Systemic Hemodynamics and Regional Blood Flow During Chronic Nitric Oxide Synthesis Inhibition in Pregnant Rats

Salah Kassab, M. Todd Miller, Robert Hester, Jacqueline Novak, Joey P. Granger

Abstract—Pregnancy-induced hypertension in women is associated with severe vasoconstriction and reductions in organ blood flow and cardiac output. Recent studies have indicated that nitric oxide (NO) synthesis inhibition during mid to late gestation in pregnant rats results in severe hypertension and proteinuria. The purpose of this study was to determine the systemic hemodynamic and regional blood flow alterations associated with chronic NO synthesis inhibition in the pregnant rat. The study was conducted in four groups of rats: virgin rats (n=6), pregnant rats (n=10), virgin rats treated with L-NAME (n=6), and pregnant rats treated with L-NAME (n=11). Rats were treated with L-NAME in drinking water at a dose of 1 mg/d for a week starting from day 13 of gestation or an equivalent time for virgins. Mean arterial pressure (MAP), cardiac output, total peripheral resistance (TPR), and regional flows were measured by tracing radiolabeled microspheres in conscious rats. Pregnant rats that were given L-NAME showed significantly higher MAP (137±6 versus 96±2 mm Hg), higher TPR (5.08±0.58 versus 2.90±0.44 mm Hg/mL/mm Hg/100 g), and lower cardiac output (87±8.4 versus 113±1.1 mL/min) than pregnant controls. Chronic NO synthesis inhibition decreased the renal blood flow in pregnant rats at a significantly greater magnitude than in virgin rats. Significant reductions in regional blood flow to the heart, lungs, liver, diaphragm, and skeletal muscles were also observed in pregnant rats treated with L-NAME. The results of this study indicate that NO may play a role in mediating the alterations in systemic hemodynamics and regional blood flow in late pregnant rats (Hypertension. 1998;31[part 2]:315-320.)

Key Words: nitric oxide • blood flow • pregnancy • hypertension

During normal pregnancy, maternal blood volume increases markedly, leading to approximately a 30% to 60% increase in cardiac output. The blood pressure falls despite the increase in cardiac output, mostly because of decreased systemic vascular resistance. Gestation in humans as well as in different animal models is also associated with significant changes in blood flow to different organs and tissues. Changes in renal hemodynamics include a 20% to 40% increase in GFR that has been correlated with increased RPF. Dramatic increases in uteroplacental blood flow also occur during pregnancy to accommodate the increasing needs of the growing fetus. Furthermore, the pressor responses to exogenously administered vasoconstrictors such as norepinephrine and angiotensin-II are attenuated in pregnant rats and humans. Recent studies have provided evidence that total body NO production as well as regional production in certain vascular beds is increased during pregnancy, indicating that NO could play a role in mediating the increased vascular reactivity and vasodilation during gestation. However, the role of NO in mediating the increased cardiac output, decreased vascular resistance, and regional blood flow alterations during pregnancy has not been elucidated.

PIH, on the other hand, is characterized by increased systemic vascular resistance, contracted blood volume, enhanced sensitivity to vasoconstrictor agents, altered renal hemodynamics, and vascular endothelial cell damage. Although the exact mechanism responsible for PIH is unclear, there is substantial evidence that maternal vascular endothelial cell dysfunction could be involved. Because NO is thought to play an important role in the control of arterial pressure and regional blood flow in nonpregnant animals, and since its levels have been reported to be elevated during pregnancy, decreased NO production has been proposed to contribute to the pathogenesis of PIH. Recent studies have indicated that chronic administration of NO synthesis inhibitors during mid to late gestation in rats leads to the development of hypertension and proteinuria. However, the hemodynamic and regional blood flow alterations in this rat model of PIH have not been fully characterized.

