Chronic NOS inhibition reverses systemic vasodilation and glomerular hyperfiltration in pregnancy

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We have previously suggested that pregnancy is accompanied by hemodynamic and body fluid alterations that are a consequence of primary peripheral arterial vasodilation (34, 35). In this condition, arterial vasodilation may lead to decreased blood pressure and enhanced cardiac output (CO) in association with decreased systemic and pulmonary vascular resistance, activation of the renin-angiotensin-aldosterone system (RAAS), increased thirst and nonosmotic vasopressin release, and renal sodium and water retention (34, 35). The changes in systemic hemodynamics and RAAS are detectable by 6-wk gestation in pregnant women (11). In contrast to other states of primary peripheral vasodilatation that are characterized by normal or decreased glomerular filtration rate (GFR), pregnancy is associated with a 30–50% increase in both GFR and renal plasma flow (RPF). In pregnant women, glomerular hyperfiltration is present as early as 6-wk gestation (11) and persists throughout pregnancy. This increase in GFR appears to be, at least in part, secondary to the increased RPF in pregnant rats (3).

Whereas the main mediator of the primary peripheral vasodilation in pregnancy is unknown, the nitric oxide (NO) system may be of seminal importance. NO is elevated in several vascular beds during pregnancy (25, 38, 39). Although several studies in pregnant animals and humans have demonstrated increased levels of plasma and urinary metabolites nitrate and nitrite (NOx) and cGMP, the second messenger of NO (8, 10, 15, 17, 30, 32), such levels do not necessarily correlate with vascular or renal NO activity (7). A recent study found no change in plasma or urinary NOx levels in pregnant women on a carefully controlled, reduced NOx diet (16). Acute (4 h) nitric oxide synthase (NOS) inhibition has been shown to reverse the systemic and renal vasodilatation response to pregnancy in rats (18), and more severe inhibition of NOS produces an experimental hypertensive model of preeclampsia, which includes proteinuria, thrombocytopenia, intrauterine growth retardation, and increased fetal loss (6, 31, 40). Chronic NOS inhibition in pregnant rats without causing hypertension was therefore necessary to examine the physiological role of NO in normal pregnancy. In the present study, we hypothesized that NO is an

Cadnapaphornchai, Melissa A., Mamiko Ohara, Kenneth G. Morris, Jr., Mladen Knotek, Boris Rogachev, Teri Latdkow, Ethan P. Carter, and Robert W. Schrier. Chronic NOS inhibition reverses systemic vasodilation and glomerular hyperfiltration in pregnancy. Am J Physiol Renal Physiol 280: F592–F598, 2001.—The chronic role of nitric oxide (NO), independent of prostaglandin synthesis, in the primary peripheral vasodilatation, increased glomerular filtration rate (GFR), and renal plasma flow (RPF) in normal pregnancy remains to be defined. The purpose of the present study was to chronically inhibit NOS to return systemic vascular resistance (SVR), cardiac output (CO), GFR, and RPF to nonpregnant values. Pregnant rats received the nitric oxide synthase (NOS) inhibitor, nitro-l-arginine methyl ester (L-NAME), orally from gestational days 7 through 14. Results were compared with nonpregnant and untreated pregnant rats. At 14 days gestation, CO significantly increased in pregnant vs. nonpregnant rats (187 ± 17 vs. 125 ± 10 ml/min, P < 0.05) as SVR decreased (0.64 ± 0.08 vs. 1.08 ± 0.08 mmHg·ml⁻¹·min, P < 0.05) and mean arterial pressure was unchanged (117 ± 5 vs. 125 ± 2 mmHg, not significant). Pregnant rats also demonstrated increased GFR (3,015 ± 6 vs. 2,165 ± 136 μl/min, P < 0.01) and RPF (7,869 ± 290 μl/min, P < 0.05) vs. nonpregnant rats. L-NAME-treated pregnant rats had values for CO (118 ± 7 ml/min), SVR (1.09 ± 0.07 mmHg·ml⁻¹·min), GFR (2,264 ± 150 μl/min), and RPF (5,777 ± 498 μl/min), which were no different than nonpregnant animals. In summary, similar to human pregnancy, primary peripheral vasodilatation occurs early in rat pregnancy. Furthermore, the hyperdynamic circulation and glomerular hyperfiltration of normal rat midterm pregnancy can be chronically reversed by NOS inhibition. These findings suggest a role for endothelial damage and decreased NO in the pathogenesis of preeclampsia.

