Pregnancy enhances G protein activation and nitric oxide release from uterine arteries

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Thompson, L. P., and C. P. Weiner. Pregnancy enhances G protein activation and nitric oxide release from uterine arteries. Am J Physiol Heart Circ Physiol 280: H2069–H2075, 2001.—We hypothesized that pregnancy modulates receptor-mediated responses of the uterine artery (UA) by altering G protein activation or coupling. Relaxation and contraction to NaF (0.5–11.5 mM), acetylcholine (10−3–10−5 M), and bradykinin (10−12–3 × 10−5 M) were measured in isolated UA of pregnant and nonpregnant guinea pigs. Responses were measured in the presence and absence of either cholera toxin (2 μg/ml) or pertussis toxin (Goαs and Goαi inhibitors, respectively). NaF relaxation was endothelium dependent and nitro-arginine sensitive (a nitric oxide synthase inhibitor). Relaxation to NaF, acetylcholine, and bradykinin were potentiated by pregnancy. Cholera but not pertussis toxin increased relaxation to acetylcholine and bradykinin in UA from nonpregnant animals, had no effect in UA from pregnant animals, and abolished the pregnancy-induced differences in acetylcholine relaxation. Cholera toxin potentiated the bradykinin-induced contraction of UA of both pregnant and nonpregnant animals, whereas pertussis toxin inhibited contraction of UA from pregnant animals only. Therefore, pregnancy may enhance agonist-stimulated endothelium-dependent relaxation and bradykinin-induced contraction of UA by inhibiting GTPase activity or enhancing Goαs but not Goαi activation in pregnant animals. Thus the diverse effects of pregnancy on UA responsiveness may result from hormonal modulation of G proteins coupled to their specific receptors.

endothelium; bradykinin; acetylcholine; cholera toxin; pertussis toxin

PREGNANCY MODULATES vascular reactivity of uterine arteries in a heterogenous manner (33). For example, pregnancy enhances endothelium-dependent relaxation of uterine arteries from human (22) and other animal species (3, 29) to acetylcholine. However, pregnancy has no effect on uterine artery relaxation to either the calcium ionophore A23187, the cGMP analog 8-bromo-cGMP, or native nitric oxide (NO), mechanisms independent of receptor-mediated activation (33). Furthermore, agonist-induced contraction of uterine arteries from pregnant animals has been shown to be both increased (9) and decreased (17, 32, 35) depending on the agonist and animal species studied. Thus differences in vascular reactivity of uterine arteries due to pregnancy may be related to the effect of pregnancy on the receptor-specific activation of the signal transduction pathway involving G proteins.

Pregnancy enhances both uterine artery NO synthase activity (31) and receptor-stimulated release of NO, although the mechanisms are poorly understood. Many receptors whose activation leads to the release of endothelium-derived relaxing factors are coupled to G proteins (11, 20). UK14,304 (an endothelium-dependent α2-adrenoceptor agonist)-induced relaxation was inhibited by pertussis toxin in the dog femoral artery, suggesting a Goαi-dependent mechanism (11, 20), whereas relaxation to acetylcholine (an endothelium-dependent muscarinic receptor agonist) was unaffected by pertussis toxin (20). There is only a single report (8) on the effect of pregnancy on the role of G protein activation in uterine artery contraction, and the role of G proteins on endothelium-dependent relaxation of uterine arteries during pregnancy has not been investigated. The purpose of this study is to test the hypothesis that the increased release of NO by agonist stimulation during pregnancy is mediated by an alteration in the G protein effector mechanism, resulting in enhanced NO release for a given level of receptor activation.

MATERIALS AND METHODS

The protocols employed for this investigation were approved by the University of Iowa Animal Care Committee. Artery preparation. Adult female nonpregnant and preg- nant (55–62 days gestation, term 60–65 days) mixed-breed guinea pigs were anesthetized with ketamine hydrochloride (80 mg/kg ip) and xylazine (3 mg/kg im), and the uterine arteries were removed with the use of microsurgical techniques. The vessels were placed into iced Krebs buffer solution composed of (in mM) 118 NaCl, 2.2 CaCl2, 4.7 KCl, 1.2 MgSO4·7 H2O, 1.21 KH2PO4, 25 NaHCO3, and 11.1 glucose. They were cleaned of loose connective tissue, cut into rings (length 3 mm) free of side branches, and suspended on two 50-μm tungsten wire triangles. One triangle was hooked to a stationary rod, and the other was hooked to a strain-gauge transducer (Grass FT 0.03, Grass Instruments; Quincy, MA).

