0022-3565/08/3272-425–431$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 327:425–431, 2008

Vol. 327, No. 2
141283/3394460
Printed in U.S.A.

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

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Received May 19, 2008; accepted August 4, 2008

ABSTRACT

The present study examined the role of cGMP in the regulation of α_1-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 the potency and the maximal response of NE-induced inositol 1,4,5-trisphosphate (IP_3) synthesis in the uterine artery. In addition, 8-bromo-cGMP significantly reduced the binding affinity of IP_3 to the IP_3 receptor. The density of IP_3 receptors was not affected. Simultaneous measurement of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) and tensions in the same tissue indicated that 8-bromo-cGMP decreased NE-induced contractions by 92% but only blocked 44% $[Ca^{2+}]_i$. In accordance, 8-bromo-cGMP significantly decreased tension generation for a given $[Ca^{2+}]_i$ ($g/R_{f340/380}$, 24.87 $\pm$ 3.43 versus 3.10 $\pm$ 0.35). In the absence of extracellular Ca^{2+}, NE produced a transient increase in $[Ca^{2+}]_i$ and contraction, 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 α_1-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 Cornwell, 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

This work was supported in part by National Institutes of Health Grants HL89012 (to L.Z.) and HD31226 (L.Z.) and by Loma Linda University School of Medicine.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.108.141283.

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 α_1-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 IP_3 signaling pathway in the uterine artery. The time- and dose-dependent IP_3 synthesis induced by norepinephrine was measured. To correlate directly IP_3 synthesis to tension development and to determine tissue sensitivity to IP_3, we developed a method to measure α_1-adrenoceptor-mediated contractile tension and IP_3 production simultaneously in the same tissue. Furthermore, we characterized IP_3 receptors in the uterine artery and determined the effect of 8-bromo-cGMP on IP_3 binding affinity to the IP_3 receptor and the receptor density. We went further to determine the effect of 8-bromo-cGMP on α_1-adrenoceptor-induced Ca^{2+} mobilization and Ca^{2+} sensitization of myofilaments in the uterine artery.

Materials and Methods

Tissue Preparation. Pregnant sheep (~140 days gestation) were anesthetized with thiamy (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 $CaCl_2$, 1.16 mM $MgSO_4$, 1.18 mM KH_2PO_4, 22.14 mM $NaHCO_3$, 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. Fourth 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. EC_{50} values for the agonist in each experiment were taken as the molar concentration at which the contraction-response curve intersected 50% of the maximal response and expressed as pD_2 ($-\log EC_{50}$) values.

Measurement of IP_3. The accumulation of IP_3 was measured by the competitive ligand binding radioreceptor 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 N_2. The tissues were then homogenized in ice-cold 16.7% trichloroacetic acid. The homogenate was centrifuged at 1500*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 with a protein assay kit (Bio-Rad, Hercules, CA). IP_3 in the supernatant was determined using a radioreceptor assay kit from PerkinElmer Life and Analytical Sciences (Waltham, MA). Values were expressed as picomole of IP_3 per milligram of protein.

Characterization of IP_3 Receptors. Saturation binding of [^{3}H]IP_3 (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% NaN_3, 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 IP_3 (Research Biomedicals Inc., Natick, MA). Saturation binding experiments used concentrations of [^{3}H] IP_3 from 0.15 to 80 nM, and nonspecific binding was determined by the addition of 12 μM ice-cold IP_3. 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 polyethylenimine-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 $[Ca^{2+}]_i$ and Tension. Simultaneous measurement of tension and free intracellular calcium concentrations ($[Ca^{2+}]_i$) 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 Ca^{2+} 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 alternatively (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 (F_{340} and F_{380}, respectively) and the ratio of these two fluorescence values ($R_{f340/380}$) were recorded with a time constant of 250 ms and stored with the force signal on a computer.

