L-Arginine Attenuates Hypertension in Pregnant Rats With Reduced Uterine Perfusion Pressure

Barbara T. Alexander, Maria T. Llinas, Walter C. Kruckeberg, Joey P. Granger

Abstract—A chronic reduction in uterine perfusion pressure in the pregnant rat is associated with significant elevations in mean arterial pressure, proteinuria, and reductions in kidney function as is chronic nitric oxide blockade, suggesting that nitric oxide deficiency may contribute to the clinical manifestations of preeclampsia. The purpose of this study was to determine whether supplementation with L-arginine, the precursor for nitric oxide, attenuates the hypertension produced in response to a chronic reduction in uterine perfusion pressure in the pregnant rat. Reduced uterine perfusion was initiated at day 14 of gestation with arterial pressure determined at day 19 of gestation in conscious, chronically instrumented rats. Arterial pressure was significantly elevated in pregnant rats with chronic reductions in uterine perfusion as compared with pregnant control rats (132±2 versus 109±2 mm Hg, P<0.01, respectively). Treatment with L-arginine (2%) in the drinking water was initiated at day 10 of gestation, L-arginine supplementation resulted in a significant decrease in arterial pressure in both pregnant rats with reduced uterine perfusion pressure (113±2 mm Hg treated, P<0.01 versus untreated pregnant with reduced uterine perfusion pressure) and pregnant control (97±3 mm Hg treated, P<0.01 versus untreated pregnant) rats. However, supplementation with L-arginine decreased blood pressure by 19 mm Hg in pregnant with reduced uterine perfusion pressure (untreated versus treated) as compared with 12 mm Hg in pregnant (untreated versus treated) rats. Thus, these results suggest that L-arginine supplementation may be beneficial in attenuating the hypertension in preeclampsia. (Hypertension. 2004;43:832-836.)

Key Words: arginine • arterial pressure • hypertension, pregnancy • nitric oxide • preeclampsia

Preeclampsia, a major cause of maternal and fetal morbidity and mortality, is estimated to affect 5% to 10% of all pregnancies in the United States.1,2 Symptoms generally associated with preeclampsia include increased responsiveness to vasoconstrictors, elevations in arterial pressure, proteinuria, reduced glomerular filtration rate (GFR), and intrauterine growth restriction.1,2 The initiating event in preeclampsia is suggested to involve reduced placental perfusion leading to maternal endothelial cell dysfunction.1,3–5 A role for endothelial dysfunction in the pathophysiology of preeclampsia is implicated, as this pregnancy-specific disorder is associated with reduced synthesis of vasodilators and increased production of vasoconstrictors.5–9 Numerous studies indicate that nitric oxide (NO) production is elevated in normal pregnancy.6,10 In the rat, plasma and urinary levels of cGMP, a second messenger of NO, and levels of urinary nitrite/nitrate, metabolites of NO and indicators of whole body NO production, are increased during pregnancy.10–12 In addition, renal protein expression of inducible and neuronal nitric oxide synthase (iNOS and nNOS, respectively) are increased by 31% and 25%, respectively, in the rat at mid-gestation.11 Further studies have demonstrated that NO plays an important role in mediating systemic hemodynamics and renal vasodilation during pregnancy.6,10–15

Chronic NO synthesis inhibition in the pregnant rat is associated with marked elevations in arterial pressure, reductions in GFR, proteinuria, and intrauterine growth restriction, many of the features observed in women with preeclampsia.14,16–18 Furthermore, these NO blockade-mediated effects are reversible by administration of L-arginine.16,19 We have previously reported that a chronic reduction in uterine perfusion pressure (RUPP) in the pregnant rat results in similar responses, as mid-gestational increases in renal vasodilation and late gestational reductions in blood pressure are attenuated and are associated with variable proteinuria and intrauterine growth restriction.20 In the RUPP pregnant rat, a significant reduction in renal nNOS protein expression is observed in late gestation as compared with the normal pregnant late-gestational rat.20 In addition, activity of the endothelium-dependent NO-cGMP pathway is reduced in the RUPP-hypertensive pregnant rat.21 Thus, as NO plays an important role in mediating systemic and renal hemodynamic changes observed during normal pregnancy, NO deficiency...
may play an important role in preeclampsia and in the rat model of reduced uterine perfusion pressure.

