ELSEVIER

European Journal of Pharmacology 367 (1999) 223–230

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Endothelial nitric oxide release in isolated perfused ovine uterine arteries: effect of pregnancy

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Received 3 November 1998; accepted 18 December 1998

Abstract

The present study was designed to determine the release of endothelial nitric oxide, measured as combined nitric oxide, nitrite and nitrate (NO_x), in isolated perfused uterine arteries obtained from nonpregnant and pregnant sheep. Noradrenaline produced concentration-dependent increases in perfusion pressure in both nonpregnant and pregnant uterine arteries with pD_2 values of 5.1 ± 0.07 and 4.6 ± 0.04, respectively. The maximum responses were 300.8 ± 8.8 mmHg for nonpregnant arteries and 86.9 ± 1.3 mmHg for pregnant ones. N^G-nitro-L-arginine increased noradrenaline-mediated maximum response in the pregnant (86.9 ± 1.3 to 144.6 ± 5.1 mmHg), but not in the nonpregnant, uterine arteries. The basal level of NO_x was significantly higher in pregnant than in nonpregnant uterine arteries (346.1 ± 63.2 vs. 86.0 ± 20.6 pmol/ml). The calcium ionophore A23187 and adenosine triphosphate produced concentration-dependent increases in NO_x release in both nonpregnant and pregnant arteries. Compared to the nonpregnant tissue, the agonist-induced increase in NO_x release was significantly enhanced in the pregnant uterine artery. In accordance, endothelial NO synthase protein expression in pregnant uterine artery was 197% of that in nonpregnant artery. These data indicate that in the uterine artery, pregnancy increases both basal and agonist-induced release of endothelial nitric oxide, which is likely to play a key role in attenuated vascular reactivity of the uterine artery to vasoconstrictors during the course of pregnancy.

Keywords: Nitric oxide (NO); Uterine artery; Pregnancy (ovine)

1. Introduction

The striking increase in uterine blood flow during the course of pregnancy optimizes the delivery of oxygen and substrates to the developing fetus via the placenta. Whereas growth of new vessels and remodeling of existing ones during early pregnancy may contribute, in part, to increased uterine blood flow, the fact that the period of greatest increase in uterine blood flow occurs after the completion of new vessel growth indicates that the maintenance of vasodilation in existing or newly-developed vessels is crucial for the increase of uterine blood flow. It has been well documented that vascular reactivity of the uterine artery to a variety of vasoconstrictors, including noradrenaline, angiotensin II, and serotonin, is reduced during pregnancy (Magness and Rosenfeld, 1986; McLaughlin et al., 1989; Weiner et al., 1991, 1992).

Many studies have been directed at determining the role of the endothelium in the changes of vascular reactivity observed during pregnancy (for a review, see Sladek et al., 1997). We (Zhang et al., 1998) and others (Conrad et al., 1993; Yang et al., 1996) have demonstrated that plasma levels of nitrate, the stable metabolite of nitric oxide (NO), are increased during pregnancy, suggesting that endogenous NO production is increased in gravid animals. Nitric oxide synthase inhibitors have been shown to raise resting blood pressure and to reverse the vascular refractoriness to vasopressor agents in pregnant rats (Ahokas et al., 1991; Molnar and Hertelendy, 1992). Studies using isolated vessels have also shown that decreased contractile responses to noradrenaline in pregnant compared with nonpregnant uterine arteries were abolished by removal of the endothelium or by blocking of NO synthesis with N^G-monomethyl-L-arginine (Weiner et al., 1991, 1992). Recent studies clearly demonstrated that endothelial isoform of NO synthase activity and protein expression in sheep uterine artery were significantly increased during pregnancy (Mag-

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00951-0

ness et al., 1997a,b). These studies also demonstrated that the endothelium contains virtually all of the NO synthase activity in the uterine vascular wall. This pregnancy-related response appears not only to be cell and isoform specific, but also unique to the uterine vasculature, because the systemic artery endothelium showed only minimal or no significant changes in NO synthase with pregnancy (Magness et al., 1997b).

