Role of neuronal nitric oxide synthase in mediating renal hemodynamic changes during pregnancy

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Abram, S. R., B. T. Alexander, W. A. Bennett, and J. P. Granger. Role of neuronal nitric oxide synthase in mediating renal hemodynamic changes during pregnancy. Am J Physiol Regulatory Integrative Comp Physiol 281: R1390–R1393, 2001.—Renal plasma flow (RPF) and glomerular filtration rate (GFR) during pregnancy are markedly increased as compared with pregnancy in rats associated with enhanced renal protein expression of neuronal nitric oxide synthase (nNOS). The purpose of this study was to determine the role of nNOS in mediating renal hemodynamic changes observed during pregnancy. To achieve this goal, we examined the effects of the nNOS inhibitor 7-nitroindazole (7-NI) on kidney function in normal conscious, chronically instrumented virgin (n = 6) and pregnant rats (n = 9) at day 16 of gestation. Infusion of 7-NI had no effect on RPF (4.7 ± 0.7 vs. 4.8 ± 0.9 ml/min), GFR (2.2 ± 0.2 vs. 2.5 ± 0.4 ml/min), or mean arterial pressure (MAP; 127 ± 7 vs. 129 ± 10 mmHg) in virgin rats. In contrast, 7-NI infused into pregnant rats decreased RPF (8.9 ± 1.6 vs. 6.5 ± 1.4 ml/min) and GFR (4.4 ± 0.7 vs. 3.3 ± 0.5 ml/min) while having no effect on MAP (123 ± 4 vs. 123 ± 3 mmHg). In summary, inhibition of nNOS in pregnant rats at midgestation results in significant decreases in RPF and GFR. NOS inhibition in virgin rats had no effect on renal hemodynamics. These data suggest that NOS may play a role in mediating the renal hemodynamic changes that occur during pregnancy.

endothelial factors; kidney; glomerular filtration rate; renal plasma flow

Gestation in humans is characterized by distinct physiological changes in maternal systemic circulation (11). Systemic vasodilation and reduced vascular reactivity typifies normal pregnancy (27). Increases in glomerular filtration rate (GFR) and renal blood flow (RBF) and decreases in mean arterial pressure occur during pregnancy in humans and in various animal models (16). Nitric oxide (NO), a simple bioactive molecule synthesized from L-arginine by several isoforms of NO synthase (NOS), has been implicated in mediating these and other dynamic responses that occur during pregnancy (2, 3, 5, 9, 20, 24, 27).

Previous studies have shown that NO synthesis is markedly elevated during pregnancy and nonselective inhibition of NO production in the pregnant animal increases arterial pressure and renal vascular resistance (RVR) and decreases GFR and RBF (6, 7, 11, 19). Although these studies suggest NO plays an important role in mediating the renal hemodynamic changes during pregnancy, the exact role of individual NOS isoforms is not completely understood. A recent study from our laboratory (1) showed that the renal hyperemia during pregnancy is associated with enhanced protein expression of neuronal NOS (nNOS). Moreover, a recent preliminary report by Santmyire and Baylis (23) indicated that the renal vasodilation during pregnancy in rats was associated with an elevation in nNOS activity. Although NOS has been suggested to play a role in the regulation of renal hemodynamics under various physiological conditions, the importance of NOS in mediating the renal hemodynamic changes during pregnancy is still unclear (1–4, 14, 15, 17, 20). Therefore, the purpose of this study was to determine the role of NOS in mediating the elevation in renal plasma flow (RPF) and GFR during pregnancy. To achieve this goal we compared the effects of the nNOS inhibitor 7-nitroindazole (7-NI) on kidney function in normal conscious, chronically instrumented pregnant and virgin rats (9, 18).

METHODS

Surgical procedures. Female Sprague-Dawley virgin (224–249 g) and time pregnant rats were purchased from Harlan Sprague Dawley Virgins, and time pregnant rats were placed in cages in a temperature-controlled room on a 12:12-h light-dark cycle and fed standard rat chow (Teklad) and water ad libitum for a period of at least 1 wk before any surgical procedures.

