Effect of cortisol on norepinephrine-mediated contractions in ovine uterine arteries

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Xiao, Daliao, Xiaohui Huang, William J. Pearce, Lawrence D. Longo, and Lubo Zhang. Effect of cortisol on norepinephrine-mediated contractions in ovine uterine arteries. Am J Physiol Heart Circ Physiol 284: H1142–H1151, 2003. First published December 12, 2002; 10.1152/ajpheart.00834.2002.—Cortisol potentiated norepinephrine (NE)-mediated contractions in ovine uterine arteries (UA). We tested the hypothesis that cortisol regulated $\alpha_1$-adrenoceptor-mediated pharmacomechanical coupling differentially in nonpregnant UA (NUA) and pregnant UA (PUA). Cortisol (10 ng/ml for 24 h) significantly increased contractile coupling efficiency of $\alpha_1$-adrenoceptors in NUA, but increased $\alpha_1$-adrenoceptor density in PUA. Cortisol potentiated NE-induced inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] synthesis in both NUA and PUA, but increased coupling efficiency of $\alpha_1$-adrenoceptors to Ins(1,4,5)P$_3$ synthesis only in NUA. Carbenoxolone alone did not affect NE-mediated Ins(1,4,5)P$_3$ production, but significantly enhanced cortisol-mediated potentiation of NE-stimulated Ins(1,4,5)P$_3$ synthesis in PUA. In addition, cortisol potentiated the NE-induced increase in Ca$^{2+}$ concentration in PUA, but increased NE-mediated contraction for a given amount of Ca$^{2+}$ concentration in NUA. Collectively, the results indicate that cortisol potentiates NE-mediated contractions differentially in NUA and PUA, i.e., by upregulating $\alpha_1$-adrenoceptor density leading to increased Ca$^{2+}$ mobilization in PUA while increasing $\alpha_1$-adrenoceptor coupling efficiency and myofilament Ca$^{2+}$ sensitivity in NUA. In addition, the results suggest that pregnancy increases type 2 11$\beta$-hydroxysteroid dehydrogenase activity in the UA.

$\alpha_1$-adrenoceptor; 11$\beta$-hydroxysteroid dehydrogenase; inositol 1,4,5-trisphosphate; calcium; pregnancy

CORTICOSTEROID HORMONES play an important role in the control of vascular smooth muscle tone by their permissive effects in potentiating vasoactive responses to catecholamines through glucocorticoid receptors. Increased cortisol response has been associated with an increase in arterial contractile sensitivity to norepinephrine (NE) and vascular resistance (5, 22, 23, 39–42). Despite the fundamental importance of cortisol in regulating sympathetically-mediated contraction of vascular smooth muscle, little is currently known about the cellular mechanisms of vascular smooth muscle in response to cortisol.

During pregnancy in several species, including humans and sheep, maternal plasma cortisol concentrations approximately double (21, 28). In sheep, cortisol plasma levels were increased from $\sim$5 ng/ml in nonpregnant animals to $\sim$10 ng/ml in pregnant animals (21). Given the importance of precise regulation of uterine blood flow for fetal growth and maternal cardiovascular well being during pregnancy, a study of the effect of cortisol on the regulation of uterine artery contraction is fully warranted. Recently, (45) demonstrated that cortisol potentiates NE-mediated contractions of ovine uterine artery by decreasing nitric oxide release and increasing NE binding affinity to $\alpha_1$-adrenoceptors. Comparison of cortisol-mediated responses in the uterine arteries obtained from nonpregnant and near-term pregnant (140 days gestation) sheep indicated that pregnancy attenuated uterine artery sensitivity to cortisol (45), which is likely to be important in maintaining a low vascular reactivity of the uterine artery to NE during pregnancy. Our finding that glucocorticoid receptors were not different between nonpregnant and pregnant uterine arteries (45), suggests that the pregnancy-associated decrease in cortisol sensitivity is not mediated by changes in glucocorticoid receptor numbers. The question arises as to whether, or to what extent, cortisol regulates NE-mediated contractile mechanisms differentially in the nonpregnant and pregnant uterine arteries.

Despite the striking physiological changes in uterine circulation during pregnancy, and the previous studies showing an important role of uterine endothelial nitric oxide (for review, see Ref. 33), little is known about the adaptation of contractile mechanisms of the uterine artery to pregnancy. It has been demonstrated that NE contracts the uterine artery by acting on $\alpha_1$-adrenoceptors and increasing inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$], which correlates well with the contractile responses in the uterine artery (20, 49). Release of intracellular Ca$^{2+}$ from the sarcoplasmic reticulum by Ins(1,4,5)P$_3$ is a major mechanism of pharmacomechanical coupling in smooth muscle (35, 48). There are two major components in receptor-mediated pharmacomechanical coupling: $I)$ an agonist-induced increase in intracellular free Ca$^{2+}$ concentration ([Ca$^{2+}$]$i$), and
agonist-mediated Ca\(^{2+}\) sensitivity of contractile myofilaments.

