Regulation of baseline Ca\(^{2+}\) sensitivity in permeabilized uterine arteries: effect of pregnancy

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Xiao, Daiiao, Xiaohui Huang, Lawrence D. Longo, William J. Pearce, and Lubo Zhang. Regulation of baseline Ca\(^{2+}\) sensitivity in permeabilized uterine arteries: effect of pregnancy. Am J Physiol Heart Circ Physiol 291: H413–H420, 2006. First published February 24, 2006; doi:10.1152/ajpheart.00103.2006.—The adaptation of contractile mechanisms of the uterine artery to pregnancy is not fully understood. The present study examined the effect of pregnancy on the uterine artery baseline Ca\(^{2+}\) sensitivity. In β-escin-permeabilized arterial preparations, Ca\(^{2+}\)-induced concentration-dependent contractions were significantly decreased in uterine arteries from pregnant animals compared with those of nonpregnant animals. Time-course studies showed that Ca\(^{2+}\) increased phosphorylation of 20-kDa myosin light chain (MLC\(_{20}\)), which preceded the tension development in vessels from both pregnant and nonpregnant animals. When compared with vessels from nonpregnant animals, there was a significant increase in the protein level of MLC\(_{20}\) and an accordance increase in the level of Ca\(^{2+}\)-induced phosphorylated MLC\(_{20}\) (MLC\(_{20}\)-P) in uterine arteries during pregnancy. Simultaneous measurements of MLC\(_{20}\)-P levels and contractions stimulated with Ca\(^{2+}\) in the same tissues demonstrated a significant attenuation in the tension-to-MLC\(_{20}\)-P ratio in uterine arteries during pregnancy. Activation of PKC with phorbol 12,13-dibutyrate (PDBu) potentiated Ca\(^{2+}\)-induced contractions in uterine arteries from nonpregnant but not pregnant animals. Accordingly, inhibition of PKC attenuated Ca\(^{2+}\)-induced contractions in uterine arteries from nonpregnant but not pregnant animals. PDBu produced contractions in the presence or absence of Ca\(^{2+}\) in the β-escin-permeabilized arteries, which were significantly decreased in uterine arteries from pregnant compared with nonpregnant animals. The results suggest that pregnancy upregulates the thick-filament regulatory pathway by increasing MLC\(_{20}\) phosphorylation but downregulates the thin-filament regulatory pathway by decreasing the contractile sensitivity of MLC\(_{20}\)-P, resulting in attenuated baseline Ca\(^{2+}\) sensitivity in the uterine artery. In addition, PKC plays an important role in the regulation of basal Ca\(^{2+}\) sensitivity, which is downregulated during pregnancy.

protein kinase C; thick filament; thin filament; myosin light chain; phosphorylation

DURING PREGNANCY, the uterine artery delivers oxygen and substrates to the developing fetus. Previous studies (5, 10, 35, 50, 51) have demonstrated a complex adaptation of uterine artery contractile and relaxation mechanisms to pregnancy. The mechanisms regulating vascular smooth muscle contraction include a variety of intracellular signaling pathways that integrate a multitude of stimuli into a coordinated contractile response. Ca\(^{2+}\) mobilization and the Ca\(^{2+}\) sensitivity of the contractile apparatus are the two major regulatory mechanisms for smooth muscle contraction. An increase in intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_i\)]) leads to activation of Ca\(^{2+}\)-dependent myosin light chain kinase, which in turn phosphorylates the 20-kDa regulatory myosin light chain (MLC\(_{20}\)), and thereby triggers contraction (17, 38). Ca\(^{2+}\) sensitivity, defined by force production per unit changes in [Ca\(^{2+}\)], is another important mechanism in the regulation of vascular contractility. Regulation of the Ca\(^{2+}\) sensitivity of the contractile apparatus involves inhibition of myosin light chain phosphatase (MLCP), resulting in an increase in the level of phosphorylated MLC\(_{20}\) (MLC\(_{20}\)-P) (12, 15, 20, 34). Moreover, many studies have demonstrated dissociation between MLC\(_{20}\)-P and crossbridge cycling rates/tension development, suggesting a thin-filament regulatory pathway of Ca\(^{2+}\) sensitivity (4, 29, 37).