Two groups of conscious chronically instrumented rats were studied: virgin rats (n = 6) and pregnant rats (n = 9) infused with 7-NI salt (2 mg·kg\(^{-1}\)·h\(^{-1}\)). Virgin and day 12 pregnant rats were anesthetized using isoflurane delivered by an anesthesia machine (Vaporizer for Forane Anesthetic, Ohio Medical Products, Madison, WI). Catheters of heat-stretched PE-50 tubing were inserted into the left femoral
artery and right femoral vein. After implantation each catheter was filled with a 50:50 solution of heparin and saline and exteriorized at the back of the neck. All rats were instrumented with a specially made Silastic catheter and steel bladder as described by Gellai and Valtin (13) for renal clearance measurements. On complete instrumentation, all animals were allowed to recover for a period of 4 days.

Experimental protocol. On the day of the experiment, virgin and pregnant (day 16 of pregnancy) rats were placed in individual restrainers that prohibited movement without external pressure (16). The arterial line was flushed with saline and connected to a pressure transducer (Cobe III Transducer CDX Sema, Birmingham, AL). A Grass model 7B polygraph (Grass Instruments) was used for continuous monitoring of mean arterial pressure. Isotonic saline, ([125I]iothalamate, Isotex Diagnostics; 0.05 mCi kg$^{-1}$ min$^{-1}$ and [131I]idohippurate, Syncor International; 0.1 mCi kg$^{-1}$ min$^{-1}$) were infused intravenously (Harvard Apparatus 22 Infusion Syringe Pump) at a rate of 2.5 ml/h. Next a Silastic connection attachment was placed on the exposed steel portion of the bladder catheter to allow for the collection of urine samples in preweighed test tubes. After a 60-min stabilization period, two 15-min urine samples along with blood reference sample. After the control measurements, 7-NI (2 mg kg$^{-1}$ h$^{-1}$) was infused for 60 min. After a 30-min equilibration period, two 15-min urine samples and a last blood reference sample (0.6 ml total) were obtained. The dose of 7-NI (Calbiochem, catalog #203912; La Jolla, CA) used in this study was derived from several previous studies that examined the chronic effects of 7-NI on hemodynamic function (8, 18, 26). 7-NI was added to hot (~80°C) saline and vigorously stirred to dissolve in solution. No obvious precipitation was noted in the syringe during the acute protocol.

Analytic methods. Kidneys were harvested and weighed. Blood reference samples in Eppendorf tubes containing two drops of heparin were centrifuged at 1,200 rpm for 4 min. One hundred microliters of plasma was pipetted into a labeled test tube. After clearance tubes were weighed to determine volume, 500 µl of urine was transferred in a similar manner. All plasma and urine samples were submitted to a gamma counter to assess 125I and 131I activity.

Statistical analysis. All data are expressed as means ± SE. Statistical comparisons between virgin and pregnant within control or 7-NI-treated groups were made by a paired Student’s t-test. A value of $P<0.05$ was considered statistically significant.

RESULTS

Figure 1 illustrates mean arterial pressure in conscious virgin and pregnant rats at day 16 of gestation under basal conditions and in response to nNOS inhibition. Notice under basal conditions there were no significant differences in arterial pressure between virgin (127 ± 7 mmHg) and pregnant (123 ± 4 mmHg) rats on day 16 of gestation. The figure also illustrates that short-term
infusion of 7-NI had no effect on arterial pressure in virgin (127 ± 7 vs. 129 ± 10 mmHg) or pregnant (123 ± 4 vs. 123 ± 3 mmHg) rats.

Figure 2 illustrates the effects of nNOS inhibition on renal vascular resistance (RVR). RVR was lower in pregnant rats than virgin under control conditions. Infusion of 7-NI resulted in increased RVR in the pregnant rat (10.6 ± 1.9 vs. 17.2 ± 4.5 mmHg) while having no effect on virgin controls (15.4 ± 3.6 vs. 14.2 ± 3.3 mmHg).

