Regulation of cGMP-induced relaxation and cGMP-dependent protein kinase in rat myometrium during pregnancy

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Word, R. Ann, and Trudy L. Cornwell. Regulation of cGMP-induced relaxation and cGMP-dependent protein kinase in rat myometrium during pregnancy. Am. J. Physiol. 274 (Cell Physiol. 43): C748–C756, 1998.—Increases in guanosine 3′,5′-cyclic monophosphate (cGMP) induced by nitric oxide (NO), nitrovasodilators, and atrial peptides correlate with relaxation of vascular smooth muscle. Relaxation of myometrial smooth muscle by increases in cGMP, however, has required unusually high concentrations of the cyclic nucleotide. We tested the hypothesis that the sensitivity of myometrium to relaxation by cGMP is increased during pregnancy. Aortic smooth muscle was more sensitive to relaxation by cGMP than myometrial tissues, and, contrary to our hypothesis, myometrium from pregnant rats was least sensitive. Although levels of cGMP were elevated after treatment with the NO donor, N-nitroso-N-acetylpenicillamine, relaxation of myometrial tissues obtained from pregnant rats occurred only at extraordinarily high concentrations. The levels of cGMP-dependent protein kinase (PKG) were significantly decreased in myometrium from pregnant rats compared with myometrium from nonpregnant cycling animals or aortic smooth muscle. Administration of estradiol to ovariectomized rats increased myometrial PKG expression, and progesterone antagonized this response. We conclude that 1) myometrial tissues from pregnant rats are not sensitive to relaxation by cGMP and 2) this insensitivity to cGMP is accompanied by progesterone-mediated decreases in the level of PKG expression.

nitrergic oxide; progesterone; guanosine 3′,5′-cyclic monophosphate phosphodiesterase; oxytocin; atrial natriuretic peptide

THE BIOCHEMICAL PROCESSES that initiate labor in women are not known. In addition, the precise mechanisms that serve to maintain uterine quiescence during pregnancy are not well understood. Because the incidence of preterm labor has remained constant for decades, and preterm labor remains a major cause of perinatal mortality and infant morbidity, modulators of myometrial function during pregnancy have been eagerly sought. Recently, it has been proposed that nitric oxide (NO) may be an endogenous uterine relaxant that contributes to uterine quiescence during pregnancy before labor (18, 31). In addition, it has been suggested that relief of NO action may lead to retreat from uterine quiescence with the onset of spontaneous uterine contractions of labor (24).

In general, the smooth muscle relaxant effects of NO, atrial peptides, and drugs that liberate NO are mediated by guanosine 3′,5′-cyclic monophosphate (cGMP). The downstream target for cGMP is believed to be the cGMP-dependent protein kinase (PKG), which mediates the vasorelaxant effects of cGMP (5, 10, 15). Whereas increases in cGMP correlate with relaxation in a time- and concentration-dependent manner in vascular smooth muscle (15), the role of cGMP as a uterine relaxant is not as well established. For example, there is no correlation between increases in cGMP and relaxation in myometrial smooth muscle (7), and increases in cGMP that effect almost complete relaxation of tonic smooth muscles have relatively little effect in uterine smooth muscle (7, 29). To resolve this apparent conundrum, several laboratories have investigated the possibility that uterine smooth muscle responds to cGMP-mobilizing agents as a function of the hormonal milieu. Bek et al. (3) reported that estradiol-primed rat myometrium relaxed in response to atrial natriuretic peptide (ANP). Potvin and Varma (22) found that myometrium from pregnant or progesterone-treated rats was refractory to ANP (21, 23). In other studies, however, it has been suggested that responses to cGMP-mobilizing agents may be enhanced during pregnancy (31). Thus, although experimental evidence supports a hormone dependence to the relaxant properties of cGMP, it remains uncertain whether pregnancy enhances or diminishes cGMP action in uterine smooth muscle.