Recently, we have demonstrated that the Ca\(^{2+}\) sensitivity is a key mechanism in the regulation of uterine artery contractility (46, 48, 50, 51). We have shown that pregnancy increases α\(_1\) adrenoceptor-induced Ca\(^{2+}\) mobilization but inhibits α\(_1\) adrenoceptor-mediated Ca\(^{2+}\) sensitivity in the uterine artery (51). Moreover, α\(_1\) adrenoceptor-stimulated contractions were regulated through both thick- and thin-filament pathways, with the thick-filament regulatory pathway, i.e., MLC\(_{20}\) phosphorylation, predominating (51). In contrast, the PKC-mediated contraction was regulated predominantly through thin-filament pathways, i.e., independent of changes in either Ca\(^{2+}\) concentrations or MLC\(_{20}\)-P (48, 51). More recently, we have shown that PKC plays a key role in the regulation of myogenic tone of the uterine artery, which is significantly decreased in uterine arteries during pregnancy (45). Myogenic contraction is an important physiological mechanism that regulates basal vascular tone and is a significant contributor to the modulation of blood flow. Our studies suggested that the Ca\(^{2+}\) sensitivity was a key component in the regulation of uterine artery myogenic tone (45). The differences in the baseline myofilament Ca\(^{2+}\) sensitivity and/or its alteration by stimuli have been shown to determine the variations in basal vascular tone and are associated with the differences in animal species, developmental age, artery size, and physiological and pathophysiological variations (1, 2, 11, 40). However, it is unknown whether or to what extent pregnancy alters the basal Ca\(^{2+}\) sensitivity in the uterine artery.

In the present study, we determined the effect of pregnancy on the baseline Ca\(^{2+}\) sensitivity in uterine artery smooth muscle and tested the hypothesis that pregnancy inhibited the basal Ca\(^{2+}\) sensitivity. By clamping Ca\(^{2+}\) concentrations, the Ca\(^{2+}\) sensitivity of myofilaments can be determined directly in permeabilized arteries (6, 23, 43). To investigate the potential...

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role of thick- and/or thin-filament pathways in the regulation of the baseline Ca\(^{2+}\) sensitivity in the uterine artery and its alteration by pregnancy, we measured Ca\(^{2+}\)-induced MLC\(_{20}\) phosphorylation and tension simultaneously in the same tissues and determined the phosphorylated MLC\(_{20}\)-to-Ca\(^{2+}\) and tension-to-phosphorylated MLC\(_{20}\) ratios. In addition, we also investigated the role of PKC in the regulation of the basal Ca\(^{2+}\) sensitivity in the uterine artery and its adaptation to pregnancy.

**METHODS**

*Tissue preparation.* Uterine arteries were isolated from nonpregnant and pregnant (\(\sim 140\) days gestation) sheep, as described previously (51). Briefly, animals were anesthetized with thiamylal (10 mg/kg), administered via the external left jugular vein. The ewes were then intubated, and anesthesia was maintained on 1.5% to 2.0% halothane in O\(_2\) throughout the surgery. An incision in the abdomen was made and the uterus exposed. The third (from nonpregnant sheep) and fourth (from pregnant sheep) branches of the main uterine arteries with a similar external diameter were separated from the surrounding tissue and cut into 2-mm ring segments in Krebs solution (pH 7.4) of the following composition (in mM): 115.21 NaCl, 4.7 KCl, 1.8 CaCl\(_2\), 1.16 MgSO\(_4\), 1.18 KH\(_2\)PO\(_4\), 22.14 NaHCO\(_3\), 0.03 EDTA, and 7.88 dextrose, oxygenated with a mixture of 95% O\(_2\)-5% CO\(_2\). After the tissues were removed, animals were euthanized with solution (T-61, Hoechst-Roussel; Somervile, 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 in the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

