONCLUSIONS—The results suggest that pregnancy selectively increases K_{ATP}, but not L-type Ca^{2+} channel activity. LTH decreases the K_{ATP} channel activity, which may contribute to the enhanced uterine vascular myogenic tone observed in pregnant sheep at high altitude hypoxia.

Keywords

Introduction

Vascular smooth muscle cells express a diverse array of ion channels that play an important role in the function of vessels in both health and disease. Potassium (K^+) channels are the dominant ion channels expressed in the plasma membrane of arterial smooth muscle cells and contribute mainly to the regulation of smooth muscle tone.[1–3] Activation of K^+ channels in vascular smooth muscle leads to decreased vascular tone, with an increase in blood flow

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and a decrease in blood pressure. Inhibition of K^+ channel activity leads to vasoconstriction. In vascular smooth muscle cells, four types of K^+ channels (K_V, K_{Ca}, K_{ATP}, and K_{IR} channels) have been identified to regulate the membrane potential, which in turn controls the activity of L-type Ca^{2+} channels and vascular tone.[2] K^+ channels may be involved in the actions of a variety of vasodilators and vasoconstrictors, and their activities and functions may be altered in patho- and/or physiological conditions.[3]

Pregnancy is associated with a significant decrease in uterine vascular tone, with a striking increase in uterine blood flow. The adaptation of uterine vascular tone to pregnancy is complex, and the mechanisms that contribute to the profound changes during pregnancy are poorly understood. Potassium channels may be the targets and the key mediators responsible for these pregnant-mediated alterations. Indeed, previous studies have demonstrated that Ca^{2+}-activated K channel (K_{Ca}) in sheep[4] and ATP-sensitive K channel (K_{ATP}) in guinea pigs[5] play important roles in the regulation of uterine blood flow during pregnancy. Previous studies also have indicated that enhanced K^+ channel activity contributed to the vascular changes associated with normal pregnancy, e.g., the fall in systemic and local vascular resistances, and the attenuated pressor responses to several vasoconstrictors.[4–7]

Chronic hypoxia during the course of pregnancy is one of the most common insults to the maternal cardiovascular system and fetal development, and is thought to be associated with increased risk of preeclampsia and fetal intrauterine growth restriction (IUGR).[8, 9] However, in our high altitude sheep animal model, chronic hypoxia alters maternal and fetal cardiovascular systems without significant IUGR, suggesting a compensatory adaptation to high altitude hypoxia in sheep, which are similar to those observed in well-adapted human populations such as Tibetans and Andeans. Previous studies have demonstrated that long-term hypoxia (LTH) has profound effects on maternal uterine artery contractility.[10–12] However, the mechanisms underlying LTH-mediated uterine contractility are poorly understood. Among the four types of K^+ channels, the K_{ATP} channel has been demonstrated to be involved in the hypoxia-mediated contractility.[13–15] Although the role of K_{ATP} channels in hypoxia-mediated contractility is well established at other tissues, to our knowledge, no studies have examined the relative roles of K_{ATP} channels in high-altitude long-term hypoxia (LTH)-mediated uterine artery contractility during pregnancy. Given that LTH enhances ovine uterine artery myogenic contractions during pregnancy,[10] we tested the hypothesis that the LTH-associated enhanced uterine artery vascular tone is secondary to altered K_{ATP} channel function during pregnancy. In addition, we also determined the L-type Ca^{2+} channel activity to test whether LTH alters this channel activity in uterine artery smooth muscle cells during pregnancy. To test our hypothesis, we used sheep as the animal model. The sheep were divided into four groups: normoxic nonpregnant sheep, normoxic pregnant sheep, LTH nonpregnant sheep, and LTH pregnant sheep. The effects of K_{ATP} channel and L-type Ca^{2+} channel on uterine vascular contractility were determined among those four groups of animals.

Materials and Methods

Tissue preparation

As previously described,[12] nonpregnant and time-dated pregnant sheep were obtained from the Nebeker Ranch in Lancaster, CA (altitude: ~300 m; arterial PaO_2: 102 ± 2 Torr). For chronic hypoxic treatment, nonpregnant and pregnant (30 days of gestation) animals were transported to the Barcroft Laboratory, White Mountain Research Station, Bishop, CA (altitude, 3,801 m; maternal PaO_2, 60 ± 2 Torr) and maintained there for ~110 days. Animals then were transported to the laboratory at Loma Linda University. Shortly after arrival, we placed a tracheal catheter in the ewe, through which N_2 flowed at a rate to maintain PaO_2 at ~60 Torr, and this was maintained until the time of the experimental study.

