Reduced Uterine Perfusion Pressure During Pregnancy in the Rat Is Associated With Increases in Arterial Pressure and Changes in Renal Nitric Oxide

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Abstract—A reduction in nitric oxide (NO) synthesis has been suggested to play a role in pregnancy-induced hypertension. We have recently reported that normal pregnancy in the rat is associated with significant increases in whole-body NO production and renal protein expression of neuronal and inducible NO synthase. The purpose of this study was to determine whether whole-body and renal NO production is reduced in a rat model of pregnancy-induced hypertension produced by chronically reducing uterine perfusion pressure starting at day 14 of gestation. Chronic reductions in uterine perfusion pressure resulted in increases in arterial pressure of 20 to 25 mm Hg, decreases in renal plasma flow (23%) and glomerular filtration rate (40%), but no difference in urinary nitrite/nitrate excretion relative to control pregnant rats. In contrast, reductions in uterine perfusion pressure in virgin rats resulted in no significant effects on arterial pressure. Renal endothelial (4%) and inducible (11%) NO synthase protein expression did not decrease significantly in the chronically reduced uterine perfusion pressure rats relative to normal pregnant rats; however, significant reductions in neuronal NO synthase were observed (30%). The results of this study indicate that the reduction in renal hemodynamics and the increase in arterial pressure observed in response to chronic decreases in uterine perfusion pressure in pregnant rats are associated with no change in whole-body NO production and a decrease in renal protein expression of neuronal NO synthase. (Hypertension. 2000;37:1191-1195.)

Key Words: preeclampsia • hypertension, pregnancy • glomerular filtration rate • renal blood flow • plasma • endothelium • nitric oxide

Preeclampsia is a multisystemic disorder of pregnancy estimated to affect 5% to 10% of all pregnancies in the United States.1,2 Although preeclampsia is one of the leading causes of maternal death and the main contributor of prenatal morbidity, the mechanisms responsible for this disorder are unclear.1,3 Preeclampsia develops during pregnancy and ceases after delivery, implicating the placenta as a primary cause.1,4 Manifestations generally associated with preeclampsia include an increased responsiveness to vasoconstrictors, increases in arterial pressure, decreases in glomerular filtration rate (GFR) and renal plasma flow (RPF), proteinuria, and vascular endothelial damage.1,2,5

The initiating event in preeclampsia is suggested to involve reduced placental perfusion, which leads to maternal endothelial cell dysfunction.1,3,4 The factors involved in mediating the hypertension during preeclampsia are unknown and may involve a delicate balance of vasoconstrictors and vasodilators of which nitric oxide (NO) may play an important role.1,4,6 Evidence indicates that NO plays an important role in mediating physiological changes during normal pregnancy.6,7 Increases in regional blood flow, RPF, and GFR during pregnancy are attenuated by systemic NO synthesis inhibition.8,9 Urinary excretion of cGMP, a second messenger of NO, and nitrite/nitrate, metabolites of NO, are increased during normal pregnancy.10 Therefore, as NO may play an important role in normal pregnancy, NO deficiency may play an important role in preeclampsia.2,4 It is unclear, however, whether NO production is reduced during preeclampsia because it is difficult to accurately assess the NO system in humans. For example, measurement of whole-body NO in clinical settings has yielded variable results caused by difficulties in controlling for nitrate intake.2 In addition, whole-body NO production may not be an accurate measure of the NO system activity in specific tissues such as the kidneys.11,12

During normal pregnancy in the rat, increases in whole-body NO production and changes in renal hemodynamics are associated with increases in renal protein expression of both neuronal (nNOS) and inducible (iNOS) nitric oxide synthase isoforms.13 Although the NO system has been well characterized during normal pregnancy in the rat, information...
regarding the activity of the NO system in animal models of pregnancy-induced hypertension (PIH) is lacking. Thus, the overall goal of this study was to assess whole-body NO production under fixed nitrate intake conditions in a rat model of PIH induced by chronic reductions in uterine perfusion pressure. In addition, because the kidneys play a major role in the long-term regulation of arterial pressure and because abnormalities in the renal pressure-natriuresis relation have been observed in all forms of hypertension examined to date, another goal of this study was to assess changes in renal hemodynamics and renal protein expression of the NOS isoforms that occur in response to reduced uterine perfusion pressure (RUPP) in pregnant rats.