Ca\(^{2+}\) buffer solutions. Two main buffer solutions were used in the present study, as previously described (1, 2, 30). The one was zero Ca\(^{2+}\) relaxing solution that contained (in mM) 110 K-acetate, 5 EGTA, 5 ATP, 6 Mg-acetate, 1 DTT, 0.01 leupeptin, 20 imidazole, and 20 HEPES, at pH 6.8 (titrated with KOH). The other contained 100 \(\mu\)M Ca\(^{2+}\) in addition to the same components in the zero Ca\(^{2+}\) buffer. As described in the previous study (1), Ca\(^{2+}\) buffer solutions were prepared by solving the multiequilibrium equations for interactions among the different ions, and solutions containing intermediate free Ca\(^{2+}\) concentrations were prepared by mixing appropriate amounts of zero Ca\(^{2+}\) relaxing solution and the maximum Ca\(^{2+}\) (100 \(\mu\)M) buffer solutions, titrated to pH 7.0 with 1 M KOH. As demonstrated in our previous studies, the accuracy of calculated free Ca\(^{2+}\) concentrations in Ca\(^{2+}\) buffer solutions was determined directly with dual-wavelength measurements of bound-to-unbound fura-2 ratios, and the calculated concentrations of free Ca\(^{2+}\) correlated well (\(r^2 = 0.97\)) with the concentrations measured with fura-2 (1).

*Permeabilization procedure.* The arterial rings were attached to isometric force transducers and bathed in Krebs solution at 37°C. Isometric tensions were measured as described previously (46, 51). 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 120 mM KCl added at each stretch level. The arterial rings were then placed into the zero Ca\(^{2+}\) relaxing solution. The permeabilization procedure was modified from previous studies (1, 6, 30). Briefly, chemical permeabilization was achieved by adding 40 \(\mu\)M \(\beta\)-escin to the relaxing solution and by allowing the rings to incubate for 20 min at 25°C. The permeabilized solution was then replaced with the relaxing solution containing 1 \(\mu\)M A-23187 to deplete Ca\(^{2+}\) from the sarcoplasmic reticulum. Concentration-response curves to Ca\(^{2+}\) were obtained by cumulative increases of Ca\(^{2+}\) concentrations in approximate one-half log increments in \(\beta\)-escin-permeabilized arterial rings. Prism software (GraphPat; San Diego, CA) was used to fit the curve and determine the pD\(_2\) values (\(-\log EC_{50}\)) and the maximum response. In certain experiments, tissues were pretreated with the PKC activator or inhibitor (or vehicle DMSO for 20 min) and then stimulated with increasing concentrations of Ca\(^{2+}\).

**Measurements of MLC\(_{20}\) phosphorylation.** MLC\(_{20}\) phosphorylation levels and contractile tensions were determined simultaneously in the same tissues, as previously described (51). Tensions developments were continuously recorded with an online computer, and arterial rings were snap frozen with liquid N\(_2\)-cooled clamps at the indicated times and rapidly immersed in a dry ice-acetone slurry containing 10% trichloroacetic acid (TCA), 5 mM NaF, and 10 mM DTT mixture. Tissues were then stored at \(-80^\circ\)C until the analysis of MLC\(_{20}\)-P. To measure MLC\(_{20}\)-P, tissues were brought to room temperature in a dry ice-acetone-TCA-DTT mixture and then washed three times with ether to remove the TCA. Tissues were then extracted in 100 \(\mu\)l of sample buffer containing 20 mM Tris base and 23 mM glycine (pH 8.6), 8.0 M urea, 10 mM DTT, 10% glycerol, and 0.04% bromophenol blue, as previously described. Samples (20 \(\mu\)l) were electrophoresed at 12 mA for 2.5 h after a 30 min prerun in 1.0 mm minipolyacrylamide gels containing 10% acrylamide-0.27% bisacrylamide, 40% glycerol, 8.0 M urea, and 20 mM Tris base (pH 8.8). Proteins were transferred to nitrocellulose membranes and subjected to Western blot analysis with a specific MLC\(_{20}\) antibody (1:500). Goat anti-mouse IgG conjugated with horseradish peroxidase was used as a secondary antibody (1:2,000). Bands were detected with enhanced chemiluminescence (ECL), visualized on Hyperfilm, and analyzed with the Kodak 1D image analysis software. Moles of inorganic phosphate per mole of MLC\(_{20}\) (fractional light chain phosphorylation) were calculated by dividing the density of the phosphorylated band by the sum of the densities of the phosphorylated plus the unphosphorylated bands. In some experiments, the apparent levels of MLC\(_{20}\)-P were calculated by multiplying the fractional MLC\(_{20}\) phosphorylation (in mol Pi/mol MLC\(_{20}\)) by the total levels of MLC\(_{20}\) determined by Western blot analysis as described below.