RPF as shown in Fig. 3 was significantly higher in pregnant rats (8.85 ± 1.60 ml/min) compared with the virgin controls (4.7 ± 0.8 ml/min). nNOS inhibition resulted in a marked reduction of RPF in pregnant rats (8.9 ± 1.6 vs. 6.5 ± 1.4 ml/min) while having no effect on RPF in virgin controls (4.7 ± 0.7 vs. 4.8 ± 0.9 ml/min).

In addition to markedly affecting RPF, 7-NI also significantly reduced GFR in pregnant rats by ~25%. Notice in Fig. 4 that under control conditions, GFR was higher in pregnant rats (4.4 ± 0.7 vs. 2.2 ± 0.3 ml/min) compared with the virgin controls. GFR (2.2 ± 0.2 vs. 2.5 ± 0.4 ml/min), was unaffected by 7-NI in virgin rats.

DISCUSSION

In the present study, we confirmed the previous finding that RPF and GFR are significantly elevated in pregnant rats at midgestation. We extend these findings by reporting that short-term infusion of an nNOS inhibitor 7-NI significantly decreases RPF and GFR in pregnant rats while having no effect on renal hemodynamics in virgin rats. These results indicate that nNOS may play an important role in mediating the renal hyperemia and hyperfiltration during pregnancy in rats.

Normal pregnancy in humans is associated with significant changes in cardiovascular and renal function (5, 22, 24, 29). Increases in RPF and GFR of ~40% are commonly observed in pregnant women (22). In the present study, we found that rats at midgestation had RPFs and GFRs that were 25–35% higher than virgin controls. In addition, RVR was 30% lower than in pregnant rats compared with virgin controls. Although the exact mechanisms that are involved in mediating these renal hemodynamic changes during pregnancy are unclear, recent studies suggest that activation of endothelial factors such as NO may play an important role (5, 6, 11, 16, 24, 25).

Pregnancy in humans and in various animal models is associated with marked increases in NO production (1, 6, 11, 29). Previous studies have also shown that nonselective inhibition of NO markedly attenuates the renal hemodynamic changes during pregnancy (6, 7, 11, 16, 19). Although these findings suggest that NO plays an important role in mediating the renal hemodynamic changes during pregnancy, the exact role of individual NOS isoforms is not completely understood. We recently reported that the increases in renal hemodynamics during pregnancy in the rat is associated with enhanced protein expression of nNOS as well as inducible NOS (1). Renal endothelial NOS expression was actually reduced during pregnancy (1). Similar findings with renal NOS activity during pregnancy have been reported (23). To examine the importance of the elevated renal nNOS in mediating the renal hemodynamics changes during pregnancy, we compared the effects of an nNOS inhibitor on renal function in conscious chronically instrumented pregnant and virgin rats. We found that short-term infusion of 7-NI reduced RPF and GFR of the pregnant rats by ~25%. In addition, 7-NI increased RVR by ~60%. In sharp contrast, infusion of 7-NI into virgin rats had no effect on RVR, RPF, or GFR. The lack of an effect of 7-NI on renal hemodynamics in normal animals is consistent with previous reports on nNOS inhibition in rats (8, 10). The fact that 7-NI markedly reduced RPF and GFR in pregnant rats while having no effect in virgin rats supports the concept that nNOS activity is upregulated during pregnancy and contributes importantly to the elevated renal hemodynamics.

Short-term blockade had no effect on mean arterial pressure in the pregnant or virgin rats. These findings are consistent with other studies examining the acute effects of 7-NI inhibition on arterial pressure (8, 10). In contrast, chronic blockade of 7-NI has been reported to cause long-term elevation in arterial pressure, possibly through its renal actions (10, 18, 21). Whether nNOS plays a role in long-term blood pressure control during pregnancy is still unknown. One would predict that sustained reductions in RPF and GFR in response to chronic 7-NI administration should lead to chronic elevations in arterial pressure in pregnant rats. Further studies will be necessary to determine whether nNOS contributes to long-term blood pressure regulation during pregnancy.

In summary, we found that short-term infusion of the nNOS inhibitor 7-NI markedly increases RVR and reduces RPF and GFR. In contrast, 7-NI had no effect on renal hemodynamics in virgin rats. These findings indicate that nNOS may play an important role in mediating the increases in renal hemodynamics observed during normal pregnancy.

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