Effect of cGMP on Pharmacomechanical Coupling in the Uterine Artery of Near-Term Pregnant Sheep

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ABSTRACT
The present study examined the role of cGMP in the regulation of α1-adrenoceptor-mediated pharmacomechanical coupling in the uterine artery of near-term pregnant sheep. The cell-permeable cGMP analog 8-bromo-cGMP produced a dose-dependent relaxation of the uterine artery and shifted norepinephrine (NE) dose-response curve to the right with a decreased maximal contraction. Accordingly, 8-bromo-cGMP significantly decreased tension generation for a given [Ca^{2+}]_i (g/R_9540/380, 24.87 ± 3.43 versus 3.10 ± 0.35). In the absence of extracellular Ca^{2+}, NE produced a transient increase in [Ca^{2+}]_i and contractions, which were inhibited by 8-bromo-cGMP by 47 and 76%, respectively. In contrast to NE-induced responses, 8-bromo-cGMP had no significant effects on KCl-induced [Ca^{2+}]_i and contractions. The results indicate that cGMP suppresses α1-adrenoceptor-mediated pharmacomechanical coupling in the uterine artery by inhibiting IP_3 synthesis and Ca^{2+} release from intracellular stores, as well as inhibiting the agonist-mediated Ca^{2+} sensitization of myofilaments, which is likely to play an important role in the adaptation of uterine artery contractility during pregnancy.

The uterine circulation during pregnancy functions as a low-resistance shunt to accommodate the large increase of uteroplacental blood flow required for normal fetal development. The mechanisms in maintaining the low uterine vascular tone in pregnancy are complex and not fully understood. A number of studies in humans and animals have demonstrated that endothelial nitric oxide plays an important role in maintaining low vascular resistance of the uterine circulation in pregnancy (Conrad et al., 1993; Sladek et al., 1997; Nelson et al., 2000; Yallampalli et al., 2002; Bird et al., 2003). Nitric oxide through the activation of guanylate cyclase increases cGMP, and elevated plasma and urinary cGMP levels are found in both human and sheep pregnancy (Kopp et al., 1977; Magness et al., 1997; Sladek et al., 1997).

Whereas it is clear that cGMP is the mediator of NO-dependent vasorelaxation, it is unknown whether and to what extent cGMP regulates vasoconstrictor-mediated contractions of the uterine artery in pregnancy. In vivo studies in pregnant sheep have demonstrated attenuated vasoconstriction of the uterine artery to norepinephrine and angiotensin II (Naden and Rosenfeld, 1981; Magness and Rosenfeld, 1986). Yet the cellular mechanisms are not fully understood. cGMP activates cGMP-dependent protein kinase and decreases intracellular free calcium concentrations leading to an inhibition of smooth muscle contraction (Lincoln and Cornwall, 1991). Although the specific substrate proteins for cGMP-dependent protein kinase are not fully understood at the present, several smooth muscle cell functions are affected by cGMP, and their modification appears to account for the effect of cGMP on the vascular reactivity (Lincoln et al., 1994). Among these, the cGMP-dependent inhibition of inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release was described previously (Murthy et al., 1993) and correlated with the phosphorylation of IP_3 receptors induced by agents known to increase intracellular cGMP concentrations (Komalavilas and Lincoln, 1994).

The present study was designed to determine the role of

ABBREVIATIONS: IP_3, inositol 1,4,5-trisphosphate; NE, norepinephrine; A23187, calcimycin; [Ca^{2+}]_i, intracellular free Ca^{2+} concentrations; cGK, cGMP-dependent protein kinase.
cGMP in the regulation of α₁-adrenoceptor-mediated pharmacomechanical coupling in pregnant uterine arteries and test the hypothesis that cGMP played an important role in the adaptation of uterine artery contractility in pregnancy. We examined the effect of 8-bromo-cGMP, a hydrolysis-resistant membrane-permeable cGMP analog, on the IP₃ signaling pathway in the uterine artery. The time- and dose-dependent IP₃ synthesis induced by norepinephrine was measured. To correlate directly IP₃ synthesis to tension development and to determine tissue sensitivity to IP₃, we developed a method to measure α₁-adrenoceptor-mediated contractile tension and IP₃ production simultaneously in the same tissue. Furthermore, we characterized IP₃ receptors in the uterine artery and determined the effect of 8-bromo-cGMP on IP₃ binding affinity to the IP₃ receptor and the receptor density. We went further to determine the effect of 8-bromo-cGMP on α₁-adrenoceptor-induced Ca²⁺ mobilization and Ca²⁺ sensitization of myofilaments in the uterine artery.