**Western blot analysis.** Uterine arteries from both nonpregnant and pregnant animals were isolated as described above and homogenized in the lysis buffer containing 150 mM NaCl, 50 mM Tris-\(\cdot\)HCl, 10 mM EDTA, 0.1% Tween 20, 0.1% \(\beta\)-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 \(\mu\)g/ml leupeptin, and 5 \(\mu\)g/ml aprotinin, pH 7.4. Homogenates were then centrifuged at 4°C for 5 min at 6,000 g, and the supernatants were collected. Protein was determined in the supernatant. Samples with equal protein were loaded on a 10% polyacrylamide gel with 0.1% SDS and were separated by electrophoresis at 100 V for 2 h. Proteins were then transferred to a nitrocellulose membrane. Nonspecific binding sites in the membranes were blocked by an overnight incubation at 4°C in a Tris-buffered saline solution containing 5% dry milk. The membranes were incubated with a specific MLC\(_{20}\) antibody, followed by a secondary antibody. Proteins were visualized with ECL reagents, and the blots were exposed to Hyperfilm. Results were quantified with the Kodak electrophoresis documentation and analysis system and Kodak 1D image analysis software.

**Materials.** Phorbol 12,13-dibutyrate (PDBu), staurosporine, monclonal antinmyosin (20-kDa light chains) antibody, \(\beta\)-escin, and other chemicals were obtained from Sigma (St. Louis, MO). Goat anti-mouse IgG conjugated with horseradish peroxidase was from Calbiochem (La Jolla, CA). Electrophoretic and immunoblotting reagents were from Bio-Rad (Hercules, CA).

**Data analysis.** Data were expressed as means \(\pm\) SE. Differences were evaluated for statistical significance (\(P < 0.05\)) by one-way ANOVA and Student’s \(t\)-test.

**RESULTS**

*Effect of pregnancy on Ca\(^{2+}\)-force relationship in permeabilized uterine arteries.* To test the permeabilization efficiency of \(\beta\)-escin in the uterine arteries of each experiment, the maximum contractile tension induced by 120 mM KCl before permeabilization was compared with the tension induced by
the buffer solution containing 10 μM free [Ca2+] after permeabilization in the same tissue. As shown in Fig. 1, the tension induced by 10 μM Ca2+ after permeabilization was not significantly different from that induced by 120 mM KCl before permeabilization. Furthermore, 120 mM KCl produced no contractile tension in the uterine artery after permeabilization (data not shown). The results suggested complete permeabilization of the tissues by β-escin.

Figure 2 shows that cumulative increases of Ca2+ produced concentration-dependent contractions of permeabilized uterine arteries from both nonpregnant and pregnant animals. The pD2 values were not significantly different between uterine arteries (P > 0.05) from nonpregnant (5.62 ± 0.14) and pregnant (5.60 ± 0.16) sheep. However, the maximum response of Ca2+-induced contractions [expressed as %120 mM KCl-induced contractions before permeabilization in the same tissue (%KCl)] was significantly decreased in uterine arteries from pregnant compared with nonpregnant sheep (87.9 ± 7.0 vs. 139.7 ± 9.4 %KCl; P < 0.05).