Ewes were anesthetized with thiamylal (10 mg/kg) administered *via* the external left jugular vein. The animals then were intubated and anesthesia was maintained with 1.5% to 2.0% halothane in oxygen throughout surgery. An incision in the abdomen was made and the uterus exposed. The uterine arteries were isolated and removed without stretching and were placed in a modified Krebs' solution (pH 7.4) of the following composition (in mM): 115.21 NaCl, 4.7 KCl, 1.80 $CaCl_2$, 1.16 $MgSO_4$, 1.18 KH_2PO_4, 22.14 $NaHCO_3$, 0.03 EDTA, and 7.88 dextrose, oxygenated with a mixture of 95% O_2-5% CO_2. After removal of the tissues, animals were killed with an overdose of the proprietary euthanasia solution, Euthasol (pentobarbital sodium 100 mg/kg and phenytoin sodium 10 mg/kg, Virbac, Ft Worth, TX). All procedures and experimental protocols were approved by the Loma Linda University Institutional Animal Care and Use Committee and adhered to the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals*.

Contraction studies

The fourth generation branches (~0.8 mm in external diameter) of main uterine arteries were separated from the surrounding tissue, and cut into 2-mm ring segments. The small branches of uterine arteries were chosen, because they are much closer in characteristics to arterioles and play a substantial role in vascular resistance. Isometric tension was measured in the Krebs solution in a tissue bath at 37°C, as described previously.[16] Briefly, each ring was equilibrated for 60 min and then gradually stretched to the optimal resting tension, as determined by the tension that developed in response to 120 mM KCl added at each stretch level. Tissues then were stimulated with cumulative additions of phenylephrine in approximate one-half log increments to generate a concentration-response curve, and contractile tensions were recorded with an online computer. After phenylephrine was washed away, tissues were relaxed to the baseline and were recovered at the resting tension for 30 min. The second concentration-response curves of phenylephrine-induced contractions then were repeated in the absence or presence of K_{ATP} channel blocker (glibenclamide, 10 μM; Research Biochemical International, Natick, MA, USA), or K_{ATP} channel opener (diazoxide, 10 μM; Research Biochemical International, Natick, MA, USA) or a L-type Ca^{2+} channel blocker (verapamil, 10 μM; Research Biochemical International, Natick, MA, USA) for 20 min. For relaxation studies, the tissues were pre-contracted with submaximal concentrations of phenylephrine, followed by diazoxide, and verapamil, respectively, added in a cumulative manner. EC_{50} values for the agonist in each experiment were taken as the molar concentration at which the contraction-response curve intersected 50% of the maximum response, and were expressed as pD2 ($-logEC_{50}$) values.

Simultaneous measurement of [Ca^{2+}]$_i$ and tension

Simultaneous recordings of contraction and [Ca^{2+}]$_i$ (fura-2 signal $R_{f340/f380}$) in the same tissue were conducted as described previously.[12] Briefly, the arterial ring was attached to an isometric force transducer in a 5-ml tissue bath mounted on a CAF-110 intracellular Ca^{2+} analyzer (model CAF-110, Jasco; Tokyo, Japan). The tissue was equilibrated in Krebs buffer under a resting tension of 0.5 g for 40 min. The ring was then loaded with 5 μM fura 2-AM for 3 h in the presence of 0.02% Cremophor EL at room temperature (25°C). After loading, the tissue was washed with Krebs solution at 37°C for 30 min to allow for hydrolysis of fura-2 ester groups by endogenous esterase. Contractile tension and fura-2 fluorescence were measured simultaneously at 37°C in the same tissue. The tissue was illuminated alternatively (125 Hz) at excitation wavelengths of 340 and 380 nm, respectively, by means of two monochromators in the light path of a 75-w xenon lamp. Fluorescence emission from the tissue was measured at 510 nm by a photomultiplier tube. The fluorescence intensity at each excitation wavelength (F_{340} and F_{380}, respectively) and the ratio of these two fluorescence values ($R_{f340/380}$) were recorded with a time constant of 250 ms and stored with the force signal on a computer.

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 (n) of experimental animals given. Differences were evaluated for statistical significance ($P < 0.05$) by two-way ANOVA followed by the Newman-Keuls post hoc test.

Results

Effect of LTH on K_{ATP}-mediated relaxation in uterine arteries

As shown in Figure 1, diazoxide, a K_{ATP} channel opener, produced dose-dependent relaxations of phenylephrine-pre-contracted uterine arteries in both normoxic and LTH sheep. In normoxic animals, diazoxide-induced vascular relaxations were more potent in pregnant than in nonpregnant uterine arteries (pD2 value: 4.8 ± 0.2 vs. 4.2 ± 0.2, $P < 0.05$). In hypoxic animals, LTH significantly decreased the potency of diazoxide-induced vascular relaxations in pregnant uterine arteries (pD2 value: 4.8 ± 0.2 vs. 3.6 ± 0.6, $P < 0.05$), but not in nonpregnant uterine arteries (pD2 value: 4.2 ± 0.2 vs. 3.5 ± 1.2, $P > 0.05$). Furthermore, LTH eliminated the difference of diazoxide-induced relaxations between pregnant and nonpregnant uterine arteries (pD2 value: 3.6 ± 0.6 vs. 3.5 ± 1.2, $P > 0.05$).

