Renal Interstitial Hydrostatic Pressure and Natriuretic Response to High Doses of Angiotensin II in Pregnant Rats

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Background: Administration of high doses of angiotensin II (Ang II) results in natriuretic and diuretic responses that are mediated by increases in renal perfusion pressure (RPP). Elevations in renal interstitial hydrostatic pressure (RIHP) caused by increases in mean arterial pressure (MAP) or RPP are associated with significant increases in urinary sodium excretion ($U_{Na,V}$) and urine flow rate ($V$). In pregnant rats the renin-angiotensin system (RAS) is activated and basal RIHP is reduced. The exact relationship among MAP, RIHP, $U_{Na,V}$, and $V$ in response to a high pressor dose of Ang II during normal pregnancy is not known.

Methods: The objective of this study was to evaluate the relationship between MAP and RIHP, and determine the role of RIHP in the natriuretic and diuretic responses to administration of high dose of Ang II in midterm pregnant (MP) and nonpregnant (NP) Sprague-Dawley (SD) rats.

Results: Intravenous infusion of Ang II (200 ng/kg/min) significantly increased MAP ($\Delta$MAP), $\Delta U_{Na,V}$, and $\Delta V$ in anesthetized MP and NP rats. The $\Delta$MAP, $\Delta U_{Na,V}$, and $\Delta V$ were 12 $\pm$ 2 mm Hg, 27.9 $\pm$ 2.7 $\mu$Eq/min, and 197 $\pm$ 23 $\mu$L/min for MP rats and were similar (15 $\pm$ 3 mm Hg, 34.1 $\pm$ 4.3 $\mu$Eq/min, and 242 $\pm$ 34 $\mu$L/min, respectively) for NP rats. The RIHP decreased significantly ($P < .05$) with Ang II infusion in NP (from 6.1 $\pm$ 0.2 to 3.9 $\pm$ 0.4 mm Hg) but not in MP (from 3.3 $\pm$ 0.3 to 4.1 $\pm$ 0.4 mm Hg) groups of rats. Acute renal decapsulation eliminated the change in RIHP mediated by high doses of Ang II infusion in the NP group, but did not affect MAP or the natriuretic and diuretic responses to Ang II in either the MP or NP groups of rats.

Conclusions: The natriuretic and diuretic responses to high doses of Ang II are not mediated by changes in RIHP in pregnant or nonpregnant rats. Am J Hypertens 2006; 19:300 –305 © 2006 American Journal of Hypertension, Ltd.

Key Words: Angiotensin II infusion, renal interstitial hydrostatic pressure, pregnancy, sodium excretion, proximal tubular reabsorption.

The renin-angiotensin system (RAS) plays an important role in regulating renal hemodynamics, sodium excretion, body fluid volumes, and blood pressure (BP). Exogenous infusion of low doses of angiotensin II (Ang II) usually cause antinatriuresis and antidiuresis, whereas infusion of high doses of Ang II result in natriuretic and diuretic responses. Administration of high doses of Ang II increase systemic arterial BP and renal perfusion pressure (RPP), inhibit proximal tubular reabsorption, and induce natriuresis and diuresis. When RPP increases, which are mediated by infusion of high doses of Ang II, are prevented from occurring, the natriuretic and diuretic responses are abolished, suggesting the increases in RPP with high doses of Ang II infusion are required for the demonstration of natriuresis and diuresis.

Although natriuretic and diuretic responses to high doses of Ang II are well studied, the possible renal mechanisms, especially those that mediate the increase in RPP by high doses of Ang II to natriuresis and diuresis, are still unclear. We have previously shown that renal interstitial hydrostatic pressure (RIHP) plays a critical role in mediating sodium and water excretion. Elevations in RIHP, which are caused by increases in RPP, acute systemic...
saline volume expansion, or by direct renal interstitial volume expansion, were associated with significant increases in urinary sodium excretion (UNaV), fractional excretion of sodium (FE\textsubscript{Na}), and urine flow rate (V).\textsuperscript{4–8} Acute bilateral renal decapsulation did not reduce basal RIHP, but blunted the increase in RIHP that is produced by increases in RPP, and abolished the pressure natriuretic and diuretic response in normotensive rats.\textsuperscript{9} Therefore, we investigated the possibility that increases in RIHP mediated by increases in RPP may play an important role in the natriuretic and diuretic responses that are caused by infusion of a high dose of Ang II.