L-arginine supplementation has been utilized for examination of the role for NO in mediating hypertension in both human and animal models of hypertension. For example, essential hypertension in humans is associated with a transient decrease in arterial pressure during infusion with L-arginine. In response to salt load, a greater reduction in arterial pressure is observed in the presence of L-arginine supplementation in salt-sensitive individuals as compared with salt-resistant individuals. In the Dahl salt-sensitive animal model of hypertension, L-arginine supplementation also attenuates sodium chloride loading increases in arterial pressure. In other animal models of hypertension, oral administration of L-arginine also prevents the rise in blood pressure in adrenocorticotropic- or mineralocorticoid-salt-induced hypertension. Thus, numerous reports indicate that L-arginine supplementation lowers blood pressure in animal models of hypertension associated with NO deficiency. Although there is evidence for NO deficiency in RUPP hypertensive rats, the effect of L-arginine supplementation in this rat model of preeclampsia is unknown. Therefore, the overall goal of this study was to determine if supplementation with L-arginine attenuates the hypertension produced in response to a chronic reduction in uterine perfusion pressure in the pregnant rat.

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 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.

Reduced Uterine Perfusion in the Pregnant Rat

A modification of the method by Eder and McDonald as previously described was used to reduce uterine perfusion by 35% to 45%. Briefly, rats were anesthetized with 2% isoflurane (W.A. Butler Co., Memphis, Tenn) delivered by an anesthesia apparatus (Vaporizer for Forane Anesthetic, Ohio Medical Products, Madison, Wis). At day 14 of gestation, a silver clip (0.203-mm ID) was placed around the lower abdominal aorta above the iliac bifurcation. Because compensation of blood flow to the placenta occurs in the pregnant rat through an adaptive increase in uterine blood flow, both right and left uterine arteries were clipped (0.100-mm ID). When the clipping procedure resulted in total reabsorption of the fetuses, rats were excluded from data analyses. Control pregnant rats were sham operated.

Measurement of Arterial Pressure in Conscious Chronically Instrumented Rats

Mean arterial pressure (MAP) was determined at day 19 of gestation in conscious, chronically instrumented rats as previously described.

Measure of Urinary Nitrite/Nitrate Excretion

Urinary nitrite/nitrate excretion rates were determined in animals as previously described.

Determination of Serum L-arginine Levels

Serum was collected and stored at −20°C for the measurement of serum L-arginine levels as previously described.

Figure 1. Measure of mean arterial pressure (MAP) at day 19 of gestation in a rat model of reduced uterine perfusion pressure. MAP was determined in conscious, chronically instrumented rats: pregnant control (pregnant), pregnant with reduced uterine perfusion pressure (RUPP), pregnant treated with 2% L-arginine administered in the drinking water (pregnant + L-ARG), and RUPP treated with 2% L-arginine (RUPP + L-ARG). *P < 0.01 vs pregnant untreated, †P < 0.01 vs RUPP untreated, ‡P < 0.01 vs RUPP + L-ARG. All data are expressed as mean ± SEM.

Experimental Design

Animals were divided into four groups: pregnant control (pregnant), pregnant control plus L-arginine (pregnant + L-ARG), pregnant with a chronic reduction in uterine perfusion pressure (RUPP), and RUPP plus L-arginine (RUPP + L-ARG). At day 10 of gestation 2% L-arginine was administered in the drinking water and animals began a low nitrite/nitrate diet (AIN76, ICN Biomedicals Inc, Aurora, Ohio). At day 14 of gestation, all rats in the RUPP group were clipped as described above. On day 17, all animals were placed in an individual metabolism cage for 24 hours for determination of whole body NO utilizing urinary nitrite/nitrate analysis. On day 18, rats were instrumented with arterial catheters as described above. MAP was measured on day 19 followed by collection of a blood sample and recording of maternal body and fetal weights. A total of 12 pregnant, 9 RUPP, 9 pregnant + L-ARG, and 9 RUPP + L-ARG rats were utilized for analysis of MAP, urinary nitrite/nitrate, and serum L-arginine determination.

Statistical Analyses

GB-STAT version 6.5 (Dynamic Microsystems Inc, Silver Spring, Md) was used for statistical analysis. All data are expressed as mean ± SEM. Comparisons of pregnant rats with RUPP rats, both treated and untreated with L-arginine, were analyzed using factorial ANOVA followed by Scheffe test. A value of P < 0.05 was considered statistically significant.

