Decreased Endothelium-Dependent Vascular Relaxation During Reduction of Uterine Perfusion Pressure in Pregnant Rat

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Abstract—Reduction in uterine perfusion and the ensuing placental ischemia during late pregnancy have been proposed to trigger increases in systemic vascular resistance and pregnancy-induced hypertension; however, the intermediary mechanisms involved are unclear. The purpose of the present study was to test the hypothesis that reduced uterine perfusion pressure during late pregnancy is associated with impaired endothelium-dependent vascular relaxation and, consequently, enhanced systemic vascular reactivity. Active stress was measured in aortic strips isolated from late pregnant Sprague-Dawley rats and a hypertensive pregnant rat model produced through the long-term reduction in uterine perfusion pressure (RUPP). Phenylephrine (Phe, 10^{-5} \text{ mol/L}) caused an increase in active stress to 4.5 \pm 0.4 \times 10^3 \text{ N/m}^2 in normal pregnant rats and a larger increase to 9.4 \pm 0.7 \times 10^3 \text{ N/m}^2 in RUPP rats. Removal of the endothelium significantly enhanced Phe-induced stress in pregnant (6.4 \pm 0.6 \times 10^3 \text{ N/m}^2) but not RUPP (9.95 \pm 0.95 \times 10^3 \text{ N/m}^2) rats. In endothelium-intact strips, acetylcholine (ACh) was more potent in inducing relaxation of Phe contraction in pregnant (6.4 \pm 0.6 \times 10^3 \text{ N/m}^2) but not RUPP (9.95 \pm 0.95 \times 10^3 \text{ N/m}^2) rats. Pretreatment of endothelium-intact strips with N^G\text{-nitro}-L-arginine methyl ester (100 \mu\text{mol/L}), to inhibit nitric oxide (NO) synthase, significantly inhibited ACh-induced relaxation and enhanced Phe-induced stress in pregnant (6.2 \pm 0.5 \times 10^3 \text{ N/m}^2) but not RUPP (9.5 \pm 0.85 \times 10^3 \text{ N/m}^2) rats. Pretreatment of endothelium-intact strips with methylene blue (10 \mu\text{mol/L}), to inhibit cGMP production in smooth muscle, also inhibited ACh-induced relaxation and enhanced Phe-induced stress in pregnant (6.9 \pm 0.65 \times 10^3 \text{ N/m}^2) but not RUPP (9.3 \pm 0.7 \times 10^3 \text{ N/m}^2) rats. In endothelium-denuded strips, relaxation of Phe contraction with the exogenous NO donor sodium nitroprusside was not significantly different between pregnant and RUPP rats. These results suggest that an endothelium-dependent relaxation pathway involving the release of NO from endothelial cells and increased cGMP production in smooth muscle is inhibited in systemic vessels of late pregnant rats with reduced uterine perfusion pressure and may in part explain the increased vascular resistance in pregnancy-induced hypertension. 

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Key Words: arteries ■ blood pressure ■ endothelium ■ nitric oxide ■ muscle, smooth, vascular

Normal pregnancy is often associated with reduction in systemic vascular resistance and arterial blood pressure and decreased vascular reactivity to circulating vasoconstrictors. The hemodynamic and vascular changes observed during normal pregnancy have been explained in part by increased nitric oxide (NO) synthesis by various cells, including vascular endothelial cells. This is supported by reports that the metabolic production and plasma level of cGMP, a second messenger of NO and a cellular mediator of vascular smooth muscle relaxation, are increased during pregnancy. In 5% to 7% of pregnancies, women develop a condition called preeclampsia that is characterized by increased intra-vascular coagulation, proteinuria, increased systemic vascular resistance, and pregnancy-induced hypertension. Although pregnancy-induced hypertension is a major cause of maternal and fetal death, the exact mechanism of this disorder has not yet been clearly identified. Because of the difficulty of performing mechanistic studies in pregnant women, several animal models of pregnancy-induced hypertension have been developed. Studies in these animal models have proposed that a reduction in the uteroplacental blood flow and the ensuing placental ischemia during late pregnancy are associated with placental release of cytokines, which eventually leads to increased systemic vascular resistance and pregnancy-induced hypertension; however, the intermediary cellular mechanisms involved are still unclear.

