Effect of Angiotensin II Synthesis Blockade on the Hypertensive Response to Chronic Reductions in Uterine Perfusion Pressure in Pregnant Rats

Barbara T. Alexander, Kathy Cockrell, Farrah D. Cline, Maria T. Llinas, Mona Sedeek, Joey P. Granger

Abstract—The purpose of this study was to examine the role of the renin-angiotensin system in mediating the hypertension in response to chronic reductions in uterine perfusion pressure (RUPP) in conscious chronically instrumented pregnant rats. Mean arterial pressure was significantly higher in pregnant rats with chronic RUPP (125±3.0 mm Hg, P<0.01, n=12) than in pregnant rats (100±2.3 mm Hg, n=17). Plasma renin activity in pregnant rats with chronic RUPP was 17.1±2.5 nmol angiotensin I -1 · h -1 compared with 21.9±3.5 nmol angiotensin I -1 · h -1 in pregnant rats. Chronic oral administration of a converting-enzyme inhibitor (enalapril, 250 mg/L for 6 days) decreased mean arterial pressure to a similar extent in pregnant rats with chronic RUPP (109±4.2 mm Hg, P<0.01, n=9) and in normal pregnant (81±1.8 mm Hg, P<0.01, n=9) rats. Blockade of the renin-angiotensin system, however, had no significant effect on the blood pressure response to chronic RUPP as differences were similar in control (∆25 mm Hg) and converting enzyme inhibitor–treated (∆27 mm Hg) groups. These findings suggest that the renin-angiotensin system does not play a major role in mediating the hypertension produced by chronic RUPP in pregnant rats. (Hypertension. 2001;38[part 2]:742-745.)

Key Words: hypertension, pregnancy ■ preeclampsia ■ renin-angiotensin system ■ enalapril ■ blood pressure

A leading theory concerning the pathophysiology of preeclampsia suggests that a pathway that starts with inadequate trophoblast invasion of maternal spiral arteries leads to decreased placental perfusion. The ensuing placental ischemia results in placental release of factors, subsequent maternal endothelial dysfunction, and systemic vasoconstriction.1,2 A variety of animal models have shown that a reduction in uterine perfusion pressure (RUPP) leads to systemic hypertension in pregnant animals3–6; however, the mechanisms involved in mediating this hypertensive response to RUPP are unknown.

The renin-angiotensin system (RAS) plays an important role in the long-term regulation of renal function and arterial pressure during a variety of both physiological and pathophysiological conditions.7 During normal pregnancy, the RAS is thought to respond to hormonal changes and contribute to maintenance of blood pressure, blood flow, and sodium balance.8 Activation of RAS during pregnancy is evident by the marked changes that occur in the RAS as plasma renin activity (PRA), plasma renin concentration, angiotensinogen, and angiotensin (Ang) II levels are all elevated, whereas vascular responsiveness to Ang II appears to be reduced.8,9 In women with preeclampsia, these same components of the RAS are altered as PRA, plasma renin concentration, angiotensinogen, plasma aldosterone, and plasma Ang II are reduced, whereas sensitivity to Ang II is increased compared with that of normotensive pregnant women.8,9 Therefore, significant changes are observed in the RAS in preeclamptic pregnancy compared with normal pregnancy.

A role for the RAS in both human preeclampsia and in mediating pregnancy-induced hypertension (PIH) in animal studies is unclear. Prorenin and angiotensinogen gene expression are present in the placenta throughout gestation,10 and characterization of renin from human placenta origin has been shown to be identical to active enzyme from the human kidney.11 As significant uterine renin release has been measured in pregnant rabbits following acute uteroplacental ischemia,12 a role for renin in mediating the hypertension produced in response to reduced placental perfusion is suggested. However, enhanced active renin production within the uteroplacental unit associated with human preeclampsia13 and with acute RUPP in pregnant rabbits14,15 is not reflected by an increase in active renin within the maternal circulation. Thus, the importance of RAS in mediating hypertension in preeclampsia and animal models of PIH has not yet been fully elucidated.

We recently reported that chronic RUPP in pregnant rats resulted in significant elevations in mean arterial pressure...
(MAP) and in reductions in kidney function. As significant alterations in vascular sensitivity to Ang II occur in women with preeclampsia, the goal of the present study was to determine the role of the RAS in mediating the hypertension produced in response to chronic RUPP. Specific aims were to determine if blockade of the RAS by use of the ACE inhibitor enalapril would attenuate 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 one per cage in a temperature-controlled room (23°C) with a 12-hour/12-hour light/dark cycle. All experimental procedures executed in this study were in accordance with Guide for the Care and Use of Laboratory Animals (NIH publication No. 93-23, revised 1985), and the Animal Care and Use Committee at the University of Mississippi Medical Center approved all protocols.