In this investigation, we compared the effects of NO donors and cGMP on contractile properties of aortic smooth muscle and on myometrial smooth muscle from nonpregnant and pregnant rats. We initially reasoned that, during pregnancy, myometrial sensitivity to cGMP may be similar to that of vascular smooth muscle, thereby providing a potential mechanism of uterine quiescence. The results of these studies, however, indicate that myometrial tissues obtained from pregnant rats are insensitive to relaxation by cGMP. Furthermore, the levels of PKG in uterine smooth muscle are significantly decreased during pregnancy, and this downregulation of PKG during pregnancy is mediated by progesterone. These latter findings provide a mechanism, at least in part, for the diminished sensitivity of uterine smooth muscle to cGMP during pregnancy.

MATERIALS AND METHODS

Source of smooth muscle tissues. Ovariectomized and late pregnant (days 17–21) Sprague-Dawley rats (180–250 g) were obtained from Sasco Laboratories. Daily subcutaneous injection of hormones was initiated at 3-wk postovariectomy and continued for a total of 3 days: 50 µg/kg 17β-estradiol, 2 mg/kg progesterone, 17β-estradiol plus progesterone or vehicle (corn oil, 200 µl). Rats were killed the day after completion of hormone treatment, and uteri were dissected.
For the late pregnant group, gestational dating was performed by vaginal smears. The first day of pregnancy was estimated by estrus pattern with sperm present. All studies were conducted in accordance with the standards of humane animal care as described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Review Board for Animal Research. Uterine and aortic tissues were placed in ice-cold physiological saline solution (PSS; in mM: 120.5 NaCl, 4.8 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄, 20.4 NaHCO₃, 2.5 CaCl₂, 10.0 d-glucose, and 1.0 pyruvate) and gassed with 95% O₂ and 5% CO₂. The tissues were then prepared immediately for experimental studies.

Evaluation of smooth muscle contractile properties. Each uterine horn was opened longitudinally along the mesenteric border. The endometrium was removed with a cotton-tip applicator and by sharp dissection using a dissecting microscope. Aortic tissues were removed, cleaned of adventitia, and opened longitudinally. Myometrial strips (1 x 3 x 0.5 mm) were cut parallel to the longitudinal muscle fibers, and aortic strips (0.5 x 1 x 0.2 mm) were cut transversely. Myometrial strips were hung from Grass FT 0.036 transducers using 6-0 braided silk suture, and aortic strips were mounted with vascular clamps. Clamps or sutures were attached to steel rods mounted on calibrated mechanical drives. Tissues were incubated in PSS (37°C), and contractions were recorded on a Grass model 7 polygraph. Optimal length for maximal force development (Lₒ) was determined by length-force relationships. Strips at Lₒ were conditioned by one contraction in PSS that contained KCl (65 mM) substituted isotonically for NaCl. Aortic smooth muscle strips were conditioned by two phenylephrine (0.1 µM)-induced contractions. In myometrial tissues, active force was quantified by digitizing the physiograph tracing of isometric force development during 10 min and computing the area of active force (30). In each strip, the area of active force after treatment with test agents was compared with changes in force development at the corresponding time intervals in separate control aortic and myometrial strips.

Radioimmunoassay of cGMP. Muscle strips were mounted for quantification of force generation (described above) and treated with vehicle or test agents for 5 min. Tissues were snap frozen with tongs precooled to the temperature of liquid nitrogen and immediately homogenized in ice-cold extract buffer that contained HCl (0.1 N) and methanol (50%, vol/vol). Samples were centrifuged at 10,000 g for 10 min, and supernatants were lyophilized, acetylated, and assayed for cyclic nucleotide content by radioimmunoassay as described (12). Precipitated protein was determined using the bichinchoninic acid protein assay (Pierce, Rockford, IL).

