Pregnant Rats With 5/6 Nephrectomy Have Normal Volume Expansion Despite Lower Renin and Kallikrein

Sofía P. Salas, Andrea Giacaman, Carlos P. Vío

Abstract—To test the hypothesis that normal volume expansion during pregnancy is impaired during chronic renal failure, we evaluated the effects of 5/6 nephrectomy (Nx) in Sprague-Dawley rats. Partial Nx was produced by ligation of 2/3 renal arteries and contralateral Nx. Control rats had a sham operation. Four weeks later, rats were assigned to nonpregnant (n=6/each) or pregnant groups (n=11 to 12/each). At day 21, blood pressure, plasma volume, renal function, hormonal levels, and reproductive outcome were determined. Statistical analysis was performed by ANOVA, and values were expressed as mean±SEM. Rats with 5/6 Nx had proteinuria and lower creatinine clearance; pregnancy did not affect these parameters. Blood pressure increased in 5/6 Nx rats and was reduced by pregnancy. Both pregnant groups had lower hematocrit and higher plasma volume values (nonpregnant control, 13.4±0.2; nonpregnant 5/6 Nx, 14.4±0.2; pregnant control, 19.1±0.7, pregnant 5/6 Nx, 19±0.9 mL, P<0.001). Pregnancy increased plasma renin activity only in control rats (nonpregnant control, 2.0±0.7; nonpregnant 5/6 Nx, 1.6±1.1; pregnant control, 36.1±7.9, pregnant 5/6 Nx, 6.1±1.8 ng AI/mL per hour, P<0.001). Serum aldosterone levels were unaffected by 5/6 Nx and were higher in pregnant than in nonpregnant rats. Urinary kallikrein activity was reduced by 5/6 Nx and not changed by pregnancy (nonpregnant control, 1499±237; nonpregnant 5/6 Nx, 346±90; pregnant control, 1595±180, pregnant 5/6 Nx, 374±95 nmol/16 hours, P<0.001). No significant differences in fetal and placental weights were observed between control and 5/6 Nx rats. Present data indicate that 5/6 Nx pregnant rats were able to normally expand plasma volume despite lower renin and kallikrein levels. (Hypertension. 2003;43[part 2]:744-748.)

Key Words: pregnancy • kidney failure • plasma volume • renin • kallikrein • aldosterone

Normal pregnancy is characterized by an initial reduction in peripheral vascular resistance that leads to several consequences such as decreased blood pressure, higher cardiac output, stimulation of the renin-angiotensin-aldosterone axis, and renal sodium and water retention, with expansion of plasma volume.1-4 These changes allow an adequate supply of blood to the placenta and the developing fetus, and their absence is associated with pregnancy complications such as preeclampsia and fetal growth restriction.5,6

To achieve positive sodium balance and cumulative plasma volume expansion, important changes in renal function also occur during pregnancy. There is a rise in glomerular filtration rate (GFR) and in renal plasma flow, the pressure natriuretic response is markedly blunted,7,8 and vasoactive hormones from renal origin, such as kallikrein and renin, are increased during pregnancy.9

On the basis of these observations, we have speculated that normal renal function and the ability of the kidney to increase the production of vasoactive hormones are important for adequate plasma volume expansion during pregnancy. To test this hypothesis, in the present study we evaluated the maternal and fetal effects of 5/6 nephrectomy (5/6 Nx) in Sprague-Dawley rats. In addition to the abnormalities characteristic of chronic renal failure, such as decreased GFR, moderate hypertension, and glomerular disease,10 5/6 Nx is associated with alterations in the renin-angiotensin-aldosterone and kallikrein-kinin systems.11-13

Methods

Experimental Design

Female Sprague-Dawley rats (160 to 190 g initial weight) were maintained at the Center for Medical Research animal care facilities in a controlled environment (22° to 24°C and a 12-hour light/dark cycle). The protocols met international guidelines for animal welfare and were reviewed and approved by the Institutional Review Board of the School of Medicine.

Partial Nx was accomplished by means of a 2-stage operation, as previously described.10 Two of three branches of the left renal artery were ligated to produce renal infarction, and contralateral nephrectomy was done 1 week later. Control animals had a sham operation. Four weeks after the last surgery, estrous cycles were monitored by cytological evaluation of vaginal smears, and 5/6 Nx and control rats were randomly assigned to nonpregnant (NP control and NP 5/6 Nx, n=6 each) or to pregnant groups (P control, n=12; P 5/6 Nx, n=11). Sperm-positive day was considered as day 0. All rats had free access to standard rat chow and tap water throughout the study period.

