Inducible Nitric Oxide Synthase Inhibition Attenuates Renal Hemodynamics During Pregnancy

Barbara T. Alexander, Kathy Cockrell, Farrah D. Cline, Joey P. Granger

Abstract—Acute, nonselective nitric oxide synthase inhibition in the pregnant rat decreases glomerular filtration rate and renal plasma flow, suggesting a role for nitric oxide in mediating renal vasodilation during pregnancy. As mid-gestation in the rat is associated with a significant increase in renal protein expression of inducible nitric oxide synthase, the aim of this study was to examine the role of inducible nitric oxide synthase in mediating renal hemodynamics changes at mid-gestation in the rat. At day 16 of pregnancy, glomerular filtration rate was significantly higher in pregnant rats compared with virgin rats (3.1±0.4 versus 2.7±0.3 mL/min, respectively; P<0.05), as was effective renal plasma flow (13.4±2.5 versus 10.9±2.2 mL/min, respectively; P<0.05). Acute administration of the inducible nitric oxide synthase selective inhibitor, AMT hydrochloride (750 nmol/h), markedly attenuated the increase in glomerular filtration rate observed in pregnant rats (2.3±0.2 mL/min, P<0.01 versus pregnant) without significantly altering glomerular filtration rate in virgin rats (2.1±0.2 mL/min). Acute AMT administration significantly decreased effective renal plasma flow in pregnant (8.9±1.8 mL/min, P<0.01 versus pregnant) and virgin rats (7.1±0.9 mL/min, P<0.05 versus virgin). Acute administration of EIT (380 nmol/h), another inducible nitric oxide synthase selective inhibitor, also attenuated pregnancy-induced increases in glomerular filtration rate (2.1±0.2, 2.8±0.3, and 2.3±0.3 mL/min; virgin, pregnant, and EIT, respectively) and effective renal plasma flow (8.5±1.1, 13.8±2.1, and 9.0±1.1 mL/min; virgin, pregnant, and EIT, respectively). Therefore, these findings suggest that inducible nitric oxide synthase may play an important role in mediating the renal hemodynamic changes that occur during normal pregnancy. (Hypertension. 2002;36[part 2]:586-590.)

Key Words: pregnancy ■ nitric oxide synthase ■ kidney ■ hemodynamics ■ rats

Normal pregnancy is associated with significant increases in renal hemodynamics as increases in glomerular filtration rate (GFR) and renal plasma flow (RPF) of >40% are observed in pregnant women.1 In the rat, GFR and RPF are increased by >20% at mid-gestation with a return to prepregnant values by late pregnancy.2,3 These increases in renal hemodynamics at mid-gestation in the rat are associated with significant increases in whole-body nitric oxide (NO) production4,5 and renal protein expression of both inducible and neuronal nitric oxide synthase (iNOS and nNOS, respectively). A role for NO in mediating renal vasodilation during pregnancy is suggested in a study by Danielson and Conrad in which acute nonselective NOS inhibition equalized GFR and effective RPF (ERPF) in virgin rats and pregnant rats at mid-gestation.5 However, nonselective inhibition of all three NOS isoforms, endothelial (eNOS), neuronal (nNOS), and inducible (iNOS), does not elucidate which specific isoform is an important source of NO during pregnancy.

Several lines of evidence suggest that NO generated by the iNOS isoform may play an important role in mediating renal function and arterial pressure under various physiological conditions, thus suggesting a role for iNOS in mediating renal hemodynamic changes during pregnancy. NO generated by iNOS is suggested to play an important role in the regulation of renal function and arterial pressure in normal Sprague-Dawley rats.6 In addition, iNOS has been suggested to play an important role in mediating salt-induced hypertension in normotensive Sprague-Dawley rats7 and the Dahl salt-resistant rat8 in mediating corticosterone-induced hypertension.9 In rats with liver cirrhosis, maintenance of GFR by iNOS.10 Thus, as the functional role of the inducible NOS isoform in mediating increases in renal hemodynamics during pregnancy is not clear, the purpose of this study was to examine the effect of iNOS selective inhibitors on renal hemodynamics in pregnant rats.

Methods

All studies were performed in virgin or 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

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animals with all protocols approved by the Animal Care and Use Committee at the University of Mississippi Medical Center.