Results

Effect of L-arginine Supplementation on Mean Arterial Pressure in Pregnant Rats With a Chronic Reduction in Uterine Perfusion Pressure

A chronic RUPP in the pregnant rat resulted in a marked increase in arterial pressure. Specifically, at day 19 of gestation, MAP averaged 132 ± 2 mm Hg in RUPP as compared with 109 ± 2 mm Hg observed in pregnant (P < 0.01, RUPP untreated versus pregnant untreated) rats (Figure 1). Supplementation with L-arginine during gestation resulted in a significant decrease in MAP in both RUPP (113 ± 2 mm Hg, RUPP + L-ARG, P < 0.01 versus RUPP untreated) and pregnant (97 ± 3 mm Hg, pregnant + L-ARG, P < 0.01 versus pregnant untreated) groups (Figure 1). Although MAP was lower in pregnant rats than in RUPP rats after supplementation with L-arginine (P < 0.01, RUPP + L-ARG versus pregnant + L-ARG), a larger decrease in blood pressure was observed in...
Changes in Serum L-arginine Levels During Pregnancy

Serum L-arginine did not differ significantly at day 19 of gestation in comparison of RUPP with pregnant (142 ± 22 versus 129 ± 29 nmol/mL, RUPP versus pregnant, respectively) rats (Figure 4). L-arginine supplementation did result in a marked increase in serum levels of L-arginine in RUPP (262 ± 51 nmol/mL, RUPP + L-ARG, P < 0.05 versus RUPP). However, serum L-arginine levels were not significantly increased in pregnant with L-arginine supplementation (281 ± 38 nmol/mL) as compared with pregnant untreated rats.

Discussion

The etiology of preeclampsia remains unknown, but one of the leading hypotheses suggests that reduced uterine perfusion in late gestation leads to placental ischemia, the release of placental factors, and subsequent maternal endothelial dysfunction. Impaired endothelium-dependent responses have been reported in vessels isolated from women with preeclampsia suggesting that a deficiency of NO may contribute to the pathophysiology of preeclampsia. Numerous studies have shown that inhibition of NO production by inhibitors specific for nitric oxide synthase (NOS) during pregnancy in the rat results in marked elevations in arterial
pressure, proteinuria, intrauterine growth restriction, and attenuation of the mid-gestational increases in renal vasodilation, all features characteristic of women with preeclampsia.\textsuperscript{14,17,18} In addition, reversal of NO-blockade mediated effects in the pregnant rat occur in treatment with L-arginine, the precursor for NO.\textsuperscript{16,19} Thus, impairment of the NO pathway may play a role in mediating the pathophysiology of preeclampsia.

In the pregnant rat, a reduction in uterine perfusion pressure initiated at day 14 of gestation attenuates late-gestational reductions in arterial pressure as MAP is elevated by an average of 20 mm Hg at day 19 of gestation as determined in the conscious, chronically instrumented rat.\textsuperscript{20} RUPP-induced hypertension in the pregnant rat is also associated with variable proteinuria, intrauterine growth restriction, and reductions in renal function.\textsuperscript{21} Furthermore, endothelial dysfunction in the RUPP hypertensive pregnant rats is evidenced because the endothelium-dependent NO-cGMP pathway is impaired.\textsuperscript{21} Thus, the purpose of this study was to determine if L-arginine supplementation would attenuate the hypertension produced in the pregnant rat in response to reduced uterine perfusion pressure. L-arginine supplementation resulted in a marked decrease in MAP in both pregnant and RUPP rats relative to their untreated counterparts. The decrease in MAP in pregnant rats supplemented with L-arginine was not unexpected, as blockade of NO results in marked elevations in MAP, suggesting a role for this vasodilator in control of arterial pressure during gestation in the rat;\textsuperscript{15–18} an effect that can be reversed by L-arginine.\textsuperscript{19} Although L-arginine supplementation resulted in marked decreases in MAP in both pregnant and RUPP subjects, the arterial pressure difference on supplementation with L-arginine was larger in the RUPP rats as compared with pregnant rats. Specifically, a 19 mm Hg difference in arterial pressure was observed between RUPP treated versus RUPP untreated rats as compared with a 12 mm Hg difference in arterial pressure observed between pregnant treated versus pregnant untreated groups.

Hypertension in women with preeclampsia is associated with markedly reduced levels of plasma L-arginine compared with normotensive pregnant women,\textsuperscript{35} suggesting a role for impairment of NO formation. In this study the significant decrease in arterial pressure in the RUPP rats on supplementation with L-arginine was associated with a significant change in serum L-arginine levels. Specifically, serum L-arginine levels, which did not differ on comparison of untreated RUPP and untreated pregnant rats, was significantly increased on supplementation with L-arginine in RUPP rats, but not pregnant rats. Thus, supplementation with L-arginine in RUPP rats led to a significant increase in serum L-arginine levels associated with marked reductions in MAP.