In the present study, we sought to examine the regulatory effects of cortisol on \(\alpha_1\)-adrenoceptor-mediated pharmacomechanical coupling at multiple steps in the signal transduction pathway in nonpregnant and pregnant ovine uterine arteries. The effect of cortisol on the density of \(\alpha_1\)-adrenoceptors was measured by a radioligand binding method. Classic pharmacological approaches were employed to evaluate the relations between receptor occupancy and contractile response and thereby determine the coupling efficiency of \(\alpha_1\)-adrenoceptors to contractions. To determine the intrinsic activity of \(\alpha_1\)-adrenoceptors in coupling to Ins\((1,4,5)P_3\) synthesis, we analyzed the relations between \(\alpha_1\)-adrenoceptors occupied and Ins\((1,4,5)P_3\) synthesis. In addition, we determined the effect of cortisol on NE-mediated intracellular Ca\(^{2+}\) mobilization and Ca\(^{2+}\) sensitivity of contractile myofilaments in the uterine arteries. Our results indicate that cortisol regulates \(\alpha_1\)-adrenoceptor-mediated pharmacomechanical coupling differentially in nonpregnant and pregnant uterine arteries.

**METHODS**

**Tissue preparation.** Nonpregnant and time-dated pregnant (~140-day gestation) sheep were obtained from Nebeker Ranch (Lancaster, CA). Animals were anesthetized with thierymal (10 mg/kg) administered via the external left jugular vein, and anesthesia was maintained with 1.5–2.0% halothane in oxygen throughout surgery. An abdominal incision was made to expose the uterus, and the uterine arteries and partial uterine vein, and anesthesia was maintained with 1.5

After cortisol pretreatment, arterial contractions were quantified in the continuous presence of cortisol in Krebs solution at 37°C, as described previously (45). Isometric tensions were measured. After 60 min of equilibration in the tissue bath, each ring was stretched to the optimal resting tension as determined by the tension developed in response to KCl added at each stretch level. One ring was used for each determination in each animal, and \(n\) represents the number of animals. Concentration-response curves were obtained by cumulative addition of NE in approximate one-half log increments. Prism software (GraphPad; San Diego, CA) was used to fit the curve and determine the apparent affinity (pD\(_2\) values (= log EC\(_{50}\)) and the maximum response. To determine the relation between receptor occupancy and response, the apparent dissociation constant (K\(_D\)) of NE to \(\alpha_1\)-adrenoceptors determined previously (45) was used. The fractional receptor occupancy was calculated from the equation [RA]/[RT] = [A]/[A] + K\(_D\)], where [RA] is the concentration of the receptor agonist complex, [RT] is the total concentration of the receptors, and [A] is the concentration of the agonist (15). To estimate the coupling efficiency of \(\alpha_1\)-adrenoceptors to Ins\((1,4,5)P_3\) synthesis, [RA]/[RT] was converted to the number of the receptors occupied by 1 or 10 \(\mu\)M NE, using the total receptor density determined by \([3H]\)prazosin. NE-elicted Ins\((1,4,5)P_3\) productions were then expressed as picomoles of Ins\((1,4,5)P_3\) per femtomole of \(\alpha_1\)-adrenoceptors occupied.

**Radioligand binding studies.** Saturation binding of \([3H]\)prazosin (DuPont-NEN; Boston, MA), an \(\alpha_1\)-adrenoceptor antagonist radioligand, was performed by a rapid filtration method, as described previously (18). Briefly, the vessels were homogenized with a homogenizer (speed setting 5.5 × 15 s; model Polytron PT10/35; Brinkman) in ice-cold 50 mM Tris-HCl (pH 7.4) buffer containing 1 mM EGTA. Nuclei and cell debris were removed by low-speed centrifugation at 1,086 g for 10 min. The supernatant was centrifuged at 50,000 g for 60 min. The microsomal pellet was resuspended in the same Tris buffer to yield ~0.2 mg/ml protein, as determined by the method of Bradford (4). Equilibrium binding was carried out at 30°C for 45 min in a 500-\(\mu\)l volume, consisting of 440 \(\mu\)l of membrane suspension, 50 \(\mu\)l of radioligand, and 10 \(\mu\)l of drug or diluent. The concentrations of \([3H]\)prazosin employed were from 0.002 to 4 nM. Nonspecific binding was determined by the addition of 10 \(\mu\)M phenolamine. All determinations were performed in triplicate. Bound and free radioligand were separated by rapid filtration of the membrane suspension over polyethyleneimine (0.5%)-pretreated filter (model GF/C; Whatman) with a Brandel cell harvester. Filters were rinsed with two 5-ml aliquots of the ice-cold Tris buffer and counted for radioactivity at 45% efficiency in liquid scintillation analyzer (model 1900CA Tri-Carb, Packard Instrument; Downers Grove, IL).