An uncomplicated normal pregnancy requires a significant and sustained increase in plasma volume, which results from renal function adaptations that increase sodium and water reabsorption and maintain the volume expansion.\textsuperscript{10,11} The RAS and aldosterone have been shown to be significantly modified during pregnancy.\textsuperscript{10,12} During normal pregnancy, plasma renin activity, plasma levels of Ang II, and aldosterone are increased,\textsuperscript{10,12,13} and the pressor response to administered Ang II is decreased.\textsuperscript{10} Also, pressure natriuresis and diuresis responses are significantly attenuated during normal pregnancy in rats.\textsuperscript{5,14} These blunted natriuretic and diuretic responses are associated with attenuated elevations in RIHP with increases in RPP in pregnant rats.\textsuperscript{5} Furthermore, basal RIHP is lower in pregnant rats and renal interstitial compliance is increased during pregnancy.\textsuperscript{5,8,15,16} However, the natriuretic and diuretic responses to volume expansion remain intact during the course of pregnancy.\textsuperscript{8,15} It is not clear whether a direct relationship exists between the increase in plasma levels of Ang II and the reduction in RIHP, and whether this relationship mediates the decreases in UNaV and V and the promotion of sodium and water retention during normal pregnancy. In pregnant rats RAS is activated and basal RIHP is decreased\textsuperscript{5,8,10,12,13,15}, however, the exact relationship among mean arterial pressure (MAP), RIHP, UNaV, and V in response to a high pressor dose of Ang II during normal pregnancy is not known.

The objective of these studies was to evaluate the relationship between MAP and RIHP, and determine the role of RIHP in the natriuretic and diuretic responses to administration of a high dose of Ang II in midterm pregnant (MP) and nonpregnant (NP) Sprague-Dawley (SD) rats. The dose of Ang II used in this study was sufficient to increase BP, when administered intravenously, and cause natriuresis and diuresis. Acute bilateral renal decapsulation, which does not alter basal RIHP, but prevents RIHP from changing,\textsuperscript{9,17} was used to determine the role of RIHP in the natriuretic and diuretic responses during infusion of a high dose of Ang II.

Methods

All rats in these studies were female SD rats purchased from Harlan Sprague Dawley (Indianapolis, IN) when they were 11 to 12 weeks old. All rats were fed Teklad Global 18% Protein Rodent Diet (Harlan, Madison, WI) and had free access to water. All protocols in these studies were in accordance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at Eastern Virginia Medical School.

Polyethylene Matrix Implantation

The implantation procedure of the polyethylene (PE) matrix has been previously described.\textsuperscript{5,7} The RIHP was measured directly and continuously through the PE matrix implanted in the left kidney when the rats were 12 to 13 weeks old.

Monitoring of Estrous Cycle and Induction of Pregnancy in Rats

Monitoring of the estrous cycle and induction of pregnancy in rats has been previously described.\textsuperscript{5,8} Approximately 1 week after PE matrix implantation, vaginal swabs were taken daily in all rats to monitor their estrous cycle. To determine the stage in the estrous cycle, female rats were manually restrained and a wet swab was inserted in the vagina and smeared on a slide. As previously described\textsuperscript{5,8} the slide was immediately fixed with 1% toluidine blue solution (with few drops of 1 N potassium hydroxide), and observed under the microscope for cells that characterize each stage of the estrous cycle. A male breeder and a female SD rat were housed together for 1 day when the female was found to be in the estrous stage. The female was tested for the presence of sperm in the vagina the next day after approximately 24 h of being in the same cage with the male breeder SD rat. The presence of sperm on the fixed slide of the vaginal smear was taken to indicate day 1 of pregnancy.

Surgical Procedure for Acute Experiments

On the day of the acute experiment, rats were anesthetized with Inactin (100 mg/kg, intraperitoneally) and catheters were placed in the trachea (PE-240), and left jugular vein (PE-50) for intravenous infusion of 1.5 mL/100 g body wt/h of saline and 1.5 mL/100 g body wt/h of a solution of 3% inulin and 6.25% bovine albumin in saline. A PE-50 catheter was implanted in the left carotid artery for MAP measurement and blood withdrawal. A PE-90 catheter with a flared tip was placed in the bladder for urine collection. The rats were allowed 1 h to stabilize after completion of the surgical procedures. Then a control period of 30 min was started. During the 30-min clearance period, MAP and RIHP were measured and recorded continuously. At the end of this period approximately 0.5 mL of blood was withdrawn from the left carotid artery for plasma electrolytes, phosphate, and inulin measurements. At this time, intravenous infusion of Ang II (200 ng/kg/min) was started. Urine was collected for 20 min starting 10 min after the initiation of the Ang II infusion. Again, during the second clearance
period, MAP and RIHP were measured and recorded continuously. At the end of this period, about 0.5 mL of blood was withdrawn from the left carotid artery for plasma electrolytes, phosphate, and inulin measurements. All rats were killed by air embolism at the end of the experiment while still under deep anesthesia, and both kidneys were excised and weighed. This method of euthanasia is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.