Materials and Methods

Tissue Preparation. Pregnant sheep (~140 days gestation) were anesthetized with thiopent (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 oxygen throughout surgery. An incision in the abdomen was made, and the uterus was exposed. The uterine arteries were isolated and removed without stretching and placed into a modified Krebs' solution (pH 7.4) of the following composition: 115.21 mM NaCl, 4.7 mM KCl, 1.80 mM CaCl₂, 1.16 mM MgSO₄, 1.18 mM KH₂PO₄, 22.14 mM NaHCO₃, and 7.88 mM dextrose. EDTA (0.03 mM) was added to suppress oxidation of amines. The Krebs' solution was oxygenated with a mixture of oxygen-carbon dioxide (95:5%). After removal of the tissues, animals were killed with euthanasia solution (T-61; Hoechst-Roussel, Somerville, NJ). All procedures and protocols were approved by the Institutional Animal Care and Use Committee of Loma Linda University and followed the guidelines by the Institute of Laboratory Animal Resources (1996).

Measurement of Arterial Tension. Four branches of the main uterine arteries were separated from the surrounding tissue and were cut into 2-mm ring segments. To determine the role of endothelium in 8-bromo-cGMP, the endothelium was removed from some arterial rings by gentle rotation of the tissue on an appropriately sized, rough-surfaced blunt hypodermic needle as described previously (Hu et al., 1996). Validation of the endothelium removal was demonstrated by the elimination of endothelium-dependent relaxation induced by the calcium ionophore A23187. Isometric tensions of arterial rings were measured in Krebs' solution in tissue baths at 37°C as described previously (Hu et al., 1996). After 40 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 three exposures of KCl (120 mM) added at different stretch levels. Concentration-response curves were obtained by cumulative additions of the agonist in approximate one-half log increments. EC₅₀ values for the agonist in each experiment were taken as the concentration at which the contraction-response curve intersected 50% of the maximal response and expressed as pD₂ (-logEC₅₀) values.

Measurement of IP₃. The accumulation of IP₃ was measured by the competitive ligand binding radioiodination assay (Hu et al., 1999). The tissues were equilibrated in Krebs' solution at 37°C for 30 min. After the treatments, tissue reactions were terminated by flash-freezing tissues in liquid N₂. The tissues were then homogenized in ice-cold 16.7% trichloroacetic acid (TCA). The homogenate was centrifuged at 100,000 g for 30 min. The supernatant was placed into a modified Krebs' solution (pH 7.5) of the following composition: 115.21 mM NaCl, 4.7 mM KCl, 1.80 mM CaCl₂, 1.16 mM MgSO₄, 1.18 mM KH₂PO₄, 22.14 mM NaHCO₃, and 7.88 mM dextrose. EDTA (0.03 mM) was added to suppress oxidation of amines. The Krebs' solution was oxygenated with a mixture of oxygen-carbon dioxide (95:5%). After removal of the tissues, animals were killed with euthanasia solution (T-61; Hoechst-Roussel, Somerville, NJ). All procedures and protocols were approved by the Institutional Animal Care and Use Committee of Loma Linda University and followed the guidelines by the Institute of Laboratory Animal Resources (1996).