**Measurement of Ins\((1,4,5)P_3\).** After treatments with or without cortisol, the tissues were equilibrated in Krebs solution at 37°C for 30 min and then stimulated with different concentrations of NE for 30 s at its peak level of Ins\((1,4,5)P_3\) production, as described previously (19). Ins\((1,4,5)P_3\) was measured by the competitive ligand binding radioreceptor assay (19). Briefly, the tissue reactions were terminated by flash freezing tissues in liquid N\(_2\). The tissues were then homogenized in ice-cold 16.7% trichloroacetic acid. The homogenate was centrifuged at 1,500 g for 10 min at 4°C. The supernatant was extracted with water-saturated diethyl ether to remove trichloroacetic acid, and the pellet was saved for protein determination by using the method of Bradford (4). Ins\((1,4,5)P_3\) in the supernatant was determined with the use of a radioreceptor assay kit from DuPont-NEN. Values were expressed as picomoles of Ins\((1,4,5)P_3\) per milligram of protein.
Simultaneous measurement of \([Ca^{2+}]\), and tension. Simultaneous recordings of contractile tension and free \([Ca^{2+}]\), in the same tissue were conducted as described previously (50). Briefly, the arterial rings were attached to an isometric force transducer in a 5-ml tissue bath mounted on an intracellular \(Ca^{2+}\) analyzer (model CAF-110, Jasco; Tokyo, Japan). The tissues were equilibrated in Krebs buffer under a resting tension of 0.5 g for 40 min and loaded under the same tension with 5 \(\mu\)M fura 2-acetoxyethyl 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 esterases. 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 (\(F_{340}\) and \(F_{380}\), respectively) and their ratio (\(R_{340/380}\)) were recorded with a time constant of 250 ms and stored with the force signal on a computer.

Data analysis. Saturation binding and concentration response curves were analyzed by computer-assisted nonlinear regression to fit the data and to determine the dissociation constant (\(K_D\)), receptor density, and \(pD_2\) with the use of Prism software. Results were expressed as means ± SE, and the differences were evaluated for statistical significance (\(P < 0.05\)) by Student’s t-test and analysis of variance.

RESULTS

NE-induced contractions. We (45) showed that cortisol (1–30 ng/ml) treatment for 24 h produces a dose-dependent increase in NE-mediated contractions in the uterine arteries. Figure 1 shows that cortisol (10 ng/ml for 24 h) significantly increased NE \(pD_2\) values (5.61 ± 0.02 → 6.36 ± 0.07, \(n = 6\), \(P < 0.05\)) and the maximal response (5.46 ± 0.05 g → 7.06 ± 0.18 g, \(P < 0.05\)) in nonpregnant uterine arteries (Fig. 1A). In pregnant uterine arteries, cortisol increased NE \(pD_2\) values (6.22 ± 0.11 → 6.55 ± 0.06, \(n = 7\), \(P < 0.05\)) without affecting the maximal response (Fig. 1B). As reported previously, the degree of cortisol-mediated potentiation of NE \(pD_2\) was significantly decreased in pregnant uterine arteries (45).

Radioligand binding studies. The effects of cortisol on the density of \(\alpha_1\)-adrenoceptors in the uterine arteries were determined by evaluating the saturation binding of \[^3H\]prazosin, a selective \(\alpha_1\)-adrenoceptor antagonist radioligand. As shown in Fig. 2, the binding of \[^3H\]prazosin to \(\alpha_1\)-adrenoceptors was specific and saturable and was best described by an interaction of the radioligand with a single class of high-affinity binding sites in both nonpregnant and pregnant uterine arteries. There was no difference in the \(K_D\) of \[^3H\]prazosin to \(\alpha_1\)-adrenoceptors between the nonpregnant (0.35 ± 0.10 nM, \(n = 5\)) and pregnant (0.23 ± 0.06 nM, \(n = 5\)) arteries. In contrast, the density of \(\alpha_1\)-adrenoceptors was significantly higher in pregnant (75.7 ± 9.6 fmol/mg protein, \(n = 5\)) than nonpregnant (30.0 ± 6.6 fmol/mg protein, \(n = 5\)) uterine arteries (\(P < 0.05\)). Cortisol did not affect the \(K_D\) of \[^3H\]prazosin in either nonpregnant (0.35 ± 0.10 → 0.19 ± 0.05 nM, \(n = 5\), \(P > 0.05\)) or pregnant (0.23 ± 0.06 → 0.54 ± 0.14 nM, \(n = 5\), \(P > 0.05\)) uterine arteries, but significantly increased the density of \(\alpha_1\)-adrenoceptors in pregnant uterine arteries (75.7 ± 9.6 → 136.2 ± 17.9 fmol/mg protein, \(n = 5\), \(P < 0.05\)). In contrast, cortisol did not change \(\alpha_1\)-adrenoceptor density in the nonpregnant arteries (30.0 ± 6.6 → 31.8 ± 5.8 fmol/mg protein, \(n = 5\), \(P > 0.05\)).