To our knowledge, there is no study yet to measure directly NO release from uterine artery endothelium, and to determine the potential differences between nonpregnant and pregnant animals. The present study was thus designed to test the hypothesis that pregnancy increases endothelial NO release in the uterine artery. Using perfused uterine arteries, coupled with a chemiluminescence detector for measurement of combined nitric oxide, nitrite and nitrate (NO_x), the specific objectives of this study were to determine: (1) if noradrenaline-induced contractions of the uterine artery decrease in response to pregnancy, (2) if N^G-nitro-L-arginine (L-NOARG), a specific NO synthase inhibitor, enhances the efficacy of noradrenaline in contracting uterine arteries, and if it has different effects on pregnant and nonpregnant animals, (3) if pregnancy increases basal and agonist-stimulated endothelial nitric oxide release, and (4) if endothelial NO synthase protein expression increases in pregnant uterine arteries.

2. Materials and methods

2.1. Materials

Noradrenaline, indomethacin, adenosine triphosphate (ATP), and N^G-nitro-L-arginine (L-NOARG) were obtained from Research Biomedicals (Natick, MA). Calcium ionophore A23187 was purchased from Sigma Chemicals (St. Louis, MO). Sodium nitrate and vanadium(III) chloride were from Aldrich Chemical (Milwaukee, WI). All drugs were prepared fresh each day and kept on ice throughout the experiment.

2.2. Tissue preparation

Nonpregnant and near-term pregnant sheep (~140 days gestation) were anesthetized with thiamylal (10 mg/kg) administered via the external left jugular vein. The ewes were then intubated and anesthesia was maintained with 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 $CaCl_2$, 1.16 $MgSO_4$, 1.18 KH_2PO_4, 22.14 $NaHCO_3$, and 7.88 dextrose. EDTA (0.03 mM) was added to suppress oxidation of amines. The Krebs solution was oxygenated with a mixture of 95% oxygen–5% carbon dioxide. The arteries were carefully cleaned of surrounding connective tissue and cut into segments of 2 cm in length.

After removal of the tissues, animals were killed with T-61 (euthanasia solution, Hoechst-Roussel, Somerville, NJ). All procedures and protocols used in the present studies were approved by the Animal Research Committee of Loma Linda University and followed the guidelines put forward in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Perfusion of uterine arteries

The uterine artery segments were cannulated at both ends and placed into a perfusion organ bath maintained at 37°C by a thermostat pump (Kent Scientific, Litchfield, CT). The artery was perfused by a peristaltic pump (Cole-Parmer Instrument, Vernon Hills, IL) with Krebs bicarbonate solution oxygenated with 95% O_2/5% CO_2 at 37°C. The flow rate (~5.0 ml/min) was determined at the beginning of the experiment to obtain an adequate perfusion pressure (30–40 mmHg) in the initial resting state and maintained constant throughout the experiment. The perfusion pressure was continuously measured with a pressure transducer (Kent Scientific, Litchfield, CT) and recorded by an on-line computer.

After a 60-min equilibration in the tissue bath at stable resting pressure (30–40 mmHg), agonists were administered into the perfusion circuit. Concentration–response curves were obtained by cumulative addition of the agonist in approximately one-half log increments. Each concentration of agonist was added at the plateau of the pressure produced by the previous concentration. EC_{50} values for the agonists in the experiments were recorded at the molar concentrations where the curves intersected the 50% level of the responses. In all experiments, indomethacin (10 μM) was added to inhibit prostacyclin (prostaglandin I_2) biosynthesis. Where appropriate, L-NOARG (100 μM), a NO synthase inhibitor, was pre-incubated in the perfusion circuit for 20 min before the addition of the agonist.

2.4. Measurement of NO_x

The perfused artery was equilibrated at the resting pressure for 60 min. Doses of calcium ionophore A23187 or ATP were administered 4 min apart. One milliliter of the perfused solution before the treatment (basal) and at the end of each dose was collected into a microcentrifuge tube and was flash frozen in liquid N_2. Samples were then stored at −80°C until measurement of NO_x.

Because of the instability of NO in oxygenated physiological solution, most of NO is converted to nitrite and further to nitrate. Nitrite and nitrate are relatively stable in the solution, and are readily reduced back to NO in vanadium(III)/HCl solution. NO was then measured by chemiluminescence method as described previously (Zhang et al., 1998). Briefly, the samples (100 μl) were injected

into the gas purge vessel containing 5 ml vanadium-(III)/HCl to react for 1 min and reduce nitrate/nitrite in the sample back to NO. To achieve high reducing efficiency, the reduction was performed at 90°C. NO in the sample was then 'stripped' into the head space of the gas purge vessel by bubbling it with helium (12 ml/min) for 60 s. NO in the head space was drawn into an NO Analyzer (Model 270B, Sievers Instruments, Boulder, CO) and mixed with ozone (O_3) in front of a cooled Hamamatsu, red-sensitive photomultiplier tube. Signals from the detector were analyzed by an on-line computer as area under the peak and presented as voltage × s (v · s). The measurement reflected the combined concentrations of nitrate, nitrite, and nitric oxide (NO_x) of each samples, which were calculated from a standard curve of 10 to 1000 pmol nitrate run in each assay.

