Cortisol-mediated regulation of uterine artery contractility: effect of chronic hypoxia

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Xiao, DaLiao, XiaoHui Huang, Soochan Bae, Charles A. Ducsay, Lawrence D. Longo, and Lubo Zhang. Cortisol-mediated regulation of uterine artery contractility: effect of chronic hypoxia. Am J Physiol Heart Circ Physiol 286: H716–H722, 2004; 10.1152/ajpheart.00805.2003.—We previously demonstrated that cortisol regulated α1-adrenoceptor-mediated contractions differentially in nonpregnant and pregnant uterine arteries. Given that chronic hypoxia during pregnancy has profound effects on maternal uterine artery reactivity, we investigated the effects of chronic hypoxia on cortisol-mediated regulation of uterine artery contractions. Pregnant (day 30) and nonpregnant ewes were divided between normoxic and hypoxic tissues. The dissociation constant of norepinephrine-α1-adrenoceptor-mediated contractions differentially in nonpregnant and pregnant arteries. In hypoxic animals, cortisol (10 ng/ml for 24 h) increased norepinephrine-induced contractions in pregnant, but not in nonpregnant, uterine arteries. The 11β-hydroxysteroid dehydrogenase inhibitor carbenoxolone did not change cortisol concentrations in nonpregnant arteries, but potentiated it in pregnant arteries. In addition, cortisol attenuated phorbol 12,13-dibutyrate (PDBu)-mediated contractions of normoxic and hypoxic uterine arteries. The regulatory effects of cortisol on α1-adrenoceptor-mediated contractions were determined in uterine arteries obtained from nonpregnant and near-term pregnant sheep exposed to high-altitude (3,820 m) hypoxia [arterial PO2 (PaO2) 60 mmHg] for 110 days. The results were compared with those in the current studies in normoxic control animals published previously (40, 41). In addition, we examined the regulatory effects of cortisol on phorbol 12,13-dibutyrate (PDBu)-mediated contractions of nonpregnant and pregnant uterine arteries from normoxic and hypoxic sheep. Our results suggest that cortisol regulates α1-adrenoceptor- and PKC-mediated contractions differentially in the uterine arteries, and chronic hypoxia attenuates uterine artery sensitivity to cortisol.

METHODS

Tissue preparation. As previously described (15, 16, 39, 41), nonpregnant and time-dated pregnant sheep were obtained from Nebeker Ranch in Lancaster, CA (altitude: ~300 m; PaO2, 102 ± 2 mmHg). Uterine arteries were obtained from nonpregnant and near-term (~140 days of gestation) pregnant sheep. For chronic hypoxia, nonpregnant and pregnant (30 days of gestation) animals were transported to Barcroft Laboratory, White Mountain Research Station, Bishop, CA (3,820 m altitude; PaO2, 60 ± 2 mmHg) and kept there for ~110 days. The animals were transported to the laboratory immediately before the studies. Ewes were anesthetized with thiamylal (10 mg/kg) administered via the external left jugular vein. The animals were then intubated and anesthesia was maintained on 1.5% to 2.0% halothane in oxygen throughout surgery. An incision in the abdomen was made and the uterus was exposed. The uterine arteries were isolated, removed without being stretched, and placed into a modified Krebs solution (pH 7.4) of the following composition (in mM): 115.21 NaCl, 4.7 KCl, 1.80 CaCl2, 1.16 MgSO4, 1.18 K2HPO4, 22.14 NaHCO3, and 7.88 dextrose. EDTA (0.03 mM) was added to suppress nonvascular smooth muscle contractions.

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oxidation of amines. The Krebs solution was oxygenated with a mixture of 95% O₂-5% CO₂. After removal of the tissues, the animals were euthanized with T-61 (euthanasia solution, Hoechst-Roussel; Somerville, NJ). All procedures and protocols used in the present study were approved by the Animal Research Committee of Loma Linda University, and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The third (nonpregnant) and fourth (pregnant) branches of the main uterine arteries with a similar external diameter (~0.8 mm) were separated from the surrounding tissue and were cut into rings of 2 mm in length. The arterial rings were maintained in Dulbecco’s modified Eagle’s medium (Mediatech Cellgro) with 1% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin, as previously described (41). The tissues were incubated at 37°C in a humidified incubator with 5% CO₂-95% air in the absence or presence of cortisol (10 ng/ml, equivalent to 27.6 nM) and/or carbeneoxolone (3 μM) (Sigma; St. Louis, MO) for 24 h.