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for the measurement of isometric force. The arteries were suspended in buffer (37°C) aerated with 95% O2-5% CO2. The pH was maintained between 7.38 and 7.45. After 60 min, the rings were stretched incrementally to their optimal passive tone, as determined by their contractile response to 40 mM KCl. At the conclusion of each experiment, the rings were opened longitudinally, and their circumference and weight were recorded after the vessel was first lightly blotted dry with a paper towel. The measured isometric force was then normalized for the cross-sectional area (in mm²), which was calculated as follows

\[
\text{Area} = 2 \times \text{wet weight/1.06} \times \text{circumference}
\]

where 1.06 represents an estimate of tissue density (in mg/mm²).

**Experimental protocols.** To determine whether pregnancy increased relaxation secondary to generalized G protein activation, endothelium-intact arteries from pregnant and nonpregnant animals were submaximally contracted with PGF₂α (5 × 10⁻⁵ M), and their relaxation to the cumulative addition of NaF (10⁻⁴–1.5 × 10⁻³ M) was measured in the presence and absence of either nitro-L-arginine (L-NNA; 10⁻³ M), an inhibitor of NO synthase, or meclofenamate (10⁻⁶ M), an inhibitor of cyclooxygenase. NaF interacts with the α-subunit of G proteins by mimicking GTP at its binding site (4, 10, 11). Responses were measured in endothelium-intact and mechanically denuded arteries. Contraction to 120 mM KCl was measured before and after the dose-response curve to determine whether the concentration of NaF used affected contractile function.

To determine the role of G proteins in mediating agonist-stimulated relaxation, responses to the cumulative addition of acetylcholine (3 × 10⁻⁹–3 × 10⁻⁴ M) were measured in the presence and absence of either pertussis or cholera toxin in submaximally contracted (PGF₂α, 5 × 10⁻⁵ M) endothelium-intact arteries from pregnant and nonpregnant animals. Cholera toxin-ADP ribosylates the Gαi subunit, reducing the intrinsic GTPase activity of the heterotrimeric G protein, thereby prolonging the duration of the activated receptor-Gαi-GTP complex and enhancing its effectiveness in interacting with its downstream effectors (36). Pertussis toxin-ADP ribosylates Gαi-subunits (36), uncoupling them from their receptors and inhibiting the response to ligand activation. To test the role of Gαo and Gαi in mediating uterine artery reactivity, rings were placed in oxygenated buffer; one set was pretreated with cholera toxin (2 μg/ml) for 2 h, and another set was pretreated with pertussis toxin (2 μg/ml), respectively. In separate vials, control arteries from the same animal were cut into rings and incubated for the same time without exposure to either toxin. Tissues were then mounted onto wires in tissue chambers containing Krebs buffer, passive tension was determined, and tissues were contracted with PGF₂α. Regardless of the toxin used, there were no differences in contractile levels to PGF₂α between control and treated arteries. We (29) have previously shown that acetylcholine-stimulated relaxation of the guinea pig uterine artery is mediated by an L-NNA-sensitive mechanism and does not involve vasodilator prostaglandins.