**Experimental Design**

Animals were divided into 4 groups: pregnant (n=17), pregnant plus enalapril (n=9), pregnant with chronic RUPP (n=12), and RUPP plus enalapril (n=9). The ACE inhibitor enalapril, (250 mg/L, Sigma-Aldrich Co) was delivered in the drinking water starting at day 14 of gestation. Enalapril was used as a tool in this study by employing a dose that has been used extensively by our laboratory and others to result in an effective blockade of the Ang II response as determined by a decrease in arterial pressure. Rats that would be in the RUPP group were clipped at day 14; normal pregnant rats were subjected to a sham operation. Rats were instrumented with arterial catheters on day 18. Arterial pressure was measured, and animal and pups weights were recorded on day 20. Water intake did not differ between pregnant and RUPP, treated or untreated, and averaged 45 to 50 mL per animal per day.

**RUPP in Pregnant Rats**

Chronic reductions in uteroplacental perfusion in rats were achieved by a method previously reported by our laboratory. Briefly, all rats undergoing surgical procedures were anesthetized with 2% isoflurane (W.A. Butler Co) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic, Ohio Medical Products). Following a midline incision, the lower abdominal aorta was isolated, and a silver clip (0.230-mm ID) was placed around the aorta above the iliac bifurcation. Because compensation of blood flow to the placenta occurs through an adaptive increase in ovarian blood flow, we also clipped branches of both the right and left ovarian arteries that supply the uterus using a silver clip (0.100-mm ID).

**Measurement of Arterial Pressure in Conscious Rats**

At day 18 of gestation under isoflurane anesthesia as described above, a catheter of heat-stretched PE-50 tubing was inserted into the carotid artery. On day 20 of gestation, rats were placed in individual restraining cages. After achieving stabilization, arterial pressure was recorded continuously for 2 hours with a pressure transducer (Cobe III Transducer), followed by collection of an arterial blood sample.

**Measurement of PRA**

PRA was measured by radioimmunoassay. A pool having an average concentration of 7.13 nmole Ang I · L⁻¹ · h⁻¹ exhibited a within-assay variation of 3.0% (n=5). Two controls having average PRAs of 1.5 and 2.3 nmole Ang I · L⁻¹ · h⁻¹ exhibited respective between-assay variations of 7.0% (n=16) and 11.4% (n=16).

**Statistical Analysis**

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

**Results**

Chronic RUPP in pregnant rats resulted in significant increases in MAP relative to that of pregnant rats. MAP averaged 100±2.3 mm Hg in pregnant rats. Arterial pressure in pregnant rats with chronic RUPP averaged 125±3.0 mm Hg, 25 mm Hg above pregnant rats (P<0.01 versus pregnant, Figure 1). Chronic oral administration of the ACE inhibitor enalapril (250 mL/L for 6 days) decreased arterial pressure to a similar extent in both RUPP (109±4.2 mm Hg, P<0.01 versus RUPP, Δ 17 mm Hg) and pregnant rats (81±1.8 mm Hg, P<0.01 versus pregnant, Δ 19 mm Hg) (Figure 1), representing a 19% and 13% decrease, respectively. Thus, blockade of the RAS had no significant effect on the blood pressure response to chronic RUPP, as differences were similar in control (Δ 25 mm Hg) and ACE inhibitor–treated (Δ 27 mm Hg) groups. No difference in PRA was observed for pregnant rats with chronic RUPP compared with pregnant rats (17.1±2.5 nmole Ang I · L⁻¹ · h⁻¹ versus 21.9±3.5 nmole Ang I · L⁻¹ · h⁻¹, respectively).

Pup weight, although not significant, tended to decrease in the RUPP rats relative to pregnant rats (3.6±0.2 g versus 3.3±0.2 g, respectively). Additionally, chronic oral treatment with the ACE inhibitor tended to decrease pup weight (3.1±0.2 grams) in pregnant-treated rats but resulted in a significant decrease in the RUPP-treated animals (2.2±0.3 g; P<0.01 versus RUPP, P<0.01 versus pregnant). In addition, at day 20 of gestation, chronic RUPP in pregnant rats was associated with a reduced litter size relative to that of pregnant rats (8.9±1.2 pups versus 12.2±0.7 pups, respectively; P<0.05). Chronic oral treatment with ACE inhibitor attenuated the decreased litter size observed in RUPP rats (10.49±1.2 pups) without altering litter size in pregnant rats (12.3±0.3 pups). Chronic RUPP resulted in decreased body weight at day 20 of gestation in RUPP rats compared with pregnant rats (297.7±6.6 g and 336.0±6.4 g, respectively; P<0.01). Chronic oral treatment with the ACE inhibitor resulted in a further decrease in body weight in both RUPP-untreated (272±6.9 grams, P<0.05 versus RUPP, P<0.01

![Figure 1. Measurements of MAP in response to chronic RUPP in pregnant rats with and without chronic oral treatment with the ACE inhibitor enalapril. All data are expressed as mean±SEM (n=17 for pregnant, n=12 for RUPP, n=9 for pregnant + enalapril, and n=9 for RUPP + enalapril). *P<0.01 vs pregnant, †P<0.01 vs pregnant, ‡P<0.01 vs RUPP.](http://hyper.ahajournals.org/content/62/5/743/F1)

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versus pregnant) and pregnant-treated (298±11.0 grams, P<0.01 versus pregnant) rats.