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Characterization of IP₃ Receptors. Saturation binding of [³H]IP₃ (PerkinElmer Life and Analytical Sciences) was performed by a rapid filtration method as described previously (Hu et al., 1999). Uterine arteries were minced and suspended in 10 volume of the buffer (composition 20 mM Tris/HCl, 20 mM NaCl, 100 mM KCl, 1 mM EDTA, and 0.02% NaN₃, pH 7.7). Tissues were then homogenized using a Polytron tissue homogenizer (Barnart Co., Barrington, IL) at a setting of 3.5 in two bursts of 15 s each. The homogenate was centrifuged at 100,000 g for 30 min. Pellets were re-homogenized in half of the original volume of the buffer at a setting of 3.5 for 15 s and recentrifuged under the same conditions. The supernatant was discarded, and the pellet was resuspended in the same buffer with pH 8.5. Radioligand binding assays were carried out in a final volume of 200 µl consisting of 175-µl membranes, 20 µl of radioligand, and 5 µl of buffer or unlabeled IP₃ (Research Biomedicals Inc., Natick, MA). Saturation binding experiments used concentrations of [³H]IP₃ from 0.15 to 80 nM, and nonspecific binding was determined by the addition of 12 µM ice-cold IP₃. Equilibrium binding was carried out at 4°C for 15 min. All determinations were performed in triplicate. Bound and free radioligand was separated by a rapid filtration of the membrane suspension over polyethyleneimine-pretreated (0.5%) filters (GF/B filters; Whatman Inc., Clifton, NJ) with a Brandel cell harvester (Brandel Inc., Gaithersburg, MD). Filters were rinsed with two 5-ml aliquots of the ice-cold buffer and counted for radioactivity at 45% efficiency in Packard 1900CA-Tri-Carb liquid scintillation analyzer (Packard Instrument Co., Downers Grove, IL). Protein was determined with a protein assay kit (Bio-Rad).

Simultaneous Measurement of [Ca²⁺]i and Tension. Simultaneous measurement of tension and free intracellular calcium concentrations ([Ca²⁺]i) in the uterine artery smooth muscle was obtained as described previously by us (Zhang and Xiao, 1998). The tissues were attached to an isometric force transducer in a 5-ml tissue bath mounted on a CAF-110 intracellular Ca²⁺ analyzer (Jasco, Tokyo, Japan) and were equilibrated in Krebs' buffer under a resting tension of 0.5 g for 40 min. The tissues were then loaded with 5 µM fura 2-acetoxymethyl ester for 2 h in the presence of 0.02% Cremophor EL at 25°C. After loading, the tissues were washed with Krebs' solution at 37°C for 30 min to allow for hydrolysis of fura 2 ester groups by endogenous esterase. The tension and fura 2 fluorescence were measured simultaneously at 37°C in the same tissue. During the stimulation with an agonist, the tissues were illuminated alternately (125 Hz) at excitation wavelengths of 340 and 380 nm, respectively, by means of two monochromators in the light path of a 75-watt xenon lamp. Fluorescence emission from the tissue was measured at 510 nm by a photomultiplier. The fluorescence intensity at each excitation wavelength (F₃₄₀ and F₃₈₀, respectively) and the ratio of these two fluorescence values (R₃₄₀/₃₈₀) 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 (Kₘ), receptor density (B_max), and pD₂ (-logEC₅₀) using Prism (GraphPad Software, Inc., San Diego, CA). Results were expressed as means ± S.E.M., and the differences were evaluated for statistical significance (P < 0.05) by analysis of variance.

Results

Effects of 8-Bromo-cGMP on NE-Induced Contractions. 8-Bromo-cGMP produced a dose-dependent relaxation of the uterine artery precontracted with NE, which was not
sion-response curves to NE were obtained in the uterine artery. B, cumulative concentrations (Fig. 2). The inhibitory effect was completely reversible (Fig. 2A). 8-Bromo-cGMP shifted the NE dose-response curve to the right (Fig. 2B) and significantly decreased the contractile sensitivity ($pD_2$) from 7.00 ± 0.08 to 5.93 ± 0.09 ($P < 0.05$) and the maximal response from 18.2 ± 0.5 to 14.2 ± 0.6 g ($P < 0.05$), respectively.