In the present study, we used a pregnant rat model with reduced uterine perfusion pressure (RUPP), which was produced by clipping the lower abdominal aorta and the main
uterine branches of both the right and left ovarian arteries during late pregnancy, to test the central hypothesis that localized reduction in uterine perfusion pressure during late pregnancy is associated with generalized impaired endothelium-dependent vascular relaxation and, consequently, enhanced systemic vascular reactivity. The purpose of the study was (1) to determine whether vascular reactivity is enhanced in pregnant rats with RUPP compared with normal pregnant rats, (2) to determine whether endothelium-dependent vascular relaxation is inhibited in RUPP rats compared with normal pregnant rats, and (3) to determine whether the pregnancy-associated reduction in vascular relaxation and enhancement of vascular reactivity involve alterations in the endothelium-dependent NO-cGMP pathway.

Methods

Animals

Time pregnant (day 12) female Sprague-Dawley rats (10 to 12 weeks of age) were purchased from Harlan Sprague-Dawley Inc, housed individually in the animal facility, and maintained on ad libitum standard rat chow and tap water on a 12-hour light/dark cycle. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee of the University of Mississippi Medical Center and the American Physiological Society.

Protocol for RUPP

On day 14 of pregnancy, pregnant rats destined to be in the RUPP group were anesthetized with isoflurane, the abdominal cavity was opened via a midline incision, the lower abdominal aorta was exposed, and a silver clip (0.203 mm ID) was placed around the aorta above the iliac bifurcation. This procedure has been shown to reduce uterine perfusion pressure in the gravid rat by ~40%. Because compensation of blood flow to the placenta occurs in pregnant rats through an adaptive increase in ovarian blood flow, a silver clip (0.229 mm ID) was also placed on the main uterine branches of both the right and left ovarian arteries. Control pregnant rats underwent sham surgery. Arterial catheters were placed in the carotid artery for the measurement of mean arterial pressure in conscious rats with a pressure transducer.

With the use of this protocol, the RUPP rats showed proteinuria, as indicated by increased urinary protein excretion; impaired renal function, as indicated by reduction in glomerular filtration rate and renal plasma flow; and decreased litter size and pup weight, as indicated by increased urinary protein excretion; impaired renal pressure transducer.

Statistical Analysis

The developed force was corrected for the cross-sectional area of each individual strip and expressed as active stress (N/m²) according to the equation: Stress = force/cross-sectional area, where cross tissue density is 1.055 g/cm³. Data were analyzed and expressed as the mean ± SEM, with n values representing the total number of experiments performed on individual aortic strips isolated from 5 to 10 different rats of each group. Data were compared with the use of ANOVA with 3 classification criteria (rat type [normal pregnant versus RUPP], condition of endothelium [intact versus denuded], and treatment [untreated versus pretreated with L-NAME or methylene blue]). Scheffé’s F test was used for comparison of multiple mean values. Student’s t test for unpaired data was used for a comparison of 2 mean values. Differences were considered statistically significant at a value of P < 0.05.

Results

On the day of the experiment (day 19 to 20 of gestation), the recorded mean arterial blood pressure in control pregnant rats was as low as 88 mm Hg in some rats and as high as 103 mm Hg in other rats, with an average arterial pressure in the entire group of 96 ± 2 mm Hg (n=8). The individual and the average arterial pressures in RUPP rats were consistently greater than those in the control pregnant rats. In RUPP rats, the recorded arterial pressure was as low as 106 mm Hg in some rats and as high as 140 mm Hg in other rats, with an average arterial pressure in the entire group of 126 ± 8 mm Hg (n=10).

In the aortic strips of both pregnant and RUPP rats, Phe caused concentration-dependent increases in active stress. In endothelium-intact strips, the Phe concentration-active stress curve in RUPP rats was enhanced compared with that in normal pregnant rats (Figure 1A). The maximal Phe-induced active stress in RUPP rats (9.4 ± 0.7 × 10³ N/m², n=32) was significantly greater than that in normal pregnant rats (4.5 ± 0.4 × 10³ N/m², n=18). Removal of the endothelium significantly enhanced the Phe-induced stress in normal
pregnant rats to a maximum of $6.4 \pm 0.6 \times 10^3$ N/m² ($n = 24$). In contrast, removal of the endothelium caused slight but insignificant increase in Phe-induced stress to a maximum of $9.95 \pm 0.95 \times 10^3$ N/m² ($n = 24$) in RUPP rats (Figure 1A).