2.5. Western analysis of NO synthase

The endothelium was gently scraped from the vessel lumen of the uterine arteries as previously described (Magness et al., 1997b). The cells were then solubilized by sonication in lysis buffer (150 mM NaCl, 50 mM Tris HCl, 10 mM EDTA, 0.1% Tween 20, 0.1% β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, and 5 μg/ml aprotinin, pH 7.4). After centrifugation, protein was quantified in the supernatant by the method of Bradford (1976). Samples with equal protein (10 μg) were loaded on a 7.0% polyacrylamide gel with 0.1% sodium dodecyl sulfate (SDS) and were separated by electrophoresis at 100 V for 2 h. Proteins were then transferred onto immobilon P membrane at 30 V for 30 min at room temperature using a semidry blotter (Bio-Rad). The immobilon P membrane was probed by mouse monoclonal antiserum for endothelial isoform of NO synthase (1:750) obtained from Transduction (Lexington, KY). The secondary antiserum was horseradish peroxidase-conjugated goat anti-mouse (1:1000) obtained from Amersham (Arlington Heights, IL). Proteins were visualized with enhanced chemiluminescence (ECL) reagents (Amersham), and the blots were exposed to hyperfilm. Results were quantified by scanning densitometer (model 670, Bio-Rad) and expressed as percent of nonpregnant values.

2.6. Data analysis

Concentration–response curves were analyzed by computer-assisted nonlinear regression to fit the data using GraphPad Prism (GraphPad software, San Diego, CA). Half-maximal effective concentration (EC_{50}) values for an 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 pD_2 ($-\log EC_{50}$) values. For nitric oxide data analysis, area under the peak was continuously integrated during sample measurement using data acquisition software WorkBench (Kent Scientific, Litchfield, CT). Linear regression of the standard curve was analyzed using GraphPad Prism. Results were expressed as means ± S.E.M., and the differences were evaluated for statistical significance ($P < 0.05$) by analysis of variance (ANOVA).

3. Results

3.1. Contraction studies

Noradrenaline produced concentration-dependent contractions of the uterine arteries and increased perfusion pressure in perfused arteries obtained from both pregnant and nonpregnant animals. Two consecutive concentration–response curves of noradrenaline-induced contractions in the same tissue were superimposed in both pregnant and nonpregnant arteries, indicating that noradrenaline-induced contractions were not changed with the time in the same tissue.

Fig. 1 illustrates the effect of L-NOARG on noradrenaline-induced contractions of the uterine arteries. In preg-

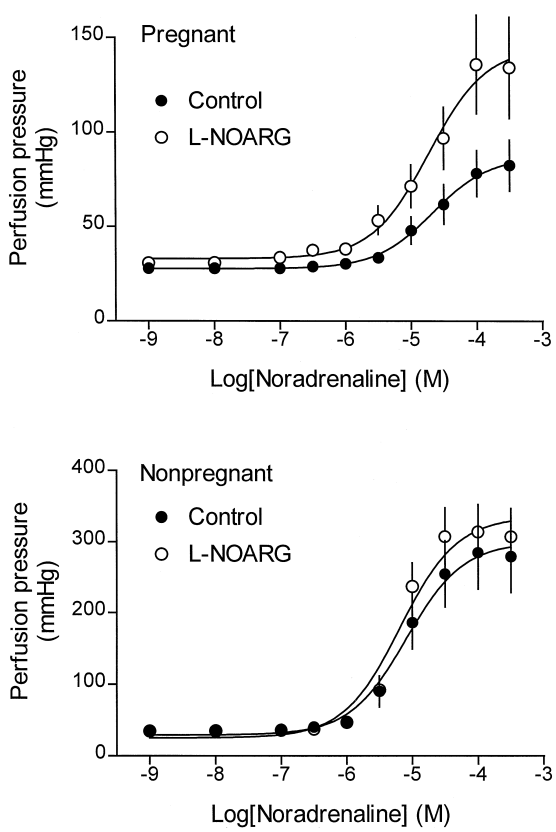

Fig. 1. Effect of L-NOARG on noradrenaline-induced contractions of the uterine arteries. Concentration-dependent responses for noradrenaline were obtained with perfused arterial segments from pregnant (lower panel) and nonpregnant (upper panel) animals before (●) and after (○) treatment with L-NOARG (100 μM, 20 min). Data are means ± S.E.M.of 8–10 animals. Mean values of pD_2 and the maximal responses to noradrenaline are presented in the text.