Receptor occupancy-contraction relation. We (45) demonstrated in sheep of similar weight and gestational age that cortisol decreased the \(K_A\) of NE to \(\alpha_1\)-adrenoceptors in nonpregnant (24.6 ± 6.5 → 6.3 ± 1.4 \(\mu\)M, \(P < 0.05\)), but not in pregnant (5.2 ± 2.0 → 2.2 ± 0.3 \(\mu\)M, \(P > 0.05\)) uterine arteries. To examine the effect of cortisol on the postreceptor mechanisms (i.e., beyond the change in receptor numbers), the \(K_A\) values determined previously were used to calculate the fraction of \(\alpha_1\)-adrenoceptors occupied (\([RA]/[RT]\)) at each NE concentration used in construction of the respective concentration-contraction curves. The respective occupancy-response relations constructed for NE-mediated contractions are presented in Fig. 3. Cortisol treatment significantly increased the NE-mediated contractions by 25% at the maximal receptor
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Fig. 2. Saturation binding of [3H]prazosin. Nonpregnant (A) and pregnant (B) uterine arteries were treated in the absence or presence of cortisol (10 ng/ml) for 24 h, and membranes were then prepared. Specific [3H]prazosin binding was defined as the arithmetic difference between total binding and nonspecific binding obtained in presence of 10 μM phentolamine. Analysis of specific binding data by nonlinear computer-based methods (fit to a rectangular hyperbola) confirmed that [3H]prazosin bound to a single class of binding sites in the vessels. Data are the means ± SE of tissues from 5 animals.

Fig. 3. NE receptor occupancy-response relation. Nonpregnant (A) and pregnant (B) uterine arteries were treated in the absence or presence of cortisol (10 ng/ml) for 24 h, and contractions were then induced by NE. Fraction of α1-adrenoceptors occupied at each NE concentration was calculated as described in METHODS. Data are the means ± SE of tissues from 5 to 7 animals.

Fig. 4. NE induced inositol 1,4,5-trisphosphate [Ins(1,4,5)P₃] production. Nonpregnant and pregnant uterine arteries were incubated with different concentrations of NE for 30 s. Data are the means ± SE of tissues from 5 animals.

occupancy in nonpregnant uterine arteries and significantly decreased the receptor occupancy required to produce 50% of the maximal response from 0.113 ± 0.007 to 0.076 ± 0.012 (P < 0.05). In contrast, cortisol did not affect the α1-adrenoceptor occupancy-contraction relation in pregnant uterine arteries (Fig. 3).

Ins(1,4,5)P₃ synthesis. NE produced a concentration-dependent increase of Ins(1,4,5)P₃ in both nonpregnant and pregnant uterine arteries with pD₂ values of 6.49 ± 0.17 and 6.83 ± 0.10 (n = 5, P > 0.05), respectively (Fig. 4). To examine the effect of cortisol on the NE-mediated Ins(1,4,5)P₃ synthesis in the uterine artery, we quantified Ins(1,4,5)P₃ production induced by 0.1 μM NE in the tissues pretreated with different concentrations of cortisol (0–30 ng/ml for 24 h). Figure 5 shows that cortisol produces a dose-dependent potentiation of NE-induced Ins(1,4,5)P₃ synthesis in both nonpregnant and pregnant uterine arteries. Given that the effect of cortisol is regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD), and that our previous findings suggested an increase in type-2 11β-HSD activity in the pregnant arteries (45), we examined the effect of 11β-HSD on the cortisol-potentiated Ins(1,4,5)P₃ synthesis in the uterine artery. Tissues were pretreated with 10 ng/ml cortisol in the absence or presence of the 11β-HSD inhibitor carbenoxolone (3 μM) for 24 h, and NE (0.1 μM)-stimulated Ins(1,4,5)P₃ production was then measured. As shown in Fig. 6, cortisol significantly potentiated NE-induced Ins(1,4,5)P₃ synthesis in both nonpregnant and pregnant uterine arteries. Carbenoxolone alone did not affect NE-mediated