Contractions studies. After cortisol pretreatment, isometric tensions of arterial contractions were measured in the presence of cortisol in Krebs solution in tissue baths at 37°C, as described previously (41). Tissues were equilibrated for 60 min, and each ring was stretched to the optimal resting tension, as determined by the tension developed in response to KCl (120 mM) added at each stretch level. Concentration-response curves were obtained by cumulative addition of agonists [norepinephrine or phorbol 12,13-dibutyrate (PDBu)] in ~one-half log increments. Prism software (GraphPad; San Diego, CA) was used to fit the curve and determine pD₂ (−log EC₅₀) values and the maximum response. The apparent dissociation constant (Kₐ) of the norepinephrine-α-adrenoceptor complex was determined as previously described (15, 40). Briefly, the concentration-response curves of norepinephrine were determined before and after the treatment of tissues with phenoxbenzamine (30 nM for 20 min) to inactivate a fraction of the receptors and reduce the maximal response to norepinephrine by ~50%. The reciprocal of the concentration of norepinephrine before phenoxbenzamine treatment (1/[A]) was then plotted against the reciprocal of the corresponding equieffective concentrations after the treatment (1/[A’]). The values for K_a and the fraction of active receptors remaining (q) were calculated as follows: 1/[A] = (1 − q)/qK_a + 1/[A’], where K_a = (slope − 1)/intercept and q = 1/slope (11).

Western blot analysis of glucocorticoid receptors. Protein levels of glucocorticoid receptors (GRs) in nonpregnant and pregnant uterine arteries obtained from normoxic control and hypoxic animals, which were separate tissues from those incubated with cortisol, were determined by Western blot analysis, as previously described (30, 40). Briefly, the same amount of proteins (25 μg) isolated from uterine arteries were loaded per lane on 7.5% SDS-PAGE, transferred to nitrocellulose membranes, and incubated with the primary antibody against GRs (Affinity Bioreagents; Neshanic Station, NJ). To minimize potential variations, samples from the four groups (normoxic nonpregnant and pregnant, hypoxic nonpregnant, and pregnant) were run in the same gel, and the percent volume density of each sample was determined. The secondary antibody was horseradish peroxidase-conjugated goat anti-rabbit (Amersham; Arlington Heights, IL). Proteins were visualized with enhanced chemiluminescence reagents, and the blots were exposed to hyperfilm. Results were quantified with Kodak Electrophoresis Documentation and Analysis System and Kodak 1D Image Analysis Software.

Simultaneous measurement of intracellular Ca²⁺ concentration and tension. Simultaneously recordings of contractile tension and free intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) in the same tissue were conducted as described previously (41, 46). Briefly, the arterial rings were attached to an isometric force transducer in a 5-ml tissue bath maintained at 37°C and were loaded with an intracellular Ca²⁺ (fura-2 AM) fluorescent indicator (model CAF-110, Jacobsen; Tokyo, Japan). The tissues were equilibrated in Krebs buffer under a resting tension of 0.5 g for 40 min, and loaded with 5 μM fura-2 AM ester (Molecular Probes; Eugene, OR) for 4 h in the presence of 0.02% Cremophor EL at 25°C. The tissues were then 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 tissues were 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 with a photomultiplier. The fluorescence intensity at each excitation wavelength (F₁₅₀₀ and F₃₈₀₀, respectively) and their ratio (F₁₅₀₀/F₃₈₀₀) 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 with a computer-assisted nonlinear regression to fit the data with the use of Prism software (GraphPad). Results were expressed as means ± SE, and the differences were evaluated for statistical significance (P < 0.05) by analysis of variance.

RESULTS

Norepinephrine-induced contractions. In the concurrent studies published previously, we have shown that in vitro cortisol (1 to 30 ng/ml) treatment for 24 h produces a dose-dependent increase in norepinephrine-mediated contractions of both nonpregnant and pregnant uterine arteries from normoxic control sheep (40). In the hypoxic animals, in vitro cortisol (10 ng/ml) treatment for 24 h potentiated norepinephrine-induced contractions in pregnant uterine arteries and increased norepinephrine pD₂ values from 5.88 ± 0.09 to 6.37 ± 0.10 (P < 0.05) (Fig. 1). In contrast, cortisol had no effect on norepinephrine-induced contractions in nonpregnant uterine arteries (pD₂: 5.74 ± 0.04 vs. 5.80 ± 0.09, P > 0.05) (Fig. 1).