Data Analysis. 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 (B_{max}), and pD_2 ($-\log EC_{50}$) 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

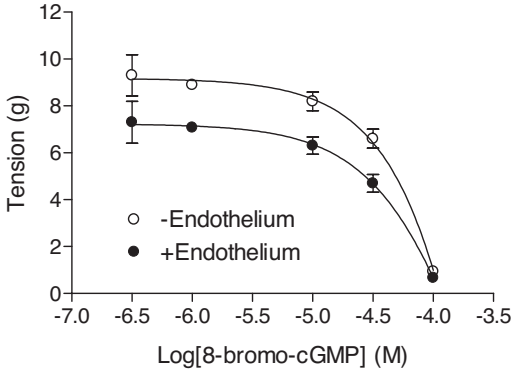
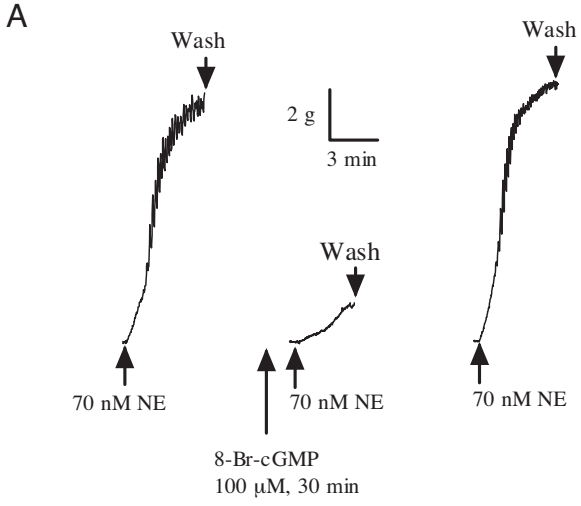


Fig. 1. 8-Bromo-cGMP-mediated relaxation of the uterine artery. Concentration-dependent relaxations induced by 8-bromo-cGMP were obtained with NE (0.1 μM)-precontracted uterine arteries with intact or denuded endothelium. Data are mean ± S.E.M. of the tissues from five animals.

significantly affected by the endothelium removal (Fig. 1). Furthermore, pretreatment of the uterine artery with 8-bromo-cGMP significantly inhibited the NE-induced contractions (Fig. 2). The inhibitory effect was completely reversible



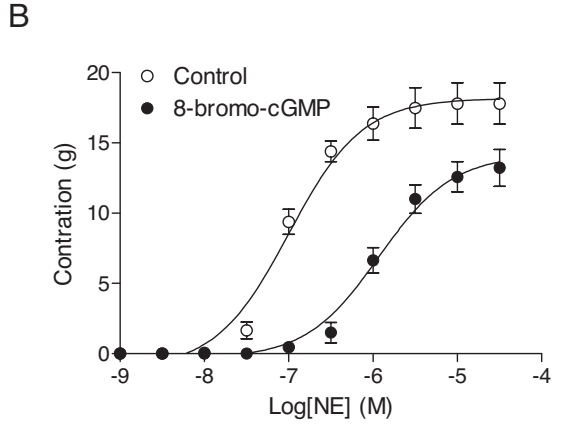


Fig. 2. 8-Bromo-cGMP-mediated inhibition of NE-induced contractions of the uterine artery. A, typical recordings of effect of 8-bromo-cGMP on the tension-induced by NE in the uterine artery. B, cumulative concentration-response curves to NE were obtained in the uterine artery in the absence or presence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

(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 ($P < 0$ 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 $[^3H]IP_3$ to tissue membranes was specific and saturable. 8-Bromo-cGMP did not significantly affect the receptor density (B_{max}, 313.6 ± 62.2 versus 356.2 ± 30.9 fmol/mg protein; $P > 0.05$) but did significantly increase the