**Effect of 8-Bromo-cGMP on NE-Induced IP$_3$ Synthesis.** The time course of NE-stimulated IP$_3$ synthesis in the uterine artery is shown in Fig. 3. NE stimulated a rapid increase in IP$_3$, which reached the peak at 30 s. At 1 min, IP$_3$ declined to a steady-state level above the basal, which lasted at least for 30 min. 8-Bromo-cGMP did not alter the time course but significantly decreased the peak level of IP$_3$ elicited by NE (79 ± 4.6 versus 159 ± 22.8 pmol/mg protein, $P < 0.05$). The elevated steady level was also decreased by 8-bromo-cGMP (Fig. 3). Figure 4 shows the concentration-dependent response of IP$_3$ synthesis induced by NE. 8-Bromo-cGMP caused an approximately 20-fold rightward shift of the concentration-response curve and decreased the $pD_2$ from 6.14 ± 0.10 to 4.81 ± 0.12 ($P < 0.05$). The maximal formation of IP$_3$ induced by NE was also decreased from 206.6 ± 6.3 to 89.1 ± 5.1 pmol/mg protein ($P < 0.05$). To determine the correlation of agonist-induced IP$_3$ synthesis and tension development in the uterine artery, we measured contractions and IP$_3$ productions simultaneously in the same tissue. As shown in Fig. 5A, there was a tight correlation between NE-induced contractions and IP$_3$ synthesis in the uterine artery. Simultaneous measurement of IP$_3$ synthesis and contractions in the same tissue also indicated that 8-bromo-cGMP-mediated inhibition of NE-induced contractions was correlated with decreases in IP$_3$ production in the same tissue (Fig. 5B).

**Effect of 8-Bromo-cGMP on the IP$_3$ Receptor.** Coupling of IP$_3$ to force generation in smooth muscle includes the binding of IP$_3$ to IP$_3$ receptors leading to the release of Ca$^{2+}$ from intracellular stores. Our previous studies have characterized the IP$_3$ receptors in the uterine artery using a radioligand binding method (Hu et al., 1999). As shown in Fig. 6A, binding of [H]$IP_3$ to tissue membranes was specific and saturable. 8-Bromo-cGMP did not significantly affect the receptor density ($B_{\text{max}}$, 313.6 ± 62.2 versus 356.2 ± 30.9 fmol/mg protein; $P > 0.05$) but did significantly increase the

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**Fig. 1.** 8-Bromo-cGMP-mediated relaxation of the uterine artery. Concentration-dependent relaxations induced by 8-bromo-cGMP were obtained with NE (0.1 μM)-precontracted uterine arteries with intact or denuded endothelium. Data are mean ± S.E.M. of the tissues from five animals.

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**Fig. 2.** 8-Bromo-cGMP-mediated inhibition of NE-induced contractions of the uterine artery. A, typical recordings of effect of 8-bromo-cGMP on the tension-induced by NE in the uterine artery. B, cumulative concentration-response curves to NE were obtained in the uterine artery in the absence or presence of 8-bromo-cGMP (100 μM, pretreatment for 30 min). Data are means ± S.E.M. of the tissues from five animals.

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**Fig. 3.** Effect of 8-bromo-cGMP on the time course of NE-induced IP$_3$ synthesis in the uterine artery. Uterine arteries were stimulated with 10 μM NE for various times in the absence or presence of 8-bromo-cGMP (100 μM, pretreatment for 30 min). IP$_3$ was measured by a competitive ligand binding radioreceptor assay as described under Materials and Methods. Data are means ± S.E.M. of the tissues from five animals.
dissociation constant ($K_{\text{D}}$, 11.3 ± 1.44 versus 5.87 ± 0.97 nM; $P < 0.05$) of IP$_3$ to its receptors (Fig. 6).