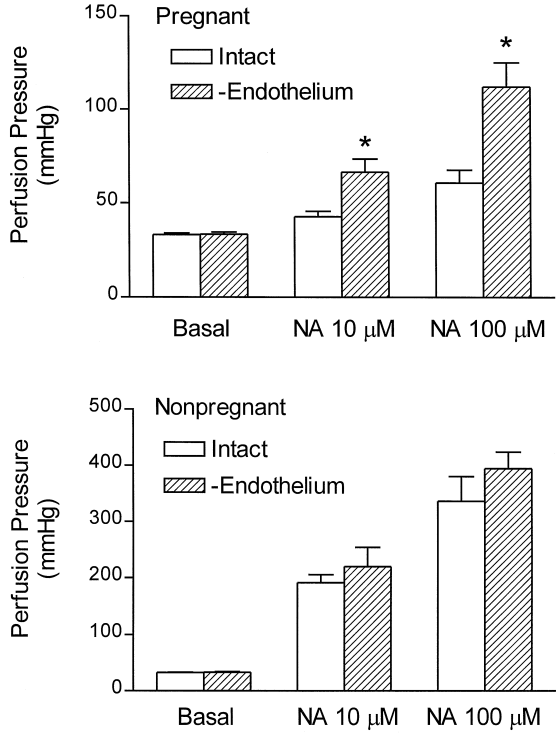

Fig. 2. Effect of the endothelium on noradrenaline-induced contractions of the uterine arteries. Concentration-dependent responses for noradrenaline were obtained with perfused, endothelium intact and denuded uterine arterial segments from pregnant (upper panel) and nonpregnant (lower panel) animals. Data are means ± S.E.M. of six animals.

nant arteries, pretreatment with L-NOARG (100 μM, 20 min) in the perfusion solution resulted in significant increases in noradrenaline-induced contractions at all concentrations and shifted the noradrenaline concentration–response curve to the left (Fig. 1, upper panel). Although the pD_2 values were not significantly different between the control (4.67 ± 0.04, $n = 10$) and L-NOARG-treated (4.72 ± 0.08, $n = 10$) tissues, L-NOARG significantly increased noradrenaline-induced contractions at each concentration and increased the maximum response from 86.9 ± 1.3 to 144.6 ± 5.1 mmHg ($P < 0.05$).

Compared to the pregnant uterine artery, noradrenaline was much more potent in stimulating contractions in nonpregnant uterine arteries (Fig. 1, lower panel). The pD_2 values of noradrenaline-mediated concentration-dependent contractions were 4.67 ± 0.04 ($n = 10$) and 5.10 ± 0.07 ($n = 8$) for pregnant and nonpregnant arteries, respectively ($P < 0.05$). The maximum contractile responses were 86.9 ± 1.3 mmHg for pregnant arteries and 300.8 ± 8.8 mmHg for nonpregnant arteries ($P < 0.05$).

In contrast to the pregnant uterine artery, noradrenaline-induced concentration-dependent increases in perfusion pressure before and after the treatment of L-NOARG were superimposed in nonpregnant uterine arteries (Fig. 1, lower panel). The pD_2 values were 5.10 ± 0.07 and 5.18 ± 0.12 for the control and L-NOARG-treated arteries, respectively. The maximum responses were 300.8 ± 8.8 mmHg and 336.6 ± 17.1 mmHg, respectively ($P > 0.05$, $n = 8$).

Similar results were obtained from experiments with intact and endothelium denuded uterine arteries. In pregnant uterine arteries, removal of the endothelium enhanced noradrenaline-induced contraction in the way similar to that obtained in the presence of L-NOARG (Fig. 2, upper panel). In contrast, removal of the endothelium failed to change noradrenaline-mediated response in nonpregnant uterine arteries (Fig. 2, lower panel).