To determine the potential effect of chronic hypoxia on 11β-hydroxysteroid dehydrogenase (11β-HSD) activity of the uterine artery, the cortisol-mediated potentiation of norepinephrine-induced contractions was examined in the absence and/or presence of the 11β-HSD inhibitor carbeneoxolone (3 μM for 24 h) as described previously (40). As shown in Table 1, carbeneoxolone potentiated norepinephrine-induced contractions of hypoxic pregnant uterine arteries by increasing the norepinephrine pD₂ values in the absence of cortisol. In the presence of carbeneoxolone, cortisol had no further effect on norepinephrine-induced contractions. In hypoxic nonpregnant uterine arteries, carbeneoxolone had no effect on norepinephrine-induced contractions in the absence or presence of cortisol (Table 1).

In normoxic animals, cortisol decreased the K_a of the norepinephrine-α-adrenoceptor complex in nonpregnant uterine arteries, but not in pregnant arteries (40). In the hypoxic animals, cortisol did not significantly change the K_a value in the nonpregnant uterine arteries, but significantly decreased it in pregnant arteries (Fig. 2).

GR protein levels. GR protein levels in uterine arteries were determined by Western blot analysis. As shown in Fig. 3, quantitative analysis of immunoreactive GR levels indicated that neither pregnancy nor chronic hypoxia affected GR protein levels in the uterine arteries.

Norepinephrine-induced Ca²⁺ mobilization. In normoxic animals, cortisol differentially regulated norepinephrine-mediated Ca²⁺ mobilization in nonpregnant and pregnant uterine arteries by increasing norepinephrine pD₂ values in pregnant, but not in nonpregnant, uterine arteries (41). In contrast, in the hypoxic animals, cortisol had no effect on norepinephrine pD₂.
values in either nonpregnant (5.61 ± 0.13 vs. 5.85 ± 0.15; P > 0.05) or pregnant (5.78 ± 0.19 vs. 5.89 ± 0.13; P > 0.05) uterine arteries (Fig. 4). However, cortisol significantly decreased the noradrenaline-mediated maximal response (fura-2 signal, R<sub>340/380</sub>) in nonpregnant (0.03 ± 0.002 vs. 0.047 ± 0.003; P < 0.05), but increased it in pregnant (0.073 ± 0.006 vs. 0.048 ± 0.003; P < 0.05) uterine arteries (Fig. 4). The [Ca<sup>2+</sup>]-tension relation depicted from the data of simultaneous measurement of [Ca<sup>2+</sup>]<sub>i</sub> and tension in the same tissue indicated that cortisol significantly increased the slope (g tension/R<sub>340/380</sub> [Ca<sup>2+</sup>]<sub>i</sub>) in nonpregnant, but not in pregnant, uterine arteries from the hypoxic animals (Fig. 5 and Table 2). Compared with the normoxic animals, chronic hypoxia significantly attenuated PDBu-induced contractions and decreased PDBu pD<sub>2</sub> values in both nonpregnant and pregnant uterine arteries (Table 3). In the hypoxic animals, cortisol showed no effect on PDBu-induced contractions in either nonpregnant or pregnant uterine arteries (Table 3).

### DISCUSSION

Our concurrent studies in normoxic sheep have demonstrated that cortisol potentiates noradrenaline-induced contractions of uterine arteries from both nonpregnant and pregnant animals (40). In the absence of exogenous cortisol, pregnant uterine arteries showed increased contractile sensitivity to α-adrenoceptor agonists compared with nonpregnant arteries (2, 7, 8, 31, 40, 42). Cortisol (10 ng/ml) increased noradrenaline-induced contractions of uterine arteries from the hypoxic animals (Table 2). In contrast, chronic hypoxia did not affect the [Ca<sup>2+</sup>]<sub>i</sub>-tension relation in nonpregnant uterine arteries (Table 2).

### PDBu-induced contractions

In the normoxic animals, in contrast to its effect on noradrenaline-induced contractions, cortisol significantly suppressed the PDBu-induced maximal contractions in both nonpregnant and pregnant uterine arteries (Table 3). Compared with the normoxic animals, chronic hypoxia significantly attenuated PDBu-induced contractions and decreased PDBu pD<sub>2</sub> values in both nonpregnant and pregnant uterine arteries (Table 3). In the hypoxic animals, cortisol showed no effect on PDBu-induced contractions in either nonpregnant or pregnant uterine arteries (Table 3).