### Experiment Protocols

**Protocol 1. Effect of Intravenous Ang II Infusion on RIHP and Sodium Excretion in Pregnant and Non-pregnant Rats**  
Experiments were performed to determine the effect of infusing a high dose of Ang II (200 ng/kg/min, intravenously) on natriuresis and on changes in RIHP. Two groups of female SD rats were studied in these experiments: a group of NP (n = 7) SD rats, and a group of MP (n = 8) SD rats. The NP rats were rats that were individually housed together for 24 h with a male breeder when they were in the estrous stage but found to be nonpregnant during the acute experiments. The MP rats were pregnant for 12 to 14 days when the acute experiments were performed. These two groups of rats were infused with Ang II (200 ng/kg/min, intravenously) after a control period was taken.

**Protocol 2. Role of RIHP in the Natriuretic Response to Ang II in Pregnant and Nonpregnant Rats**  
The NP (n = 5) and MP (n = 7) female SD rats were prepared identically to those in protocol 1, except that both renal capsules were gently removed during the surgical procedures of the acute terminal experiments in both groups of rats in this protocol. Acute bilateral renal decapsulation prevents the changes in RIHP, but does not have a significant effect on basal level of RIHP, glomerular filtration rate (GFR), or the excretory responses of the kidney as determined by sodium and potassium excretions, and urine osmolality.9,17

### Laboratory Analyses

The GFR was calculated from the clearance of inulin, and inulin concentrations were measured by the anthrone method.18 Sodium concentrations in plasma and urine were measured using flame photometry (model 943; Instrumentation Laboratory, Lexington, MA). Phosphate concentrations in plasma and urine were measured according to the method of Chen et al.19 Because phosphate is almost exclusively reabsorbed in the proximal tubule,20 it has been used in these studies as an index for proximal tubular reabsorption.

### Statistical Analyses

The data were analyzed with standard paired Student t tests for comparisons between the first and second clearance periods in the same group of rats, or with a one-way analysis of variance (ANOVA) followed by a post-hoc Bonferroni correction for group comparisons at equivalent periods. All data are reported as means ± SE, and P < .05 was accepted as a statistically significant difference.

### Results

**Natriuretic Response to Intravenous Infusion of High Dose Ang II**

Infusion of Ang II (200 ng/kg/min, intravenously) significantly increased MAP in both NP and MP groups of rats (Table 1). As shown in Fig. 1, U_{Na}V and V were significantly increased during Ang II infusion as compared with the control period. These increases in U_{Na}V and V (Fig. 1) were also associated with a significant increase in fractional excretion of phosphate (FEPi) (Table 1) in both groups of rats, suggesting a significant inhibition of proximal tubule reabsorption with intravenous infusion of Ang II. The GFR increased in the MP group but not in the NP group studied in response to Ang II infusion (Table 1). No significant differences were found in the increases in MAP, U_{Na}V, V, and FEPi produced by intravenous Ang II
infusion between NP and MP groups of rats (Table 1 and Fig. 1).

RIHP in the Mechanisms of Ang II Natriuresis in Pregnant and Nonpregnant Rats

As shown in Fig. 1, basal RIHP was significantly lower ($P < .05$) in MP ($3.3 \pm 0.3$ mm Hg) as compared to NP rats ($6.1 \pm 0.2$ mm Hg). Although Ang II infusion increased MAP in both NP and MP groups (Table 1), RIHP decreased significantly in the NP group (to $3.9 \pm 0.4$ mm Hg), whereas it remained unchanged in pregnant rats ($4.1 \pm 0.4$ mm Hg) with the intravenous infusion of Ang II (Fig. 1). Renal decapsulation eliminated the change in RIHP that is produced by a high dose of Ang II infusion (Fig. 2), but did not affect MAP (Table 1) or the natriuretic and diuretic responses to intravenous infusion of Ang II (Fig. 2). There were no significant differences in the increases in MAP, $U_{Na}V$, $V$, and $FE_{Pi}$ produced by Ang II infusion between NP and MP of rats with bilateral renal decapsulation (Table 1 and Fig. 2).