The aim of this study was to determine the systemic hemodynamic and regional flow changes during normal pregnancy and in a rat model of PIH caused by chronic NO
Regional Flow in Pregnancy-Induced Hypertension

**Selected Abbreviations and Acronyms**

- NO = nitric oxide
- PIH = pregnancy-induced hypertension
- MAP = mean arterial pressure
- L-NAME = N\(^\text{\textmd{-}}\)nitro-L-arginine methyl ester
- L-NMMA = N\(^\text{\textmd{-}}\)monomethyl-L-arginine
- L-NNA = N\(^\text{\textmd{-}}\)nitro-L-arginine
- RBF = renal blood flow
- RPF = renal plasma flow
- RVR = renal vascular resistance
- GFR = glomerular filtration rate
- ANG II = angiotensin II
- ET = endothelin
- cAMP = cyclic adenosine monophosphate
- CAMP = cyclic adenosine monophosphate
- PIH = pregnancy-induced hypertension
- MAP = mean arterial pressure
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**Methods**

Virgin female Sprague Dawley rats were purchased from Harlan Sprague Dawley, Inc. Animals were placed two in a cage in a temperature-controlled room (23°C) with a 12:12 hour light/dark cycle and fed a standard rat chow (Teklad) and water ad libitum. All experimental procedures in this study were executed in accordance with the National Institutes of Health guidelines for the use and care of animals, and the protocols were approved by the Animal Care and Use Committee at the University of Mississippi Medical Center.

The study was conducted on four groups of rats: virgin rats (n=6), pregnant rats (n=11), pregnant rats (n=10) that received the NO synthesis inhibitor L-NAME in drinking water for 7 prenatal days (days 13 to 19 gestation), and virgin rats that received L-NAME (n=6) in drinking water for one week before the experiment. L-NAME (Sigma Chemical Co.) was dissolved in distilled water at a dose of 50 mg/L ad libitum. On the basis of a preliminary study in our laboratory using this concentration of L-NAME in distilled water, each rat received approximately 1 mg of L-NAME per day. We also found that no difference in water intake was observed between virgin and pregnant rats until day 20 of pregnancy. A sperm-positive vaginal smear from females was considered day 1 of pregnancy. Pregnant rats were then housed individually in cages from the first day of pregnancy until the time of experimentation.

**Surgical Procedures**

On the morning of the experiment, rats were anesthetized with isoflurane that was delivered to the rat by using a portable anesthesia machine for rodents modified from a model described by Henry and Casto. A catheter of heat-stretched PE 50 tubing was inserted into the right carotid artery and advanced 3.5 to 4.5 cm into the left ventricle of the heart. The location of the catheter was determined operatively by the left ventricular pulse pressure tracing and was confirmed by postmortem examination. Another length of PE-50 tubing was inserted into the left femoral artery for measurement of arterial pressure and for blood withdrawal of the reference sample. The catheters were filled with heparin, and animals were allowed to recover for approximately 4 hours. Each rat was placed in a Plexiglas restrainer, the size of which was chosen so that it did not allow the rat to move around without external pressure. Furthermore, rats were pretrained before the experiment to minimize the stress of the restraining procedure. The femoral catheter was connected to a Statham pressure transducer, and MAP was continuously recorded on a Grass Model 7B polygraph (Grass Instruments Co.) for a period of 30 minutes before the experiment.

**Microsphere Injection**

Microspheres radiolabeled with \(^{46}\text{Sc}\) (New England Nuclear) with a mean diameter of 15.2 ± 3.2 \(\mu\)m were purchased dry and suspended in a 1:3 specific gravity dextrose solution with one drop of 0.05% tween 80 to prevent clumping of microspheres as previously described. The microsphere solution was then dispersed by using an ultrasonic bath (Radigraph) for 5 minutes and then mixed with a vortex shaker for 3 minutes. Precollected PE-50 tubing (72 cm long, 0.2 mL filling volume) was filled with the microsphere solution, which was adjusted to a dilution to contain \(\sim300,000\) microspheres per coil. The coils were sealed with metal plugs at both ends, and radioactivity was determined by using a gamma counter (Series 1185). Each rat was prepared for microsphere injection in the conscious state by interposing the preloaded coil between the left ventricular and catheter and a Gilford infusion pump and connecting the femoral catheter to a withdrawal pump of the same type. Microspheres were injected into the left femoral and flushed with 0.6 mL of saline over a period of \(\sim20\) seconds. The withdrawal pump was adjusted to collect a 1-mL blood sample from the femoral artery over a period of 90 seconds starting at the same time as the microsphere injection.