Methods
All studies were performed in timed pregnant Sprague-Dawley rats purchased from Harlan Sprague-Dawley Inc. Animals were housed 3 to a cage in a temperature-controlled room (23°C) with a 12:12-hour light/dark cycle. All experimental procedures executed in this study were in accordance with National Institutes of Health Guidelines for Use and Care of Animals, and the Animal Care and Use Committee at the University of Mississippi Medical Center approved all protocols.

RUPP Model in Rat
Chronic reductions in uteroplacental perfusion in rats, previously reported by Eder and McDonald,14 reduced uterine perfusion pressure by 35% to 45% during mid to late gestation by placing a silver clip or a silk ligature around the aorta below the renal arteries. We used a modification of this model to examine the role of NO in mediating hypertension during chronic reductions in uterine perfusion pressure. All rats undergoing surgical procedures were anesthetized with 2% isoflurane.8 Pregnant rats entering the RUPP group underwent the following clipping procedure at day 14 of gestation. After a midline incision, the lower abdominal aorta was isolated and underwent the following clipping procedure at day 14 of gestation. Similar surgical procedures for monitoring of blood pressure in pregnant rats through an adaptive increase in ovarian blood flow, we

Measure of Renal Hemodynamics and Arterial Pressure in Conscious Rats
Renal hemodynamics and arterial pressures were determined in conscious control pregnant (n=12) and RUPP (n=9) animals at day 19 of gestation. Similar surgical procedures for monitoring of blood pressure were performed in virgin control and virgin clipped rats. Measurements of renal hemodynamics and arterial pressures in conscious rats were performed as previously described by the authors.13

Results

Figure 1. Measurement of MAP in model of RUPP in pregnant rats. All data are expressed as mean±SEM.

Measure of Urinary Nitrite/Nitrate Excretion
Urinary nitrite/nitrate excretion rates were determined in both control pregnant (n=15) and RUPP (n=18) animals fed a low nitrite/nitrate diet (AIN76, ICN Pharmaceuticals Inc) for 5 days before collection. Measure of urinary nitrite/nitrate was performed as previously described by the authors.13

Determination of Urinary Protein Levels
Urinary excretion of protein was measured in both control pregnant (n=15) and RUPP (n=18) animals with 24-hour urine collection and storage of a final sample at −20°C. Protein concentration was determined with a Sigma Protein Determination kit (P5656, Sigma Chemical Co).15

Measure of Plasma Estradiol 17-β and Progesterone Levels
Plasma estradiol and progesterone concentrations were determined by chemiluminescence immunoassay with the Chiron ACS-180 autoanalyzer (Chiron Diagnostics Corp). The assay sensitivity for estradiol and progesterone was 10 pg/mL and <0.1 ng/mL, respectively. The within-assay precision (%CV) was ±5.0% and the between-assay precision was ±9.2%.

Isolation of Total Cellular Proteins and Western Blot Analyses
Kidneys were removed from control pregnant (n=8) and RUPP (n=10) rats at day 19 of pregnancy, quick-frozen in liquid nitrogen, then stored at −80°C. Isolation of total cellular proteins and Western blot analyses of the renal NOS isoforms were performed as previously described by the authors.13

Statistical Analysis
All data are expressed as mean±SEM. Comparisons of control pregnant rats with RUPP rats and comparisons between virgin control and virgin clipped rats were analyzed by factorial ANOVA followed by Scheffé’s test. A value of P<0.05 was considered statistically significant.