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Ins(1,4,5)P3 synthesis, but significantly enhanced cortisol-mediated potentiation of NE-stimulated Ins(1,4,5)P3 synthesis in the pregnant arteries. In contrast, cortisol-mediated potentiation of NE-induced Ins(1,4,5)P3 synthesis in the nonpregnant arteries was not affected by carbenoxolone (Fig. 6). To estimate the coupling efficiency of α1-adrenoceptors to Ins(1,4,5)P3 synthesis, NE-elicited Ins(1,4,5)P3 productions were expressed as picomoles of Ins(1,4,5)P3 per femtomole of α1-adrenoceptors occupied, as described in METHODS. As shown in Fig. 7, cortisol significantly increased the coupling efficiency in nonpregnant (P < 0.05), but not pregnant, arteries.

[Ca2+]i and [Ca2+]tension relation. NE produced a dose-dependent increase of free [Ca2+]i in both nonpregnant and pregnant uterine arteries with pD2 values of 5.78 ± 0.11 and 5.24 ± 0.06 (n = 4, P < 0.05), respectively (Fig. 8). Cortisol treatment did not significantly affect NE-induced [Ca2+]i in nonpregnant arteries (pD2: 5.78 ± 0.11 → 6.05 ± 0.10, n = 4, P > 0.05) but significantly increased pD2 of NE-stimulated [Ca2+]i in pregnant (5.24 ± 0.06 → 5.96 ± 0.16, n = 4, P < 0.05) uterine arteries. To examine whether cortisol-mediated potentiation of [Ca2+]i responses in pregnant uterine arteries was due to increased coupling efficiency of Ins(1,4,5)P3 and Ca2+ release, we evaluated the relation between Ins(1,4,5)P3 production and [Ca2+]i responses. As shown in Fig. 9, NE-evoked Ins(1,4,5)P3 production correlated significantly with increased [Ca2+]i in the uterine arteries, implicating a key role of Ins(1,4,5)P3 in Ca2+ mobilization. There
was no significant difference between the slopes of the Ins(1,4,5)P3,[Ca\(^{2+}\)] relationship determined in control and cortisol-treated pregnant arteries (0.00098 ± 0.00017 vs. 0.00013 ± 0.00002, P < 0.05; Fig. 9), suggesting that cortisol did not affect the apparent coupling efficiency of Ins(1,4,5)P3 to Ca\(^{2+}\) mobilization in the pregnant arteries. In contrast, cortisol caused a significant decrease in the slope in the nonpregnant arteries (0.00091 ± 0.00018 vs. 0.00017 ± 0.00002, P < 0.05; Fig. 9).

The [Ca\(^{2+}\)]-tension relation depicted from the data of simultaneous measurement of [Ca\(^{2+}\)] and tension in the same tissue indicated that there was a positive correlation between these two parameters in the presence of cumulative concentrations of NE in both nonpregnant and pregnant uterine arteries (Fig. 10). There was a significant increase in the slope (g tension/R\(_{340/380}\) [Ca\(^{2+}\)]) from nonpregnant (16.6 ± 2.5) to pregnant (29.9 ± 2.3) uterine arteries (n = 4, P < 0.05). Cortisol significantly increased the slope in the nonpregnant (16.6 ± 2.5 → 45.6 ± 7.9, P < 0.05) but not pregnant (29.9 ± 2.3 → 24.1 ± 2.9, P > 0.05) uterine arteries (Fig. 10).

**DISCUSSION**

The present study demonstrated clearly that cortisol regulated \(\alpha_1\)-adrenoceptor-mediated pharmacomechanical coupling differentially in nonpregnant and pregnant uterine arteries. In the absence of cortisol, the basal levels of Ins(1,4,5)P3 and NE-induced contractions were increased in pregnant uterine arteries. Although the attenuation of cortisol-potentiated contractions of pregnant uterine arteries may be due in part to elevated endogenous cortisol in pregnant animals, there are striking differences in signaling pathways between nonpregnant and pregnant uterine arteries in response to cortisol. In nonpregnant arteries, cortisol did not affect \(\alpha_1\)-adrenoceptor numbers, but significantly enhanced its coupling efficiency by increasing both Ins(1,4,5)P3 and agonist-mediated Ca\(^{2+}\) sensitivity of contractile myofilaments. Pregnancy abolished the effects of cortisol on \(\alpha_1\)-adrenoceptor coupling efficiency, and, instead, cortisol upregulated \(\alpha_1\)-adrenoceptor numbers, leading to increased Ins(1,4,5)P3 and Ca\(^{2+}\) mobilization.