To test whether uterine artery smooth muscle became more sensitive to nitric oxide during pregnancy and thereby generate more cGMP, we examined the relaxation response produced by sodium nitroprusside in pregnant and nonpregnant uterine arteries. As shown in Fig. 3, there was no difference in sodium nitroprusside-induced relaxation of pregnant and nonpregnant uterine arteries.

3.2. Nitric oxide release

Fig. 4 shows a typical calibration curve for nitrate standards from 10 to 1000 pmol of nitrate injected. The repeatability for nitrate measurements was generally better than ±5%, and the response of the analyzer was always linear within the range. Whereas the calibration of the nitric oxide analyzer was usually stable for several weeks, the standard curve was always performed for each assay.

Basal levels of nitric oxide release were significantly increased in pregnant (346.1 ± 100.1 pmol/ml, $n = 7$) compared with nonpregnant (86 ± 24.3 pmol/ml, $n = 6$) uterine arteries ($P < 0.05$). As shown in Fig. 5, calcium ionophore A23187 produced concentration-dependent increases in nitric oxide release in both pregnant and nonpregnant uterine arteries. The A23187-induced release of nitric oxide was significantly higher in the pregnant uterine

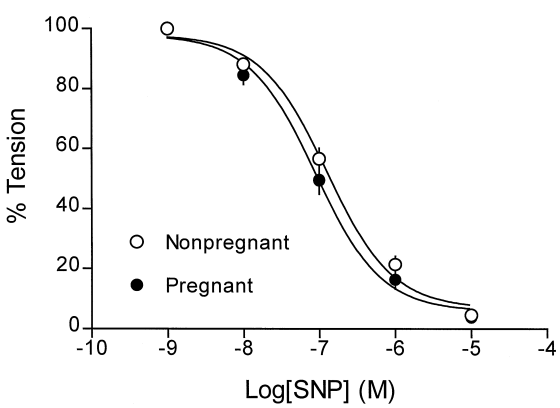

Fig. 3. Sodium nitroprusside (SNP)-mediated relaxation of the uterine arteries. Concentration-dependent relaxation induced by SNP was obtained with phenylephrine (10 μM) pre-contracted uterine arteries from pregnant (●) and nonpregnant (○) animals. Data are means ± S.E.M. of 18 animals.

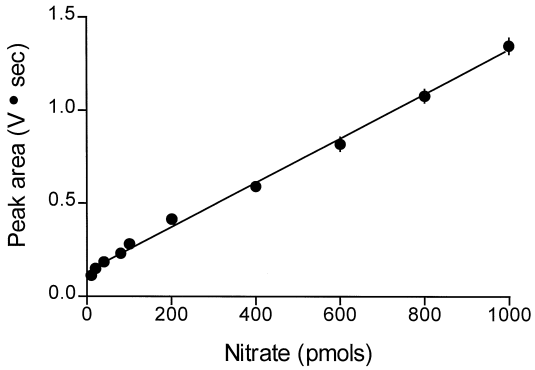

Fig. 4. Calibration curve of nitrate using the chemiluminescence method. Sodium nitrate standards were injected into the reaction vessel in the amounts indicated. Signals from the detector were analyzed by an on-line computer as area under the peak and presented as voltage×s (v·s). Symbols show the means±S.E.M. for seven determinations for each amount of nitrate.

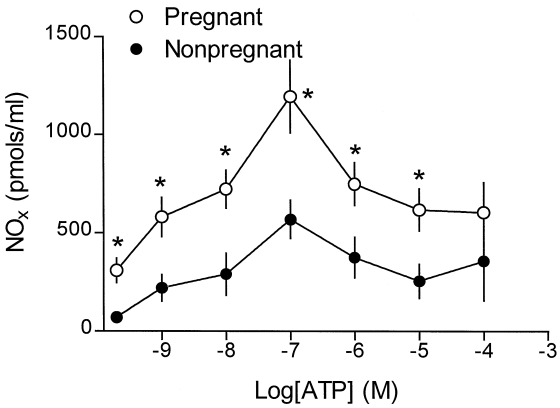

Fig. 6. ATP-stimulated endothelial nitric oxide releases in perfused uterine arteries from nonpregnant and pregnant animals. Nitric oxide was measured as indicated in Section 2. Data are means±S.E.M. of tissues from four pregnant and four nonpregnant animals. $* P < 0.05$, comparison of the corresponding values between pregnant and nonpregnant uterine arteries.

artery than that in the nonpregnant artery at all concentrations used. Fig. 6 shows ATP-induced nitric oxide release in pregnant and nonpregnant uterine arteries. Similar to the calcium ionophore A23187, ATP-induced nitric oxide release was concentration dependent, but it reached the maximum level at 0.1 μM. The nitric oxide release was then decreased as the concentration of ATP increased in both groups. In agreement with the finding with A23187, ATP-induced nitric oxide release was significantly elevated at all concentrations in pregnant compared with nonpregnant uterine arteries.