#### Table 1. Effect of carbenoxolone on cortisol-mediated effects on noradrenaline pD<sub>2</sub> in hypoxic uterine arteries

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Pregnant</th>
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<tr>
<td></td>
<td>−Carbenoxolone</td>
<td>+Carbenoxolone</td>
</tr>
<tr>
<td>Control</td>
<td>5.82 ± 0.09</td>
<td>5.78 ± 0.13</td>
</tr>
<tr>
<td>Cortisol</td>
<td>5.85 ± 0.19</td>
<td>5.98 ± 0.18</td>
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Values are means ± SE; n = 4–6 arteries *P < 0.05 vs. control; †P < 0.05 vs. −carbenoxolone.
norepinephrine contractile sensitivity in nonpregnant uterine arteries and eliminated the difference between pregnant and nonpregnant uterine arteries (40). This suggests that increased norepinephrine contractile sensitivity in pregnant compared with nonpregnant uterine arteries may be mediated, in part, by an increase in endogenous cortisol binding to GRs in pregnant uterine arteries due to elevated cortisol levels in pregnancy. In pregnant sheep, plasma levels of cortisol were 11011 to 43 ng/ml (12, 21).

However, because of corticosteroid-binding globulin in vivo, only a fraction of the total cortisol is free. Therefore, the in vitro levels of cortisol used in this study are more comparable to stress-induced levels of free plasma cortisol. In hypoxic sheep, we found that compared with normoxic animals, norepinephrine-induced contractions of uterine arteries are decreased in pregnant but not in nonpregnant animals. This is in agreement with our previous findings (15, 16). The finding that in hypoxic animals there is no difference in norepinephrine contractile sensitivity between pregnant and nonpregnant uterine arteries in the absence of exogenous cortisol would suggest that chronic hypoxia attenuates endogenous cortisol-mediated potentiation of norepinephrine-induced contractions in pregnant uterine arteries. This may not be due to changes in GRs in vascular smooth muscle, because immunoreactive GR protein levels estimated by Western blotting in the present study showed no difference in the uterine arteries between the normoxic control and hypoxic animals. However, because immunoreactive GRs include both cytosolic and nuclear receptors, future studies are needed to examine whether cytosolic GR availability and binding affinity are altered by chronic hypoxia in the uterine artery.

Table 2. Effects of cortisol on slope of norepinephrine-induced [Ca\textsuperscript{2+}]\textsubscript{i}-tension relation in uterine artery

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th></th>
<th>Hyoxia</th>
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<tr>
<td></td>
<td>Control</td>
<td>Cortisol</td>
<td>Control</td>
<td>Cortisol</td>
</tr>
<tr>
<td>Nonpregnant</td>
<td>5,957±762.1</td>
<td>18,020±2,281*</td>
<td>5,367±690.5</td>
<td>7,559±472.3*</td>
</tr>
<tr>
<td>Pregnant</td>
<td>6,466±719.7</td>
<td>6,422±538.6</td>
<td>3,997±487.8†</td>
<td>3,275±220.0</td>
</tr>
</tbody>
</table>

Values are means ± SE (in g tension/ratio of fluorescence at 340 to 380 nm). [Ca\textsuperscript{2+}]\textsubscript{i}, intracellular Ca\textsuperscript{2+} concentration. *P < 0.05 vs. control; †P < 0.5 vs. normoxia.
regulate local levels of cortisol in the absence of changes in HSD activity in the uterine artery, which suggests an endogenous protective mechanism, and counteract the pregnant animals. Progesterone has antiglucocorticoid effects in pregnant uterine arteries by increasing norepinephrine-mediated Ca2+ mobilization in pregnant uterine arteries. In normoxic animals, our previous studies (41) showed that cortisol-mediated enhancement of norepinephrine-mediated Ca2+ mobilization in pregnant uterine arteries was preserved in the hypoxic animals. This is likely to play a key role in the adaptation of the uterine artery to the stress of chronic hypoxia in pregnant animals by protecting the uterine artery and limiting the effect of cortisol under hypoxic stress. In the study of healthy men, acute exposure to high altitude (7,000 m) decreased the activity of the renal 11B-HSD2 (13). In sheep, both increased and decreased 11B-HSD2 mRNA levels in the fetal kidney induced by sustained hypoxia have been reported, with either no change or decreased activity of 11B-HSD2 (3, 28, 43).