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Materials. Sprague-Dawley female rats aged 12–14 wk (Harlan Sprague Dawley, Indianapolis, IN) were used. The rats were housed in a controlled environment and kept in filter-top microisolators. Animals were allowed free access to tap water and food (0.44% sodium and 22.5% protein, ProLab 3000, Agway, Syracuse, NY) until the time of study. Two female animals were housed with one male in the same cage for 2 days. Observation of the vaginal plug was used to determine gestational day 1. If no vaginal plug was noted, the second day of mating was defined as gestational day 1, and animals were monitored serially for evidence of weight gain consistent with pregnancy.

Study Design

Baseline hemodynamic parameters were established in nonpregnant rats and in pregnant rats at days 7, 14, and 20 of gestation. In subsequent studies, pregnant and nonpregnant rats were divided into four groups: nonpregnant control rats (NP-CTRL); pregnant control rats (Preg-CTRL); pregnant rats treated with the nonspecific NOS inhibitor, nitro-L-arginine-methyl-ester (L-NAME; Sigma, St. Louis, MO) in drinking water from days 7 through 14 gestation (Preg + NAME); and nonpregnant rats treated with L-NAME in drinking water for 7 days (NP + NAME). On gestational day 7 or the equivalent time in nonpregnant rats, animals were housed individually and daily water intake was monitored.

Experimental Protocols

Protocol I: baseline systemic hemodynamic parameters and plasma NOx levels in pregnant rats. Pregnant animals were maintained on ad libitum food and water intake. Animals were studied at days 7, 14, or 20 of gestation. All surgical procedures were conducted under sterile conditions. On the day before study, animals underwent placement of vascular catheters under anesthesia with ketamine (40 mg/kg body wt, IM) and xylazine (5 mg/kg body wt, IM). A 1.5-cm incision was made in the right anterior cervical neck region. A polyethylene catheter (PE-50, Intramedic, Clays Adams, Parsippany, NJ) was inserted 2 cm into the right carotid artery to obtain adequate flow. A second PE-50 catheter was placed 2 cm into the right jugular vein to allow creation of a carotid artery-to-jugular vein loop for measurement of CO. A third PE-50 catheter was placed into the right jugular vein to allow injection of indocyanine green dye (0.05 ml) into the PE-50 right jugular vein catheter with pumping of blood at 3 ml/min from the carotid artery through a Waters cuvette into the right jugular vein (total extracorporeal volume ~0.1–0.2 ml). Three sequential CO were measured over a few-minute period, calculated by computer, and averaged. The animal’s blood was returned at the end of the measurements. Systemic vascular resistance (SVR) was calculated as MAP/CO; MAP, CO, and SVR measurements were compared with age-matched nonpregnant rats. At the conclusion of the experiment, animals were killed by decapitation. Trunk blood was collected for measurement of plasma NOx levels.

Protocol II: determination of l-NAME dose needed to normalize systemic hemodynamics in midpregnant rats. From days 7 through 14 of pregnancy, female Sprague-Dawley rats received l-NAME at different oral doses (4, 6, or 8 mg/l) in drinking water. Four animals were studied per dose. Daily urine volumes were recorded. On gestational day 14, rats underwent placement of vascular catheters as described above. On gestational day 14, MAP and CO were measured in conscious animals. Measurements were compared with age-matched nonpregnant rats receiving tap water. The dose of l-NAME that returned CO in day 14 pregnant rats to nonpregnant levels without inducing hypertension was 6 mg/l, thus this dose was used for the remainder of the studies.