3.3. Protein expression of endothelial isoform of NO synthase

Fig. 7 shows the effect of pregnancy on endothelial NO synthase protein expression in uterine arteries. The representative Western immunoblot showed that the monoclonal antibody for endothelial isoform of NO synthase detected a single band at the expected size of 145 kDa (Fig. 7, upper panel). There was an enhancement of NO synthase protein expression in pregnant uterine artery endothelial cells. Quantitative densitometry for seven independent experiments revealed that endothelial NO synthase protein expression in pregnant uterine artery endothelial cells was increased to 197.7% of nonpregnant values (Fig. 7, lower panel).

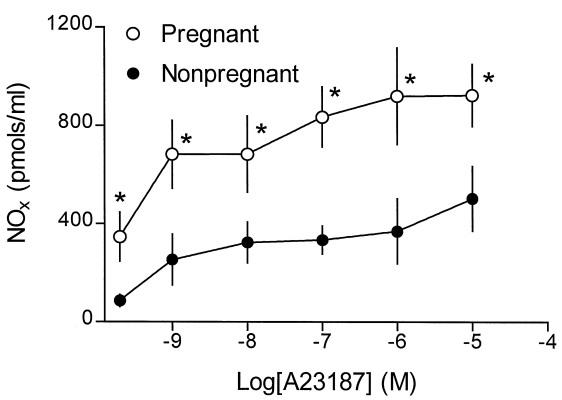

Fig. 5. Calcium ionophore A23187-stimulated endothelial nitric oxide releases in perfused uterine arteries from nonpregnant and pregnant animals. Nitric oxide was measured as indicated in Section 2. Data are means±S.E.M. of tissues from seven pregnant and six nonpregnant animals. $* P < 0.05$, comparison of the corresponding values between pregnant and nonpregnant uterine arteries.

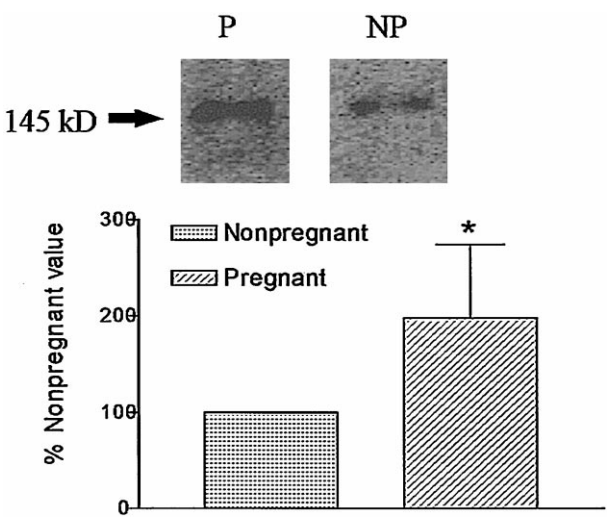

Fig. 7. Endothelial NO synthase protein expression in uterine arteries. Immunoblot analysis of endothelial NO synthase was performed in endothelial cells obtained from pregnant (P) and nonpregnant (NP) animals. In the representative immunoblot shown in the upper panel the protein was detected by monoclonal antibody at the expected size of 145 kDa, and there was enhanced protein expression in the cells from pregnant animals. Summary findings for quantitative densitometry in seven animals are shown in the lower panel. $* P < 0.01$ vs. nonpregnant.

4. Discussion

The present study has demonstrated that both basal and agonist-stimulated endothelial nitric oxide release in the uterine artery are increased in response to pregnancy. It is unlikely that the basal release of nitric oxide plays an important role in modulating noradrenaline-mediated contractions in nonpregnant uterine arteries. In contrast, increased basal nitric oxide release in pregnant uterine arteries significantly attenuated noradrenaline-induced contractions.