Effect of LTH on L-type Ca^{2+} channel activity in uterine arteries

As shown in Figure 2, an L-type Ca^{2+} channel blocker, verapamil produced dose-dependent relaxations of phenylephrine-pre-contracted uterine arteries in both normoxic and LTH sheep. Verapamil-induced relaxations had no significant difference between nonpregnant and pregnant uterine arteries in either normoxic (pD2 value: 5.6 ± 0.2 vs. 5.9 ± 0.09, $P > 0.05$) or LTH (pD2 value: 6.1 ± 0.2 vs. 6.1 ± 0.05, $P > 0.05$) animals. However, LTH significantly enhanced verapamil-induced relaxations in nonpregnant (pD2 value: 5.6 ± 0.2 vs. 6.1 ± 0.2, $P < 0.05$) but not in pregnant (pD2 value: 5.9 ± 0.09 vs. 6.1 ± 0.05, $P > 0.05$) uterine arteries.

Role of K_{ATP} channel in α_1-adrenoceptor-mediated contractions of uterine arteries

As shown in Figure 3, inhibition of K_{ATP} channel by glibenclamide showed no significant effect on α_1-adrenoceptor agonist, phenylephrine-induced contractions in either pregnant or nonpregnant uterine arteries from either normoxic or LTH animals. However, activation of K_{ATP} channel by diazoxide differentially affected phenylephrine-induced contractions of both pregnant and nonprenant uterine arteries from normoxic and LTH animals (Figure 4). As show in Figure 4, pretreatment with diazoxide significantly inhibited the maximal responses (E_{max}) of phenylephrine-induced concentration-response curves in pregnant but not in nonpregnant uterine arteries from normoxic animals. In contrast to normoxic animals, diazoxide did not significantly inhibit the E_{max} in pregnant but inhibited it in nonpregnant uterine arteries from LTH animals. Figure 4 also shows the pD_2 values of phenylephrine-induced concentration- response curves of uterine arteries in absence (control) and presence of diazoxide. Diazoxide significantly inhibited the pD_2 values in both pregnant and nonpregnant uterine arteries from both normoxic and LTH animals.

Role of L-type Ca^{2+} channel in α_1-adrenoceptor-mediated contractions of uterine arteries

As shown in Figure 5, pretreatment with verapamil significantly inhibited the E_{max} of phenylephrine-induced concentration-response curves in both pregnant and nonpregnant uterine arteries from both normoxic and LTH animals. However, verapamil selectively inhibited the pD_2 values in normoxic pregnant (pD_2 value: 6.6 ± 0.1 vs. 5.9 ± 0.13, $P < 0.05$), LTH pregnant (pD_2 value: 6.0 ± 0.1 vs. 4.7 ± 0.1, $P < 0.05$), and LTH nonpregnant

(pD$_2$ value: 5.7 ± 0.2 vs. 4.8 ± 0.1, $P < 0.05$), but not in normoxic nonpregnant (pD$_2$ value: 6.0 ± 0.2 vs. 5.6 ± 0.3, $P > 0.05$) uterine arteries.

Relation of K$_{ATP}$-mediated relaxation and Ca^{2+} mobilization in pregnant uterine artery

Figure 6 shows the simultaneous measurement of intracellular free calcium concentration ([Ca^{2+}]$_i$) (R$_{f340/f380}$) and contractile tensions in response to diazoxide in the same phenylephrine-contracted pregnant uterine artery smooth muscle cells. As shown in Figure 6 (upper panel), diazoxide produced concentration dependent uterine vasorelaxation and decrease in R$_{f340/f380}$. The decreased R$_{f340/f380}$ was superimposed with the vasorelaxation. Figure 6 (lower panel) shows a positive correlation between vasorelaxation and [Ca^{2+}]$_i$ decreased by cumulative additions of diazoxide in precontracted pregnant uterine arteries.

Comment

The present study offers the following key observations: 1) although basal K$_{ATP}$ channel activity may not play a great role in α_1-adrenoceptor-mediated contraction in the uterine artery, K$_{ATP}$ channel activation by diazoxide decreased uterine vascular tone by decreasing [Ca^{2+}]$_i$; 2) pregnancy enhanced K$_{ATP}$ channel-mediated relaxation; 3) LTH decreased K$_{ATP}$ channel activity and eliminated the pregnancy-mediated responses; 4) basal L-type Ca^{2+} channel activity plays a key role in regulating uterine vascular tone, and while this was not altered by pregnancy; 5) LTH enhanced the effect of L-type Ca^{2+} channel activity in α_1-adrenoceptor mediated contractions of uterine artery in both pregnant and nonpregnant sheep.