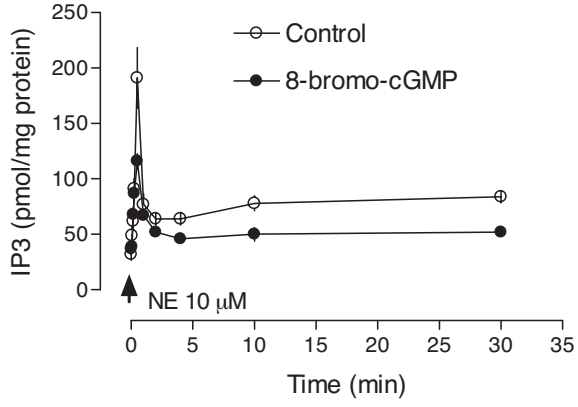


Fig. 3. Effect of 8-bromo-cGMP on the time course of NE-induced IP_3 synthesis in the uterine artery. Uterine arteries were stimulated with 10 μM NE for various times in the absence or presence of 8-bromo-cGMP (100 μM, pretreatment for 30 min). IP_3 was measured by a competitive ligand binding radioreceptor assay as described under *Materials and Methods*. Data are means ± S.E.M. of the tissues from five animals.

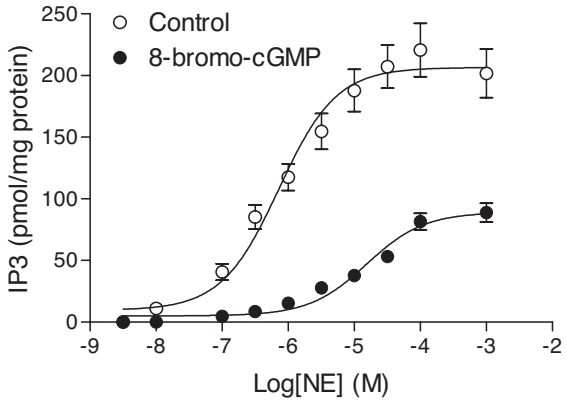


Fig. 4. 8-Bromo-cGMP-mediated inhibition of NE-stimulated IP_3 synthesis in the uterine artery. Uterine arteries were stimulated with various concentrations of NE for 30 s in the absence or presence of 8-bromo-cGMP (100 μM, pretreatment for 30 min). IP_3 was measured by a competitive ligand binding radioreceptor assay as described under *Materials and Methods*. Data are means ± S.E.M. of the tissues from five animals.

dissociation constant (K_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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ ($R_{f340/380}$, 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; $P \geq 0.05$) or $[Ca^{2+}]_i$ ($R_{f340/380}$, 0.07 ± 0.022 versus 0.058 ± 0.017; $P \geq 0.05$) 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 development observed in the presence of extracellular Ca^{2+}, NE produced a transient increase in both $[Ca^{2+}]_i$ 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/$R_{f340/380}$) by 45% in the absence of extracellular Ca^{2+} (Fig. 7B).