Effect of pregnancy on Ca2+-induced MLC20 phosphorylation. Figure 3 shows the time courses of Ca2+ (10 μM)-induced increases in MLC20 phosphorylation and contractions in permeabilized uterine arteries. In the uterine arteries from both pregnant and nonpregnant sheep, Ca2+ produced time-dependent increases in MLC20 phosphorylation, which preceded contractions. Total protein levels of MLC20 were deter-

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Fig. 1. KCl- (top, left) and Ca2+-mediated (top, right) contractions before and after permeabilization of uterine arteries. Uterine arteries were contracted with 120 mM KCl and 10 μM Ca2+ before and after permeabilization with β-escin as described in methods (bottom). Data are means ± SE of tissues from 14 pregnant and 8 nonpregnant animals.

Fig. 2. Effect of pregnancy on basal Ca2+ sensitivity in β-escin-permeabilized uterine arteries. Concentration-response curves of Ca2+-induced contractions were measured in β-escin-permeabilized uterine arteries from nonpregnant and pregnant animals. Data are expressed as percentage of maximal KCl-induced contractions of the same tissue before permeabilization to normalize tissue size. Data are means ± SE of tissues from pregnant (n = 11) and nonpregnant (n = 7) animals. pD2 values and maximal response are presented in results.

Fig. 3. Ca2+-induced time courses of 20-kDa myosin light chain (MLC20) phosphorylation and contractions in β-escin-permeabilized uterine arteries. β-escin-permeabilized uterine arteries from nonpregnant (top) and pregnant (bottom) animals were stimulated with 10 μM Ca2+. Contractile tension (○) and phosphorylated MLC20 (MLC20-P; ●) were measured simultaneously in the same tissues at indicated time points. Data are means ± SE of tissues from pregnant (n = 5) and nonpregnant (n = 5) animals.
mined by Western blot analysis. Figure 4 shows that the MLC20 levels were significantly increased in uterine arteries from pregnant compared with nonpregnant sheep (19.23 ± 1.45 vs. 5.77 ± 1.35, arbitrary density units; *P < 0.05). As shown in Fig. 5, Ca2+ produced dose-dependent increases in the levels of MLC20-P (Fig. 5, top) in the uterine arteries. The apparent levels of MLC20-P induced by Ca2+ were significantly higher in uterine arteries from pregnant versus nonpregnant sheep at the doses of 10 μM Ca2+ (11.2 ± 0.56 vs. 2.28 ± 0.25 mol Pi; *P < 0.05) and 30 μM Ca2+ (9.32 ± 1.33 vs. 2.57 ± 0.18 mol Pi; *P < 0.05), respectively. However, the ratios of tension to MLC20-P (g/MLC20-P), obtained from the simultaneous measurements of MLC20-P and tension in the same tissues, were significantly decreased in uterine arteries from pregnant compared with nonpregnant sheep at the doses of 10 μM Ca2+ (0.55 ± 0.11 vs. 1.63 ± 0.38; *P < 0.05) and 30 μM Ca2+ (0.74 ± 0.10 vs. 1.89 ± 0.44; *P < 0.05), respectively (Fig. 5, bottom).

Effect of PKC activation on Ca2+ sensitivity. Figure 6 shows the effect of the PKC activator PDBu on tension development in permeabilized uterine arteries in the absence and presence of submaximal Ca2+ concentrations (1 μM). In the presence of Ca2+, the maximal concentration of PDBu (10 μM) induced tension development that was significantly greater in uterine arteries from nonpregnant than during pregnancy (30.8 ± 2.8 vs. 18.1 ± 3.2 %KCl; *P < 0.05) (Fig. 6). In the absence of Ca2+ in the Ca2+-free relaxing solution, PDBu-induced contractions were significantly decreased, but the difference between uterine arteries from nonpregnant and pregnant sheep remained (14.5 ± 1.9 vs. 7.3 ± 2.0 %KCl; *P < 0.05) (Fig. 6). To determine the effect of PKC activation on Ca2+-force relations, we compared [Ca2+]i-dependent contractions in the absence and presence of PDBu in permeabilized uterine arteries. As shown in Fig. 7, pretreatment with the subthreshold concentration of PDBu (0.3 μM) did not change the pD2 (5.68 ± 0.12 vs. 5.56 ± 0.06, *P > 0.05) but significantly potentiated the maximum response of Ca2+-induced contractions (168.9 ± 8.4 vs. 129.2 ± 3.9 %KCl; *P < 0.05) in uterine arteries from nonpregnant sheep (Fig. 7, top). In contrast, neither the pD2 (5.79 ± 0.12 vs. 5.70 ± 0.16, *P > 0.05) nor the maximum response (106.9 ± 5.6 vs. 89.1 ± 6.7 %KCl, *P > 0.05) of Ca2+-induced contractions was significantly changed in the presence of PDBu in uterine arteries during pregnancy (Fig. 7, bottom).