Protocol III: effect of chronic NOS inhibition on systemic and renal hemodynamics in midpregnant rats. Pregnant and nonpregnant animals received tap water or l-NAME, 6 mg/l in drinking water, as described above. On day 13, rats were anesthetized and vascular catheters were placed. Additionally, a cannula (PE-50) was placed in the bladder through a 1-cm transverse abdominal incision. The bladder catheter was plugged and tunneled subcutaneously under the abdominal skin. Interrupted sutures were placed to close the skin layer and to facilitate later exteriorization of the bladder catheter. Animals were able to void spontaneously through the urethra until the time of the experiment. All surgical procedures were conducted under sterile conditions. Subsequent studies were performed on conscious animals. On day 14, MAP, CO, and HR were measured in animals receiving l-NAME. Systemic hemodynamics were also measured simultaneously in some pregnant and nonpregnant control animals and compared with previously obtained values (protocol I) to verify consistency of technique. The animal’s urethra was blocked with medical adhesive with no observed evidence of discomfort. The bladder catheter was exteriorized by removal of 1–2 interrupted sutures and unplugged for timed urine collection before study. The animal was placed in a 12 × 6-inch cage for renal hemodynamic studies. A 50-μl blood sample was removed through the arterial line for measurement of hematocrit. Subsequently, an infusion of inulin (70 mg/l) and p-amminohippurate (PAH; Sigma, St. Louis, MO) (20 mg/l) was given intravenously at a rate of 2 ml/h after a loading dose. After an equilibration time of 60 min, three 20-min urine collections were performed and the urinary volumes measured. One hundred fifty-microliter arterial blood samples were taken at the midpoint of each urine collection. After centrifugation, the red cells were diluted with sterile 0.9% saline and reinfected. Inulin concentrations in plasma and urine samples were measured by the anthrone method (23). GFR was calculated as inulin clearance; RPF was calculated as PAH clearance corrected for PAH extraction (RPF = PAH clearance/0.85) (37).
Baseline systemic hemodynamic parameters and plasma NOx levels in normal rat pregnancy. MAP and CO were measured in rats at days 7, 14, and 20 of pregnancy (Table 1). MAP was not decreased until late in rat pregnancy; however, CO began to rise early in gestation, with significant increases noted by day 14 and persisting through day 20. As anticipated, calculated SVR was mildly decreased in early pregnancy with further decreases noted at mid- to late gestation. Plasma NOx levels were increased during pregnancy, with statistically significant elevations at early and midpregnancy (Fig. 1).

Determination of L-NAME dose needed to normalize systemic hemodynamics in midpregnant rats (Table 2). L-NAME was administered in pregnant rats (n = 4/group) in drinking water at doses of 4, 6, or 8 mg/l from day 7 to 14 gestation. Systemic hemodynamics were measured and compared with NP-CTRL. The average daily dose of L-NAME differed significantly between the treatment groups. Pregnant rats demonstrated a significant increase in MAP on the 8 mg/l dose of L-NAME. Treatment of pregnant rats with L-NAME at a dose of 4 mg/l did not return CO to nonpregnant levels. As expected, calculated SVR was mildly decreased in the 4 mg/l L-NAME dose group and significantly increased in the 8 mg/l L-NAME dose group compared with nonpregnant controls. Therefore, the dose of L-NAME that returned CO in day 14 pregnant rats to nonpregnant levels without inducing hypertension was 6 mg/l; thus this dose was used for the remainder of the studies.

Water intake and L-NAME dosing in midpregnant rats. Daily water intake from gestational days 7 to 14 (and for a comparable time period in nonpregnant rats) was used to assess the L-NAME dose received. Average daily water intake was increased in pregnant control (n = 5) and pregnant treated (n = 13) rats compared with nonpregnant controls (n = 14) (NP-CTRL 33 ± 1 ml/day; Preg-CTRL 43 ± 2 ml/day, P < 0.05 vs. NP-CTRL; Preg + NAME 44 ± 2 ml/day, P < 0.001 vs. NP-CTRL). Water intake in L-NAME-treated nonpregnant rats (n = 4) did not differ from the other study groups [NP-NAME 38 ± 5 ml/day, P not significant (NS)]. There was no difference in mean daily L-NAME dose in the treatment groups (Preg + NAME 0.27 ± 0.01 mg/day, NP + NAME 0.23 ± 0.03 mg/day, P NS).