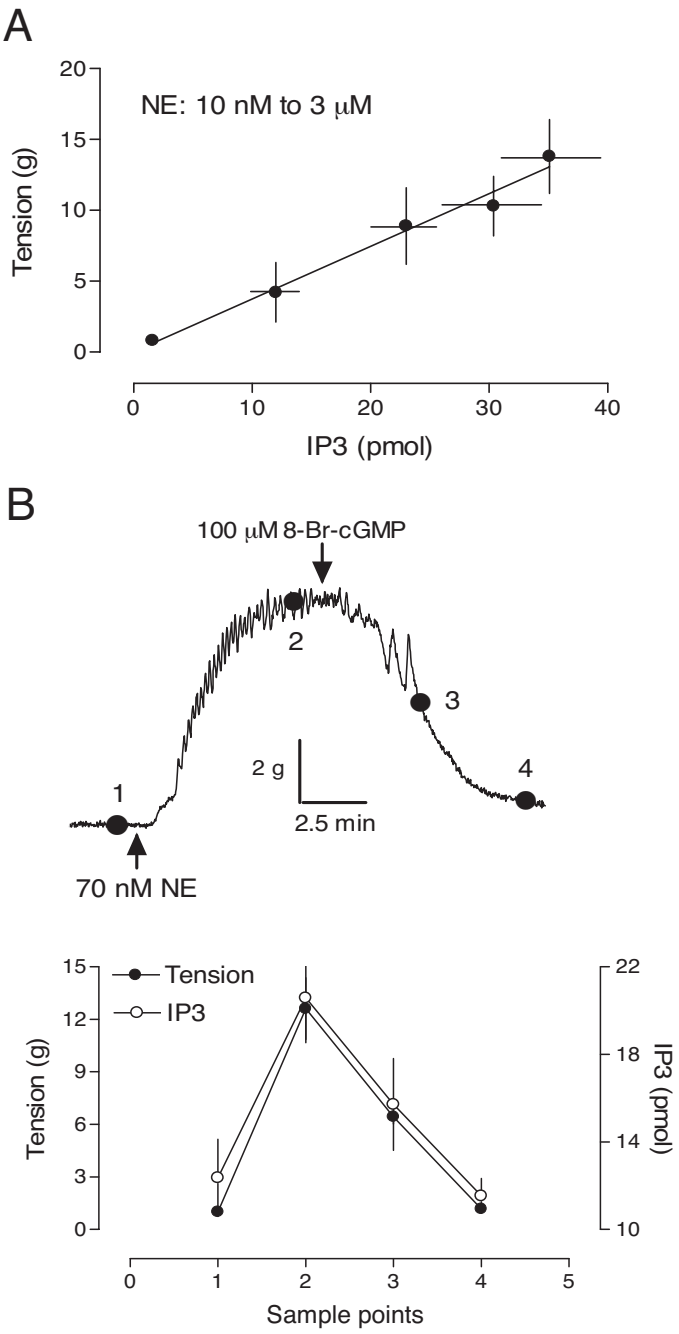


Fig. 5. Effect of 8-bromo-cGMP on NE-stimulated tension and IP_3 synthesis in the uterine artery. A, NE-induced IP_3 synthesis and tension development measured simultaneously in the same tissue. B, uterine arteries were stimulated with NE in the organ baths. After the contraction reached the plateau, 8-bromo-cGMP was added. Contractile tension and IP_3 were measured simultaneously in the same tissue at four different stages as shown in the trace. Tensions were recorded at the indicated sample points, and the tissues were snap-frozen in liquid N_2 at corresponding points for measurement of the IP_3 as described under *Materials and Methods*. Data are means ± S.E.M. of the tissues from five animals.

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 nonpregnant 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-