Bradykinin was also selected to measure the role of G proteins in mediating agonist-induced responses of the uterine artery. However, bradykinin causes both relaxation and contraction of the guinea pig uterine artery depending on the concentration. To determine whether bradykinin-stimulated relaxation or contraction is coupled to pertussis or cholera toxin-sensitive G proteins, responses to the cumulative addition of bradykinin (10⁻¹²–3 × 10⁻⁵ M) were measured in submaximally contracted (PGF₂α, 5 × 10⁻⁵ M) endothelium-intact uterine arteries from pregnant and nonpregnant animals pretreated with both toxins (2 μg/ml for 2 h) separately. Analyses. Results are expressed as means ± SE. Relaxation to NaF, acetylcholine, and bradykinin were expressed as the percentage of the PGF₂α-induced tone. The EC₅₀ was defined as the concentration producing 50% of the maximal response and was extrapolated from the linear portion of the sigmoid dose-response curve (InPlot rel. 4, GraphPad Software; San Diego, CA) and expressed as negative log concentration (pD₂) values. The effects of pregnancy and the various interventions were tested using paired and unpaired t-tests where appropriate. Dose-response analyses also employed a two- or three-way random block repeated measures analysis of variance (ANOVA) with relaxation or contractile force as dependent variables and dose, pregnancy status, and treatment as independent variables (blocking by animal). If the mean values for the ANOVA were found to differ significantly (P < 0.05), a Tukey’s test was applied.

**RESULTS**

NaF caused a dose-dependent relaxation of uterine arteries from both nonpregnant and pregnant animals (Fig. 1). Pregnancy caused a leftward shift of the dose-response curve to NaF in uterine arteries from pregnant animals. Endothelium denudation completely inhibited relaxation responses in uterine arteries from both animal groups (Fig. 2). However, L-NNA significantly reduced relaxation responses to the maximal concentration in arteries from nonpregnant animals only. Furthermore, meclofenamate had no significant effect on NaF-induced relaxation of arteries from either group despite the apparent upward shift in the dose-response curve of uterine arteries from nonpregnant animals. Additionally, the exposure to high concentrations of NaF did not alter vascular smooth muscle function, because the contraction to 120 mM KCl was similar before and after the NaF dose-response curve.

Relaxation to acetylcholine of uterine arteries from pregnant animals was significantly greater (P < 0.05) than nonpregnant animals at all concentrations > 1 × 10⁻⁹ M.
$10^{-6}$ M (Fig. 3). EC$_{50}$ values could not be obtained from arteries of nonpregnant animals because maximal responses were not achieved even at the highest concentration tested. In the presence of cholera toxin, relaxation was significantly increased in arteries from nonpregnant animals at each concentration tested (Fig. 4). In contrast, relaxation responses of arteries from pregnant animals were unaffected by cholera toxin, and the pD$_2$ values were not significantly different ($P = 0.07$) ($-4.58 \pm 0.12$ vs. $-4.95 \pm 0.06$ in control vs. treated arteries, respectively). Pertussis toxin had no effect on relaxation of arteries from either nonpregnant or pregnant animals to any concentration of acetylcholine tested (pD$_2$ values: $-4.58 \pm 0.12$ vs. $-4.39 \pm 0.07$, $P < 0.22$, in control vs. treated arteries, respectively). Figure 4 also illustrates that cholera toxin eliminates the difference in responsiveness to acetylcholine between the two groups, with no differences in pD$_2$ values ($-4.95 \pm 0.14$ vs. $-4.67 \pm 0.15$ in arteries from pregnant vs. nonpregnant animals, respectively, $P = 0.19$), whereas this difference persists in the presence of pertussis toxin. PGF$_{2\alpha}$-induced contraction of uterine arteries was similar regardless of the toxin treatment.

Bradykinin causes both relaxation and contraction of guinea pig uterine arteries depending on the concentration tested (Fig. 5). Bradykinin relaxed uterine arteries at concentrations $<1 \times 10^{-7}$ M and contracted arteries greater than this concentration. At concentrations $>3 \times 10^{-6}$ M, contractile responses declined below the peak value. Pregnancy had variable effects on both bradykinin-induced relaxation and contraction.
between the different experimental series. In the cholera toxin series, pregnancy had little effect on either maximal relaxation or contraction or sensitivity, whereas in the pertussis toxin series, pregnancy enhanced both maximal relaxation and contraction. The combined average responses showed no significant differences in maximal responses or sensitivity between the groups. Regardless, cholera toxin caused a shift in the dose-response curve for both the relaxation and contraction responses of arteries independent of pregnancy (Fig. 5). In contrast, pertussis toxin reduced the maximum contraction to bradykinin of submaximally contracted arteries from pregnant animals but had no effect on bradykinin-stimulated relaxation (Fig. 6). Pertussis toxin had no significant effect on the dose-response relationship to bradykinin of arteries from nonpregnant animals.