**Effect of 8-Bromo-cGMP on Ca$^{2+}$ Homeostasis.** To examine the effect of 8-bromo-cGMP on NE-mediated Ca$^{2+}$ homeostasis in the uterine artery, NE-induced contractions and [Ca$^{2+}$], were measured simultaneously in the same tissue as described under Materials and Methods. As shown in Fig. 7A, 8-bromo-cGMP significantly decreased NE-stimulated increases in [Ca$^{2+}$]$_i$, ($R_{340/380}$, 0.046 ± 0.011 versus 0.082 ± 0.013; $P < 0.05$) and contraction (0.143 ± 0.022 versus 2.04 ± 0.38 g; $P < 0.05$) in the same tissue. The reduction of tension was significantly greater than that of [Ca$^{2+}$]$_i$. In accordance, the contractile tension of the uterine artery at a given amount of [Ca$^{2+}$]$_i$, induced by NE was significantly decreased by 8-bromo-cGMP ($g/R_{340/380}$, 3.10 ± 0.35 versus 24.87 ± 3.43; $P < 0.05$). In contrast to NE-induced responses, 8-bromo-cGMP had no significant effect on either the tension (1.37 ± 0.29 versus 1.57 ± 0.30 g; $P > 0.05$) or [Ca$^{2+}$]$_i$ ($R_{340/380}$, 0.07 ± 0.022 versus 0.058 ± 0.017; $P > 0.05$) induced by KCl (Fig. 7C).

To determine the role of 8-bromo-cGMP on NE-induced Ca$^{2+}$ release from intracellular stores, NE-stimulated [Ca$^{2+}$]$_i$ and contractions were determined in the absence of extracellular Ca$^{2+}$. In contrast to the sustained increases in [Ca$^{2+}$]$_i$ and tension in the uterine artery in the absence of extracellular Ca$^{2+}$. In agreement with the findings in the presence of extracellular Ca$^{2+}$, 8-bromo-cGMP significantly decreased the NE-induced tension by 76% and [Ca$^{2+}$]$_i$ by 47% and, accordingly, decreased the NE-mediated Ca$^{2+}$ sensitization ($g/R_{340/380}$) by 45% in the absence of extracellular Ca$^{2+}$ (Fig. 7B).

**Discussion**

In the present study, we have demonstrated that KCl-induced Ca$^{2+}$ mobilization and contractions in the uterine artery from near-term pregnant sheep are not altered by 8-bromo-cGMP. Similar findings were obtained in rabbit aorta in which sodium nitroprusside did not affect KCl-evoked contractions (Karaki et al., 1984, 1986). Consistently, KCl-stimulated [Ca$^{2+}$]$_i$ and contractions were not significantly different between uterine arteries isolated from non-pregnant and pregnant sheep (Xiao and Zhang, 2004), albeit uterine cGMP levels were significantly increased during pregnancy in sheep (Magness et al., 1997; Sladek et al., 1997). These findings suggest that cGMP has a minimal role in the regulation of L-type Ca$^{2+}$ channels and electrome-
mechanical coupling of uterine artery contractions. Although K⁺ channels activation is a main mechanism induced by cGMP, in KCl-induced contractions, these channels may not be activated.

In contrast to the lack of effect on KCl-induced contractions, 8-bromo-cGMP attenuated α₁-adrenoceptor-mediated contractions by inhibiting both Ca²⁺ mobilization and the Ca²⁺ sensitivity of contractile myofilaments in the uterine artery. cGMP decreased NE-induced Ca²⁺ release from intracellular stores by inhibiting NE-mediated IP₃ synthesis and IP₃ binding affinity to the IP₃ receptor. There are two major components in G protein-coupled receptor-mediated pharmacomechanical coupling: 1) agonist-induced increase in intracellular free Ca²⁺ concentration ([Ca²⁺]ᵢ); and 2) agonist-induced increase in the Ca²⁺ sensitivity of contractile myofilaments. We have demonstrated that NE contracts the uterine artery by acting on α₁-adrenoceptors (Zhang et al., 1995b). Activation of α₁-adrenoceptors stimulated a rapid increase of IP₃, which correlated well with the contractile responses in the uterine artery (Zhang et al., 1995b). Release of intracellular Ca²⁺ from the sarcoplasmic reticulum by IP₃ is a major mechanism of pharmacomechanical coupling in smooth muscle (Somlyo and Somlyo, 1994; Zhang et al., 1995a). In the present study, we demonstrated that 8-bromo-cGMP significantly inhibited NE-induced contractions of the uterine artery and shifted NE dose-response curve to the