It has been shown that K$_{ATP}$ channel inhibition leads to vasoconstriction with increased vascular tone in vascular beds such as the coronary,[17,18] mesenteric,[19] and guinea pig uterine[5] arteries both *in vitro* and *in vivo*. The present finding that glibenclamide had no significant effect on phenylphrine-induced contractions in ovine uterine arteries, is in agreement with previous studies that glibenclamide did not affect basal tone in the renal, cerebral and pulmonary arteries.[20–24] It is likely that the role of K$_{ATP}$ channels in the regulation of baseline vascular tone is tissues and/or animal species dependent. In the present studies, although the baseline vascular tone was not significantly affected by K$_{ATP}$ channels, the K$_{ATP}$ channel opener, diazoxide caused significant vasodilation and attenuated phenylphrine-induced contractions, suggesting a functional role of K$_{ATP}$ channels in the ovine uterine artery. Similar studies have demonstrated that K$_{ATP}$ channels are not involved in regulating basal tone in cerebral artery, but K$_{ATP}$ channels play an important role in vasodilation caused by changes in metabolic state and by endogenous substances or synthetic K$_{ATP}$ channel openers.[3,25] Hypoxia, adenosine, and synthetic K$_{ATP}$ channel openers have been shown to induce dilation in cerebral and pulmonary arteries.[15,21] Taken together, our results support the idea that K$_{ATP}$ channel activation is involved in uterine artery contractility. K$_{ATP}$ channel activation plays a major role in modulating membrane potential, L-type Ca^{2+} channel activity, intracellular Ca^{2+} concentration ([Ca^{2+}]$_i$), and vascular tone.[3] Simultaneous measurement of R$_{f340/f380}$ and contractile tensions in the same tissue of uterine artery smooth muscle shows the K$_{ATP}$ channel opener, diazoxide, caused a positive relationship in decrements of R$_{f340/f380}$ and tension. This suggests that K$_{ATP}$ channel activation leads to decreased vascular tone by decreasing [Ca^{2+}]$_i$.

In vascular smooth muscle cells, previous studies have demonstrated that K$_{ATP}$ channel activation is closely regulated by several pathophysiological stimuli, and altered K$_{ATP}$ channel function has been detected in various pathological conditions including hypertension, diabetes, and ischemia.[2,26,27] In addition, the role of K$_{ATP}$ channels also is dependent on physiologic and/or pathologic conditions. For example, in pulmonary arteries K$_{ATP}$ channels are not involved in regulating basal tone in either normoxia or moderate

hypoxia.[24] However, K_{ATP} channels play an important role in pulmonary vasorelaxation in sustained and severe pulmonary hypoxia. In agreement with previous studies showing that pregnancy augments K_{ATP} channel activity in guinea pig uterine vascular bed,[5] our current studies have shown that diazoxide-induced relaxations are significantly higher in pregnant than those in nonpregnant uterine arteries, and in pregnant but not nonpregnant uterine arteries, diazoxide pretreatment significantly inhibited the maximal responses of phenylephrine-induced contractions. These observations suggest that pregnancy up-regulates K_{ATP} channel activity, resulting in decreased uterine vascular resistance and reduced contractile efficacy of α_1-adrenoceptor-mediated vasocontractile responses. This may contribute greatly to the increased uterine blood flow during normal pregnancy. In contrast to the potential effect of pregnancy on K_{ATP} channel activity in normoxic animals, the present studies indicated that LTH decreased diazoxide-induced relaxation in pregnant uterine arteries and eliminated pregnancy-mediated responses. Recently, we have shown that LTH increases uterine vascular tone during pregnancy.[10] Thus, the present observations suggest that LTH-mediated enhanced uterine vascular tone may be attributed, in part, to a decrease in K_{ATP} channel activity. The inhibited patterns of pD_2 value by diazoxide in phenylephrine-induced contractions were not affected by pregnancy and LTH, suggesting that pregnancy and LTH-mediated changes of α_1-adrenoceptor-mediated sensitivity (pD_2 value) of contractions may not be associated with this altered K_{ATP} channel activity.

In contrast to the potential effect of pregnancy on K_{ATP} channel activity, verapamil-induced relaxations of uterine arteries were not significant different between nonpregnant and pregnant animals. This suggests that L-type Ca^{2+} channel activity is not regulated by pregnancy, and is supported by previous studies showing KCl-induced uterine artery contractions are similar in nonpregnant and pregnant animals.[12] We also examined the effect of L-type Ca^{2+} channel inhibition on α_1-adrenoceptor-mediated contraction. Phenylephrine-induced E_{max} values of uterine artery smooth muscle cells were significantly inhibited by verapamil, suggesting an important role of L-type Ca^{2+} channel in the regulation of α_1-adrenoceptor-mediated contraction. The finding that inhibition of L-type Ca^{2+} channel activity by verapamil significantly attenuated phenylephrine-induced pD_2 values of uterine arteries in pregnant, but not in nonpregnant, animals, suggests enhanced coupling of L-type Ca^{2+} channel activity to α_1-adrenoceptor-mediated contraction of uterine artery during pregnancy.