Coupling of \(\alpha_1\)-adrenoceptors to contractile responses can be modulated at several steps in the signal transduction pathway, including receptor density, agonist affinity, and coupling efficiency of the receptor. The finding that cortisol upregulated \(\alpha_1\)-adrenoceptor density in pregnant arteries is consistent with previous studies (16) showing that adrenalectomy caused a 40% decrease in \(\alpha_1\)-adrenoceptor density in the rat aorta, which was restored by dexamethasone replacement.
Whereas the present study did not examine \(\alpha_1\)-adrenoceptor subtypes, previous studies (29) showed that glucocorticoids upregulated expression of \(\alpha_{1\beta}\)-adrenoceptors in vascular smooth muscle cells by increasing the rate of gene transcription. These studies suggest that glucocorticoids play a key role in the regulation of \(\alpha_1\)-adrenoceptor density in vascular smooth muscle. In contrast to the pregnant uterine artery, cortisol did not affect \(\alpha_1\)-adrenoceptor density, but instead increased NE efficiency in contracting nonpregnant uterine arteries, indicating that mechanisms beyond the agonist-receptor interaction are also regulated by cortisol. This increased coupling efficiency may be mediated by multiple mechanisms, including receptor coupling to Ins(1,4,5)P_3 synthesis, Ins(1,4,5)P_3 efficiency in Ca^{2+} mobilization, and Ca^{2+} sensitivity of contractile myofilaments.

In the present study, basal Ins(1,4,5)P_3 levels were elevated in pregnant uterine arteries. Although it is possible that elevated cortisol during pregnancy may play a role, we found that NE sensitivity (pD_2 values) in stimulating Ins(1,4,5)P_3 production was not significantly different between nonpregnant and pregnant uterine arteries. Cortisol potentiated NE-induced Ins(1,4,5)P_3 synthesis in both nonpregnant and pregnant uterine arteries. Glucocorticoid-mediated potentiation of Ins(1,4,5)P_3 production has been reported in vascular smooth muscle for angiotensin II, arginine vasopressin, endothelin-1, and catecholamines (24, 30, 43). The role of Ins(1,4,5)P_3 as the messenger of pharmacomechanical Ca^{2+} mobilization in smooth muscle has been firmly established (35). We have demonstrated that Ins(1,4,5)P_3 is the messenger of pharmacomechanical coupling for \(\alpha_1\)-adrenoceptor-mediated contractions in the uterine artery (49). Although \(\alpha_1\)-adrenoceptor-mediated increase in intracellular Ca^{2+} may result from both Ca^{2+} release from intracellular stores and Ca^{2+} influx through receptor- and voltage-operated Ca^{2+} channels, the initial signal is dependent on Ins(1,4,5)P_3-mediated Ca^{2+} release from intracellular stores. In the present study, we measured Ca^{2+} by its peak, rather than the area under the curve, which provided a reasonable estimation of Ca^{2+} release from intracellular stores. It has been suggested that both the mobilization of Ca^{2+} from internal stores, as well as the entry of external Ca^{2+}, are critically dependent on the formation of Ins(1,4,5)P_3 (3, 48). By using permeabilized vascular smooth muscle preparations, Somlyo et al. (34) demonstrated that photolytically released Ins(1,4,5)P_3 from the caged Ins(1,4,5)P_3 stimulated a rise in free [Ca^{2+}], that correlated closely with the force development.

The finding that the 11\(\beta\)-HSD inhibitor carbenoxolone selectively enhanced cortisol-mediated potentiation of Ins(1,4,5)P_3 synthesis in the pregnant uterine artery is intriguing and suggests an increase in type \(\beta\) 11\(\beta\)-HSD activity in this vessel. This is in agreement with our previous finding that carbenoxolone selectively potentiated NE-induced contraction in the pregnant, but not in nonpregnant, uterine arteries (45). The effect of glucocorticoids on vascular reactivity is regulated with the use of 11\(\beta\)-HSD (39). Two 11\(\beta\)-HSD isozymes catalyze the interconversion of cortisol and cortisone. The type 1 11\(\beta\)-HSD has bidirectional activity, whereas the type 2 enzyme mainly converts cortisol into cortisone, the biologically inactive form. Both type 1 and type 2 11\(\beta\)-HSD have been found in vascular smooth muscle (6, 42). Several studies (5, 22, 39, 42) have demonstrated that inhibition of 11\(\beta\)-HSD with inhibitors such as carbenoxolone increases cortisol-mediated potentiation of vascular response to NE. Although under normal conditions, the type 1 isoform dominates functioning in the oxo-reductase mode that converts cortisone to cortisol in vascular smooth muscle, the two major isoforms are compartmentalized discretely and regulated differentially by steroids such as estrogen and progesterone (36). In human pregnancy, placental type 2 11\(\beta\)-HSD activity increases markedly in the third trimester of pregnancy, at a time when maternal circulating levels of glucocorticoid are rising, which serves as a protective mechanism for the fetus (32). Our results suggest an increase in the activity of type \(\beta\) 11\(\beta\)-HSD in pregnant uterine arteries, which is likely to play an important role in the local regulation of cortisol concentration by limiting cortisol effects on the uterine artery, and protecting it from elevated cortisol levels during pregnancy.