Experimental Design

The identical protocol was followed for both iNOS selective inhibitors. Animals were divided into two groups, virgin and pregnant, with each group serving as its own control. Inducible nitric oxide synthase inhibitors, AMT (hydrochloride) (4H-1,3-Thiazin-2-amine, 5,6-dihydro-6-methyl-, [monohydrochloride], Cayman Chemicals) administered at a dose of 0.125 mg/h or 750 nmol/h, virgin (n=8) and pregnant (n=9); and EIT (S-Ethylisothiourea, Cayman Chemicals), 0.375 mg/h or 380 nmol/h, virgin (n=10) and pregnant (n=11), were administered acutely with each animal serving as its own control. All drugs were made fresh the day of the study. Dosage for each drug was based on studies published in the literature.8,11 At day 14 of gestation, pregnant rats were instrumented with catheters; virgin rats on day 1 of the protocol. On day 16 of gestation, renal function and arterial pressure measurements were performed in pregnant rats; virgin rats on day 3 of the protocol.

Measurement of Renal Hemodynamics and Arterial Pressure in Conscious Rats

In brief, all rats undergoing surgical procedures were anesthetized with 2% isoflurane delivered by an anesthesia apparatus and surgically instrumented with catheters in the femoral vein, carotid artery, and bladder as previously described.12 Renal function and arterial pressure measurements were performed in conscious rats with glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) calculated from radioactivity of sodium 125Iothalamate and concentration of para-aminohippurate, respectively, as described previously.12 Specifically, for determination of the acute effects of selective iNOS inhibition on the renal hemodynamics during pregnancy in the rat, after a 60-minute stabilization period, a 20-minute urine collection was obtained followed by collection of a blood sample. After the control measurement, iNOS selective inhibitor was administered for 30 minutes. Following a 30-minute stabilization period, another 20-minute urine collection was obtained followed by the final blood sample collection.

Statistical Analysis

All data are expressed as mean±SEM. Statistical analysis was performed using GB-Stat. Comparisons were made using analysis of variance for repeated measures with a value of P<0.05 considered statistically significant.

Results

GFR was significantly increased in pregnant rats at day 16 of gestation as compared with virgin rats (3.1±0.4 versus 2.7±0.3 mL/min, pregnant versus virgin, respectively; P<0.05) (Figure 1). Acute inhibition of iNOS by use of the iNOS selective inhibitor, AMT (0.125 mg/h), resulted in a significant decrease in GFR in the pregnant treated rats compared with pregnant untreated rats (2.3±0.2 mL/min, P<0.01) at day 16 of gestation. However, selective inhibition of iNOS using AMT resulted in no significant change in GFR in the virgin treated rats compared with virgin untreated rats (2.1±0.2 mL/min) (Figure 1).

As shown in Figure 2, ERPF was also significantly increased in pregnant rats at day 16 of gestation compared with virgin rats (13.4±2.5 versus 10.9±2.2 mL/min, pregnant versus virgin, respectively; P<0.05) (Figure 2). Acute inhibition of iNOS using the iNOS selective inhibitor, AMT (0.125 mg/h), resulted in a significant decrease in ERPF in both the pregnant treated (8.9±1.8 mL/min, P<0.01 versus pregnant untreated, both at day 16 of gestation) and virgin treated rats (7.1±0.9 mL/min, P<0.05 versus virgin untreated).

The iNOS selective inhibitor, AMT, was administered acutely at a dose that resulted in significant alterations in GFR in pregnant rat at mid-gestation but not virgin rats. This dose did not significantly alter mean arterial pressure (MAP) in virgin treated rats as compared with the virgin untreated rats (126±3 versus 129±2 mm Hg, virgin treated versus virgin untreated, respectively) (Figure 3). However, at day 16 of gestation, MAP was significantly altered in the pregnant treated rats relative to pregnant untreated rats, as MAP
increased from 123±3 mm Hg in the pregnant untreated rats to 130±5 mm Hg in the pregnant treated rats (P<0.05) (Figure 3a). In addition, MAP did not differ significantly on comparison of pregnant to virgin rats. Renal vascular resistance (RVR), although not significant, was reduced at day 16 in pregnant rats as compared with virgin rats (9±2 versus 11±3 mm Hg/ml per min, pregnant versus virgin, respectively) (Figure 3b). RVR was not significantly increased in virgin rats on acute iNOS inhibition (12±2 mm Hg/ml per min, virgin treated), but it was significantly increased in pregnant rats (14±3 mm Hg/ml per min, pregnant treated, P<0.05 versus pregnant untreated).