When compared with normoxic animals, there was an increase in norepinephrine pD2 from 5.24 (41) to 5.89 in stimulating Ca2+ mobilization in pregnant uterine arteries from the hypoxic animals. In contrast, norepinephrine-mediated Ca2+ sensitivity was significantly decreased in the hypoxic pregnant uterine arteries, compared with that in normoxic animals. Given the finding that norepinephrine-induced contractions were decreased in the pregnant arteries in hypoxic animals, these results suggest that the regulation of Ca2+ sensitivity dominates the hypoxic-mediated changes in the agonist-induced contractions. Chronic hypoxia had no effect on either the agonist-induced Ca2+ mobilization or Ca2+ sensitivity in nonpregnant uterine arteries. This is consistent with the finding that norepinephrine-induced contractions of nonpregnant uterine arteries were the same between the normoxic and hypoxic animals. In normoxic animals, our previous studies (41) showed that cortisol regulated α1-adrenoceptor-mediated pharmacomechanical coupling differentially between nonpregnant and pregnant uterine arteries. In nonpregnant arteries, cortisol enhanced norepinephrine-mediated Ca2+ sensitivity of contractile myofilaments, whereas it increased norepinephrine-induced Ca2+ mobilization in pregnant uterine arteries. Although the effect of cortisol on the agonist-induced Ca2+ mobilization in pregnant uterine arteries was preserved in hypoxic animals, cortisol-mediated enhancement of norepinephrine-mediated Ca2+ sensitivity in nonpregnant arteries was somewhat inhibited in the hypoxic animals. The cortisol-mediated increase in norepinephrine-induced Ca2+ mobilization in hypoxic nonpregnant uterine arteries may counteract its remaining positive effect on the agonist-mediated Ca2+ sensitivity, leading to no changes in norepinephrine-induced contractions.

In contrast to its potentiation effect on norepinephrine-mediated contractions, cortisol suppressed PDBu-mediated contractions in both nonpregnant and pregnant uterine arteries in normoxic animals, suggesting a striking difference between α1-adrenoceptor- and PKC-mediated signaling pathways in response to cortisol. PKC activation of smooth muscle con-
contraction has been well demonstrated from studies showing that phorbol esters, known to activate PKC, induce slow sustained contractions in many types of vascular smooth muscle (5, 9, 17, 18, 23, 32, 34). In our previous studies in the uterine artery, we showed that PDBu increased PKC activity and induced contractions (42). The present finding that PKC-mediated contractions were downregulated in pregnant uterine arteries is consistent with our previous results (42). Although α1-adrenoceptor-mediated contractions are regulated predominately through the thick filament pathway, i.e., increases in intracellular Ca2+ and myosin light chain phosphorylation, many studies have shown a dissociation between myosin light chain phosphorylation and tension development in response to phorbol esters, suggesting an additional thin filament regulation in PKC-mediated smooth muscle contractions (10, 14, 22, 27, 35). We have recently demonstrated that PDBu-induced contractions are independent of changes in either [Ca2+]i or myosin light chain phosphorylation in uterine arteries (D. Xiao and L. Zhang, unpublished observations). This suggests that in the uterine artery PKC-induced contraction is mediated predominately through thin filament regulatory pathways. Taken together, these results would suggest that cortisol modulates contractile force by a dual regulation of thick and thin filaments in the uterine artery. Cortisol may potentiate the thin filament regulatory pathway by enhancing myosin light chain phosphorylation via increases in [Ca2+]i, and/or Ca2+ sensitivity. In contrast, cortisol may attenuate the thin filament regulatory pathway and suppress contractions independent of changes in myosin light chain phosphorylation in the uterine artery. The finding that chronic hypoxia decreased PDBu contractile sensitivity in the uterine arteries suggests an inhibitory effect of hypoxia on the thin-filament regulatory pathways. Unlike norepinephrine-induced contractions, in which the cortisol-mediated potentiation was preserved in the hypoxic pregnant uterine arteries, exogenous cortisol had no effect on PDBu-induced contractions in either nonpregnant or pregnant uterine arteries in hypoxic animals. This suggests an adaptation of the thin filament regulatory pathways of the uterine artery to elevated endogenous cortisol under hypoxic stress, which is not affect by pregnancy.

In summary, the results demonstrate that cortisol potentiates α1-adrenoceptor-mediated contractions, but inhibits PKC-mediated contractions in the uterine arteries, and suggest a differential regulation of the thick and thin filament regulatory pathways in vascular smooth muscle in response to cortisol. Chronic hypoxia suppresses uterine artery sensitivity to cortisol, which may play an important role in the adaptation of uterine vascular tone and blood flow in response to chronic stress of hypoxia during pregnancy.

GRANTS

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