The present study demonstrated that for a given number of \(\alpha_1\)-adrenoceptors occupied, cortisol increased Ins(1,4,5)P_3 production in nonpregnant uterine arteries, suggesting that the intrinsic activity of the receptor was enhanced. The mechanisms underlying this enhanced coupling efficiency of \(\alpha_1\)-adrenoceptors to Ins(1,4,5)P_3 synthesis are not clear at present, but can occur at multiple levels. For example, heterotrimeric guanine nucleotide-binding proteins (G-proteins) are physiological targets of glucocorticoids in vivo (31). It has been shown that glucocorticoids increase G_{\text{q/11\(\beta\)}} protein expression and phospholipase C activity in rat osteoblastic cells (10). In addition, glucocorticoids have been shown to play a crucial role in maintaining coupling of \(\alpha_1\)-adrenoceptors to G proteins, by regulating the amounts of G-proteins in the rat aorta (16, 17). Given the finding that pregnant women increased inhibitory G protein activation/coupling in uterine arteries to certain agonists (37), it is speculated that the increased adrenoceptor binding induced by cortisol treatment in this study may be due to increased G protein receptor coupling, which augments ligand/receptor binding. Taken together, these studies suggest an important mechanism by which glucocorticoids regulate receptor-G protein coupling, and hence transmembrane signaling pathways, in vascular smooth muscle. Future studies are needed to determine whether cortisol treatment for 24 h increases G proteins expression and activity in the uterine artery. Alternatively, cortisol may enhance the coupling of \(\alpha_1\)-adrenoceptors to Ins(1,4,5)P_3 synthesis by increasing phosphoinositide-specific phospholipase C activity. It has been demonstrated that dexamethasone increases phospholipase C activity and mRNA/protein expression of the phospholipase C-\(\beta\) isoforms in the rat brain (12).
The finding that cortisol did not affect the coupling efficiency of \( \alpha_1 \)-adrenoceptors to Ins\((1,4,5)\)P\(_3\) synthesis in the pregnant arteries is consistent with the results that cortisol did not change the intrinsic efficiency of NE in contracting these vessels. In addition, cortisol did not affect Ins\((1,4,5)\)P\(_3\) efficiency in Ca\(^{2+}\) mobilization in the pregnant artery. These results suggest that cortisol-induced potentiation of NE-stimulated Ca\(^{2+}\) mobilization is mediated predominantly by the upregulation of \( \alpha_1 \)-adrenoceptor numbers in the pregnant artery. The apparent loss of the ability of cortisol in regulating \( \alpha_1 \)-adrenoceptor coupling efficiency in the pregnant uterine artery may be due in part to pregnancy-mediated alterations in G protein levels and GTPase activity (7–9, 11, 37). In uterine arteries, pregnancy inhibited stimulatory \( G_\alpha \) GTPase activity and the decreased \( G_\alpha \) cycling rate, but increased inhibitory \( G \) protein activation/coupling (7, 37).

The finding that cortisol did not increase NE-induced Ca\(^{2+}\) mobilization in nonpregnant uterine arteries is somewhat surprising, given that cortisol potentiated \( \alpha_1 \)-adrenoceptor-mediated Ins\((1,4,5)\)P\(_3\) synthesis in the nonpregnant arteries. Nevertheless, cortisol significantly decreased the coupling efficiency of Ins\((1,4,5)\)P\(_3\) to Ca\(^{2+}\) mobilization in the nonpregnant arteries, which may counteract the effect of increased Ins\((1,4,5)\)P\(_3\). The coupling of Ins\((1,4,5)\)P\(_3\) to Ca\(^{2+}\) mobilization involves the binding of Ins\((1,4,5)\)P\(_3\) to Ins\((1,4,5)\)P\(_3\) receptors. Our previous studies (19, 51) demonstrated that hypoxic stress altered Ins\((1,4,5)\)P\(_3\) binding affinity and Ins\((1,4,5)\)P\(_3\) receptor density in the uterine and cerebral arteries, respectively. Other studies (26) suggested that dexamethasone caused a decrease in Ins\((1,4,5)\)P\(_3\) affinity to Ins\((1,4,5)\)P\(_3\) receptors in NIH3T3 cells. Given the previous finding that glucocorticoids did not alter any of three isoforms of Ins\((1,4,5)\)P\(_3\) receptors in the rat brain (15), it is speculated that cortisol-mediated decrease in the coupling of Ins\((1,4,5)\)P\(_3\) to Ca\(^{2+}\) mobilization in the uterine artery is due to decreased Ins\((1,4,5)\)P\(_3\) binding affinity.