The iNOS selective inhibitor, EIT, was also administered to virgin and pregnant rats at day 16 of gestation to confirm that the changes in renal hemodynamics observed on iNOS blockade by AMT were specific to the iNOS isoform. GFR was significantly increased in pregnant rats relative to virgin rats (2.8±0.3 versus 2.1±0.2 ml/min, pregnant versus virgin, respectively; P<0.05) as was ERPF (13.8±2.1 versus 9.0±1.1 ml/min, pregnant versus virgin, respectively; P<0.05). Acute administration of EIT led to a decrease in GFR in pregnant rats (2.3±0.3 ml/min) without altering GFR in virgin (2.2±0.2 ml/min) rats. On acute EIT administration, ERPF was reduced in pregnant (8.5±1.1 ml/min, P<0.05 versus pregnant) and virgin (5.5±0.5 ml/min, P<0.05 versus virgin) rats. In this study, there was a marked decrease in MAP in pregnant rats as compared with virgin rats (124±3 versus 136±3 mm Hg, pregnant versus virgin, respectively; P<0.05). MAP was increased on acute EIT administration in both pregnant treated (144±4 mm Hg, P<0.05 versus pregnant) and virgin treated (151±5 mm Hg, P<0.05 versus virgin) rats. RVR was significantly increased in both virgin (12±2 versus 20±3 mm Hg/ml per min, virgin versus virgin untreated; P<0.05) and pregnant (8±1 versus 16±4 mm Hg/ml/min, pregnant versus pregnant untreated; P<0.05) rats on short-term iNOS blockade using the selective iNOS inhibitor, EIT.

Discussion

Mid-gestation in the rat is associated with significant increases in GFR and ERPF of approximately 20% to 30%. In addition, renal protein expression of the iNOS isoform is significantly increased at mid-gestation in the rat by approximately 30% as compared with prepregnant value. As this increase in renal protein expression of iNOS is associated with an increase in renal hemodynamics, a role for the iNOS isoform in mediating pregnancy-induced renal vasodilation is suggested. The purpose of this study, therefore, was to examine the importance of the iNOS isoform in mediating the changes in renal hemodynamics that occur during midgestation in the rat.

To examine the role of the iNOS isoform in mediating pregnancy-induced increases in renal hemodynamics, we used reversible, competitive inhibitors of the inducible NO synthase, AMT and EIT. AMT and EIT are approximately 10- to 40-fold more selective for the iNOS isoform than the nNOS or eNOS isoforms. As selectivity for the iNOS isoform may be lost at higher doses, short-term blockade by AMT was administered at a dose that resulted in a significant decrease in GFR in pregnant rats without increasing systemic blood pressure in virgin rats. In addition, this dose has previously been shown by others to not block acetylcholine-induced vasodilation, suggesting that effects produced are specific to the iNOS isoform and not due to blockade of the eNOS isoform. AMT was also administered at a higher dose that resulted in a significant increase in MAP in both pregnant and virgin but still attenuated the significant increase in both GFR and ERPF observed in pregnant rats as compared with virgin rats (data not shown).

A second iNOS selective inhibitor, EIT, was also used to discern that the effects of acute AMT blockade were specific to the iNOS isoform. In this study, however, MAP was increased on acute administration of EIT in both pregnant and virgin rats. Although EIT was administered at varying concentrations, concentrations of EIT that resulted in a change in renal hemodynamics also resulted in an increase in MAP within both pregnant and virgin animals. Thus, a difference due to specificity between the two iNOS selective inhibitors, EIT and AMT, was evident and may account for the observed variations in blood pressure.

Significant increases in renal hemodynamics at mid-gestation in the pregnant rat were attenuated on acute selective iNOS blockade as GFR was decreased by an average of 22% and ERPF by an average of 36%. Specifically, administration of AMT resulted in a decrease in GFR of 26% and a decrease in ERPF of 33%. Similar effects were observed in pregnant rats at mid-gestation on acute administration of EIT (GFR, 18%; ERPF, 39%).

Acute iNOS blockade by the iNOS selective inhibitor, AMT, resulted in no effect on GFR in virgin treated rats. In addition, AMT was administered acutely at a dose that resulted in no significant change in MAP in virgin treated rats but a significant increase in MAP in pregnant treated rats. Within this study, there was no significant difference in MAP on comparison of untreated pregnant with untreated virgin rats, an observation that is consistent with other studies in which decreases in MAP are not usually observed until term in pregnant rats. Although not significant, RVR was lower in pregnant rats as compared with virgin rats. RVR increased by approximately 45% in the mid-pregnant rats on acute AMT administration, with no significant effect on RVR observed in the virgin rats treated. In the rat, RVR has been shown to decrease at mid-gestation and return to prepregnant values at term. Thus, in this study, pregnant rats were more responsive to acute iNOS blockade by AMT as compared with virgin rats. Within the kidney, inducible NO synthase may be both constitutively and differentially expressed, suggesting its importance as a mediator of renal hemodynamics under normal physiological conditions and capable of induction in response to physiological stimuli. Steady-state production of iNOS within the outer medulla, particularly within the medullary thick ascending limb, has been suggested in a study by Morrissey, et al. Furthermore, observation of a 2- to 3-fold induction of iNOS mRNA expression in the outer medulla by endotoxin indicates that iNOS is also capable of induction on stimulation. In addition, NO generated by iNOS has been shown to play an important role in the maintenance
of renal hemodynamics and in the long-term control of arterial pressure under both normal physiological conditions and in response to pathophysiological conditions.\textsuperscript{6,7,8,9,10} Therefore, as iNOS may be both constitutively and inducibly expressed within the kidney, this data suggests that the renal hemodynamic changes that occur during pregnancy may be mediated in part by the iNOS isoform.