Received May 12, 2003; first decision June 12, 2003; revision accepted June 27, 2003.
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Hypertension is available at http://www.hypertensionaha.org
DOI: 10.1161/01.HYP.0000085783.96609.0C

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TABLE 1. Renal Function in Nonpregnant and Pregnant Rats With 5/6 Nephrectomy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonpregnant</th>
<th>Pregnant</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5/6 Nx</td>
</tr>
<tr>
<td></td>
<td>(n=6)</td>
<td>(n=6)</td>
</tr>
<tr>
<td>Urine volume, mL/16 h</td>
<td>7.6±2.2</td>
<td>13.6±1.9</td>
</tr>
<tr>
<td>Urinary protein, mg/16 h</td>
<td>8.4±5.9</td>
<td>144±26*</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.5±0.04</td>
<td>1.1±0.14*</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>1.6±0.2</td>
<td>0.7±0.1*</td>
</tr>
<tr>
<td>Urinary to serum osmolality ratio</td>
<td>5.7±1.2</td>
<td>2.3±0.3*</td>
</tr>
<tr>
<td>Urinary Na, mEq/16 h</td>
<td>0.5±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Urinary K, mEq/16 h</td>
<td>1.6±0.3</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td>Fe Na⁺</td>
<td>0.23±0.06</td>
<td>0.55±0.1</td>
</tr>
<tr>
<td>Fe K⁺</td>
<td>26.5±3.4</td>
<td>50.9±8</td>
</tr>
</tbody>
</table>

Values represent the mean±SEM. Nx indicates nephrectomy; Fe Na⁺, fractional sodium excretion; Fe K⁺, fractional potassium excretion.

* Differences between control and 5/6 Nx; P<0.05, ANOVA.

At day 20 of pregnancy, or in the corresponding day in nonpregnant rats, animals were individually placed in metabolic cages with free access to food and water from 5 PM to 9 AM. The next morning, systolic blood pressure was measured by tail-cuff plethysmography. The rats were then anesthetized with xylazine (5 mg/kg) plus ketamine (40 mg/kg) IP. Plasma volume was measured by the Evans blue dye dilution technique, and blood samples were obtained from the abdominal aorta. An aliquot was used for microhematocrit determination, and the remaining was centrifuged and the plasma frozen for further analysis. Net maternal body weight (after removal of major organs and the uterus) and litter size and individual weights of fetuses and placentas were recorded. Weights and aliquoted as described. Only 5/6 Nx rats with a corresponding control rats, but only a nonsignificant increment was observed in nonpregnant rats. Pregnancy did not modify urine volume. Both groups of 5/6 Nx rats had significant proteinuria, which was not affected by pregnancy. Serum creatinine was significantly increased and creatinine clearance decreased by 5/6 Nx to nearly 50% of control values. Urinary osmolality was reduced, whereas serum osmolality remained unchanged by 5/6 Nx (not shown); in consequence, urinary to serum osmolality ratio was significantly reduced by 5/6 Nx (Table 1). Pregnancy had no effect on these parameters (Table 1). Plasma sodium was reduced in control pregnant rats, and not modify by 5/6 Nx (NP control, 143±1.9; NP 5/6 Nx, 142±1.8; P control, 138±0.8; P 5/6 Nx, 139±0.6 mEq/L, P<0.05). Serum potassium was not modified by 5/6 Nx or by pregnancy (not shown). Urinary sodium and potassium excretions and fractional sodium excretion were similar in all groups, whereas fractional potassium excretion was significantly higher in pregnant 5/6 Nx rats when compared with control rats (Table 1).

Hormonal and Biochemical Measurements

Plasma renin activity (PRA) was determined by radioimmunoassay of generated angiotensin I, under control conditions. Plasma aldosterone was measured by radioimmunoassay, with the use of a commercial kit Diagnostic Products Corporation. Urinary kallikrein activity was determined by the amidase method, with the use of the synthetic substrate DL-Val-Leu-arginine-p-nitroanilide (Sigma). Plasma and urinary electrolytes were measured by flame photometry (IL 343). Plasma and urinary creatinine levels were measured with a Beckman Autoanalyzer. Urinary protein concentration was determined by Bradford’s method (Bio-Rad protein assay). Serum and urinary osmolality were determined by freezing point (Advanced Instruments, Model 3DII).

Statistical Analysis

Statistical analysis was performed by either an ANOVA, by an unpaired 2-tailed Student t test, or by χ² test when appropriate, using the computer program Stat View II (Abacus Concepts Inc). Statistical significance was accepted at a level of P<0.05. All data were expressed as mean±SEM.

Results

As shown in Table 1, 5/6 Nx induced a moderate chronic renal failure, both in nonpregnant and in pregnant rats. Urine output was higher in 5/6 Nx pregnant rats than in their corresponding control rats, but only a nonsignificant increment was observed in nonpregnant rats. Pregnancy did not modify urine volume. Both groups of 5/6 Nx rats had significant proteinuria, which was not affected by pregnancy. Serum creatinine was significantly increased and creatinine clearance decreased by 5/6 Nx to nearly 50% of control values. Urinary osmolality was reduced, whereas serum osmolality remained unchanged by 5/6 Nx (not shown); in consequence, urinary to serum osmolality ratio was significantly reduced by 5/6 Nx (Table 1). Pregnancy had no effect on these parameters (Table 1). Plasma sodium was reduced in control pregnant rats, and not modify by 5/6 Nx (NP control, 143±1.9; NP 5/6 Nx, 142±1.8; P control, 138±0.8; P 5/6 Nx, 139±0.6 mEq/L, P<0.05). Serum potassium was not modified by 5/6 Nx or by pregnancy (not shown). Urinary sodium and potassium excretions and fractional sodium excretion were similar in all groups, whereas fractional potassium excretion was significantly higher in pregnant 5/6 Nx rats when compared with control rats (Table 1).