**Cardiac Output and Regional Flow Measurements**

At the end of the experiment, rats were euthanized by intraventricular injection of pentobarbital. Individual placentas, uterus, heart, lungs, liver, stomach, intestines, kidneys, brain, and a tissue sample from the skeletal muscle of the forelimbs and hindlimbs were carefully separated and weighed. The arterial reference sample, along with tissues and organs, were counted in a gamma counter that was set at the photopeak of the isotope that was used. The residual activity in the microsphere injection coil and plugs was determined and subtracted from the radioactivity of the coil before injection for calculation of the real amount of radioactivity that was infused in each rat. The uniformity of distribution of microspheres was considered adequate on the basis of a <5% difference in the number of microspheres entrapped in the right and left kidneys.

Cardiac output was calculated as follows:

\[
\text{CO (mL/min)} = \frac{\text{arterial reference flow (mL/min)} \times \text{number of microspheres injected}}{\text{number of microspheres in arterial reference sample}}
\]

Tissue and organ flows were determined by the ratio of the number of microspheres in the tissue to the total number of injected microspheres times total cardiac output divided by the organ weight and expressed as mL/min/100 g of tissues.

**Statistical Analysis**

All data in the experiment are expressed as mean ±SEM. Differences between groups were determined by using factorial ANOVA followed by Scheffé's test. Differences in placental flow and resistance between pregnant rats and rats treated with L-NAME were analyzed by using unpaired t-test. A value of \(P<0.05\) was considered statistically significant.

**Results**

**Changes in Systemic Hemodynamics in Virgin and Pregnant Rats in Response to Chronic NO Synthesis Inhibition**

Fig 1 illustrates the systemic hemodynamics in virgin and pregnant rats and in rats chronically-treated with L-NAME. In pregnant rats, MAP was 25% lower (96 ± 2 versus 120 ± 1 mm Hg, \(P<0.05\)) and cardiac output was 30% higher (113 ± 11 versus 86 ± 10 mL/min, \(P<0.05\)) than in virgin rats. Although TPR was 14% lower in pregnant rats than in virgin rats, this difference...
Figure 1. Mean arterial pressure, cardiac output, and total peripheral resistance in virgin and pregnant rats and in those chronically treated with L-NAME (1 mg/d) for 1 week. Data are mean±SEM. *P<0.05 in control rats compared with rats treated with L-NAME. tP<0.05 in virgin versus pregnant rats.

Changes in Regional Hemodynamics in Virgin and Pregnant Rats in Response to Chronic NO Synthesis Inhibition

Fig 2 illustrates the renal hemodynamic changes in virgin and pregnant rats and in virgin and pregnant rats treated with L-NAME for 1 week. RBF in pregnant rats was 44% higher (14.59±3.92 versus 10.12±1.29 mL/min) and RVR was 12% lower (10.33±1.62 versus 11.73±1.70 mmHg/mL/min) than in virgin rats. However, the renal hemodynamics in virgin and pregnant rats were not statistically different. In L-NAME-treated pregnant rats, RBF was significantly lower (6.06±0.83 versus 14.59±3.92 mL/min, P<0.01) and RVR was significantly higher (28.25±3.61 versus 10.33±1.62 mmHg/mL/min, P<0.01) compared with pregnant controls. On the other hand, RBF in L-NAME-treated virgin rats was not statistically different from that in virgin controls despite the increase in RVR.

Table 1 shows the regional blood flow (mL/min/g) and regional vascular resistance (mmHg/mL/min/g tissue) in different vascular beds (other than reproductive organs) in virgin and pregnant rats and in those chronically treated with L-NAME. Chronic inhibition of NO synthesis in virgin rats led to a significant decrease in pulmonary blood flow with no significant effects on blood flow to other vascular beds. However, in pregnant rats chronically treated with L-NAME, significant reductions in blood flow occurred in a wide variety of vascular beds, including heart, lungs, liver, and skeletal muscles (P<0.05).

Table 2 shows the regional blood flow (mL/min) and vascular resistance (mmHg/mL/min) in the uterus, ovary, and placenta in each group of rats. Pregnancy was associated with a 17-fold increase in uterine flow and a 22-fold decrease in uterine vascular resistance. Ovarian blood flow was also significantly higher and resistance was significantly lower in pregnant rats than in virgin rats and was not significantly altered in response to chronic NO synthesis inhibition in both groups. Uterine and placental flows in pregnant rats that were chronically treated with L-NAME were not statistically different from its values in the control pregnant group whether the data are expressed in total flow or flow per fetus.