When the Phe response was presented as a percent of the maximum Phe contraction, the ED$_{50}$ value for Phe in endothelium-intact aortic strips of pregnant rats was $3.5 \pm 0.4 \times 10^{-8}$ mol/L ($n = 18$) (Figure 1B). Phe was markedly more potent in causing contraction in endothelium-denuded aortic strips of pregnant rats (ED$_{50}$ $1.4 \pm 0.2 \times 10^{-8}$ mol/L, $n = 24$) than in endothelium-intact strips of pregnant rats. In contrast, Phe was only slightly more potent in causing contraction in endothelium-denuded aortic strips of RUPP rats (ED$_{50}$ $2.0 \pm 0.2 \times 10^{-8}$ mol/L, $n = 24$) than in endothelium-intact strips of RUPP rats (ED$_{50}$ $2.6 \pm 0.3 \times 10^{-8}$ mol/L, $n = 32$) (Figure 1C).

In endothelium-intact strips, pretreatment with L-NAME (100 μmol/L) for 30 minutes, to inhibit NO synthase, significantly enhanced the Phe-induced stress in pregnant rats to a maximum of $6.2 \pm 0.5 \times 10^3$ N/m² ($n = 32$) (Figure 2A). In addition, in L-NAME–pretreated aortic strips of pregnant rats, Phe was more potent in causing contraction (ED$_{50}$ $1.2 \pm 0.3 \times 10^{-8}$ mol/L, $n = 32$) than in untreated strips of pregnant rats (Figure 2C). In contrast, in L-NAME–pretreated aortic strips of RUPP rats, the maximal Phe-induced stress ($9.5 \pm 0.85 \times 10^3$ N/m², $n = 24$) and the ED$_{50}$ value of Phe ($2.1 \pm 0.2 \times 10^{-8}$ mol/L, $n = 24$) were not significantly different from those in untreated aortic strips of RUPP rats (Figures 2B and 2D).

Similarly, in endothelium-intact strips, pretreatment with methylene blue (10 μmol/L) for 30 minutes, to inhibit cGMP production in smooth muscle, enhanced Phe-induced stress in normal pregnant rats to a maximum of $6.9 \pm 0.65 \times 10^3$ N/m² ($n = 24$) (Figure 2A). In addition, in methylene blue–pretreated aortic strips of normal pregnant rats, Phe was more potent in causing contraction (ED$_{50}$ $1.6 \pm 0.2 \times 10^{-8}$ mol/L, $n = 24$) than in untreated aortic strips (Figure 2C). In contrast, in methylene blue–pretreated aortic strips of RUPP rats, the maximal Phe-induced stress ($9.3 \pm 0.7 \times 10^3$ N/m², $n = 29$) and the ED$_{50}$ value of Phe ($2.0 \pm 0.2 \times 10^{-8}$ mol/L, $n = 29$) were not significantly different from those in untreated aortic strips of RUPP rats (Figures 2B and 2D).

**Figure 1.** Phe-induced contraction in normal pregnant (Preg) and RUPP rats. Endothelium-intact (+ Endo) and endothelium-denuded (−Endo) rat aortic strips were incubated in normal Krebs’ solution (2.5 mmol/L Ca$^{2+}$) and then stimulated with increasing concentrations of Phe. Phe contraction was measured and presented as active stress (A) or percent of maximum Phe contraction (B and C). Data points represent mean±SEM of measurements in 18 to 32 aortic strips from 6 to 8 rats of each group.