Effects of Chronic Reductions in Uterine Perfusion Pressure on Mean Arterial Pressure and Protein Excretion
Chronic reductions in uterine perfusion pressure in pregnant rats resulted in significant increases in arterial pressure relative to normal pregnant rats. Figure 1 illustrates the increase in arterial pressure that was present in RUPP animals as compared with control pregnant animals. Mean arterial pressure (MAP) averaged ~126±7 mm Hg (P<0.05) in the RUPP rats at day 19 of pregnancy. This was a significant increase as compared with an average MAP of
103±4 mm Hg observed in control pregnant rats. In contrast, reductions in uterine perfusion pressure in virgin rats resulted in no significant effects on arterial pressure relative to control virgin rats (Figure 2). MAP averaged 113±3 mm Hg in the clipped virgin rats and 112±10 mm Hg in the virgin control rats.

Although not significant, an increase in urinary protein excretion was also observed in the RUPP model when compared with normal pregnancy (108±15 and 15±2 mg/24 hours, respectively) (Figure 3). Intrauterine growth restriction, another hallmark of human preeclampsia, was also evident as pup weights were slightly decreased in RUPP animals relative to control pregnant animals (2.68±0.22 and 3.18±0.19 g, respectively). In addition, at day 19 of pregnancy, the RUPP model was also associated with decreased litter size (7.3±0.48 and 10.438±0.63 pups, respectively, P<0.05) as well as decreased body weight (248.4±7.14 and 307.9±0.36 g, respectively, P<0.05).

**Effects of Chronic Reductions in Uterine Perfusion Pressure on Renal Hemodynamics**

The renal hemodynamic changes observed in the RUPP animals relative to normal pregnant animals are illustrated in Figure 4. Both GFR (1.29±0.42 and 2.21±0.14 mL/min, respectively, P<0.05) and RPF (4.57±1.17 and 5.91±0.67 mL/min, respectively) decreased in the RUPP model relative to normal pregnancy at day 19, with a significant reduction occurring in GFR.

**Effects of Chronic Reductions in Uterine Perfusion Pressure on Urinary Nitrite/Nitrate Levels**

Urinary nitrite/nitrate excretion was measured to estimate whole-body production of NO. At day 19 of pregnancy, excretion of nitrite/nitrate in the RUPP model (49.8±6.4 µmol/24 hours) did not differ significantly compared with normal pregnancy (46.4±5.3 µmol/24 hours) (Figure 5).

**Effects of Chronic Reductions in Uterine Perfusion Pressure on Plasma Levels of Estradiol 17-β and Progesterone**

No significant changes were observed in plasma hormone levels in the RUPP model. A slight but nonsignificant decrease in plasma estradiol 17-β was observed in the RUPP animals relative to normal pregnant animals (49.9±6.1 versus 62.4±8.9 pg/mL, respectively), whereas a slight but nonsignificant increase in plasma progesterone was observed (107.7±12.9 versus 83.4±8.5 ng/mL, respectively).

**Effects of Chronic Reductions in Uterine Perfusion Pressure on Renal Protein Expression of NOS Isoforms**

Comparable levels of renal endothelial NOS (eNOS) protein expression were found at day 19 of pregnancy in both the RUPP and control pregnant animals (Figure 6). However, renal protein expression of both iNOS and nNOS were decreased at day 19 of pregnancy in the RUPP animals relative to control pregnant animals (Figure 6). Renal iNOS expression decreased by 11% in the RUPP model as com-
A major goal of this study was to determine whether whole-body and renal NO production is reduced in the RUPP model. Although a deficiency in NO production has been suggested to play a role in PIH, clinical assessment of whole-body NO production in preeclampsia has given rise to varied results caused by differences in dietary intake, exercise, and duration of urine collection. In our study, 24-hour urine collections representing steady-state levels of NO production on animals fed a low nitrite/nitrate diet were measured to provide a more accurate measure of whole-body NO synthesis. No change in whole-body NO production as determined by urinary nitrite/nitrate excretion was observed in the RUPP animals. However, whole-body NO production may not be indicative of the NO synthesis in specific tissues such as the kidney.

Because the kidneys play a major role in the long-term regulation of arterial pressure and abnormalities in the renal pressure-natriuresis relation have been observed in all forms of hypertension examined to date, another goal of this study was to assess renal NO production in the RUPP model. As a means of investigating the NO system in the kidney, we examined the renal protein expression of the NOS isoforms in the RUPP model. We observed no difference in renal eNOS protein expression at day 19 of pregnancy in the RUPP rats as compared with control pregnant rats. However, renal iNOS protein expression in RUPP animals was decreased by 11% as compared with normal pregnancy and renal nNOS by 31%. Thus, renal nNOS protein expression in the RUPP rats was significantly decreased, and this decrease was associated with a significant decrease in GFR of 40% and a decrease in RPF of 23%.