Discussion

The main finding in this study is that chronic NO synthesis inhibition during mid to late gestation in rats caused a significant increase in arterial pressure associated with a significant reduction in cardiac output, increased TPR, and reduction in blood flow to different vascular beds such as the kidneys, heart, lungs, liver, and skeletal muscles. Moreover, the hemodynamic alterations in virgin rats treated with the same dose of L-NAME for the same period of time either were not observed or were significantly less than those observed in pregnant rats.
The data presented here indicate that NO may play an important role in mediating the high-output, low-resistance, low-pressure state that is a hallmark of rat gestation. Nathan et al. recently reported that in ganglion-blocked pregnant rats, inhibition of NO synthesis led to an increase in arterial pressure that was significantly greater in late pregnant rats than in nonpregnant rats. Molnar et al. similarly observed a greater increase in blood pressure in pregnant rats given L-NAME chronically during late gestation than in virgin rats. In humans, a greater reduction in forearm flow was observed in late pregnant than in nonpregnant or early pregnant women during infusion of L-NMA into the brachial artery. Therefore, it appears that NO plays an important role in gestational dilatation and decreased blood pressure during late stages of pregnancy but not during early or mid pregnancy. Other investigators, however, reported that the pressor responses to NO synthesis inhibition in late pregnant and nonpregnant rats were not statistically different. A major cause of the discrepancy between the results of these studies could be the doses used in the previous studies, which were high enough to cause marked hemodynamic changes in both virgin and pregnant rats.

Multiple mechanisms have been proposed to explain enhanced systemic vasoconstriction and accentuated arterial pressure response to NO synthesis blockade in pregnant rats. Edwards et al. recently reported that long-term NO synthesis inhibition during mid to late gestation causes sustained hypertension and elevated levels of plasma ET-1. However, whether elevated ET-1 levels are a cause of increased blood pressure or a consequence of the endothelial injury caused by hypertension due to NO synthesis blockade is still unclear. Another possible mechanism is that NO synthesis blockade in pregnant rats unmasks the vasoconstrictor effects of circulating ANG II, which is known to be elevated during pregnancy. Whether increased vascular smooth muscle tone during chronic NO synthesis inhibition in pregnant rats is due to a direct effect of reduced NO availability or indirectly due to enhanced activity of endogenous vasoconstrictors is still unclear and requires further investigation.

In addition to the peripheral vasoconstrictor effects of NO synthesis inhibition in pregnant rats, we observed a significant decrease in RBF and increase in RVR that were greater in
pregnant than in virgin rats. NO has been previously shown to play an important role in the control of renal hemodynamics in the rat. A possible role for NO in mediating the gestational increases in renal blood flow is supported by studies by Danielson and Conrad, who reported that acute blockade of NO synthesis abrogates the renal vasodilation and hyperfiltration in pregnant rats. The same group of investigators, however, demonstrated that chronic blockade of NO (48 hours) failed to prevent the renal vasodilation during midgestation. In pregnant rats mainly because of the compensatory increase in renal prostaglandins, since the vasodilation was prevented by meclofenamate. The difference between these results and the present study may be due to the gestational age when rats were examined or the dose, route, and duration of administration of L-NAME. Although the mechanism through which chronic NO inhibition increases renal vascular smooth muscle tone is unknown, it is unlikely that this effect is mediated through overexpression or increased sensitivity of endothelial vasoconstrictors, since we recently reported that L-NAME-treated pregnant rats exhibited the same degree of renal vascular response to exogenously administered ANG II as control pregnant rats. Therefore, it appears that NO may play an important role in mediating the gestational increases in RBF, further investigation is required to elucidate the mechanisms through which NO mediates renal vasodilation in pregnant rats.