L-type Ca^{2+} channel regulation by O_2 tension is a physiologically important and widespread phenomenon.[28] In agreement with previous reports that hypoxia up-regulated L-type Ca^{2+} channel activity,[29–31] our findings that verapamil-induced relaxations of uterine arteries were significantly enhanced in LTH nonpregnant, but not pregnant, animals compared to normoxic controls, suggest that LTH may up-regulate basal L-type Ca^{2+} channel activity in uterine arteries. Consistent with the effect of LTH on basal L-type Ca^{2+} channel activity, our findings that in both nonpregnant and pregnant uterine arteries, the inhibited patterns by verapamil in phenylephrine-induced contractions were enhanced by LTH, suggest that LTH altered the coupling of L-type Ca^{2+} channel to α_1-adrenoceptor-mediated contraction. These results present a novel regulation of L-type Ca^{2+} channel activity under hypoxic conditions.

Although the present studies have demonstrated that pregnancy and LTH differentially regulate uterine artery K_{ATP} channel and L-type Ca^{2+} channel activities, the underlying mechanisms are not understood. Previous studies have demonstrated that K_{ATP} channels and L-type Ca^{2+} channels activities are regulated through a number of endogenous vasodilators and vasocontrictors,[2,3,19] an important enzyme in this regard being protein kinase C (PKC). In vascular smooth muscle, several vasoconstricting hormones and neurotransmitters inhibit KATP channels function via PKC activation.[2,32,33] Given the fact that uterine artery PKC activity is significantly attenuated by pregnancy[34–36] and enhanced by chronic hypoxia,[10]

the pregnancy-mediated increase and LTH-mediated a decrease in K_{ATP} channel activity may be regulated through the alteration of PKC signaling system. Thus, it provides a new direction for us to study the role of PKC in regulation of K_{ATP} channel activity in our future studies. In addition, we will further explore whether pregnancy and chronic hypoxia directly regulate K_{ATP} channel gene expression which may contribute to the altered K_{ATP} channel activity in uterine vasculatures.

In conclusion, both K_{ATP} and L-type Ca^{2+} channels play key roles in the regulation of uterine artery contractility. Pregnancy selectively increases K_{ATP} channel activity, but has no significant effect on the L-type Ca^{2+} channel activity *per se*. This increased K_{ATP} channel activity is likely to contribute to the decreased uterine artery resistance and the increased uterine blood flow in pregnancy. High altitude long-term hypoxia decreases the K_{ATP} channel activity but increases the L-type Ca^{2+} channel activity, which may provide a underlying mechanism for the enhanced myogenic tone observed in uterine arteries of pregnant sheep acclimatized to high altitude long-term hypoxia. The potential role of the sex steroid hormones and other factors in regulating the channel activities in the adaptation of uterine artery contractility to pregnancy and chronic hypoxia remains to be investigated.