**Figure 2.** Effect of L-NAME and methylene blue (Meth Blue) on Phe-induced contraction in endothelium-intact aortic strips of normal pregnant (Preg) and RUPP rats. Rat aortic strips incubated in normal Krebs’ solution (2.5 mmol/L Ca$^{2+}$) were either untreated or pretreated with L-NAME (100 μmol/L) or methylene blue (10 μmol/L) for 30 minutes and then stimulated with increasing concentrations of Phe. Phe contraction was measured and presented as active stress (A and B) or percent of maximum Phe contraction (C and D). Data points represent mean±SEM of measurements in 18 to 32 aortic strips from 6 to 8 rats of each group.
In endothelium-intact aortic strips of both normal pregnant and RUPP rats, ACh caused concentration-dependent relaxation of Phe (3×10^{-7} mol/L)-induced contraction. The ACh-induced relaxation of Phe contraction was significantly greater in normal pregnant rats than in RUPP rats (Figure 3A). Similarly, bradykinin (10^{-7} mol/L) caused 38.6±2.7% relaxation of Phe (3×10^{-7} mol/L)-induced contraction in aortic strips of normal pregnant rats compared with only 9.7±1.9% relaxation in aortic strips of RUPP rats. When the ACh-induced response was presented as a percent of the maximal ACh-induced relaxation, ACh was more potent in inducing relaxation in normal pregnant rats (ED_{50} = 0.1±0.04×10^{-6} mol/L, n=22) than in RUPP rats (ED_{50} = 1.2±0.06×10^{-6} mol/L, n=18) (Figure 2B). Because the aortic strips of RUPP rats showed greater vascular reactivity than those of normal pregnant rats, control experiments were performed on strips from RUPP rats in which the initial Phe concentration was lowered to 1×10^{-7} mol/L to produce a submaximal contraction that is roughly equal in magnitude to the contraction observed in strips of normal pregnant rats precontracted with 3×10^{-7} mol/L Phe. These experiments showed that the ED_{50} value of ACh in aortic strips of RUPP rats precontracted with 1×10^{-7} mol/L Phe (1.1±0.07×10^{-6} mol/L) was not significantly different from that in strips precontracted with 3×10^{-7} mol/L Phe (1.2±0.06×10^{-6} mol/L). Pretreatment of endothelium-intact strips with L-NAME (100 µmol/L) or methylene blue (10 µmol/L) significantly inhibited the ACh-induced relaxation of Phe contraction in normal pregnant rats but not in RUPP rats (Figure 3A). Removal of the endothelium completely inhibited the ACh-induced relaxation of Phe contraction in both normal pregnant and RUPP rats.

Discussion

The main findings of the present study are that (1) the mean arterial pressure in late pregnant rats with RUPP is significantly greater than that in normal pregnant rats, (2) vascular reactivity is greater in RUPP rats than in normal pregnant rats, (3) endothelium-dependent vascular relaxation is less in RUPP rats than in normal pregnant rats, and (4) the activity of an endothelium-dependent NO-cGMP pathway is reduced in RUPP rats compared with normal pregnant rats.

We and others\(^2\) have previously shown that the mean arterial pressure and the vascular reactivity to various vasoconstrictors are reduced in pregnant rats compared with virgin rats. On the other hand, the present study showed that the mean arterial pressure, as well as the vascular reactivity in response to the α-adrenergic agonist Phe, is enhanced in a pregnant rat model of RUPP compared with normal pregnant rat. These findings in the RUPP rat are consistent with previous studies from our laboratory and others that have shown that the arterial blood pressure and the vascular reactivity in response to vasoconstrictors are enhanced in other animal models of hypertension during late pregnancy.\(^1,4,16,21,22\) In search for the possible intermediary cellular mechanisms involved in the observed enhanced vascular...
reactivity in the RUPP rat, we found that removal of the endothelium significantly enhanced the Phe contraction in normal pregnant rats but had minimal effects in RUPP rats. In addition, the ACh-induced relaxation was less in RUPP rats than in normal pregnant rats. These results suggest that an endothelium-dependent relaxation pathway is intact in normal pregnant rats but possibly impaired in the RUPP rats.

The vascular endothelium releases several vasodilators, including endothelium-derived relaxing factor, and several studies have suggested that endothelium-derived relaxing factor is NO.23–26 The reduced ACh-induced relaxation in RUPP rats could be due either to a decrease in the synthesis and release of NO from endothelial cells or to a change in the sensitivity of vascular smooth muscle to relaxation by NO. The sensitivity of vascular smooth muscle to relaxation by NO could be evaluated on the basis of its sensitivity to relaxation by exogenous NO donors such as sodium nitroprusside. The observation that relaxation of endothelium-denuded vascular strips by sodium nitroprusside was not significantly different for normal pregnant and RUPP rats provided evidence that the endothelium-independent mechanisms of vascular relaxation and the sensitivity of vascular muscle to relaxation by NO are not impaired in RUPP rats and thereby suggest that the impaired ACh-induced relaxation in RUPP rats is most likely due to a decrease in the synthesis or release (or both) of NO from endothelial cells.