Immunoblot analysis of PKG. Proteins in tissue extracts were separated by electrophoresis on sodium dodecyl sulfate (SDS)-polyacrylamide gels (7%). Proteins were transferred to nitrocellulose at 90 mA for 14–16 h in the presence of methanol (20%) and SDS (0.1%). Blots were treated with TBST-milk buffer [10 mM tris(hydroxymethyl)aminomethane, 150 mM NaCl, 0.05% Tween 20, and 0.3% powdered milk] for 1 h and then incubated overnight with TBST-milk that contained a polyclonal antibody against bovine lung PKG (1:1,000) at 4°C. Thereafter, the blots were washed three times with TBST (5 min each) and incubated with goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (1:10,000). After an extensive washing with TBST, the blots were developed with a chemiluminescent detection system (enhanced chemiluminescence Western blotting detection system). Relative amounts of PKG were quantified using an LKB 2202 Ultrosan laser densitometer. Quantification was obtained from blots in which densitometric units were linear with respect to amounts of purified PKG standards and protein loaded on the gel.

Assay for PKG. PKG activity was determined from crude soluble extracts. Incorporation of [γ-32P]ATP into histone F2b was monitored using a filter paper assay (16).

Materials. S-nitroso-N-acetyl-penicillamine (SNAP) was purchased from Research Biochemicals International (Natick, MA). The monophosphorothioate activator of PKG, 8-(4-chlorophenylthio)guanosine 3',5'-cyclic monophosphate (8-CPT-cGMP) was obtained from Biolog (La Jolla, CA). M2056 was obtained from LC Laboratories (Woburn, MA). Radiolabeled compounds ([γ-32P]ATP and [125I]-labeled cGMP) were from DuPont NEN (Boston, MA), and the bichinchoninic acid protein assay reagents were from Pierce. The enhanced chemiluminescence Western blotting detection system was obtained from Amersham (Arlington Heights, IL). All other reagents were from Sigma (St. Louis, MO) or Fisher Biochemicals (Pittsburgh, PA).

Statistics. Data are expressed as means ± SE. For multiple comparisons, data were analyzed using a nonparametric Kruskal-Wallis analysis followed by χ² approximation. Differences between aorta and myometrium were determined with Mann-Whitney’s U test for two independent variables.

RESULTS

Effect of activators of guanylate cyclases and cGMP phosphodiesterase inhibitors on smooth muscle contractility. To compare the effects of cGMP in vascular and uterine smooth muscles, we treated aortic and myometrial tissues from pregnant rats with sodium nitroprusside (SNP), a prototype of NO donors known to increase cellular levels of cGMP (Fig. 1). Strips were precontracted with submaximal concentrations of phenylephrine (0.1 µM) or oxytocin (1 nM) to effect similar degrees of oxytocin-induced contractions in myometrial tissues (Figs. 1D and 2). This refractoriness to SNP in myometrial tissues was not unique to oxytocin. Myometrial tissues precontracted with 5-hydroxytryptamine were also insensitive to relaxation by SNP (data not shown). The effects of SNP on contractile force of both tissues are summarized in Fig. 2. Maximal relaxation of myometrial tissues (70%) was effected by 1 x 10⁻⁴ M and half-maximal effects at 3 x 10⁻⁵ M. Although high concentrations of SNP (>100 µM) abolished contractile activity in oxytocin-contracted myometrial tissues, these effects were irreversible and spontaneous activity did not resume after several rinses with PSS or after depolarization with KCl (40 mM). Thus, compared with aortic smooth muscle, SNP was less effective in relaxing myometrium from pregnant rats. Similar results were obtained with the NO donor SNAP (Fig. 2, right). The concentration of SNAP required to effect 50% inhibition of contractile force in aortic smooth muscle was significantly less than that required for myometrium (4.2 ± 0.2 x 10⁻⁸ M, aorta compared with 2.2 ± 0.4 x 10⁻⁴ M in myometrium, P < 0.01).
The effect of SNP on spontaneous contractions in myometrium from nonpregnant and pregnant rats was also determined (Fig. 3). Although treatment with SNP resulted in very small decreases in the amplitude of force development in both tissues, the predominant effect of SNP was to decrease the frequency of spontaneous contractions (Fig. 3, top). Myometrium from nonpregnant animals in estrus was more sensitive to relaxation by SNP than that from pregnant animals (Fig. 3, bottom). A comparison of these data, along with those presented in Figs. 1 and 2, clearly indicates that myometrial tissues are less responsive to SNP than aortic smooth muscle, and myometrium from pregnant animals is even less sensitive than myometrium from nonpregnant animals. The apparent increased relaxation of oxytocin-induced contractions (70%, Fig. 2) compared with spontaneous contractions (10%, Fig. 3) is due to the irreversible abolishment of contractions in some oxytocin-contracted tissues treated with high concentrations of SNP.