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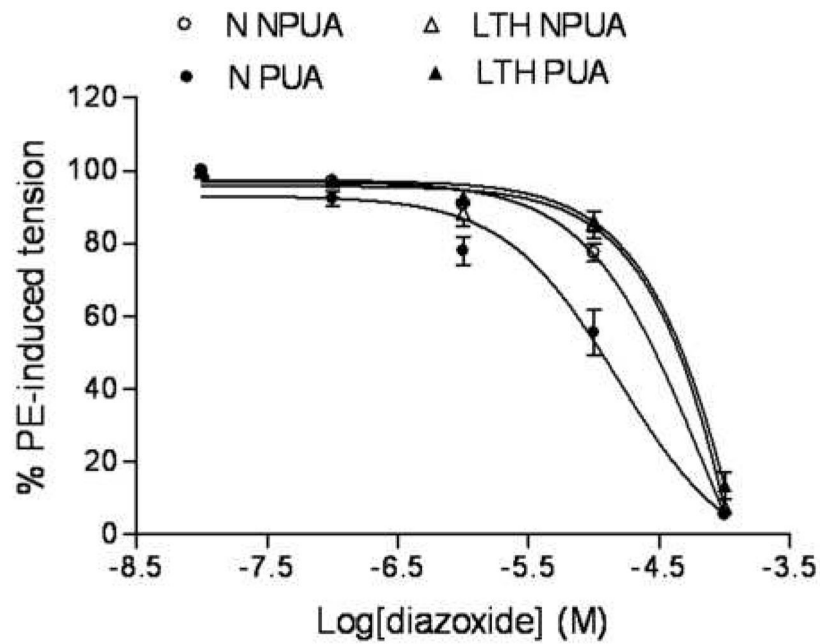
Figure 1. Diazoxide-mediated relaxation of uterine ateries
Concentration-dependent relaxation induced by the K_{ATP} channel opener, diazoxide were obtained with phenylephrine (1 μM)-precontracted uterine arteries in normoxic nonpregnant (N NPUA), pregnant (N PUA), LTH nonpregnant (LTH NPUA) and LTH pregnant (LTH PUA) sheep. Concentration-response curves were analyzed by computer-assisted nonlinear regression to fit the data using GraphPad Prism (GraphPad software, San Diego, CA). Data are means ± SEM of the tissues from 5 to 13 animals.
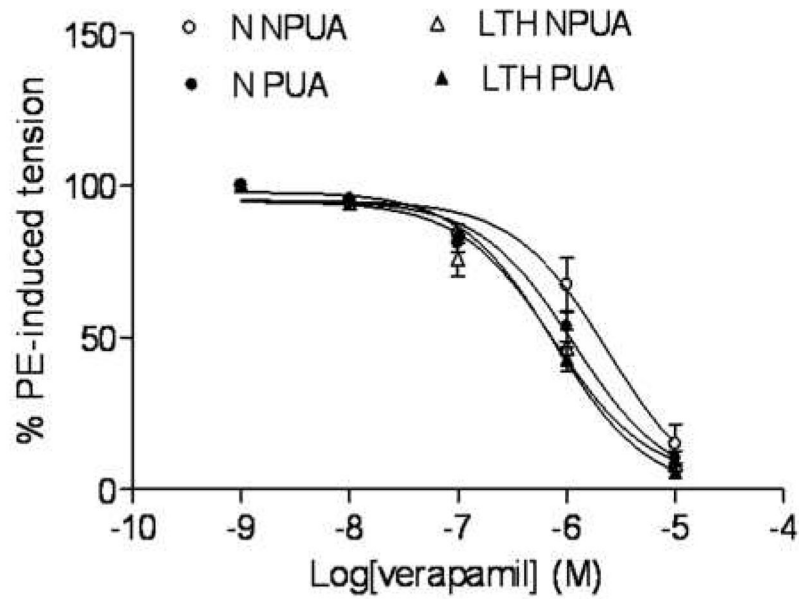
Figure 2. Verapamil-mediated relaxations of uterine ateries
Concentration-dependent relaxation induced by the L-type Ca^{2+} channel blocker, verapamil were obtained with phenylephrine (1 μM)-precontracted uterine arteries in normoxic nonpregnant (N NPUA), pregnant (N PUA), LTH nonpregnant (LTH NPUA) and LTH pregnant (LTH PUA) sheep. Concentration-response curves were analyzed by computer-assisted nonlinear regression to fit the data using GraphPad Prism (GraphPad software, San Diego, CA). Data are means ± SEM of the tissues from 6 to 13 animals.