Assessment of whole body NO production in women with preeclampsia has yielded variable results on comparison to normotensive pregnant women.\textsuperscript{36–39} Evaluation of NO formation in preeclampsia by supplementation with L-arginine has also generated inconsistent results. For example, acute infusion of L-arginine in women during weeks 31 to 32 of gestation led to a reduction in diastolic blood pressure in both normal pregnant and preeclamptic women, with the percent change greater in women with preeclampsia.\textsuperscript{40} Yet in women with preeclampsia, the L-arginine-induced reduction in blood pressure was not associated with increased whole body NO production as noted by serum nitrite and total L-citrulline.\textsuperscript{40} However, another study reported that acute infusion of L-arginine between 36 to 37 weeks of gestation in women with preeclampsia was associated with increased whole body production of NO as indicated by increased levels of plasma cGMP, yet arterial pressure was not altered.\textsuperscript{41} We have previously shown that measurement of whole body production of NO is not significantly altered in the RUPP hypertensive pregnant rats relative to control pregnant rats.\textsuperscript{20} However, determination of whole body NO may not reflect possible abnormalities in NO production as we have previously shown that basal and stimulated release of NO from isolated vascular strips is significantly reduced in RUPP as compared with pregnant rats.\textsuperscript{21} Supplementation with L-arginine did lead to a significant increase in whole body NO production in both RUPP and pregnant rats as determined by urinary nitrite/nitrate excretion. However, supplementation with L-arginine resulted in a more than 2-fold increase in whole body NO production in the RUPP-treated rats as compared with RUPP untreated rats, whereas pregnant treated rats exhibited an increase of approximately 1.5 fold as compared with pregnant untreated. Thus, supplementation with L-arginine may result in excess L-arginine and increased NO synthesis, alterations that may occur in specific vascular beds in a manner differential on comparison of RUPP and pregnant groups.

Supplementation with L-arginine did result in a differential effect on placental weight between RUPP and pregnant rats. Placental weight, which did not differ between untreated RUPP and untreated pregnant rats, was increased significantly on supplementation with L-arginine in RUPP rats, but not in pregnant rats. Intrauterine growth restriction, a known risk factor in preeclampsia,\textsuperscript{2–5} was present in RUPP as compared with control. However, supplementation with L-arginine did not significantly improve fetal weights in either group. Thus, L-arginine supplementation significantly increased placental weights but not fetal weights in the RUPP, suggesting a differential effect in response to L-arginine supplementation on vascular beds of the uteroplacental unit in the presence of chronic RUPP.

Supplementation with L-arginine in both pregnant and RUPP rats resulted in a reduction in arterial pressure associated with an increase in whole body production of NO. The difference in the MAP reduction was greater in RUPP as compared with control group as was the increase in whole body NO. Whether these differences are significant and indicative of enhanced sensitivity to L-arginine by RUPP is unknown. However, a deficiency in NO production or impairment in the NO pathway is suggested, as elevations in serum L-arginine were significant in response to excess L-arginine in the RUPP.

**Perspectives**

NO deficiency is thought to play a role in the pathophysiology of preeclampsia. The present study examines the importance of NO in the rat model of reduced uterine perfusion pressure. Treatment with L-arginine (2%) resulted in a significant decrease in arterial pressure (\(\Delta19\) mm Hg) in RUPP pregnant and normal pregnant rats (\(\Delta12\) mm Hg). In addition, whole body production of NO was increased in both RUPP
and pregnant rats on supplementation with L-arginine. However, significant increases in serum L-arginine levels were observed only in RUPP-treated rats, suggesting de novo synthesis of NO may be stimulated in this rat model by the presence of excess L-arginine. Thus, L-arginine supplementation may be beneficial in attenuating the hypertension in preeclampsia as impairment of the NO pathway may play an important role in mediating the pathophysiology of preeclampsia.

Acknowledgments

This work was supported by National Institutes of Health grants HL38499 and HL51971. The NIH National Research Service Award HL10137–01 supported Dr. Alexander. The authors thank Kathy Cockrell for her excellent technical assistance.

References

l-Arginine Attenuates Hypertension in Pregnant Rats With Reduced Uterine Perfusion Pressure
Barbara T. Alexander, Maria T. Llinas, Walter C. Kruckeberg and Joey P. Granger

Hypertension. 2004;43:832-836; originally published online February 9, 2004;
doi: 10.1161/01.HYP.0000119192.32360.a9

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/43/4/832

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/