Endothelin Type A Receptor Blockade Attenuates the Hypertension in Response to Chronic Reductions in Uterine Perfusion Pressure

Barbara T. Alexander, A. Nicole Rinewalt, Kathy L. Cockrell, Matthew B. Massey, William A. Bennett, Joey P. Granger

Abstract—A chronic reduction in uterine perfusion pressure in pregnant rats is associated with a significant elevation in mean arterial pressure (MAP) and reduction in kidney function. The purpose of this study was to examine the role of endothelin in mediating the hypertension in response to chronic reductions in uterine perfusion pressure in conscious, chronically instrumented, pregnant rats. MAP in pregnant rats with chronic reductions in uterine perfusion pressure (123.0 ± 1.8 mm Hg) was significantly higher than that in control pregnant rats (101.3 ± 4.0 mm Hg). Renal expression of preproendothelin mRNA as determined by ribonuclease protection assay was also significantly elevated in the medulla (45%, P < 0.05) and in the cortex (22%, P < 0.05) of the pregnant rats with chronic reductions in uterine perfusion pressure compared with control pregnant rats. Chronic administration of the selective endothelin type A receptor antagonist (ABT-627, 5 mg/kg per day for 10 days) markedly attenuated the increase in MAP observed in the pregnant rats with chronic reductions in uterine perfusion pressure (103.3 ± 5.6 mm Hg, plus endothelin antagonist; P < 0.05). However, endothelin type A receptor blockade had no significant effect on blood pressure in the normal pregnant animals (96.0 ± 2.7 mm Hg, plus endothelin antagonist). These findings suggest that endothelin plays a major role in mediating the hypertension produced by chronic reductions in uterine perfusion pressure in pregnant rats. (Hypertension. 2001;37[part 2]:485-489.)

Key Words: preeclampsia ■ hypertension, pregnancy ■ endothelial growth factors ■ endothelin ■ receptors, endothelin

Hypertensive disorders of pregnancy, such as preeclampsia, occur in 6% to 8% of all pregnancies.1,2 Despite being one of the leading causes of maternal death and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for the pathogenesis of preeclampsia are unknown.1-3 The hypertension associated with preeclampsia develops during pregnancy and abates after delivery, implicating the placenta as a central culprit in this disease.1,3 The initiating event in preeclampsia has been postulated to involve reduced placental perfusion, which leads to widespread dysfunction of the maternal vascular endothelium by mechanisms that are unknown and may involve a delicate balance of vasodilators and vasoconstrictors, of which endothelin may play an important role.1-3

Endothelin, an endothelium-derived peptide, is a potent vasoconstrictor.4 Because endothelial damage is a known stimulus for endothelin synthesis,5 increases in the production of endothelin may participate in preeclampsia. Evidence indicates that endothelin may play an important role in mediating the physiological changes that occur during preeclampsia.6 A number of studies have found increased plasma concentrations of endothelin in women with preeclampsia compared with normotensive pregnant women.7-9 Elevation of the circulating levels of endothelin in pregnant sheep resulted in a significant increase in mean arterial pressure (MAP), renal vascular resistance, and proteinuria,10 all features observed in women with preeclampsia.1,2 An endothelin type A (ETₐ) selective receptor antagonist significantly attenuated the hypertension induced by a chronic infusion of the NO synthase inhibitor N⁵-nitro-L-arginine methyl ester in pregnant rats, thus supporting a role for endothelin in mediating the hypertension in this animal model of pregnancy-induced hypertension.11 Whether increased synthesis of endothelin occurs within the kidney during preeclampsia remains unknown, inasmuch as some studies have found a decrease in urinary excretion of endothelin, a measure of local renal synthesis, in preeclamptic women compared with normotensive pregnant women.12,13

We recently reported that chronic reduced uterine perfusion pressure (RUPP) in pregnant rats resulted in significant

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From the Departments of Physiology and Obstetrics and Gynecology, Center for Excellence in Cardiovascular-Renal Research Center, University of Mississippi Medical Center, Jackson.
Correspondence to Joey P. Granger, PhD, Department of Physiology and Biophysics, University of Mississippi Medical Center, 2500 North State St, Jackson, MS 39216-4505. E-mail jgranger@physiology.umsmed.edu
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elevations in MAP and reductions in kidney function.\textsuperscript{14} Because the kidneys play a major role in the long-term regulation of arterial pressure, the first aim of the present study was to determine whether increases in renal preproendothelin mRNA expression were associated with the hypertension produced by chronic RUPP in pregnant rats. Subsequently, the next aim was to test the hypothesis that blockade of the ETA receptor would prevent the hypertension produced by chronic RUPP in pregnant rats. Therefore, the overall goal of the present study was to assess the role that endothelin plays in mediating the hypertension produced by chronic RUPP in pregnant rats.