To further investigate the possible role of NO synthesis and release in the proposed impaired endothelium-dependent relaxation pathway in the RUPP rats, we found that pretreatment of the vascular strips with L-NAME, which is known to block NO synthesis, significantly inhibited vascular relaxation by ACh and significantly enhanced the vascular reactivity to Phe in normal pregnant rats but had minimal effects in RUPP rats. These results suggest that NO synthesis by endothelial cells is intact in pregnant rats but is significantly impaired during reduction of uterine perfusion pressure in late pregnant rats.

The NO produced by endothelial cells is known to promote vascular relaxation by activating guanylate cyclase and increasing cGMP production in vascular smooth muscle.26 We found that methylene blue, which is known to inhibit guanylate cyclase and to decrease cGMP production in smooth muscle,11 significantly inhibited the endothelium-dependent vascular relaxation by ACh and significantly enhanced the vascular reactivity to Phe in endothelium-intact strips of normal pregnant rats but not RUPP rats. These results further support the hypothesis that NO production or release by endothelial cells, and thereby the activity of the NO-cGMP pathway in vascular smooth muscle, is reduced in RUPP rats compared with normal pregnant rats. However, the results of the methylene blue experiments should be interpreted with caution because methylene blue may also inhibit NOS.

It is important to emphasize the following cautionary remarks regarding the above interpretations. First, although the present results suggest that the decrease in endothelial cell function and the increase in vascular reactivity observed in the RUPP rats could contribute to the observed increase in blood pressure, these results should be interpreted with caution because the changes in endothelial cell function and vascular reactivity may also be secondary to blood pressure elevation. An analysis of the time course of the changes in vascular reactivity and the increase in blood pressure should help determine whether the relationship between these 2 parameters is causal or associative in nature and should, therefore, represent an important area for future investigations. Second, the vascular endothelium has been shown to release other vasodilator substances, in addition to NO, such as endothelium-derived hyperpolarizing factor and prostacyclin.27 This may explain why in the aortic strips of RUPP rats, some relaxation to ACh was still observed and was not completely inhibited by L-NAME or methylene blue. On the other hand, the complete absence of ACh-induced relaxation in endothelium-denuded strips of RUPP rats still supports the contention that the ACh-induced relaxation is endothelium dependent. Third, although the present results provided evidence that the enhanced vascular reactivity in the RUPP rats may involve inhibition of an endothelium-dependent NO-cGMP pathway, we cannot rule out the possibility that an increase in the release of contracting factors from the endothelium or an increase in the sensitivity of vascular smooth muscle to endothelium-derived contracting factors also occurs. This is supported by a recent report that long-term inhibition of NO synthesis during mid to late gestation in rats is associated with elevated plasma levels of endothelin-1.28 This is also supported by the present observation that removal of the endothelium or pretreatment of aortic strips of the normal pregnant rats with L-NAME or methylene blue caused an enhancement of Phe-induced vascular reactivity to levels that were still less than that observed in the RUPP rats. Thus, the reduction in uterine perfusion pressure during late pregnancy in rats may be associated with additional alterations in the cellular mechanisms of vascular smooth muscle contraction and should be an interesting area for future experiments. Fourth, because the present study was performed on strips of thoracic aorta, we cannot make a definite conclusion regarding whether the observed changes in vascular reactivity also occur in resistance vessels, which should represent an important area for future investigations.

The question remains of how a localized reduction in uterine perfusion pressure during late pregnancy would cause generalized impaired endothelium-dependent vascular relaxation, and consequently, enhanced systemic vascular reactivity. It has been hypothesized that reduction in the uteroplacental blood flow and the ensuing placental ischemia during late pregnancy may be associated with placental release of cytokines, which may then cause generalized endothelial cell damage.29,30 This is supported by recent reports that cytokines such as tumor necrosis factor-α significantly depress endothelium-dependent vascular relaxation.31 This is also supported by preliminary observations from our laboratory suggesting that chronic infusion of tumor necrosis factor-α is associated with increased vascular resistance and arterial pressure in late pregnant rats.32

In conclusion, the present results suggest that an endothelium-dependent relaxation pathway involving the production and release of NO from endothelial cells and increased cGMP production in smooth muscle is inhibited in a pregnant rat model of RUPP. The RUPP rat could be useful as an animal
model for a specific form of hypertension associated with endothelial dysfunction and should be helpful in understanding the cellular mechanisms of the increased vascular resistance in pregnancy-induced hypertension.

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References


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