An alternative mechanism known to elevate cGMP levels was also tested. Natriuretic peptides elevate cGMP by binding to extracellular receptors that are transmembrane guanylate cyclases. Stimulation of particulate guanylate cyclase by ANP (0.1 µM) resulted in significant relaxation (85%) in phenylephrine-induced contractions in aorta (Fig. 4). Precontracted myometrial tissues from pregnant rats, however, were insensitive to relaxation by ANP (Fig. 4). Thus myometrium from pregnant rats was not sensitive to activators of either particulate or soluble guanylate cyclase.
guanylate cyclases. Insensitivity to agents that synthesize cGMP may be due to increased cellular levels of cGMP phosphodiesterase activity. To test this hypothesis, we treated myometrial and aortic smooth muscles with a selective inhibitor of cGMP phosphodiesterase (M2056, 10 µM). Inhibition of cGMP phosphodiesterase resulted in 47 ± 10% relaxation of aortic smooth muscle within 10 min (n = 3, Fig. 5A). The same concentration of M2056 had no effect on oxytocin-induced myometrial contractions (Fig. 5B). In addition, preincubation of myometrial tissues with M2056 for up to 20 min did not alter the relaxation response to SNAP (10−6 M).

Production of cGMP in myometrial and aortic tissues. Production of cGMP may be impaired in myometrial tissues, thereby providing an explanation for the apparent refractoriness of this tissue to activators of guanylate cyclases. To ensure that cellular levels of cGMP were increased in myometrial tissues by NO donors and cGMP phosphodiesterase inhibitors, myometrial and aortic tissues were treated with SNAP (1 µM), ANP (0.1 µM), M2056 (10 µM), or SNAP + M2056 for 5 min and homogenized, and the tissue content of cGMP was determined by radiomunoassay. In both aortic and myometrial tissues, levels of cGMP increased significantly after treatment with SNAP, M2056, or SNAP + M2056 (Table 1). In contrast, ANP resulted in increased...
tissue levels of cGMP in aortic but not myometrial tissues from pregnant animals (P \leq 0.06).

Effect of cGMP analogs on smooth muscle contractility. We utilized two poorly hydrolyzed analogs of cGMP to assess the sensitivity of vascular and myometrial smooth muscles to cGMP, thereby circumventing any requirement for tissue guanylate cyclases and avoiding the metabolism of cGMP by tissue phosphodiesterases. We quantified the effects of 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) on oxytocin-induced contractions in myometrium and aorta (Fig. 6). Precontracted aortic (Fig. 6A) and myometrial (Fig. 6B) smooth muscle strips were treated with increasing concentrations of 8-BrcGMP (10^{-6} to 10^{-4} M), and the decrease in force generation was quantified over time. Whereas 8-BrcGMP (10^{-4} M) resulted in significant inhibition of phenylephrine-induced contractions in aortic smooth muscle (86 \pm 2.7\%, n = 5), precontracted myometrial tissues from pregnant rats responded to 8-BrcGMP (10^{-4} M) with only small decreases in both amplitude (17 \pm 6\%) and frequency (7 \pm 6\%) of contraction. Similar results were obtained with 8-CPT-cGMP, an analog of cGMP that is more resistant to cGMP hydrolysis than 8-BrcGMP (10). Myometrial tissues from nonpregnant rats in estrus, however, were relaxed with 8-BrcGMP, thereby demonstrating a sensitivity similar to aortic smooth muscle (Fig. 7). Although myometrial tissues from nonpregnant rats in estrus were relaxed by 10^{-5} M 8-BrcGMP (Fig. 7A), myometrial tissues from rats in diestrus or from rats treated with progesterone were like those of pregnant rats and insensitive to 8-BrcGMP (Fig. 7B). Even high concentrations of 8-BrcGMP (10^{-4} M) were not effective in relaxing progesterone-dominated myometrium.