Methods
All studies were performed in timed pregnant Sprague-Dawley rats purchased from Harlan Sprague Dawley Inc (Indianapolis, Ind). Animals were housed 1 to a cage in a temperature-controlled room (23°C) with a 12/12-hour light/dark cycle. All experimental procedures executed in the present 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 in Pregnant Rats
A chronic reduction in uteroplacental perfusion in rats was achieved by a method previously reported by our laboratory.\textsuperscript{14} In brief, all rats undergoing surgical procedures were anesthetized with 2% isoflurane.\textsuperscript{13} 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 a silver clip (0.203-mm internal diameter) was placed around the aorta above the iliac bifurcation. Because compensation of blood flow to the placenta occurs in pregnant rats through an adaptive increase in ovarian blood flow, we also clipped branches of both the right and left ovarian arteries that supply the uterus with the use of a silver clip (0.100-mm internal diameter). When the clipping procedure resulted in total reabsorption of the fetuses, rats were excluded from data analyses.

Measurement of Renal Hemodynamics and Arterial Pressure in Conscious Rats
During isoflurane anesthesia, as described above, rats at day 16 of pregnancy were surgically instrumented with catheters (PE-50 tubing) in the femoral vein and carotid artery for blood sampling and blood pressure monitoring. A midline lower abdominal incision was made, and the bladder was cannulated with flare-tipped PE-90 tubing for urine collection. All catheters were tunneled to the back of the neck and exteriorized. On day 19 of pregnancy, the rats were placed in modified restraining cages for renal function measurements. The femoral vein catheter was connected to an infusion pump that delivered isotonic saline containing sodium iothalamate (Glofil [125 I], 0.05 mCi · kg\textsuperscript{-1} · min\textsuperscript{-1}, Cypros) and para-aminomethylurea (PAH, 24 mg/mL, Sigma Chemical Co) at a fixed rate of 3 mL/h. Arterial pressure was monitored in conscious rats with a pressure transducer connected to a Grass model 7B chart recorder (Grass Instrument Co) for continuous recording. After a 60-minute stabilization period, two 20-minute urine collections were obtained, followed by collection of blood samples. Urine volume was determined gravimetrically. Sodium and potassium concentrations in plasma and urine were measured by flame photometry (IL-943, Instrumentation Laboratory). Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were calculated from the radioactivity of 131I and concentration of PAH, respectively, in plasma and urine. PAH concentration was determined colorimetrically.

Isolation of Total Cellular RNA
The kidneys were removed and sliced into cortical and medullary sections, quick-frozen in liquid nitrogen, and stored at −80°C. After each kidney was ground with use of a liquid nitrogen–chilled mortar and pestle, total cellular RNA was isolated by use of the acid guanidinium thiocyanate–phenol–chloroform procedure of Chomczynski and Sacchi\textsuperscript{16} (TOtALLY RNA kit, Ambion). Total RNA concentration and purity were determined spectrophotometrically by using absorbance (A) ratios A260 and A260/A280, respectively. Total RNA integrity was checked by using 1% agarose gel electrophoresis with a 0.4 mol/L Tris-acetate and 0.001 mol/L EDTA buffer.

Ribonuclease Protection Assay
The cDNA for rat preproendothelin, a gift from Dr Ernesto L. Schiffrin\textsuperscript{17} (The University of Montreal, Montreal, Canada), was linearized with XhoI (New England Biolabs). An antisense internal control template for β-actin was obtained from Ambion. Antisense RNA probes were synthesized and labeled with [α-32P]UTP (Dupont NEN) by use of a MAXiScript IV TRO Transcription kit (Ambion) according to the manufacturer’s instructions. Full-length probes were separated on denaturing 5% acrylamide gels. Ribonuclease protection assays (RPAs) were performed with an Ambion RPA III kit as described by the manufacturer. Protected fragments were quantified with use of a Molecular Analyst Imager System (Bio-Rad), and quantified with use of the Molecular Analyst Imager System (Bio-Rad). An equivalent amount of total RNA (20 μg) was used from each rat kidney. In each individual RPA, the RNA concentration was varied to confirm that the probe was in excess and that the response was linear. Where indicated, the error bars represent the SEM from at least 3 separate determinations per kidney. Quantification represents a ratio of transcript levels of preproendothelin to actin.