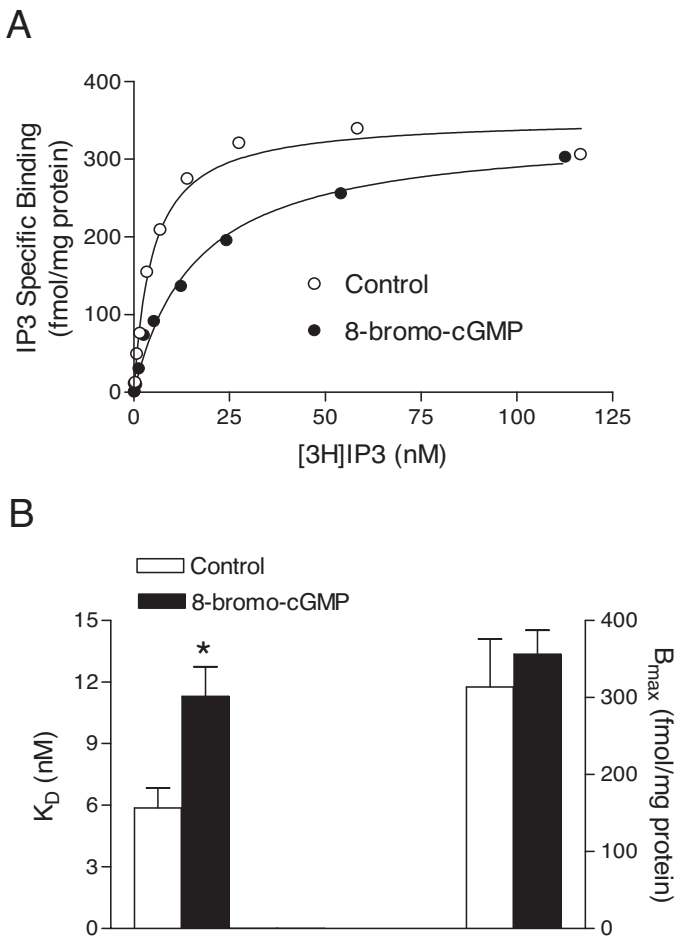


Fig. 6. Effect of 8-bromo-cGMP on the IP_3 receptor in the uterine artery. Uterine arteries were treated with 100 μM 8-bromo-cGMP for 30 min. Saturation binding of [^{3}H]IP_3 to membranes prepared from the uterine arteries was conducted as described under *Materials and Methods*. A, the specific binding curves for [^{3}H]IP_3. Data points represent means of triplicate determinations in a single experiment. B, IP_3 binding dissociation constant (K_D) and IP_3 receptor density (B_{max}) determined in the saturation binding. Data are means ± S.E.M. of the tissues from five animals. *, $P < 0.05$.