Despite the finding of a striking increase in uterine blood flow and well-documented attenuated responsiveness of the uterine artery to noradrenaline in pregnant animals in vivo (Magness and Rosenfeld, 1986; McLaughlin et al., 1989), observations comparing the sensitivity of uterine arteries from pregnant and nonpregnant animals to vasoconstriction by adrenergic agents in vitro are controversial. The contractile responses of the uterine arteries to noradrenaline and phenylephrine were either increased (Annibale et al., 1989), decreased (Weiner et al., 1991), or unchanged (Jovanovic et al., 1995) in response to pregnancy. Almost all of these in vitro studies used either arterial ring or strip preparations. To our knowledge, the present study is the first one to use perfused ovine uterine artery segments to compare adrenergic responsiveness in pregnant and nonpregnant animals. We clearly demonstrated that noradrenaline-mediated pressor response of perfused uterine arteries was significantly attenuated in near-term pregnant sheep compared with nonpregnant ones. This is consistent with previous in vivo studies. In contrast, Annibale et al. (1989) reported that the contractile sensitivity of isolated ovine uterine artery to adrenergic agonist was increased in response to pregnancy. Whereas the cause(s) of the discrepancy between the previous finding and the present results are not entirely clear at present, they may be due, in part, to differences in preparation. The previous study (Annibale et al., 1989) used arterial strips with the endothelium removed, whereas the intact perfused arterial segments used in the present study were likely to preserve physiological endothelial–smooth muscle relationships and interactions.

The finding that L-NOARG, a potent inhibitor of endothelial nitric oxide biosynthesis (Moore et al., 1990), significantly increased noradrenaline-induced contractions of pregnant uterine arteries suggests that basal release of nitric oxide plays an important role in regulating vascular reactivity of the uterine artery in pregnant animals. In contrast, failure of L-NOARG in enhancing noradrenaline-mediated contractions in nonpregnant arteries suggests that nitric oxide is not involved in regulating basal vascular tone of nonpregnant uterine arteries. These conclusions are further supported by the findings that removal of the endothelium potentiates noradrenaline-induced contractions in pregnant, but not in nonpregnant uterine arteries. These results suggest either an increase in basal nitric oxide synthesis/release or (and) some other changes in downstream mechanisms of nitric oxide pathway have occurred in the uterine artery during pregnancy. For example, uterine artery smooth muscle may become more sensitive to nitric oxide during pregnancy and thereby generate more cGMP. Alternatively, the contractile machinery of the vascular smooth muscle may become more sensitive to inhibition by cGMP-dependent protein kinase. The findings that relaxation responses to sodium nitroprusside were similar in uterine arteries from pregnant and nonpregnant rats (Ni et al., 1997), women (Nelson and Suresh, 1988), and sheep (the present study) indicate that the signal transduction pathways distal to nitric oxide are unchanged by pregnancy.

Previous studies have evaluated the effect of pregnancy on nitric oxide release by means of endothelium removal and NOS inhibitors on either vasoconstrictor responses or vasorelaxation responses of precontracted vessels (Sladek et al., 1997). No attempt has been made to measure directly nitric oxide release from isolated uterine arteries. Our recent studies (Zhang et al., 1998) and those of others (Conrad et al., 1993; Yang et al., 1996) demonstrated a significant increase in plasma nitrate levels in pregnant animals suggesting that pregnancy increases endogenous nitric oxide synthesis and/or decreases clearance of nitrate in the circulation. The present study provides direct evidence, for the first time, that pregnancy increases basal release of endothelial nitric oxide, measured NO_x, in the uterine artery. Previously, we (Zhang et al., 1998) and others (Magness et al., 1997a) demonstrated, indirectly, that pregnancy augmented basal uterine artery nitric oxide production by showing a fivefold increase in vascular smooth muscle cGMP production. This is in agreement with the present finding of more than a fourfold increase in basal release of nitric oxide in pregnant uterine arteries compared with nonpregnant arteries. Cyclic GMP has been used as an indicator of nitric oxide production in numerous studies in which proportional increases in cGMP concentrations have been demonstrated in systemic plasma and urine obtained from pregnant vs. nonpregnant animals and humans (Kopp et al., 1977; Conrad and Vernier, 1989; Sladek et al., 1997). It has been demonstrated that basal specific activity of NO synthase in uterine artery endothelium is elevated in pregnant vs. nonpregnant guinea pigs (Weiner et al., 1994) and sheep (Li et al., 1996). Magness et al. (1997a) reported that the NO synthase specific activity was 1.8-fold greater in uterine arteries from pregnant compared with nonpregnant sheep. This increase was specific to the uterine artery because no difference was noted in NO synthase activity among omental arteries due to pregnancy. It was suggested that the endothelium contained virtually all of the NO synthase activity in the uterine vascular wall (Magness et al., 1997a). Recent studies clearly demonstrated that the expression of endothelial NO synthase protein as determined by immunohistochemistry and Western immunoblots was increased by