Accordingly, in the presence of the PKC inhibitor staurosporine, both the pD2 value (5.10 ± 0.10 vs. 5.60 ± 0.07; *P < 0.05) and the maximum response (66.7 ± 3.9 vs. 145.8 ± 5.3 %KCl; *P < 0.05) of Ca2+-induced concentration-dependent contractions were significantly decreased in uterine arteries from nonpregnant sheep (Fig. 8, top). However, staurosporine did not significantly affect the pD2 value (5.30 ± 0.11 vs. 5.62 ± 0.20; *P > 0.05) and the maximum response (85.0 ± 5.1 vs. 116.6 ± 12.3 %KCl; *P > 0.05) in uterine arteries during pregnancy (Fig. 8, bottom).

**DISCUSSION**

The major findings of the present study are that in permeabilized uterine arteries, I) the baseline Ca2+ sensitivity was
significantly increased in uterine arteries during pregnancy; 2) MLC20 phosphorylation preceded Ca2+ -induced contractions in uterine arteries from both nonpregnant and pregnant sheep; 3) the Ca2+ sensitivity of MLC20 phosphorylation was significantly increased in uterine arteries during pregnancy; 4) the sensitivity of MLC20-P in contractions was significantly decreased in uterine arteries during pregnancy; and 5) PKC modulated the baseline Ca2+ sensitivity in the uterine arteries, which was significantly attenuated in pregnant animals.

It has been well demonstrated that the Ca2+ sensitivity is a key mechanism in the regulation of vascular contractility. In the present study, we examined the effect of pregnancy on the baseline Ca2+ sensitivity in the uterine artery using β-escin-permeabilized arterial preparations, the method that has been widely used to study the myofilament Ca2+ sensitivity by many investigators in a variety of smooth muscles (24, 33, 43), including our own in cerebral and coronary arteries (1, 2, 11, 30). In agreement with previous studies (1, 2), the present study showed that, in the β-escin-permeabilized uterine arteries, the buffer solution containing 10 μM free [Ca2+] produced the equivalent level of contractions to that of the maximum KCl contractions in the same intact vessels before permeabilization. This suggests that the β-escin permeabilization was complete and that smooth muscle contractile apparatus remained intact. The finding that the Ca2+ -force relationship was significantly depressed in uterine arteries during pregnancy suggests that pregnancy decreased the baseline Ca2+ sensitivity in the uterine artery. This is consistent with our previous findings in the intact arterial preparations that pregnancy attenuated α1-adrenoceptor-mediated myofilament Ca2+ sensitivity in the uterine artery (51). The idea that the baseline Ca2+ sensitivity is regulated both physiologically and pathophysiologically is further supported by previous findings of differences in basal Ca2+ sensitivity associated with the artery type, arterial size, developmental age, and oxygen tension (1, 2, 11, 40). Our recent studies demonstrated that the Ca2+ sensitivity played an important role in the regulation of uterine artery myogenic tone (45). The present finding of attenuated baseline Ca2+ sensitivity in uterine arteries during pregnancy suggests a key mechanism in the decreased myogenic tone in the same arteries (41, 45).