DISCUSSION

The present study demonstrates that pregnancy increases endothelium-dependent relaxation of guinea pig uterine arteries to NaF and acetylcholine via modification of heterotrimeric G protein activation. NaF activates the α-subunit of multiple G proteins (e.g., Gs, Gi, and Gq) by forming a trifluoroaluminate complex (with Al3+ contained in the glass walls of the tissue bath) and combines with the GDP bound to the G protein (4). In the presence of exogenously added Al3+ (data not shown), we observed the same differences in NaF relaxation between arteries from pregnant and nonpregnant animals. NaF-induced relaxation of uterine arteries of nonpregnant animals is endothelium dependent and NO mediated. Pregnancy increases NaF-induced relaxation, although the relative contribution of NO appears reduced. Thus pregnancy enhances G protein activation in the uterine artery in response to NaF independent of NO and vasodilator prostaglandins. This may reflect a differential effect of pregnancy on different G proteins, resulting in opposing actions on the modulation of NO release. Several studies have shown that NaF causes either contraction (1, 6) or endothelium-dependent relaxation (7, 11, 27) by activation of G proteins. This is the first study to show that, in the intact uterine artery, the receptor-G protein signaling mechanism may be a target site for modulation by sex hormones during pregnancy.

To examine the effect of pregnancy on receptor-specific activation of G proteins, we measured relaxation to both acetylcholine and bradykinin. We (29) have previously shown that acetylcholine-induced relaxation in the guinea pig uterine artery is mediated by endothelium-dependent and l-NNA-sensitive mechanisms and unaltered by meclofenamate (29). Pregnancy enhanced acetylcholine-induced relaxation of the uterine artery in the present study, confirming previous results (18, 22, 33). Cholera toxin enhanced the acetylcholine-induced relaxation of uterine arteries of nonpregnant animals but had no effect on arteries...
from pregnant animals. Thus enhanced NO release by acetylcholine stimulation may be mediated by pregnancy-induced alterations in G protein activation, which couples the receptor to the downstream effector NO synthase. Pretreatment with cholera toxin-ADP ribosylates the Go-s subunit of heterotrimeric G proteins in the active state and inhibits the intrinsic GTPase activity (12, 14), thus prolonging activation of the receptor-G protein signaling mechanism. Because inhibition by cholera toxin eliminated the difference in uterine artery relaxation between the two groups, it suggests that pregnancy downregulates heterotrimeric GTPase activity as a mechanism for enhancing acetylcholine-induced endothelium-dependent relaxation. Because GTPase activity is regulated by a variety of mechanisms, including GTPase-activating proteins (37), the mechanisms by which pregnancy alters activity remain unclear. It is thought that receptors can couple to multiple G proteins, producing an amplification of the intracellular signaling mechanism involving multiple downstream effectors (26). Therefore, pregnancy may increase the amplification of the receptor-coupled signal by increasing the number of G proteins involved in addition to decreasing GTPase activity. A recent preliminary study (5) from our lab demonstrated that basal high-affinity GTPase activity of plasma membranes of the uterine artery from pregnant guinea pigs is reduced compared with arteries from nonpregnant animals, thus providing more direct evidence that GTPase activity is decreased during pregnancy. However, the current study does not exclude enhanced G protein activation as an additional mechanism. It appears, however, that the Go-s protein subunit is not involved in acetylcholine-induced relaxation of the guinea pig uterine artery, because pertussis toxin had no effect on uterine artery responses from either nonpregnant or pregnant animals. This is consistent with other studies reporting no effect of pertussis toxin on endothelium-dependent relaxation to acetylcholine in dog femoral arteries (20). We speculate that pregnancy modifies the receptor-G protein signaling mechanism induced by acetylcholine, causing increased release of NO and enhanced endothelium-dependent relaxation of the uterine artery. Our data also suggest that muscarinic receptors are coupled to Go-s in the uterine artery of the guinea pig. While our evidence is indirect, muscarinic receptors have been shown in other tissues to be coupled to this subunit (10, 19, 23, 24).

