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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 α_1 -adrenoceptor-mediated pharmacomechanical coupling in the uterine artery of near-term pregnant sheep. The cell permeable cGMP analogue 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% of $[Ca^{2+}]_i$. In accordance, 8-bromo-cGMP significantly decreased tension generation for a given $[Ca^{2+}]_i$ ($g/R_{f340/380}$: 24.87 ± 3.43 vs. 3.10 ± 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.

Introduction

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 are 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 (IP₃)-induced Ca²⁺ release was described (Murthy et al., 1993) and was correlated with the phosphorylation of IP₃ receptors induced by agents known to increase intracellular cGMP concentrations (Komalavilas and Lincoln, 1994).

The present study was designed to determine the role of 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 analogue, 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 α_1 -adrenoceptor-mediated contractile tension and IP₃ production simultaneously in the same tissue. Additionally, 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 α_1 -adrenoceptor-induced Ca²⁺ mobilization and Ca²⁺ sensitization of myofilaments in the uterine artery.

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 exposed. The uterine arteries were isolated and removed without stretching and placed into a modified Krebs solution (pH 7.4) of the following composition (in mM): 115.21 NaCl, 4.7 KCl, 1.80 Ca Cl₂, 1.16 MgSO₄, 1.18 KH₂PO₄, 22.14 NaHCO₃, and 7.88 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 T-61 (euthanasia solution, Hoechst-Roussel, Somerville, NJ, USA). All procedures and protocols were approved by the Institutional Animal Care and Use Committee of Loma Linda University and followed the guidelines by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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's effect, 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 molar concentration at which the contraction-response curve intersected 50% of the maximum response, and were expressed as pD₂ (-logEC₅₀) values.

Measurement of IP₃

The accumulation of IP₃ 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. Following 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 1,500g for 10 min at 4 °C. The supernatant was extracted with water-saturated diethyl ether to remove TCA, and the pellet was saved for protein determination with a protein assay kit (Bio-Rad). IP₃ in the supernatant was determined using a radioreceptor assay kit from DuPont-NEN (Boston, MA). Values were expressed as pmol IP₃/mg protein.

Characterization of IP₃ Receptors

Saturation binding of [³H]IP₃ (Dupont-NEN, Boston, MA) 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, 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 at setting 3.5 in two bursts of 15 s each. The homogenate was centrifuged at 100,000g for 30 min. Pellets were re-homogenized in half the original volume of the buffer at setting 3.5 for 15 s, and recentrifuged under the same conditions. The supernatant was discarded and the pellet was re-suspended 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 radioligand, and 5 µl 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 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 polyethylenimine (0.5%)-pretreated filters (GF/B: Whatman Inc., Clifton, NJ) with a Brandel cell harvester. 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 (fura 2-AM) 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-w Xe 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 ($R_{f340/380}$) 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 GraphPad Prism (GraphPad Software, San Diego, CA). Results were expressed as means $\pm$ SEM 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 pre-contracted with NE, which was not significantly affected by the endothelium removal (Fig. 1). Additionally, pretreatment of the uterine artery with 8-bromo-cGMP significantly inhibited the NE-induced contractions (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 g 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 vs. 159 ± 22.8 pmol/mg protein, $P < 0.05$). The elevated steady level was also decreased by 8-bromo-cGMP (Fig. 3). Fig. 4 shows the concentration-dependent response of IP_3 synthesis induced by NE. 8-Bromo-cGMP caused about 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 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 vs. 356.2 ± 30.9 fmol/mg protein, $P > 0.05$), but significantly increased the dissociation constant (K_D : 11.3 ± 1.44 vs. 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 in the *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 vs. 0.082 ± 0.013 , $P < 0.05$) and contraction (0.143 ± 0.022 vs. 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 vs. 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 vs. 1.57 ± 0.30 g, $P > 0.05$) or $[Ca^{2+}]_i$ ($R_{f340/380}$: 0.07 ± 0.022 vs. 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 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 the NE-mediated Ca^{2+} sensitization ($g/R_{f340/380}$) 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., 1996; Rosenfeld et al., 1996; Sladek et al., 1997). These findings suggest that cGMP has minimum role in the regulation of L-type Ca^{2+} channels and electromechanical 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 IP₃ formation by inhibiting phospholipase C and/or receptor/phospholipase C coupling (Hirata et al., 1990).