Biochemical measurements. Plasma NOx levels were measured by using a Sievers NO chemiluminescence analyzer (Model 270B, Sievers, Boulder, CO). Plasma NOx levels were not evaluated in L-NAME-treated animals, as readings became uninterpretable due to the presence of L-NAME or its derivatives in the plasma samples (26).

Statistical analysis. Statistical analysis was performed by using unpaired Student’s t-test or ANOVA test followed by Tukey’s post hoc test. P < 0.05 was considered significant. Results are expressed as means ± SE.

**RESULTS**

**Table 1. Systemic hemodynamic measurements during rat pregnancy**

<table>
<thead>
<tr>
<th></th>
<th>MAP, mmHg</th>
<th>n</th>
<th>CO, ml/min</th>
<th>n</th>
<th>SVR, mmHg·ml⁻¹·min</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>NP</td>
<td>120 ± 2</td>
<td>14</td>
<td>125 ± 10</td>
<td>14</td>
<td>1.11 ± 0.08</td>
<td>14</td>
</tr>
<tr>
<td>Pregnant day 7</td>
<td>134 ± 2</td>
<td>8</td>
<td>148 ± 12</td>
<td>6</td>
<td>0.80 ± 0.08</td>
<td>6</td>
</tr>
<tr>
<td>Pregnant day 14</td>
<td>117 ± 4</td>
<td>5</td>
<td>187 ± 17‡</td>
<td>4</td>
<td>0.64 ± 0.08‡</td>
<td>4</td>
</tr>
<tr>
<td>Pregnant day 20</td>
<td>101 ± 4*</td>
<td>6</td>
<td>192 ± 5‡</td>
<td>3</td>
<td>0.48 ± 0.03‡</td>
<td>3</td>
</tr>
</tbody>
</table>

Values are means ± SE. n, No. of rats; nonpregnant, NP; mean arterial pressure, MAP; cardiac output, CO; and calculated systemic vascular resistance (SVR) were studied at days 7, 14, and 20 of gestation and compared with nonpregnant values. *P < 0.001 vs. NP; ‡P < 0.01 vs. NP; †P < 0.05 vs. NP.

**Fig. 1. Plasma nitrate and nitrite (NOx) levels are elevated in normal rat pregnancy.** Study groups include nonpregnant (NP), day 7 pregnant, day 14 pregnant, and day 20 pregnant rats. Values are reported as means ± SE. *P < 0.01 vs. NP.

Water intake and L-NAME dosing in midpregnant rats. Daily water intake from gestational days 7 to 14 (and for a comparable time period in nonpregnant rats) was used to assess the L-NAME dose received. Average daily water intake was increased in pregnant control (n = 5) and pregnant treated (n = 13) rats compared with nonpregnant controls (n = 14) (NP-CTRL 33 ± 1 ml/day; Preg-CTRL 43 ± 2 ml/day, P < 0.05 vs. NP-CTRL; Preg + NAME 44 ± 2 ml/day, P < 0.001 vs. NP-CTRL). Water intake in L-NAME-treated nonpregnant rats (n = 4) did not differ from the other study groups [NP-NAME 38 ± 5 ml/day, P not significant (NS)]. There was no difference in mean daily L-NAME dose in the treatment groups (Preg + NAME 0.27 ± 0.01 mg/day, NP + NAME 0.23 ± 0.03 mg/day, P NS).

Systemic hemodynamics during chronic NOS inhibition (Fig. 2). There was no difference in MAP among the study groups. As previously noted, CO was significantly increased at midgestation compared with nonpregnant levels. Thus calculated SVR was significantly decreased at midpregnancy. These alterations in CO and SVR were reversed with L-NAME treatment. Seven days of L-NAME treatment in nonpregnant rats (NP + NAME) did not affect systemic hemodynamics (MAP 127 ± 2 vs. 125 ± 2 mmHg, P NS; CO 96 ± 4 vs. 125 ± 10 ml/min, P NS; SVR 1.33 ± 0.04 vs. 1.08 ± 0.08 mmHg·ml⁻¹·min, P NS) compared with nonpregnant controls.