pregnancy in the ovine uterine, but not systemic, artery endothelium (Magness et al., 1997b). The present study confirmed the finding of increased NO synthase protein expression in pregnant uterine artery endothelial cells and directly supports the interpretation that increased endothelial NO synthase protein expression in the uterine artery during pregnancy is associated with elevations of nitric oxide release.

Not only was basal nitric oxide release elevated, but agonist-stimulated release of endothelial nitric oxide was also increased by pregnancy in the uterine artery. In the present study, we have chosen two different agonists to study the effect of pregnancy on agonist-mediated nitric oxide release. Potent actions of ATP on vascular endothelial cells leading to release of nitric oxide and vasodilatation have been described in many vessels (Barnard et al., 1994; Burnstock, 1997). ATP, acting on P_{2y} purinergic receptors, stimulates inositol 1,4,5-trisphosphate production and intracellular Ca^{2+} mobilization, which in turn activates endothelial NO synthase in the endothelium. We have shown that ATP produces concentration-dependent increases in nitric oxide release in perfused uterine arteries. It has been demonstrated that ATP is a very potent vasodilator and can cause vascular relaxation at concentrations as low as 10 pM (Burnstock, 1993). The present observation of decreased nitric oxide release at higher concentrations of ATP is likely due to the receptor desensitization. The increased ATP-induced nitric oxide release by pregnancy in the uterine artery may be due, in part, to increased interactions between ATP and P_{2y} receptors. The findings that calcium ionophore A23187-stimulated nitric oxide release was also significantly enhanced in pregnant uterine arteries, as well as that uterine artery endothelial NO synthase protein expression was increased, suggest that changes at the level of endothelial NO synthase play a key role in pregnancy-associated increase in nitric oxide release. By measuring cGMP production, Magness et al. (1997a) also demonstrated that pregnancy augmented angiotensin II-stimulated uterine artery nitric oxide production in sheep. The increased cGMP levels are directly correlated with uterine, but not systemic, artery endothelial NO synthase protein expression (Magness et al., 1997b).

Whereas it is apparent that pregnancy increases endothelial nitric oxide release that is likely to play an important role in pregnancy-associated attenuation of uterine artery contractility, the present finding that noradrenaline-induced contractions of pregnant uterine arteries in the presence of L-NOARG are still significantly attenuated compared with those of nonpregnant arteries indicates that increased nitric oxide may not be the only factor induced by pregnancy. This is in contrast with the previous findings that NO synthase inhibitors increased adrenergic agent-induced contractions and fully reversed the blunted contractile responses of noradrenaline on uterine arteries of pregnant to that of nonpregnant guinea pigs (Weiner et al., 1991) and rats (Ni et al., 1997). This discrepancy may be due in part to differences in species and/or tissue preparations used in the present and previous studies. It is unlikely that vasodilating prostanoids are involved in the attenuated contractions of pregnant uterine arteries after NO synthase blockade because cyclooxygenase was inhibited by indomethacin in these preparations. Given the findings that pregnancy decreased α_1-adrenoceptor density (Shaul et al., 1990), attenuated phosphoinositide turnover and inositol 1,4,5-trisphosphate synthesis (Conrad et al., 1991), and inhibited potential-operated calcium channels (St-Louis et al., 1995) in vascular smooth muscle, we speculate that pregnancy also attenuates receptor-mediated excitation–contraction coupling and signal transduction in the uterine artery by inhibiting either electromechanical and/or pharmacomechanical coupling. These potential mechanisms are under current investigations.

Acknowledgements

We thank Drs. Ronald R. Magness and Ian M. Bird for their technical consultation on Western analysis of NO synthase. This work was supported in part by NIH grants HL-54094 and HL-57787 (LZ), HD-31226 (WJP), Grant-in-Aid from the American Heart Association No. 96007560 (LZ), and by Loma Linda University School of Medicine.

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