Not only does Ca\(^{2+}\) play an important role in the regulation of smooth muscle contraction, Ca\(^{2+}\) sensitivity also provides a key determinant of smooth muscle contraction, which is modulated physiologically and pathophysiological in the uterine arteries (46, 50). In the present study, we have shown that NE-induced Ca\(^{2+}\) mobilization is decreased by pregnancy. In contrast, NE-mediated Ca\(^{2+}\) sensitivity was increased. To our knowledge, this is the first study to demonstrate the differential adaptation of Ca\(^{2+}\) homeostasis in the uterine artery to pregnancy. Although few studies examined the effects of pregnancy and/or steroid hormones on contractile mechanisms in the uterine artery, studies in human myometrium demonstrated that adaptation to pregnancy included 1) cellular mechanisms that preclude the development of high levels of myosin light chain phosphorylation during contraction; and 2) an increase in the stress-generating capacity for any given level of myosin light chain phosphorylation, suggesting a decrease in Ca\(^{2+}\) mobilization and an increase in Ca\(^{2+}\) sensitivity (44). Although the mechanisms for this differential adaptation of Ca\(^{2+}\) mobilization and Ca\(^{2+}\) sensitivity to pregnancy are not entirely clear at present, it has been shown that progesterone decreases Ca\(^{2+}\) mobilization in myometrial smooth muscle cells (14). On the other hand, an increase in RhoA/Rho kinase (27) and a decrease in myosin light chain phosphatase (38) may play an important role in pregnancy-mediated increase in Ca\(^{2+}\) sensitivity. In addition, an increase in contractile proteins of actin and myosin in the pregnant uterine artery (2) may also contribute to the increased Ca\(^{2+}\) sensitivity.

In the present study, despite a decrease in NE-induced Ca\(^{2+}\) mobilization in pregnant uterine arteries, NE-mediated contractions were increased in pregnant compared with nonpregnant uterine arteries (1, 46). In pregnant uterine arteries, NE had ~10 times lower sensitivity in Ca\(^{2+}\) mobilization (pD\(_2\): 5.24) than tension generation (pD\(_2\): 6.22). This suggests that changes in Ca\(^{2+}\) sensitivity play a predominant role in the regulation of uterine artery contractility during pregnancy. The finding that cortisol increased Ca\(^{2+}\) sensitivity in the nonpregnant uterine artery is intriguing and suggests that cortisol may play an important role in the pregnancy-induced increase in Ca\(^{2+}\) sensitivity in the uterine artery, given that maternal plasma cortisol concentrations significantly increase during pregnancy (21, 28). Because progesterone and/or estrogen treatment inhibits agonist- and GTP\(_\gamma\)S-induced Ca\(^{2+}\) sensitization of smooth muscle by increasing Rnd1 expression, which inhibits the RhoA-dependent pathways (25), and progesterone has antiglucocorticoid effects and binds to glucocorticoid receptors at a physiological concentration, we propose that cortisol counteracts with progesterone and/or estrogen in regulating Ca\(^{2+}\) sensitivity of the uterine artery during pregnancy.

In summary, the present results indicate that cortisol enhances \( \alpha_1 \)-adrenoceptor coupling efficiency and agonist-mediated myofilament Ca\(^{2+}\) sensitivity in the nonpregnant uterine artery, whereas it increases \( \alpha_1 \)-adrenoceptor density, leading to an increase in Ca\(^{2+}\) mobilization in the pregnant artery. To our knowledge, this is the first study of the effect of cortisol on agonist-mediated pharmacomechanical coupling in vascular smooth muscle in general and on the regulation of Ca\(^{2+}\) homeostasis in the uterine artery in particular. Although the mechanisms underlying the differential regulatory effects of cortisol on Ca\(^{2+}\) mobilization and Ca\(^{2+}\) sensitivity in pregnant and nonpregnant uterine arteries remain to be elucidated, the present study suggests an important role of cortisol in the regulation of Ca\(^{2+}\) homeostasis in the uterine artery during pregnancy. Our recent study (46) demonstrated that pregnancy altered the ERK/protein kinase C pathway in Ca\(^{2+}\) handling in the uterine artery. The potential interaction of glucocorticoids with the ERK/protein kinase C pathway in the regulation of uterine artery contractility presents an intriguing avenue for future investigation.
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