Experimental Design
The animals were divided into 4 groups: normal pregnant (NP group), normal pregnant plus ETA receptor antagonist (NP+ET\textsubscript{A} group), pregnant with chronic RUPP (RUPP group), and pregnant with chronic RUPP plus ETA receptor antagonist (RUPP+ET\textsubscript{A} group). An ET\textsubscript{A}-selective receptor antagonist (ABT-627, Abbott Laboratories) was administered in the drinking water at a dose of 5 mg/kg per day, and treatment was initiated at day 10 of pregnancy. At day 14, all rats were instrumented with bladder catheters, and animals destined to enter the RUPP group were clipped as described above. On day 16, the rats were instrumented with arterial and venous catheters as described above for the measurement of renal function and arterial pressure, which were performed on day 19. A total of 8 NP, 8 RUPP, 8 NP+ET\textsubscript{A}, and 9 RUPP+ET\textsubscript{A} rats were used for analysis of renal preproendothelin mRNA expression. A total of 14 NP, 12 RUPP, 17 NP+ET\textsubscript{A}, and 16 RUPP+ET\textsubscript{A} rats were used for renal and systemic hemodynamic analyses. In all groups, animal and pup weights were recorded on day 19 of pregnancy.

Statistical Analysis
All data are expressed as mean±SEM. Comparisons of control pregnant rats with RUPP rats, both treated and untreated, were analyzed by use of factorial ANOVA, followed by the Scheffé test. A value of P<0.05 was considered statistically significant.

Results
MAP in Pregnant Rats With Chronic RUPP
Chronic RUPP in pregnant rats resulted in significant increases in arterial pressure relative to that in NP rats. MAP averaged 101±4 mm Hg in NP rats (Figure 1a). Arterial pressure in the pregnant rats with chronic RUPP averaged 123±2 mm Hg, which was 22 mm Hg above the level in NP rats (P<0.05, RUPP versus NP; Figure 1a). Pretreatment with ABT-627 (+ET\textsubscript{A}) markedly attenuated the increase in arterial pressure in the RUPP rats. Arterial pressure in RUPP rats pretreated with the ETA receptor antagonist averaged 103±6 mm Hg and was not significantly different from that
in NP rats \((P<0.05, \text{RUPP versus RUPP+ET} \alpha \text{ groups})\). Pretreatment with the ET \( \alpha \) receptor antagonist in NP animals did not significantly alter MAP \((96.2 \pm 3 \text{ mm Hg, NP+ET} \alpha \text{ group; Figure 1b})\).

Chronic RUPP resulted in a decreased body weight at day 19 of gestation in the RUPP rats relative to NP rats \((295.1 \pm 5.5 \text{ versus } 351.3 \pm 9.1 \text{ g, respectively; } P<0.05)\). Pretreatment with the ET \( \alpha \) receptor antagonist did not attenuate the decrease in body weight observed in the RUPP rats \((301.6 \pm 9.2 \text{ g, RUPP+ET} \alpha \text{ group; } P<0.05 \text{ relative to NP control group})\), nor did it alter body weight in NP rats \((349.0 \pm 6.8 \text{ g, NP+ET} \alpha \text{ group})\). Pup weight was also decreased in RUPP rats relative to NP rats \((3.2 \pm 0.2 \text{ versus } 6.8 \pm 0.3 \text{ g, RUPP+ET} \alpha \text{; Figure 3a})\). ERPF was increased by 11% in the RUPP rats compared with NP rats \((44.1 \pm 6.3 \text{ versus } 24.0 \pm 2.6 \text{ densitometric units, respectively; } P<0.05)\). Also, RUPP kidneys were significantly elevated by 22% in the cortex of RUPP rats compared with NP rats \((37.7 \pm 2.4 \text{ versus } 29.4 \pm 2.8 \text{ densitometric units, respectively; } P<0.05)\).

Effects of an ET \( \alpha \)-Selective Receptor Antagonist on Renal Hemodynamics in Pregnant Rats With Chronic RUPP

Although nonsignificant, both GFR and ERPF decreased by 15% in the RUPP rats compared with NP rats \((GFR, 1.9 \pm 0.2 \text{ versus } 2.3 \pm 0.3 \text{ mL/min for RUPP versus NP, respectively; ERPF, 5.5 \pm 0.3 \text{ versus } 6.3 \pm 0.8 \text{ mL/min for RUPP versus NP, respectively})\) (Figure 3). Although ERPF was increased by 11% in the RUPP rats pretreated with the ET \( \alpha \) antagonist \((6.2 \pm 0.9 \text{ mL/min, RUPP+ET} \alpha \text{ group; Figure 3b})\), ET \( \alpha \) antagonist pretreatment did not attenuate the GFR response to chronic RUPP \((1.8 \pm 0.2 \text{ mL/min, RUPP+ET} \alpha \text{ group; Figure 3a})\).

Discussion

One of the leading theories concerning the etiology of pregnancy-induced hypertension suggests a pathway starting with inadequate trophoblast invasion of maternal spiral arteries, which leads to decreased placental perfusion. This, in turn, leads to placental ischemia, which results in placental release of factors, subsequent maternal endothelial dysfunction, and then systemic vasoconstriction. Chronic RUPP in pregnant animals has been used by numerous investigators to study the potential mechanisms of human preeclampsia as...
it initiates the disorder at a early step in the cascade described above. We recently reported that chronic RUPP in pregnant rats resulted in significant elevations in MAP, proteinuria, intrauterine growth restriction, and reductions in kidney function. In the present study, we confirmed that chronic RUPP in pregnant rats resulted in elevations in MAP, intrauterine growth restriction, and reductions in kidney function. We have extended our previous findings in the present study by showing that the endothelin system plays an important role in mediating the hypertension produced by chronic RUPP in pregnant rats.