Levels of PKG in aortic and myometrial smooth muscles. We next examined the expression of the major receptor protein for cGMP, PKG, in myometrial and aortic tissues. Because myometrial smooth muscle from pregnant rats was relatively insensitive to relaxation

### Table 1. Tissue content of cGMP in aortic and myometrial tissues

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Myometrium</th>
<th>n</th>
<th>Aorta</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.34 ± 0.03</td>
<td>10</td>
<td>0.38 ± 0.04</td>
<td>9</td>
</tr>
<tr>
<td>SNAP (1 μM)</td>
<td>0.75 ± 0.08*</td>
<td>8</td>
<td>0.78 ± 0.06</td>
<td>3</td>
</tr>
<tr>
<td>ANP (0.1 μM)</td>
<td>0.39 ± 0.05</td>
<td>6</td>
<td>1.07 ± 0.42†</td>
<td>3</td>
</tr>
<tr>
<td>M2056</td>
<td>0.84 ± 0.19*</td>
<td>6</td>
<td>1.79 ± 0.61*</td>
<td>3</td>
</tr>
<tr>
<td>SNAP + M2056</td>
<td>0.96 ± 0.04*</td>
<td>6</td>
<td>1.46 ± 0.17*</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are means ± SE of n (no. of tissues) from 2 animals. Myometrial and aortic tissues were treated with vehicle, S-nitroso-N-acetylpenicillamine (SNAP; 1 μM), or atrial natriuretic peptide (ANP; 0.1 μM) for 5 min and snap frozen in liquid N₂, and the tissue content of guanosine 3',5'-cyclic monophosphate (cGMP) was quantified by radioimmunoassay. *P < 0.05 compared with control; †P = 0.06 compared with control and same treatment in myometrium.

Fig. 6. Effect of 8-bromoguanosine 3',5'-cyclic monophosphate (8-BrcGMP) on force of contraction in aortic and myometrial smooth muscle. A and B: representative tracings of the effect of 8-BrcGMP on contractions in aortic (A) and myometrial (B) tissues from pregnant rats (day 17). 8-BrcGMP was more effective in relaxing contractions of aortic smooth muscle. C: concentration-response curves to 8-BrcGMP in aortic (○) and in myometrial tissues from pregnant rats (●) and from nonpregnant rats in estrus (▲). Myometrium from pregnant rats was less responsive to the effects of 8-BrcGMP than aorta or myometrium from nonpregnant animals in estrus. Data represent inhibition of the area of isometric force developed during 10 min as described in MATERIALS AND METHODS.
by cGMP, we analyzed myometrial extracts from nonpregnant, pregnant, and postpartum rats for PKG by Western blot analysis (Fig. 8). Utilizing a polyclonal antibody to PKG, we found a single immunoreactive species of 78 kDa in all tissues examined. Myometrial extracts prepared from nonpregnant cycling rats contained the highest level of PKG, whereas enzyme levels were significantly reduced in extracts prepared from term myometrium (Fig. 8). Compared with nonpregnant myometrium, levels of PKG were three- to fivefold lower in myometrium obtained from pregnant animals at all gestational days tested (days 12, 15, 17, 18, and 20). Levels of PKG increased in the postpartum period and by the third postpartum day were similar to levels observed in nonpregnant animals (Fig. 8). PKG levels were similar in uterine horns from unilateral pregnant rats, indicating that regulation of PKG expression may be due to the hormonal milieu rather than mechanical factors induced by fetal occupancy (data not shown).