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Fig. 6. Effect of 8-bromo-cGMP on the IP₃ receptor in the uterine artery. Uterine arteries were treated with 100 µM 8-bromo-cGMP for 30 min. Saturation binding of [³H]IP₃ to membranes prepared from the uterine arteries was conducted as described under Materials and Methods. A, the specific binding curves for [³H]IP₃. Data points represent means of triplicate determinations in a single experiment. B, IP₃ binding dissociation constant (Kₐ) and IP₃ receptor density (Bₘₐₓ) determined in the saturation binding. Data are means ± S.E.M. of the tissues from five animals.

Fig. 7. Effect of 8-bromo-cGMP on NE- and KCl-stimulated [Ca²⁺]ᵢ and contractions in the uterine artery. NE- (3 µM) and KCl-induced (60 mM) [Ca²⁺]ᵢ (R₁₃₄₀/₃₈₀, ratio of F₃₄₀–F₃₈₀) and contractions were measured simultaneously in the same tissue in the absence or presence of 8-bromo-cGMP (100 µM, pretreatment for 30 min). A, NE-induced response in the presence of extracellular Ca²⁺. B, NE-induced response in the absence of extracellular Ca²⁺. C, KCl-induced response in the presence of extracellular Ca²⁺. Data are normalized to control and are means ± S.E.M. of tissues from five to seven animals. * P < 0.05.
right. The finding of excellent correlation between 8-bromocGMP-induced decreases in NE-mediated tension development and IP$_3$ production measured simultaneously in the same tissue strongly suggests that reduced IP$_3$ synthesis plays an important role in cGMP-mediated inhibition of NE-induced contractions in the uterine artery. Whereas 8-bromocGMP did not affect the time course of NE-induced IP$_3$ synthesis, it significantly decreased the potency and the maximal response of NE-induced IP$_3$ production in the uterine artery. In agreement, it has been shown previously in cultured vascular smooth muscle cells that cGMP decreases vasopressin-mediated IP$_3$ formation by inhibiting phospholipase C and/or receptor/phospholipase C coupling (Hirata et al., 1990).

Given that intracellular responses to IP$_3$ are mediated by the IP$_3$ receptor, the density of IP$_3$ receptors and IP$_3$ binding affinity to the IP$_3$ receptor are key determinants in explaining coupling between IP$_3$ and intracellular Ca$^{2+}$ release. In the present study, we demonstrated that 8-bromocGMP significantly decreased IP$_3$ binding affinity without changing the IP$_3$ receptor density in the uterine artery. It has been demonstrated that cGMP, through activation of cGMP-dependent protein kinase (cGK), induces phosphorylation of the IP$_3$ receptor in vascular smooth muscle and decreases the IP$_3$ binding affinity (Komalavilas and Lincoln, 1994). However, whether phosphorylation of the purified IP$_3$ receptor in constituted systems potentiates or inhibits its ability to release Ca$^{2+}$ from intracellular stores remains controversial (Supattapone et al., 1988; Nakade et al., 1994). It has been demonstrated in intact megakaryocytes that activation of cGK inhibits Ca$^{2+}$ release from the IP$_3$-sensitive stores (Tertyshnikova et al., 1998). In agreement with this finding, the present study demonstrated that 8-bromocGMP inhibited NE-induced transient increase in [Ca$^{2+}$]$_i$, in the absence of extracellular Ca$^{2+}$, suggesting that activation of cGK in the uterine artery decreased Ca$^{2+}$ release from intracellular stores. To our knowledge, the present study is the first to demonstrate cGK-mediated inhibition of Ca$^{2+}$ release from the IP$_3$-sensitive stores in the intact vessel. It has been shown in microsomal smooth muscle membranes that the cGK pathway regulates IP$_3$-mediated Ca$^{2+}$ release by the phosphorylation of not only IP$_3$ receptors but also a regulatory protein named IP$_3$ receptor-associated cGMP kinase substrate (Schloßmann et al., 2000). Furthermore, in cultured vascular smooth muscle cells, 8-bromo-cGMP inhibited IP$_3$-stimulated Ca$^{2+}$ release induced by angiotensin II (Saito et al., 1993). These findings indicate that the effect of cGMP may not be specific to NE but to other vasoconstrictors as well that are coupled to the IP$_3$ pathway and suggest an important role of cGMP in the attenuated vasoconstriction of the uterine artery to both NE and angiotensin II in pregnancy.