We also observed a reduction in blood flow to other organs, including the heart, lungs, liver, and skeletal muscles, confirming the importance of NO in regulating regional blood flow during pregnancy. NO has been previously reported to play an important role in long-term regulation of regional blood flow in virgin rats. However, the state of NO production and its role in regulating regional blood flow during pregnancy have been studied mostly by using in vitro preparations. Using in situ blood-perfused mesenteric vessels, Chu and Beilin reported a comparable potentiation of basal perfusion pressure and vascular reactivity to l-NNA in late pregnant and nonpregnant rats. In isolated mesenteric vessels, the vasoconstrictor response to phenylephrine was attenuated in late pregnant rats compared with nonpregnant rats, and l-NNAME caused a twofold potentiation of the vasoconstrictor response in both groups. In isolated hindlimb preparations, vascular reactivity to ANG II, but not to NE, was attenuated in late pregnant rats and was enhanced by LNMA to nonpregnant levels. Although these data support our observation that NO does not play a role in regulating mesenteric blood flow in pregnant rats, the pathological significance of NO in mediating the flow alterations to other vascular beds still needs to be investigated.

During the third trimester, a dramatic increase in blood flow to the uterus and placenta occurs to fulfill the nutritional needs of the rapidly growing fetus. Recent studies have presented evidence that NO production increases not only systemically during pregnancy but also in the uterine arteries in different animal species. Total nitrite production and cGMP levels are markedly elevated in rat uterine tissue during late gestation. Furthermore, calcium-dependent NO synthase activity has been shown to be elevated during pregnancy in guinea pig uterine arteries. A recent in vitro study has also demonstrated that the basal or acutely activated endothelial-derived vasodilation is augmented in uterine arteries of late pregnant compared with nonpregnant rats, mainly because of enhanced release of NO.

On the basis of these and other studies, it has been proposed that diminished NO synthesis in the uteroplacental circulation will cause a reduction in placental perfusion. Surprisingly, we did not observe a decrease in uterine or placental blood flow after chronic NO synthesis blockade in pregnant rats. In fact, uteroplacental flow tended to be higher in l-NAME-treated pregnant rats than in controls. This observation could be explained by different mechanisms. First, chronic l-NAME administration could induce the expression of inducible NO synthase, an enzyme that is known to produce a large amounts of NO and is less susceptible to inhibition by l-NAME. Second, since placental vessels lack the autoregulation, it is possible that the increased systemic arterial pressure in l-NAME-treated pregnant rats is transmitted to the placental circulation, overcoming the lack of vasodilation due to NO deficiency. Third, it is possible that a compensatory mechanism is present in the placenta to maintain placental perfusion by increasing synthesis (or enhancing the tissue sensitivity) of other vasodilators such as prostaglandins. This explanation is supported by recent studies demonstrating that pregnancy is associated with a twofold to threefold increase in uterine artery PG12 from both the endothelium and vascular smooth muscle. Furthermore, alterations in basal uterine cAMP production are related directly to alterations in PG12 production, and NO synthesis blockade does not alter either cAMP or PG12 production. Further regional flow studies using combined NO

### TABLE 2. Regional Blood Flow (mL/min) and Vascular Resistance (mm Hg/mL/min) in Reproductive Organs in Virgin and Pregnant Rats and in Those Chronically Treated With L-NAME (1 mg/d) for 1 Week. Data Are Mean±SEM

<table>
<thead>
<tr>
<th>Organ</th>
<th>Virgin</th>
<th>L-NAME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virgin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.03±0.10</td>
<td>...</td>
</tr>
<tr>
<td>L-NAME</td>
<td>0.51±0.12</td>
<td>...</td>
</tr>
<tr>
<td>Pregnant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.69±0.84*</td>
<td>0.62±0.08</td>
</tr>
<tr>
<td>L-NAME</td>
<td>9.99±0.14</td>
<td>0.75±0.14</td>
</tr>
</tbody>
</table>

*P<0.01 in virgin versus pregnant rats

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and prostaglandin blockade or servocontrolled uterine perfusion pressure may be needed to clarify this point.

In summary, we confirm our previous observations that chronic NO synthesis inhibition during mid to late gestation in rats is associated with a significantly greater increase in arterial pressure than in virgin rats. Chronic NO synthesis inhibition in pregnant rats was also associated with significant reduction in cardiac output and an increase in total peripheral resistance. l-NNAME also caused significant reductions in blood flow to the kidneys, heart, lungs, liver, and skeletal muscles in pregnant rats. The changes in systemic and regional hemodynamics in response to NO synthesis inhibition in virgin rats either were not observed or were significantly less than in pregnant rats. The results of this study indicate that NO plays a role in mediating the alterations in systemic hemodynamics and regional flow in late pregnant rats.

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