The myofilament Ca2+ sensitivity is regulated through multiple mechanisms. Both thick- and thin-filament regulatory pathways contribute to the regulation of Ca2+ sensitivity (4, 15, 29, 48). In the present study, we measured Ca2+ -induced MLC20 phosphorylation and contractions simultaneously in the same tissues of permeabilized uterine arteries. By examining the relationships of Ca2+ and MLC20 phosphorylation as well as MLC20 phosphorylation and contractions, we were able to separate the thick-filament mechanism, i.e., Ca2+ sensitivity of MLC20 phosphorylation, and the thin-filament mechanism, i.e., the sensitivity of MLC20 phosphorylation of contractions, in the regulation of baseline Ca2+ sensitivity in the uterine artery, and their adaptations to pregnancy. Consistent with previous studies (3), we found significantly increased protein levels of...
findings suggest that pregnancy increases the baseline Ca$^{2+}$ significantly increased in the uterine artery during pregnancy. These apparent contradictory findings, i.e., increased baseline Ca$^{2+}$ sensitivity of MLC$_{20}$ phosphorylation and decreased $\alpha_1$-adrenoceptor-mediated Ca$^{2+}$ sensitivity of MLC$_{20}$ phosphorylation in uterine arteries during pregnancy, are intriguing and suggest different mechanisms in the agonist-induced Ca$^{2+}$ and basal Ca$^{2+}$ sensitivity. It has been well documented that the activity of MLCP plays a key role in the regulation of the Ca$^{2+}$ sensitivity of MLC$_{20}$ phosphorylation (39). MLCP consists of three subunits: 110- to 130-kDa regulatory subunit [myosin phosphate target-protein-1 (MYPT-1)], ~37-kDa catalytic subunit (PP1c), and 20-kDa subunit of unknown function (13, 39). One of the major mechanisms in inhibition of MLCP is through phosphorylation of MYPT-1 (16). A key event in agonist-induced Ca$^{2+}$ sensitization is G protein-dependent phosphorylation of MYPT-1, which leads to inhibition of MLCP (16, 24). At least two phosphorylation sites of MYPT-1 have been identified at Thr$^{696}$ and Thr$^{850}$ (39). MYPT-1/Thr$^{696}$ is constitutively phosphorylated by kinases other than Rho kinase, and phosphorylation of MYPT-1/Thr$^{696}$ does not respond to agonists (22, 32, 39). In contrast to Thr$^{696}$, MYPT-1/Thr$^{850}$ is phosphorylated in response to agonists (9, 22, 23, 39). Our recent studies of the uterine artery demonstrated that phenylephrine stimulated phosphorylation of MYPT-1/Thr$^{850}$ and that the time course of MYPT-1/Thr$^{850}$ phosphorylation resembled that of phenylephrine-induced MLC$_{20}$ phosphorylation and preceded contractions, suggesting that phenylephrine-mediated Ca$^{2+}$ sensitization was regulated through phosphorylation of MYPT-1/Thr$^{850}$, resulting in an inhibition of MLCP activity in the uterine artery (46, 48). In contrast, the basal levels of phosphorylated MYPT-1/Thr$^{696}$ in the uterine artery were not altered by phenylephrine (46). Taken together, these findings suggest the exciting hypothesis that the different phosphorylation sites of MYPT-1 may regulate MLCP activities differently at basal and agonist-stimulated states in the adaptation of the uterine artery to pregnancy.

The finding that Ca$^{2+}$-mediated contractions at given levels of MLC$_{20}$ phosphorylation were significantly decreased in uterine arteries from pregnant compared with nonpregnant sheep suggests that pregnancy attenuates the thin-filament pathway in the regulation of baseline Ca$^{2+}$ sensitivity in the uterine artery. Given that pregnancy upregulates the thick-regulatory pathway, i.e., the Ca$^{2+}$ sensitivity of MLC$_{20}$ phosphorylation, the data suggest that the downregulation of the thin-filament regulatory pathway in response to pregnancy dominates in the uterine artery, resulting in decreased baseline Ca$^{2+}$ sensitivity of contractions. Consistent with the present findings, our previous studies demonstrated that pregnancy significantly depressed the thin-filament regulatory pathway in response to the activation of $\alpha_1$-adrenoceptors and PKC in the uterine artery (51). In contrast to the thick-filament pathway, the effect of pregnancy on the thin filaments was the same in both basal and agonist-mediated Ca$^{2+}$ sensitivities in the uterine artery, suggesting that its downregulation is a major mechanism in the adaptation of decreased uterine artery contractility to pregnancy.