Discussion

The results of the present study show that intravenous infusion of a high dose of Ang II (200 ng/kg/min) caused a significant increase in MAP in both the NP and MP groups of rats (Table 1). Also, $U_{Na}V$ and $V$ significantly increased with the infusion of Ang II and these increases were similar and were associated with a significant increase in $FE_{Pi}$ in both NP and MP groups of rats, suggesting a significant inhibition in proximal tubule reabsorption with the intravenous infusion of Ang II (Table 1 and Fig. 1). Infusion of a high dose of Ang II results in increases in systemic BP, RPP, and renal sodium and water excretions, and inhibition of proximal tubular reabsorption. It has been demonstrated by Olsen et al. that the natriuretic and diuretic responses caused by the infusion of a high dose of Ang II are mediated by the increases in RPP because when RPP is prevented from increasing the natriuretic and diuretic responses are abolished. The results of the present study showed that the natriuretic and diuretic responses of infusion of a high dose of Ang II were associated with a significant increase in MAP and thus RPP in both NP and MP groups of rats (Table 1). Furthermore, in the present study we investigated the possible role of RIHP in mediating the natriuretic and diuretic responses of a high dose of Ang II in NP and MP rats. As shown Fig. 1, basal RIHP was significantly lower in MP as compared to NP rats. The Ang II infusion increased MAP in both NP and MP groups (Table 1). However, RIHP decreased significantly in the NP group and remained unchanged in the MP group of rats with the intravenous infusion of Ang II (Fig. 1). Because the increases in $U_{Na}V$ and $V$ produced by intravenous infusion of a high dose of Ang II were similar in the NP and MP groups of
rats (Fig. 1), we conclude that the natriuretic and diuretic responses to infusion of Ang II are not mediated by changes in RIHP in either NP or MP rats. It is reasonable to suggest that in pregnant rats the inability to transmit the increase in MAP to the renal interstitium during Ang II infusion may be due to the resulting renal vasoconstriction. In addition to the increase in renal interstitial compliance during pregnancy, the blunted renal vasoconstriction to Ang II may explain why RIHP does not decrease by as much in pregnant as compared to nonpregnant rats (Fig. 1).

Acute bilateral renal decapsulation prevents the changes in RIHP, but does not have a significant effect on basal level of RIHP. In the current study, renal decapsulation eliminated the change in RIHP that is produced by a high dose of Ang II infusion in NP rats (Fig. 2), but did not affect MAP or the natriuretic and diuretic responses to intravenous infusion of Ang II in either NP or MP groups of rats (Table 1 and Fig. 2). The increases in MAP, \( U_{\text{Na}} \), V, and \( FE_{\text{p}} \) produced by intravenous infusion of Ang II were similar in NP and MP groups of rats with bilateral renal decapsulation (Table 1 and Fig. 2). Taken together, these results further show that the natriuretic and diuretic responses, and the inhibition of proximal tubular reabsorption that result from intravenous infusion of a high dose of Ang II are not mediated by changes in RIHP in either NP or MP rats. It should be noted that during normal pregnancy both sodium-retentive hormones, such as deoxycorticosterone, Ang II, and aldosterone, and natriuretic hormones, such as atrial natriuretic peptide and progesterone, are increased. This suggests that the plasma volume regulation during pregnancy is likely to be multifactorial and is not the result of a simple increase in the levels of one or more of hormones (such as Ang II) or factors (such as a decrease in RIHP) that can cause sodium retention. During normal pregnancy Ang II levels are increased and basal RIHP is reduced. Therefore, one of the objectives of this study was to determine the relationship among MAP, RIHP, and sodium and water excretions in response to a high pressor dose of Ang II during normal pregnancy. Our results showed that the natriuretic and diuretic responses to high doses of Ang II are not mediated by changes in RIHP in pregnant rats.

In summary intravenous infusion of a high pressor dose of Ang II resulted in significant increases in MAP, sodium and water excretion in both pregnant and nonpregnant groups of rats. The RIHP decreased significantly with Ang II infusion in nonpregnant but not in pregnant rats, whereas proximal tubular reabsorption was significantly inhibited in both groups. Renal decapsulation eliminated the change in RIHP mediated by high doses of Ang II infusion in nonpregnant rats, but did not affect MAP or the natriuretic and diuretic responses to Ang II in either pregnant or nonpregnant groups of rats. We conclude that the natriuretic and diuretic responses to high doses of Ang II are not mediated by changes in RIHP in pregnant or nonpregnant rats.
References