Evidence from human studies indicates that endothelin may play an important role in mediating the pathophysiological changes that occur during preeclampsia. Some, but not all, studies have found increased plasma concentrations of endothelin in women with preeclampsia compared with normal pregnant women. Whether increased synthesis of endothelin occurs within the kidney during preeclampsia remains unknown, inasmuch as some studies have found a decrease or no significant change in urinary excretion of endothelin in preeclamptic women compared with normotensive pregnant women. Because the kidneys play a major role in the long-term regulation of arterial pressure and because abnormalities in the renal pressure–natriuresis relationship have been observed in all forms of hypertension examined to date, we examined whether increases in renal preproendothelin mRNA expression were associated with the hypertension produced in response to chronic RUPP in pregnant rats. We observed a significant increase in preproendothelin mRNA expression in the cortex and medulla of RUPP hypertensive rats. Preproendothelin mRNA levels were significantly elevated in the medulla of RUPP rats compared with NP rats by approximately 45%. Preproendothelin mRNA levels were also significantly elevated by 22% in the cortex of RUPP rats compared with NP rats. Therefore, hypertension produced in response to chronic RUPP in pregnant rats is associated with a significant increase in renal expression of preproendothelin mRNA.

To test the hypothesis that blockade of the endothelin receptor would prevent the hypertension observed in the pregnant rats with chronic RUPP, we pretreated rats with a selective ETA receptor antagonist. We found that pretreatment with the ETA receptor antagonist markedly attenuated the increase in arterial pressure in the RUPP rats. Arterial pressure in RUPP rats pretreated with the ETA receptor antagonist was not significantly different from that in NP rats. This effect of the receptor antagonist appears to be specific to the RUPP rats, inasmuch as pretreatment with the ETA receptor antagonist in NP animals did not significantly alter MAP. Whether endothelin type B activation plays a role in mediating the decrease in arterial pressure observed on ETA receptor blockade in the RUPP rats is unknown because this aspect has not yet been examined by our laboratory. The increase in MAP observed in the RUPP rats was associated with slight, but nonsignificant, decreases in both GFR and ERPF compared with the reaction in NP rats. Although pretreatment with the ETA receptor antagonist tended to improve renal function in the RUPP rats, statistical significance was not reached because of variability in the response.

Animal studies indicate that disruptions in endothelin-1 expression or blockade of endothelin-1 activation through the endothelin receptor results in abnormal fetal development and growth, thus indicating that use of endothelin receptor blockade as a mode of antihypertensive treatment during early pregnancy is contraindicated. However, specific animal studies regarding the teratogenicity of ETA-selective antagonists are lacking. As preeclampsia develops closer to term, risks of adverse effects indicated for early stages of development may not be applicable, and studies are needed to determine both developmental and long-term fetal risk regarding the use of ETA-selective antagonists in the treatment of preeclampsia.

Although reductions in blood flow to the uteroplacental unit are known to result in cardiovascular and renal abnormalities consistent with the pathophysiological features of human pregnancy-induced hypertension, the mechanisms linking placental ischemia with the abnormalities in endothelial function and enhanced synthesis of endothelin are unclear. Several lines of evidence support the hypothesis that the ischemic placenta contributes to endothelial cell activation/dysfunction of the maternal circulation by enhancing the synthesis of cytokines, such as tumor necrosis factor-α and interleukin-1. Tumor necrosis factor-α and interleukin-1 are inflammatory cytokines that have been shown to induce structural as well as functional alterations in endothelial cells. These inflammatory cytokines also enhance the in vitro formation of a number of endothelial cell substances, such as endothelin. Also supporting a potential role of cytokines in preeclampsia are findings that plasma levels of tumor necrosis factor-α are significantly elevated in women with preeclampsia by 2-fold. Whether chronic and modest increases in plasma cytokines in response to chronic RUPP can stimulate endothelin synthesis is unknown and requires further investigation.

In summary, we found that chronic RUPP in the pregnant rat was associated with significant increases in arterial pressure and renal expression of preproendothelin mRNA in both the medulla and the cortex. Chronic administration of the selective ETA receptor antagonist ABT-627 markedly attenuated the increase in MAP observed in the pregnant rats with chronic RUPP. However, ETA receptor blockade had no
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significant effect on blood pressure in the NP rats. These results suggest that endothelin may play a major role in mediating the hypertension produced by chronic RUPP.

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References

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