Renal hemodynamics during chronic NOS inhibition (Fig. 3). Day 14 pregnant rats demonstrated glomerular hyperfiltration and renal vasodilation compared with nonpregnant animals. Treatment with L-NAME returned GFR and RPF to nonpregnant levels. Nonpregnant animals treated with L-NAME demonstrated a decrease in both GFR (1,382 ± 109 vs. 2,165 ± 136 ul/min, P < 0.01) and RPF (3,915 ± 336 vs. 5,507 ± 290 ul/min, P NS) compared with nonpregnant controls.
DISCUSSION

Preeclampsia is the leading cause of morbidity and mortality in the mother and the fetus, yet the mechanisms underlying preeclampsia have not been well defined. It is essential to understand the physiology of normal pregnancy to better address the pathological alterations that result in preeclampsia. Experiments in the present study were designed to evaluate the role of NO in the hyperdynamic circulation of normal pregnancy.

The pregnant rat has been used as a model for the study of human pregnancy. There are, however, some apparent differences in the time course of hemodynamic changes in human and rat pregnancy. Pregnant women demonstrate decreased blood pressure in the first trimester of pregnancy (11). Decreased blood pressure does not occur until the last trimester of pregnancy in the rat (24, 36). This suggests that the primary vasodilation may occur at profoundly different times in human and rat pregnancy. It is conceivable, however, that primary arterial vasodilation also occurs early in rat gestation but is compensated for by an early rise in CO, which stabilizes blood pressure. In this regard, we observed an early trend of decreased SVR and increased CO in pregnant rats (Table 1). The observed alterations in systemic hemodynamics at day 7 of rat gestation did not reach statistical significance compared with the nonpregnant state. This is consistent with prior reports that did not find a significant increase in CO or decrease in SVR at day 7 of rat pregnancy using the Fick methodology (24). A significant rise in CO at day 8 of rat pregnancy using an electromagnetic flow probe measurement has, however, been found (36). In our study, CO and SVR at day 14 of gestation were statistically different from the nonpregnant rat and remained altered through late pregnancy. These data support an early onset of primary arterial vasodilation in rat pregnancy between 8–14 days gestation, an observation similar to that of human pregnancy.

Rising plasma NOx concentrations during rat pregnancy suggest a potential role for NO in primary arterial vasodilation. In the present study, plasma NOx levels were most dramatically elevated in early to midgestation in the rat (Fig. 1) and were sustained, albeit to a lesser degree, into late gestation. A prior report has documented an increase in 24-h urinary excretion of NOx by day 6 of gestation in the rat; plasma NOx concentrations were examined and found to be elevated at mid and late gestation (15). The present results at day 7 of gestation demonstrate the earliest elevation in plasma NOx levels observed in rat pregnancy. It must be emphasized, however, that plasma measurements of NOx may not directly correlate with local renal or vascular NO activity (7).

Several studies have evaluated NOS inhibition in pregnancy (18, 19, 22, 29). In these experiments, NOS inhibition prevented the renal and systemic vasodilation response to pregnancy. However, NOS inhibition was associated with significant hypertension, thus producing a potential model of preeclampsia rather than normal pregnancy. To investigate the physiological role of NO, we performed a preliminary dose-response study to determine the dose of l-NAME that would normalize CO for 7 days without inducing an elevation in blood pressure. With an l-NAME dose of 6 mg/l in drinking water, we were able to reverse peripheral vasodilation in pregnant animals (Table 2; Fig. 2).

Primary peripheral vasodilation is often associated with a decline in GFR, as in patients with cirrhosis (33, 34). In contrast, pregnancy is the unique state where systemic arterial vasodilation is associated with glomerular hyperfiltration. In normal human pregnancy, GFR and RPF increase by 30–50% over nonpregnant values. In preeclampsia, however, renal hemodynamics are relatively decreased in association with proteinuria and hypertension, with GFR being more significantly affected than RPF (27, 28). As noted previously, NOS inhibition produces several maternal/fetal manifestations of preeclampsia in animals (6, 31, 40). Our data support a function for NO in the increased renal hemodynamics of normal pregnancy. We observed increases of ~40% in both GFR and RPF during midgestation in the rat, and NOS inhibition in pregnant rats returned the renal hemodynamics to nonpregnant levels (Fig. 3).