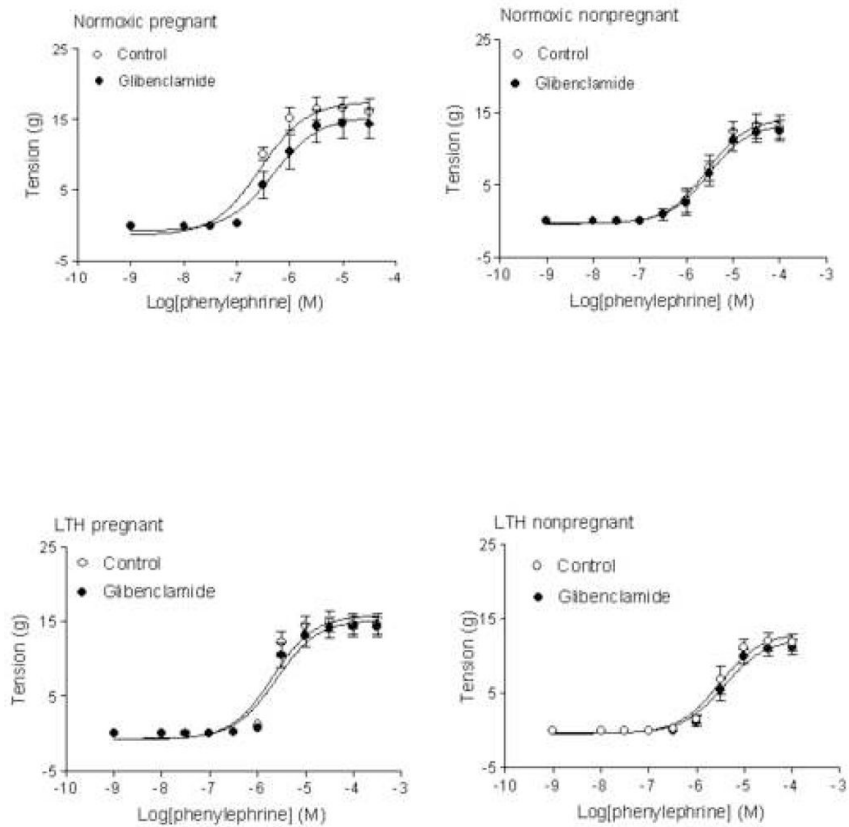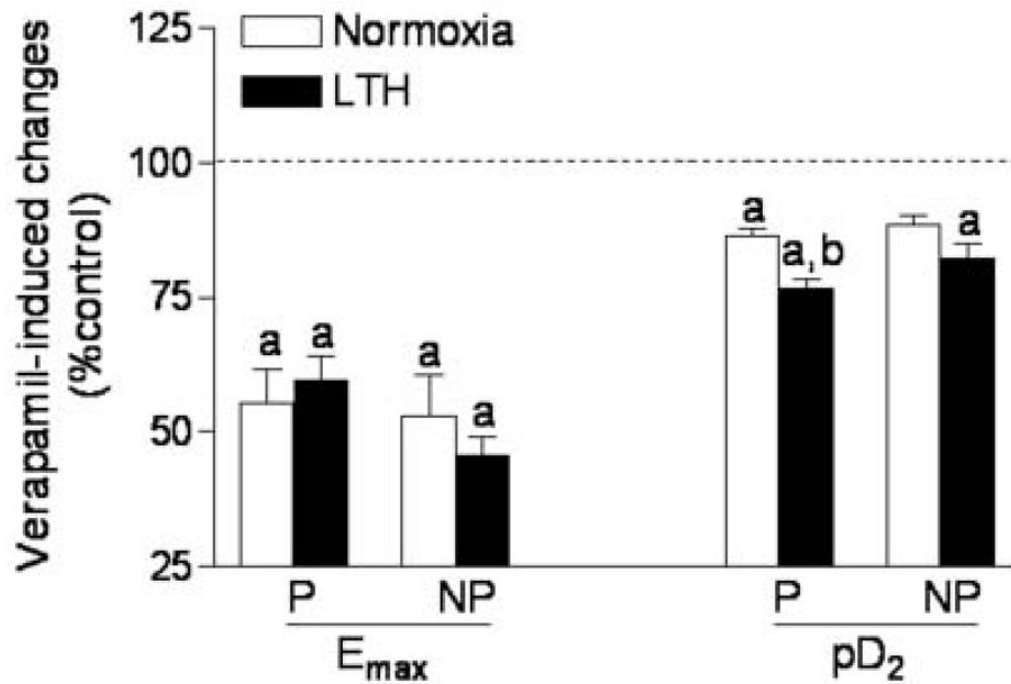
Figure 3. Effect of glibenclamide on phenylephrine-induced contraction
Arterial rings were pretreated in the absence or presence of 10 μM glibenclamide for 20 min, and then subjected to the cumulative addition of phenylephrine in the tissue bath. Data are means ± SEM of the tissues from 6 to 7 animals.
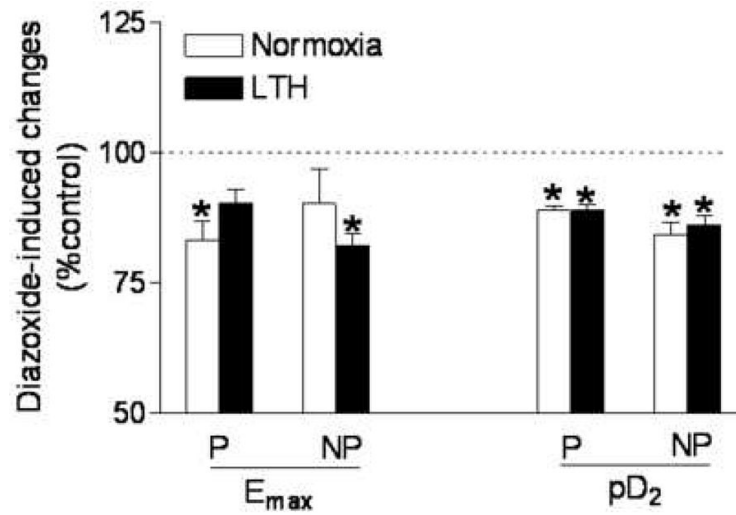
Figure 4. Effect of diazoxide on phenylephrine-induced E_{max} and pD_2
Arterial rings were pretreated in the absence or presence of 10 μM diazoxide for 20 min, and then subjected to the cumulative addition of phenylephrine in the tissue bath. The maximal response (E_{max}) and potency (pD_2) values were determined from the phenylephrine concentration-response curve analyzed by computer-assisted nonlinear regression to fit the data using GraphPad Prism. P, pregnant; NP, nonpregnant. Data are expressed percentage of their own control and are means ± SEM of the tissues from 5 to 11 animals. $^*P < 0.05$, control vs. diazoxide treated group.