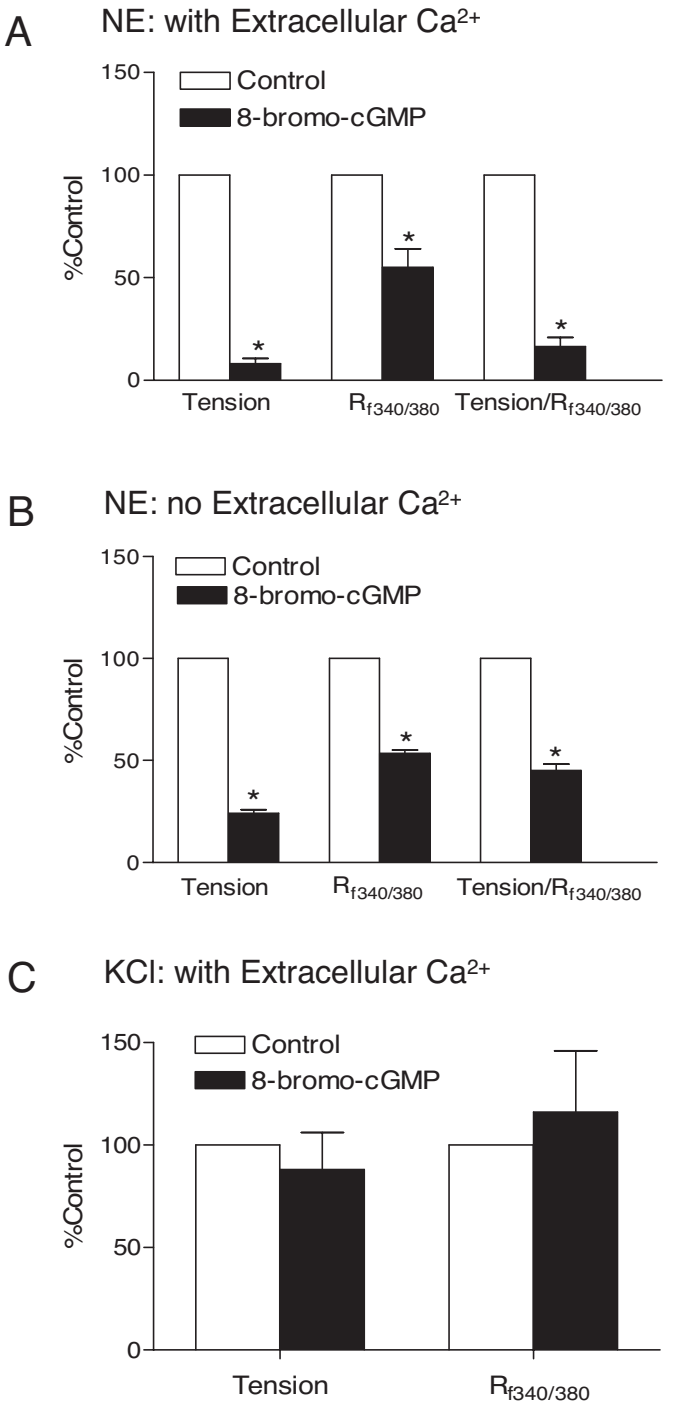


Fig. 7. Effect of 8-bromo-cGMP on NE- and KCl-stimulated $[Ca^{2+}]_i$ and contractions in the uterine artery. NE- (3 μM) and KCl-induced (60 mM) $[Ca^{2+}]_i$ ($R_{f340/380}$, ratio of F_{340}–F_{380}) and contractions were measured simultaneously in the same tissue in the absence or presence of 8-bromo-cGMP (100 μM, pretreatment for 30 min). A, NE-induced response in the presence of extracellular Ca^{2+}. B, NE-induced response in the absence of extracellular Ca^{2+}. C, KCl-induced response in the presence of extracellular Ca^{2+}. Data are normalized to control and are means ± S.E.M. of tissues from five to seven animals. *, $P < 0.05$.

chanical 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 α_1-adrenoceptor-mediated contractions by inhibiting both Ca^{2+} mobilization and the Ca^{2+} sensitivity of contractile myofilaments in the uterine artery. cGMP decreased NE-induced Ca^{2+} release from intracellular stores by inhibiting NE-mediated IP_3 synthesis and IP_3 binding affinity to the IP_3 receptor. There are two major components in G protein-coupled receptor-mediated pharmacomechanical coupling: 1) agonist-induced increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$); and 2) agonist-induced increase in the Ca^{2+} sensitivity of contractile myofilaments. We have demonstrated that NE contracts the uterine artery by acting on α_1-adrenoceptors (Zhang et al., 1995b). Activation of α_1-adrenoceptors stimulated a rapid increase of IP_3, which correlated well with the contractile responses in the uterine artery (Zhang et al., 1995b). Release of intracellular Ca^{2+} from the sarcoplasmic reticulum by IP_3 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

right. The finding of excellent correlation between 8-bromo-cGMP-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-bromo-cGMP 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-bromo-cGMP 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-bromo-cGMP 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 sympathetic innervation may

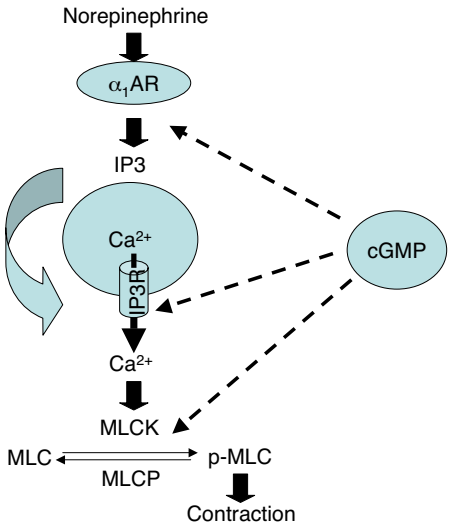


Fig. 8. cGMP-mediated inhibition of α_1-adrenoceptor-mediated pharmacomechanical coupling in the uterine artery. cGMP inhibits α_1-adrenoceptor-mediated IP_3 synthesis and reduces IP_3 binding affinity to the IP_3 receptor, leading to a decreased Ca^{2+} release from Ca^{2+} stores. Furthermore, cGMP inhibits the Ca^{2+} sensitivity of myofilaments possibly by activating myosin light chain phosphatase activity. α_1AR, α_1-adrenoceptor; IP_3, inositol 1,4,5-trisphosphate; IP3R, inositol 1,4,5-trisphosphate receptor; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; MLC, myosin light chain; p-MLC, phosphorylated myosin light chain.

sensitize postsynaptic α_1-adrenoceptor signaling pathways, the present finding of the inhibitory effect of cGMP on α_1-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 α_1-adrenoceptor-mediated signaling pathway between uterine arteries from nonpregnant and pregnant animals presents an intriguing area for the future investigation.

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