Bradykinin causes both relaxation and contraction of the guinea pig uterine artery. The effect of pregnancy on bradykinin responses is difficult to assess because of the variable responses between the two series. However, because cholera toxin enhanced both relaxation and contraction in pregnant and nonpregnant animals, the regulation of GTPase activity of the Go-s subunit is important in mediating bradykinin-induced responses of guinea pig uterine arteries. There are both similarities and differences in how cholera toxin and pertussis toxin affected bradykinin responsiveness compared with acetylcholine. Similar to acetylcholine relaxation, pertussis toxin had no effect on bradykinin-induced relaxation of either arteries from pregnant or nonpregnant animals. Because bradykinin induces NO release (21), this is consistent with a prior report (13) that the release of NO by cultured endothelial cells is coupled to a cholera but not a pertussis toxin-sensitive G protein. However, pertussis toxin significantly inhibited contractile responses to bradykinin in pregnant animals only. The mechanism by which the bradykinin receptors are coupled to their effector mechanisms has been explored previously (2, 13, 15, 16, 25, 38). There are at least two subtypes of bradykinin-2 (Bk-2) receptors in the guinea pig uterine artery: one, for contraction, linked to a pertussis toxin-sensitive G protein and the other, for relaxation, coupled to a cholera toxin-sensitive G protein. Our results suggest that the contractile portion of the Bk-2 receptor is coupled to a pertussis toxin-sensitive G protein. In a preliminary study (30), we have shown that both bradykinin-induced relaxation, which is endothelium dependent, and contractions of guinea pig uterine arteries are inhibited by the Bk-2 receptor antagonist HOE-140 (d-Arg-Hyp\(^5\), Thr\(^5\), d-Tic, Oic\(^8\)) but not the Bk-1 receptor antagonist des-Arg\(^9\)-Leu\(^8\)-bradykinin. Thus, while relaxation and contraction to bradykinin are mediated by the same receptor subtype, they are located on different cell types (i.e., the endothelium and vascular smooth muscle, respectively). Cholera toxin had a greater effect on bradykinin-induced contraction in pregnant compared with nonpregnant animals, an effect similar to that with acetylcholine. However, in contrast to the acetyl-
choline response, cholera toxin enhanced bradykinin relaxation in arteries from both nonpregnant and pregnant animals in a similar manner. The relative differences in the effect of cholera toxin on agonist-induced responses may be related to how pregnancy affects the receptor coupling and/or G protein activation to its multiple downstream effectors. Thus the effect of pregnancy may be dependent on the predominant alteration of individual G proteins mediating the response. This is consistent with our hypothesis that pregnancy may differentially affect specific receptor-G protein complexes in the uterine artery of the guinea pig.

We (31) have previously shown that pregnancy increases Ca\(^{2+}\)-dependent NO synthase activity in the isolated guinea pig uterine artery. In association with this increase, we (28, 32, 35) observed that pregnancy decreases the contractile responses to norepinephrine, thromboxane, and serotonin. Interestingly, increased NO release appears to explain the decreased response to norepinephrine only (20). We (29) also demonstrated that the stimulated release of NO by agonists such as acetylcholine is increased by pregnancy. Yet, despite the increase in NO synthase activity, non-receptor-stimulated release of NO by agents such as A23187 is unaltered by pregnancy (28). Each of the aforementioned receptors share in common coupling to a G protein effector system. In addition, pregnancy reduced constrictor responsiveness to a variety of agents, such as thromboxane (34, 35) and angiotensin II (17), but did not inhibit (32), and in some cases enhanced (9), constrictor responses to phenylephrine. Thus a pregnancy effect on G proteins provides a potential common mechanism to explain these diverse responses.

In conclusion, pregnancy may reduce the GTPase activity of receptor-specific G proteins as a mechanism for modifying uterine artery reactivity. Thus the receptor-G protein complex may be an important target site in the uterine circulation for steroid hormone modulation during pregnancy.

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