Next, we compared the levels of PKG in aorta and myometrium from nonpregnant animals during defined phases of the estrus cycle (Fig. 9). Levels of PKG were greatest in aortic tissues. Myometrial extracts prepared from nonpregnant animals in proestrus or estrus contained relatively lower levels of the enzyme, yet these levels were greater than those found during late pregnancy (35% of aorta). Myometrial tissues from animals in diestrus (high progesterone-to-estrogen ratio) contained lower levels of PKG than myometrium from animals in estrus, and progesterone treatment of nonpregnant animals resulted in further decreases in enzyme immunoreactivity (30% of aorta). These results suggested that PKG was regulated by ovarian hormones. To test this hypothesis, we determined the relative amounts of PKG in aorta or myometrium from pregnant rats and in myometrium from ovariectomized rats treated with vehicle, estradiol, progesterone, or estradiol and progesterone (Fig. 10). The average densities of the immunoreactive bands from Western blots utilizing extracts from four groups of animals are also presented in Fig. 10. The same aortic extract was included in all blots to serve as an internal control. Estradiol administration resulted in marked increases in PKG expression in myometrial tissues to levels similar to those of aortic tissues. Although progesterone treatment did not affect the low baseline levels of PKG in myometrium of ovariectomized animals, progesterone significantly inhibited the estradiol-induced increase in myometrial PKG expression. The levels of PKG in myometrium from pregnant animals were
similar to those in myometrium from ovariectomized animals or animals treated with progesterone. Activity of PKG in myometrial extracts from nonpregnant and pregnant rats. PKG assays were conducted to determine whether the decrease in immunoreactive PKG in myometrium from pregnant animals correlated with a decrease in the levels of active enzyme. Rat myometrial extracts were prepared and assayed for cGMP-stimulated histone kinase activity. Myometrial extracts prepared from nonpregnant rats \( n = 3 \) contained high levels of histone kinase activity, both in the absence or presence of cGMP (without cGMP, \( 225 \pm 45 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \); with cGMP, \( 827 \pm 92 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \)).

PKG-induced PKG activities in extracts from pregnant rats \( n = 3 \), days 12–20 were significantly lower (without cGMP, \( 62 \pm 14 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \); with cGMP, \( 137 \pm 66 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1} \), \( P < 0.05 \). The meaning of the decreased basal histone kinase activity is not known because many cellular kinases are capable of phosphorylating histone F2b. Decreased cGMP-induced activity in myometrium from pregnant rats, however, indicates decreased PKG enzymatic activity. Thus the decreased enzymatic activity in pregnant myometrium parallels the decrease in PKG immunoreactivity by immunoblot analysis.

**DISCUSSION**

It is perhaps ironic that some of the earliest investigations with cGMP were conducted with uterine smooth muscle; however, more than 20 years later, we have a better understanding of the role of cGMP in vascular smooth muscle than in myometrial smooth muscle. In the vasculature, endothelial NO or atrial peptides signal smooth muscle relaxation by intracellular in-
contractions in pregnant rats (31). In each instance, however, very high concentrations of SNP (≥1 mM) were required to produce an effect. In comparison, aortic smooth muscle strips are maximally relaxed with concentrations 10,000-fold less than that required for myometrium (i.e., 0.1 μM). The effects of SNP in uterine tissue may be due to nonspecific or even toxic effects of the drug. In the current study, we found that SNP-induced “relaxation” was irreversible and spontaneous activity did not resume after removal of the drug from the bathing solution. Thus responses to high concentrations of SNP (≥1 mM) are not likely to be mediated by cGMP.

The essential role of PKG in mediating smooth muscle relaxation has been established for vascular and bronchiolar smooth muscle (5, 10, 15). In the current study, the gestational insensitivity of uterine smooth muscle to cGMP was also correlated with decreased levels of immunoreactive PKG and lower levels of PKG enzyme activity compared with tissues that relaxed in response to increases in cGMP. Thus the sensitivity of smooth muscle to cGMP (aorta > nonpregnant myometrium > pregnant myometrium) parallels the levels of immunoreactive PKG and PKG enzyme activity. This is the first report of specific hormonal regulation of PKG expression in cells.