Figure 5. Effect of verapamil on phenylephrine-induced E_{max} and pD_2
Arterial rings were pretreated in the absence or presence of 10 μM verapamil for 20 min, and then subjected to the cumulative addition of phenylephrine in the tissue bath. E_{max} and pD_2 values were determined from the phenylephrine concentration-response curve analyzed by computer-assisted nonlinear regression to fit the data using GraphPad Prism. P, pregnant; NP, nonpregnant. Data are expressed percentage of their own control and are means ± SEM of the tissues from 6 to 12 animals. [a]$P < 0.05$, control vs. verapamil; [b]$P < 0.05$, normoxia vs. LTH group.
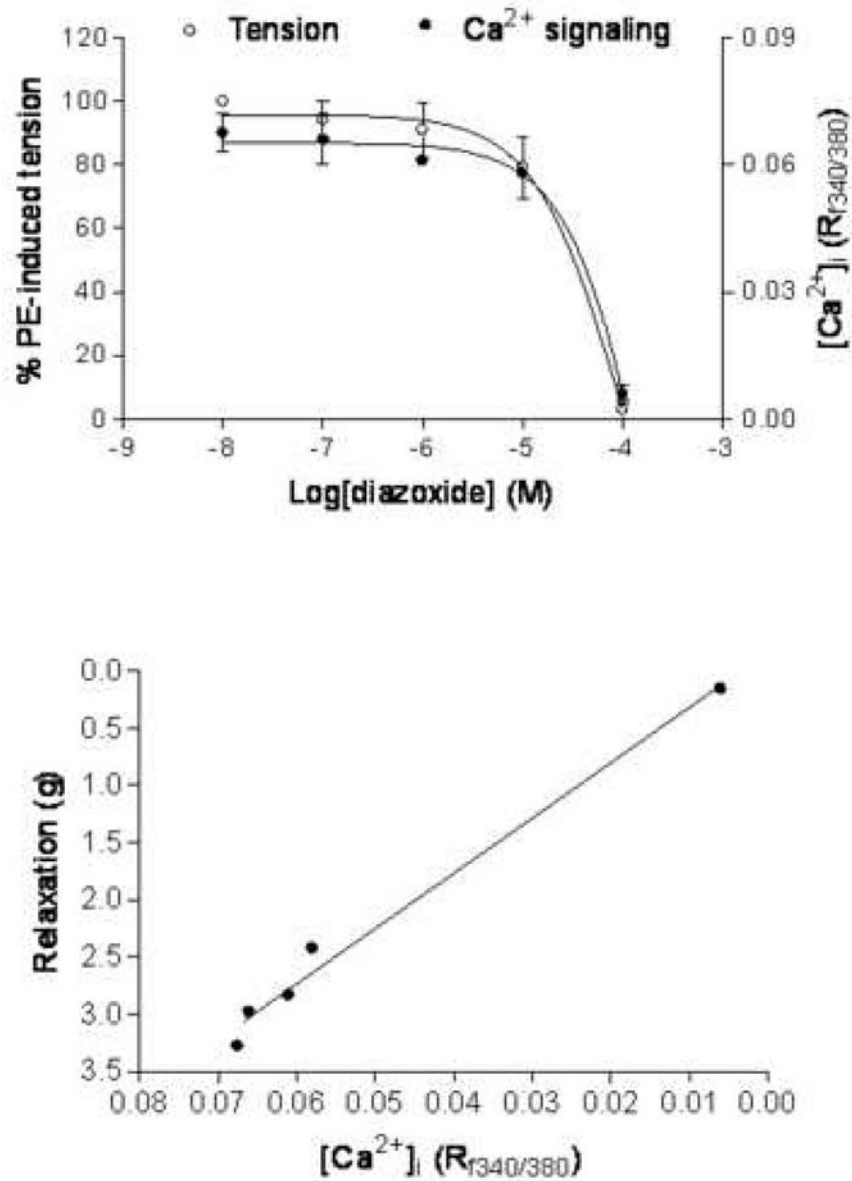
Figure 6. Diazoxide-induced reduction of $[Ca^{2+}]_i$ and tension
Smooth muscle $[Ca^{2+}]_i$ and tension were measured simultaneously in the same tissue of normoxic pregnant uterine arterial rings loaded with fura 2. Upper panel: dose-response curves of the diazoxide-mediated reduction of $[Ca^{2+}]_i$ ($R_{f340/380}$) and tension in the arterial rings precontracted with 10 μM of phenylephrine. Lower panel: Diazoxide-induced $[Ca^{2+}]_i$-tension relationships obtained with phenylephrine-precontracted arterial rings. Data are means ± SEM of the tissues from 4 animals.