As NO may play an important role in normal pregnancy, NO deficiency may play an important role in preeclampsia.\textsuperscript{17,18} Preeclampsia, a pregnancy specific disorder that develops during pregnancy and ceases after delivery, is generally associated with increases in arterial pressure, decreases in GFR and RPF, increased responsiveness to vasoconstrictors, and proteinuria.\textsuperscript{17,19} Chronic nonselective NOS inhibition in the pregnant rat produces hypertension, proteinuria, and a reduction in kidney function, suggesting that NO deficiency may contribute to the clinical manifestations of preeclampsia.\textsuperscript{14} In a rat model of preeclampsia or pregnancy-induced hypertension produced in response to a chronic reduction in uterine perfusion pressure in the pregnant rat, alterations in renal protein expression of the NOS isoforms are associated with hypertension, variable proteinuria, and reductions in renal hemodynamics.\textsuperscript{20} Specifically, renal iNOS protein expression is decreased by 11\%, and renal protein expression of nNOS is significantly decreased by 31\% in response to a chronic reduction in uterine perfusion pressure in the pregnant rat,\textsuperscript{20} thus suggesting a role for the NOS isoforms in mediating hypertension in this pregnancy-specific disorder. A role for the nNOS isoform in mediating systemic and renal hemodynamic changes during normal pregnancy is also suggested as renal expression of nNOS is significantly increased by approximately 25\% at mid-gestation in the rat.\textsuperscript{20} Additionally, acute inhibition of nNOS by use of the selective nNOS inhibitor, 7-nitroindazole, significantly decreases GFR and ERPF at mid-gestation in pregnant rats at a dose that has no effect in virgin rats.\textsuperscript{21} Thus, regulation of renal hemodynamic changes that occur during normal pregnancy may be mediated in part by both the nNOS and the iNOS isoforms; however, the specific role for each of these isoforms remains unknown.

Meditators of NOS isoform expression during pregnancy also remain unknown. During pseudopregnancy in the rat kidney vasodilation and urinary excretion of nitrite are increased, implicating involvement of a maternal factor in the absence of a fetoplacental unit.\textsuperscript{2,22} Relaxin, a peptide hormone product of the female reproductive organs, has been suggested to have many roles, including stimulation of NO generation.\textsuperscript{23} During human pregnancy, the maximum concentration of circulating relaxin occurs during the first trimester.\textsuperscript{24} Within the rat, relaxin is not detected until day 10 of gestation and increases steadily during mid-gestation with a significant peak occurring at day 21.\textsuperscript{25} A role for relaxin in mediating renal hemodynamic changes during pregnancy is suggested in a study by Danielson and Conrad wherein a chronic infusion of a physiologically relevant dose of human recombinant relaxin induces renal hemodynamic changes in nonpregnant rats comparable to that observed in mid-term pregnant rats.\textsuperscript{26} Furthermore, as acute nonselective NOS inhibition prevents the relaxin-induced renal vasodilation response, a role for NO in mediating relaxin-induced renal vasodilation is suggested.\textsuperscript{26} Thus, relaxin may serve as a mediator of renal vasodilation during pregnancy through interactions with the NOS isoforms. However, the exact relationship between relaxin and the NOS isoforms is yet to be elucidated.

In summary, we found that GFR and ERPF were significantly higher in pregnant rats as compared with virgin rats. Acute administration of the iNOS selective inhibitor, AMT, markedly attenuated the increase in GFR observed in pregnant rats without significantly altering GFR in virgin rats. Acute AMT administration also significantly decreased ERPF in pregnant rats. In addition, acute AMT administration resulted in a significant increase in arterial pressure and RVR in pregnant rats at mid-gestation with no significant effect in virgin rats. Therefore, these findings suggest that the inducible NO synthase isoform may play an important role in mediating the renal hemodynamic changes that occur during normal pregnancy.

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