There is evidence to support an interaction between NO and vasodilatory prostaglandins in the renal vasodilation of pregnancy. Whereas cyclooxygenase inhibition alone does not decrease GFR and RPF in pregnant animals (2, 4, 13), a study by Danielson and Conrad (19) indicated that prostaglandins can be recruited to maintain renal vasodilation and hyperfiltration during 48 h of NOS inhibition in pregnant rats. We have utilized a more chronic (7 day) model of pregnancy with NOS inhibition. In these studies, NOS inhibition returned GFR and RPF to nonpregnant levels without prostaglandin inhibition. These results support a role...
for NO as a mediator of glomerular hyperfiltration and renal vasodilation in rat pregnancy.

The comparison of systemic and renal hemodynamics in pregnancy with and without NOS inhibition was the primary purpose of the present experiments. It should be stated, however, that although L-NAME-treated nonpregnant rats may not be appropriate controls because of the markedly different hormonal milieu in pregnant vs. nonpregnant rats, a decrease in GFR and RPF was observed in nonpregnant rats receiving L-NAME. Thus whereas the present studies support the role of NO in systemic and renal vasodilation in pregnant rats, the results do not exclude additional factors.

The results of this study therefore emphasize a role for the NO system in the peripheral vasodilation and glomerular hyperfiltration of normal pregnancy. Recent studies provide some information about systems influencing NO action in pregnancy. For example, in vitro studies have shown that estrogen can stimulate NO synthesis in vascular endothelium. Although the underlying molecular mechanisms are not well understood, estrogen may activate endothelial NOS (12). In addition, estrogen increases gene expression for inducible NOS in vascular smooth muscle denuded of endothelium (9). It has been reported that this NOS isoform is increased in whole kidney during rat pregnancy (1) and thus could be important in increased renal hemodynamics of pregnancy. Endothelin-1 (ET-1) has also been suggested to mediate renal vasodilation via the NO system. A recent study found that ET-B receptor blockade in pregnant rats returned GFR and RPF to nonpregnant levels while inducing significant hypertension (14). These physiological changes were similar to those seen in rats receiving L-NAME alone or the combination of L-NAME and ET-B receptor blockade, suggesting that ET-1 and NOS might act via a common vasodilatory pathway. There is also evidence to suggest that the ovarian hormone relaxin induces renal vasodilation and glomerular hyperfiltration through endothelin and NO (20, 21).

In summary, we have demonstrated a role for NO in the maintenance of peripheral arterial vasodilation in normal pregnancy. Chronic NOS inhibition also reversed glomerular hyperfiltration and renal vasodilation in midterm pregnant rats independent of prostaglandin inhibition. The present results are compatible with endothelial damage leading to impaired NO synthesis as a pathogenetic factor in preeclampsia. However, further studies are necessary to support this hypothesis, and there are no doubt factors in addition to NO deficiency involved in the preeclamptic state.

Fig. 2. Chronic nitric oxide synthase (NOS) inhibition returns cardiac output (CO) and systemic vascular resistance (SVR) in day 14 pregnant rats to nonpregnant levels. Values are means ± SE for mean arterial pressure (MAP; A), CO (B), and calculated SVR (C). *P < 0.05 vs. NP-control (CTRL) and vs. pregnant (Preg) + nitro-L-arginine methyl ester (NAME).

Fig. 3. Chronic NOS inhibition reverses glomerular hyperfiltration and renal vasodilation in day 14 pregnant rats. A: glomerular filtration rate (GFR); B: renal plasma flow (RPF). *P < 0.05 vs. NP-CTRL and vs. Preg + NAME; **P < 0.01 vs. NP-CTRL.
REFERENCES