Therefore, the significant reductions in renal hemodynamics observed in the RUPP model in the rat were associated with significant reductions in renal expression of the nNOS isoform. Recent studies suggest that NO produced by either eNOS or iNOS does not play a significant role in mediating the renal hemodynamic changes observed during normal pregnancy. However, NO generated from nNOS has been implicated in mediating the increase in hemodynamics observed during normal pregnancy. Whether the decrease in renal nNOS protein expression is involved in mediating the decrease in renal hemodynamics in the RUPP model is unclear. We have recently reported, however, that inhibition of nNOS with 7-nitroindazole significantly reduces RPF and GFR in pregnant rats but not virgin rats. Therefore, NO generated by nNOS in the kidney may be involved in the long-term control of renal hemodynamic changes that occur during normal gestation, and variations in renal nNOS expression may contribute to changes in renal hemodynamics during preeclampsia.

Summary

We found that a chronic reduction in uterine perfusion pressure in the pregnant rat was associated with significant increases in arterial pressure and decreases in GFR. In addition, decreases in RPF, proteinuria, and intrauterine growth restriction were also observed in the RUPP animals. Thus, the RUPP model in the rat closely resembles many of the features observed in women with PIH. Although hyper-

Discussion

One of the leading theories concerning the cause of pregnancy-induced hypertension suggests a pathway starting with inadequate trophoblast invasion of maternal spiral arteries leading to decreased placental perfusion. This in turn leads to placental ischemia, resulting in placental release of factors, subsequent maternal endothelial dysfunction, and then systemic vasoconstriction. Chronic reductions in uterine perfusion pressure in pregnant animals has been used by numerous investigators to study potential mechanisms of human preeclampsia because it initiates the disorder at an early step in the cascade described above. Elevations in arterial pressure and proteinuria in response to chronic reductions in uteroplacental perfusion in pregnant rats has been previously reported by Eder and MacDonald. They reduced the uterine perfusion pressure by 30% to 35% during mid to late gestation by placing a silk ligature around the aorta below the renal arteries. In this study, we used a modification of this model to examine the role of NO in mediating hypertension during chronic reductions in uterine perfusion. We placed a clip on the abdominal aorta to reduce uterine perfusion pressure by 40 mm Hg in rats at mid-gestation. Because compensation of blood flow to the placenta occurs in pregnant rats through an adaptive increase in ovarian blood flow, we also placed silver clips on branches of both the right and left ovarian arteries that supply the uterus. In this study, we report that the RUPP model in the conscious rat is characterized by increases in arterial pressure and urinary protein excretion, decreases in GFR and RPF, and intrauterine growth restriction. Thus, the RUPP model in the rat has many features similar to those of humans with PIH.

Figure 6. Renal protein expression of renal NOS isoforms in rat model of RUPP: Representative Western blot (A) and quantification of renal NOS isoform protein expression (B). NOS protein expression levels are normalized relative to renal actin protein expression levels. Standard error is calculated from ≥3 determinations from ≥3 independent experiments. All data are expressed as mean±SEM.

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Comparison of renal protein expression of renal NOS isoforms in rat model of RUPP. Representative Western blot (A) and quantification of renal NOS isoform protein expression (B). NOS protein expression levels are normalized relative to renal actin protein expression levels. Standard error is calculated from ≥3 determinations from ≥3 independent experiments. All data are expressed as mean±SEM.
tension in the RUPP model was not associated with significant reductions in whole-body NO synthesis, as assessed by urinary excretion of nitrite/nitrate, significant reductions in renal protein expression of the nNOS isoform were found. These changes in renal nNOS expression may contribute to changes in renal hemodynamics and possibly the hypertension observed during chronic reductions in uterine perfusion pressure in the rat.

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