The simultaneous measurement of [Ca$^{2+}$]$_i$, with tension in the same intact tissue allowed us to determine directly the precise relationship between [Ca$^{2+}$]$_i$ and tension development in the uterine artery and thus to estimate Ca$^{2+}$ sensitivity of myofilaments with unimpaired excitation-contraction coupling processes and retained regulatory targets for second messenger pathways. In present study, the finding that addition of 8-bromo-cGMP nearly completely inhibited NE-induced contractions but decreased only 44% of the increased [Ca$^{2+}$]$_i$, suggests that not only does cGMP regulate Ca$^{2+}$ mobilization, it also inhibits Ca$^{2+}$ sensitivity of myofilaments in the uterine artery. This is consistent with the previous findings that sodium nitroprusside produced a greater inhibition on agonist-induced contractions than its inhibition on intracellular Ca$^{2+}$ concentrations in vascular smooth muscle (Karaki et al., 1986; McDaniel et al., 1992; Xiao et al., 2001). In vascular smooth muscle, the sensitivity of contractile apparatus to Ca$^{2+}$ is modulated by two major pathways: thick filament regulatory pathway and thin filament regulatory pathway (Horowitz et al., 1996). The thin filament regulatory pathway involves regulatory proteins caldesmon and calponin. The thick filament regulatory pathway is mediated by myosin light chain phosphatase that dephosphorylates myosin light chain. It has been demonstrated that cGMP-mediated vascular relaxation is characterized by both a reduction of intracellular Ca$^{2+}$ concentrations and by an activation of myosin light chain phosphatase leading to a decrease in the sensitivity of contractile apparatus to intracellular Ca$^{2+}$ (Lincoln and Cornwell, 1993; Lee et al., 1997; Pfeifer et al., 1998; Sauzeau et al., 2000).

In summary, the present study demonstrates that cGMP inhibits α$_1$-adrenoceptor-mediated pharmacomechanical coupling and contractions in the uterine artery of near-term pregnant sheep by suppressing both Ca$^{2+}$ mobilization and the Ca$^{2+}$ sensitivity of myofilaments (Fig. 8). From the physiological perspective, the uterine circulation during pregnancy functions as a low-resistance shunt to accommodate the large increase of uteroplacental blood flow required for normal fetal development. In addition to growth and remodeling of vessels, the decreased uterine artery resistance is accomplished by increased endothelial nitric oxide release, decreased myogenic response, and a reversible sympathetic denervation of the uterine artery. Although decreased sympathetically innervation may
sensitize postsynaptic α₁-adrenoceptor signaling pathways, the present finding of the inhibitory effect of cGMP on α₁-adrenoceptor-mediated contractions in the pregnant uterine artery reveals another important mechanism in maintaining the low uterine vascular tone in pregnancy given that uterine cGMP levels are elevated during pregnancy. Whereas it is not the goal of the present study, a comparison of cGMP mechanism in the regulation of α₁-adrenoceptor-mediated signaling pathway between uterine arteries from nonpregnant and pregnant animals presents an intriguing area for the future investigation.

References


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