It has been demonstrated that PKC is able to modulate the Ca$^{2+}$ sensitivity via the thin-filament pathway in vascular smooth muscle (19, 36). Our previous studies in the intact uterine arteries demonstrated that PKC-induced contractions were independent of changes in intracellular Ca$^{2+}$ concentrations and MLC$_{20}$ phosphorylation, indicating that PKC-induced contractions were mediated predominately through thin-filament regulatory pathways in the uterine artery (46, 51). In the present study, we demonstrated that activation of PKC with
PDBu enhanced Ca\(^{2+}\)-mediated contractions in membrane-permeabilized uterine arteries. In addition, inhibition of PKC with staurosporine attenuated Ca\(^{2+}\)-induced contractions, suggesting an important role of endogenous PKC in the regulation of baseline Ca\(^{2+}\) sensitivity in the uterine artery. Our previous studies demonstrated that PDBu stimulated PKC activity, which was inhibited by staurosporine in the uterine artery (50). In the present study, the finding that PDBu induced contractions in the absence of Ca\(^{2+}\) is intriguing and suggests a Ca\(^{2+}\)-independent isozyme of PKC in the regulation of basal Ca\(^{2+}\) sensitivity in the uterine artery. This contrasts with the previous findings in ovine cerebral arteries, in which PDBu failed to produce contractions in permeabilized arteries at zero Ca\(^{2+}\) (2). In agreement with the present finding, phorbol ester-induced Ca\(^{2+}\)-independent contractions were demonstrated in ferret and rabbit aorta (28, 42). These studies suggest a tissue specificity of PKC isozymes in the regulation of basal Ca\(^{2+}\) sensitivity. It remains to be determined which isozyme of PKC is involved in the regulation of basal Ca\(^{2+}\) sensitivity in the uterine artery. Consistent with our recent finding of a primary role of PKC in pregnancy-associated decrease in uterine artery myogenic tone (45), the present study demonstrated that PKC-mediated modulation of baseline Ca\(^{2+}\) sensitivity was significantly attenuated in uterine arteries during pregnancy. The effects of PDBu and staurosporine on [Ca\(^{2+}\)]m response curves did not reach the significant levels in uterine arteries from nonpregnant sheep. Nevertheless, it is apparent that the effects of PDBu and staurosporine are much greater in uterine arteries from nonpregnant sheep, suggesting a key role of the decreased PKC pathway in the adaptation of basal Ca\(^{2+}\) sensitivity and, hence, vascular tone of the uterine artery during pregnancy. In agreement with the present findings, it has been demonstrated that pregnancy attenuates the PKC activity and decreases PKC-mediated contractions of the uterine artery (8, 26, 50, 51).

In conclusion, we have demonstrated that pregnancy down-regulates the baseline Ca\(^{2+}\) sensitivity in the uterine artery, which is mediated primarily through the inhibition of thin-filament regulatory pathways. PKC plays an important role in the adaptation of uterine artery thin-filament function and baseline Ca\(^{2+}\) sensitivity to pregnancy. Phosphorylation of the actin-associated proteins calponin and caldesmon and actin polymerization have been proposed in the mechanisms of PKC in the regulation of Ca\(^{2+}\) sensitivity independent of MLC\(_{20}\) phosphorylation, and, hence, their involvement in the adaptation of basal Ca\(^{2+}\) sensitivity of the uterine artery during pregnancy presents an intriguing area of further investigation. Given that basal Ca\(^{2+}\) sensitivity plays a key role in the regulation of myogenic tone and, hence, basal vascular resistance and blood flow, decreased baseline Ca\(^{2+}\) sensitivity is likely to play an important role in the adaptation of uterine vascular hemodynamics during pregnancy.

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