Given that intracellular responses to IP₃ are mediated by the IP₃ receptor, the density of IP₃ receptors and IP₃ binding affinity to the IP₃ receptor are key determinants in explaining coupling between IP₃ and intracellular Ca²⁺ release. In the present study, we demonstrated that 8-bromo-cGMP significantly decreased IP₃ binding affinity without changing the IP₃ receptor density in the uterine artery. It has been demonstrated that cGMP, through activation of cGMP-dependent protein kinase (cGK), induces phosphorylation of the IP₃ receptor in vascular smooth muscle and decreases the IP₃ binding affinity (Komalavilas and Lincoln, 1994). However, whether phosphorylation of the purified IP₃ receptor in constituted systems potentiates or inhibits its ability to release Ca²⁺ 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 Ca²⁺ release from the IP₃-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 [Ca²⁺]_i in the absence of extracellular Ca²⁺, suggesting that activation of cGK in the uterine artery decreased Ca²⁺ release from intracellular stores. To our knowledge, the present study is the first to demonstrate cGK-mediated inhibition of Ca²⁺ release from the IP₃-sensitive stores in the intact vessel. It has been shown in microsomal smooth muscle membranes that the cGK pathway regulates IP₃-mediated Ca²⁺ release by the phosphorylation of not only IP₃ receptors but also a regulatory protein named IP₃ receptor-associated cGMP kinase substrate (IRAG) (Schlossmann et al., 2000). Additionally, in cultured vascular smooth muscle cells, 8-bromo-cGMP inhibited IP₃-stimulated Ca²⁺ release induced by angiotensin II (Saito et al., 1993). These findings indicate that the effect of cGMP may not be specific to NE but also to other vasoconstrictors coupled to the IP₃ 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 [Ca²⁺]_i with tension in the same intact tissue allowed us to determine directly the precise relationship between [Ca²⁺]_i and tension development in the uterine artery and thus to estimate Ca²⁺ 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 [Ca²⁺]_i, suggests that not only does cGMP regulate Ca²⁺ mobilization, but it also inhibits Ca²⁺ 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 Ca²⁺ concentrations in vascular smooth muscle (Karaki et al., 1986; McDaniel et al., 1992; Xiao et al., 2001b). In vascular smooth muscle, the sensitivity of contractile apparatus to Ca²⁺ 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 Ca²⁺ concentrations and by an activation of myosin light chain phosphatase leading to a decrease in the sensitivity of contractile apparatus to intracellular Ca²⁺ (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 α₁-adrenoceptor-mediated pharmacomechanical coupling and contractions in the uterine artery of near-term pregnant sheep by suppressing both Ca²⁺ mobilization and the Ca²⁺ 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 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 non-pregnant and pregnant animals presents an intriguing area for the future investigation.

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List of non-standard abbreviations

NE	norepinephrine
IP3	inositol 1,4,5-trisphosphate
[Ca²⁺]_i	intracellular free Ca ²⁺ concentrations

Figure 1. 8-Bromo-cGMP-mediated relaxation of the uterine artery

Concentration-dependent relaxations induced by 8-bromo-cGMP were obtained with NE (0.1 μ M)-pre-contracted uterine arteries with intact or denuded endothelium. Data are mean $\pm$ SEM of the tissues from five animals.

Figure 2. 8-Bromo-cGMP-mediated inhibition of NE-induced contractions of the uterine artery
Panel A: Typical recordings of effect of 8-bromo-cGMP on the tension-induced by NE in the uterine artery. **Panel 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 ± SEM of the tissues from five animals.

Figure 3. Effect of 8-bromo-cGMP on the time course of NE-induced IP₃ synthesis in the uterine artery
 Uterine arteries were stimulated with 10 μ M NE for various times in the absence or presence of 8-bromo-cGMP (100 μ M, pretreatment for 30 min). IP₃ was measured by a competitive ligand binding radioreceptor assay as described in the Methods. Data are means $\pm$ SEM of the tissues from five animals.

Figure 4. 8-Bromo-cGMP-mediated inhibition of NE-stimulated IP₃ 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 μ M, pretreatment for 30 min). IP₃ was measured by a competitive ligand binding radioreceptor assay as described in the Methods. Data are means $\pm$ SEM of the tissues from five animals.

Figure 5. Effect of 8-bromo-cGMP on NE-stimulated tension and IP₃ synthesis in the uterine artery
Panel A. NE-induced IP₃ synthesis and tension development measured simultaneously in the same tissue. **Panel 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 IP₃ 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 N₂ at corresponding points for measurement of the IP₃ as described in Methods. Data are means $\pm$ SEM of the tissues from five animals.

Figure 6. Effect of 8-bromo-cGMP on the IP₃ receptor in the uterine artery

Uterine arteries were treated with 100 μ M 8-bromo-cGMP for 30 min. Saturation binding of [³H]IP₃ to membranes prepared from the uterine arteries was conducted as described in Methods. **Panel A** shows the specific binding curves for [³H]IP₃. Data points represent means of triplicate determinations in a single experiment. **Panel B** shows IP₃ binding dissociation constant (K_D) and IP₃ receptor density (B_{max}) determined in the saturation binding. Data are means $\pm$ SEM of the tissues from five animals. * $P < 0.05$

Figure 7. Effect of 8-bromo-cGMP on NE- and KCl-stimulated $[\text{Ca}^{2+}]_i$ and contractions in the uterine artery

NE (3 μM)- and KCl (60 mM)-induced $[\text{Ca}^{2+}]_i$ ($R_{f340/380}$, ratio of F_{340} to 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). **Panel A.** NE-induced response in the presence of extracellular Ca^{2+} ; **Panel B.** NE-induced response in the absence of extracellular Ca^{2+} . **Panel C.** KCl-induced response in the presence of extracellular Ca^{2+} . Data are normalized to control and are means $\pm$ SEM of tissues from 5-7 animals, * $P < 0.05$.

Figure 8. cGMP-mediated inhibition of α_1 -adrenoceptor-mediated pharmacomechanical coupling in the uterine artery

cGMP inhibits α_1 -adrenoceptor-mediated IP₃ synthesis and reduces IP₃ binding affinity to the IP₃ receptor, leading to a decreased Ca^{2+} release from Ca^{2+} stores. Additionally, cGMP inhibits the Ca^{2+} sensitivity of myofilaments possibly by activating myosin light chain phosphatase activity. α_1 AR, α_1 -adrenoceptor; IP₃, 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.