Very little is known about the mechanisms that regulate expression of PKG. The finding that myometrial PKG levels vary during pregnancy, the postpartum period, and the estrus cycle suggests that enzyme expression is regulated by ovarian hormones. Our studies in ovariectomized animals indicate that estradiol upregulates myometrial PKG and that this estradiol-induced increase in PKG expression is suppressed by progesterone. The low levels of estrogen and progesterone in the immediate postpartum period in the rat are consistent with low levels of PKG expression in postpartum animals (days 1 and 2) with restoration of PKG levels by postpartum day 3. The finding that the low levels of PKG in myometrium from ovariectomized animals were not downregulated further by progesterone is not surprising because progesterone receptor expression is regulated by estradiol. Estradiol has also been reported to increase the levels of cGMP in uterine tissues (27). Increased levels of cGMP in conjunction with elevated levels of PKG would ensure increased PKG activity in estrogen-primed uterine tissues.

The physiological relevance of this regulatory pathway is unknown. PKG may mediate thus far uncharacterized physiological responses in uterine smooth muscle. For example, it is possible that the massive uterine smooth muscle cell hypertrophy that accompanies pregnancy may involve downregulation of cGMP action. Antiproliferative properties have been assigned to cGMP based on studies in mesangial (11) and arterial (6) smooth muscle cells. Alternatively, cGMP, perhaps through PKG, may also play a role in remodeling of the extracellular matrix, as it does in other tissues (19, 26).

In addition to PKG, there are other proteins in reproductive tissues that are regulated in opposing directions by estrogen and progesterone. For example, oxytocin receptors, the major gap junction protein, connexin 43, and interstitial collagenase are upregulated by estradiol and downregulated by progesterone (28). In most species at term, progesterone withdrawal, together with increasing levels of estrogen, leads to increased expression of oxytocin receptors, gap junctions, and cervical collagenase, thereby providing effective contractions of labor and cervical ripening. It has also been suggested that the contractile phenotype of uterine smooth muscle is increased by estradiol treatment and decreased by progesterone (2). The concept that estrogen promotes a “contractile” state whereas progesterone gives rise to “quiescence” suggests that the tissue functions as one or the other of these physiological states, depending on the hormonal milieu. It implies that procontractile mechanisms are expressed and functioning under estrogen domination and prorelaxant mechanisms are acting under the influence of progesterone. We speculate that estradiol, through PKG expression, may serve to increase proteins of the contractile phenotype in uterine smooth muscle. A similar role for PKG has been described in vascular smooth muscle (4). Progesterone, through suppression of PKG expression, may decrease the contractile phenotype of the cell, whereas estrogen facilitates smooth muscle function, i.e., force generation and relaxation.

Previously, we reported that, compared with smooth muscle from bovine trachealis, myometrial tissues from nonpregnant women were relatively insensitive to relaxation by cGMP (29). In the current investigation, we expanded these studies to demonstrate that, during pregnancy, myometrium from pregnant rats is even less sensitive to relaxation by cGMP than is myometrium from nonpregnant animals. The lack of sensitivity to relaxation is correlated with decreased expression of PKG in myometrial tissues. These results are consistent with those reported in pregnant human uterine smooth muscle where neither L-arginine nor inhibitors of NO synthase result in any alterations of contractility (14a). These in vitro studies do not support a role for cGMP in the maintenance of uterine quiescence during pregnancy and are consistent with results obtained in vivo. In vivo, NO synthase inhibitors do not affect uterine contractility, and neither NO synthase inhibitors nor NO donors alter the timing of parturition (1, 8, 17). Together, these data provide evidence that the NO-cGMP-signaling pathway is reduced and unavailable to mediate uterine quiescence during pregnancy. Downregulation of PKG may result in phenotypic alterations of myometrial cells that facilitate other adaptations of uterine smooth muscle during pregnancy such as cellular hypertrophy and remodeling of the extracellular matrix.
REGULATION OF cGMP EFFECTS IN PREGNANT MYOMETRIUM

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