Discussion

The initiating event in preeclampsia is suggested to involve reduced placental perfusion, leading to maternal endothelial cell dysfunction and subsequent hypertension.\(^1\) In pregnant animals has been used by numerous investigators to study potential mechanisms of human preeclampsia, as it initiates the disorder at an early step in the cascade described above.\(^2\) We recently reported that chronic RUPP in pregnant rats resulted in significant elevations in MAP, proteinuria, intrauterine growth restriction (IUGR), and reductions in kidney function, all features characteristic of human preeclampsia.\(^3\) Nonpregnant animals with comparable RUPP experienced no significant changes in MAP.\(^4\) In the present study, we again confirmed that chronic RUPP in the pregnant rat resulted in elevations in MAP (Figure 1). However, in this study we extended our previous findings to show that the RAS does not play an important role in mediating the hypertension produced in response to chronic RUPP in pregnant rats.

The RAS is activated in human pregnancy\(^5\) and also in the pregnant rat.\(^6\) However, activity of the RAS appears to be normal or suppressed during preeclampsia as PRA, plasma renin concentration, angiotensinogen, and Ang II are decreased in women with preeclampsia compared with normotensive pregnant women.\(^8\) whereas the sensitivity to pressor effects of Ang II is increased.\(^1\) This increase in Ang II sensitivity may not be suggestive of the importance of endogenous Ang II in mediating the hypertension in experimental models of PIH, as it may be indicative of decreas of endogenous Ang II. In a study by Losonczy et al., acute infusion of an Ang II receptor antagonist in pregnant rabbits 24 hours after clipping did not alter the hypertension produced in response to RUPP, suggesting that RUPP-induced hypertension is not mediated by Ang II. In a study by Woods and Brooks, however, Ang II levels did not decrease in response to acute RUPP in the pregnant dog. Thus, the role of the RAS in the regulation of arterial pressure in PIH is unclear.

In the present study, we observed no significant difference in PRA between hypertensive chronic RUPP rats and normotensive pregnant rats (Figure 2). PRA was also unchanged in response to acute RUPP in pregnant dogs in the study by Woods and Brooks.\(^6\) In addition, they also concluded that RUPP-induced hypertension in pregnancy was independent of the RAS as acute RUPP-induced increases in arterial pressure were not attenuated in pregnant dogs with RAS that was fixed by infusion of captopril and Ang II before acute RUPP. However, the mechanisms resulting in acute RUPP-induced hypertension may not be the same as those that contribute to chronic RUPP-induced hypertension.

In the present study, the role of the RAS in mediating the hypertension produced in response to chronic RUPP was examined by use of the ACE inhibitor enalapril. We found that chronic treatment with the ACE inhibitor markedly attenuated the hypertension produced in response to chronic RUPP in pregnant rats; however, pregnant rats pretreated with the ACE inhibitor also experienced a comparable decrease in arterial pressure (Figure 1). Therefore, the increase in arterial pressure in the pregnant rats with chronic RUPP is not due to the RAS as blood pressure differences were similar in control (Δ25 mm Hg) and ACE inhibitor–treated (Δ27 mm Hg) groups.

Data from human and animal studies indicate that administration of ACE inhibitors during pregnancy results in abnormal fetal development and growth.\(^9\) In a study by Valdes et al., treatment with enalapril during the second half of gestation resulted in marked reductions in pup weight; however, IUGR was also evident in animals receiving enalapril treatment in the first half of gestation. Within this study, ACE inhibitor treatment during the third week of gestation resulted in IUGR within both pregnant-treated and RUPP-treated animals with a marked decrease in pup weight observed in the RUPP-treated animals. Thus, use of ACE inhibitors, such as enalapril, as a mode of antihypertensive treatment during pregnancy or PIH is contraindicated.

In summary, we found that chronic RUPP in the pregnant rat was associated with significant increases in arterial pressure but with no change in PRA. Chronic administration of the ACE inhibitor enalapril markedly attenuated the increase in MAP observed in the pregnant rats with chronic RUPP; however, ACE inhibition also significantly reduced blood pressure in pregnant rats to a similar extent as observed in the RUPP rats. Therefore, as arterial pressure differences were similar in control and ACE inhibitor–treated animals, these results suggests that RAS does not play a major role in mediating the hypertension produced by chronic RUPP.

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