Weight at day 0 of pregnancy, or in the corresponding day in nonpregnant rats, was similar in all groups. As expected, pregnancy increased final weight and also net maternal weight, whereas 5/6 Nx did not have any detrimental effect on weight gain. Systolic blood pressure was significantly reduced in both groups of pregnant rats, but values were higher in 5/6 Nx than in control rats. Nonpregnant rats with 5/6 Nx had a moderate but nonsignificant increase in blood pressure. Hematocrit levels were significantly lower in both groups of pregnant rats and were not affected by 5/6 Nx. Plasma volume increased significantly and to a similar extent in both groups of pregnant rats, 5/6 Nx had no effect on volume levels (Table 2). When all groups were combined, a significant negative correlation was observed between plasma volume and hematocrit levels (r=-0.6, P<0.001).

As expected, PRA levels were significantly higher in control pregnant rats than in nonpregnant control rats; however, 5/6 Nx pregnant rats had only a moderate and nonsignificant rise in PRA. Thus, PRA values in this group were much lower than those observed in control pregnant rats. 5/6 Nx did not change PRA in nonpregnant rats. In contrast to what could be expected according to PRA levels, both groups...
of pregnant rats had higher aldosterone levels than their corresponding nonpregnant groups, and 5/6 Nx did not significantly affect aldosterone levels. Aldosterone but not PRA had a significant positive correlation with plasma volume (r=0.55, P<0.01). Urinary kallikrein activity was significantly reduced by 5/6 Nx and was not affected by pregnancy (Figure). When kallikrein values were expressed per creatinine excretion, the difference between control rats and 5/6 Nx groups persisted (NP control, 2.0±0.1; NP 5/6 Nx, 0.4±0.1; P control, 1.7±0.1; P 5/6 Nx, 0.4±0.1, P<0.001 for differences between 5/6 Nx and corresponding control rats).

Reproductive outcome was not altered by 5/6 Nx. All groups had a normal 4-day estrous cycle; those with a sperm-positive vaginal smear did not have early or late fetal loss, and fetuses from both groups were alive when extracted from the uterus. Although fetal weight was slightly reduced by 5/6 Nx (control, 5.1±0.1 g versus 5/6 Nx, 4.9±0.1 g), this difference was not statistically significant. The number of pups below the 10th centile was 10 of 120 in control rats and 17 of 93 in 5/6 Nx (χ²=NS). Placental weight was also similar in both groups (control, 0.47±0.01 g versus 5/6 Nx, 0.48±0.01 g); in consequence, placental to fetal ratio was not affected by 5/6 Nx.

Discussion

The results presented in this study demonstrate that pregnant rats with 5/6 Nx are able to achieve a normal plasma volume expansion despite renal mass reduction, lower PRA and urinary kallikrein activity, and higher blood pressure levels.

This model of reduction of functional renal mass is characterized by an initial rise in filtration rate followed by an increased scarring of the kidney, accompanied by progressive azotemia, proteinuria, and arterial hypertension. Our nonpregnant rats had most of the changes in renal function described for this model, such as higher serum creatinine, lower creatinine clearance, and proteinuria. However, blood pressure rise was modest, and they failed to have the increment on aldosterone described in this model. These discrepancies can be attributed either to gender differences, since most of previous studies were performed in male instead of female rats, or to different time intervals after surgery.

Normal volume expansion in rats with 5/6 Nx was an unexpected finding. It is unlikely that this was caused by methodological problems in volume determination. Plasma volume was measured by intravenous injection of a dye (Evans blue, T-1824), which mixes completely within a few minutes and circulates, bound to albumin. This method has been extensively used in pregnancy and in nephrotic syn-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nonpregnant</th>
<th>Pregnant</th>
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<tbody>
<tr>
<td></td>
<td>Control (n=6)</td>
<td>5/6 Nx (n=6)</td>
</tr>
<tr>
<td>Initial weight, g</td>
<td>247±2.2</td>
<td>243±1.5</td>
</tr>
<tr>
<td>Final weight, g</td>
<td>257±6.2</td>
<td>255±7.1</td>
</tr>
<tr>
<td>Net weight, g</td>
<td>194±4.2</td>
<td>192±4.9</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>127±3.0</td>
<td>149±7.9</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.39±0.005</td>
<td>0.39±0.012</td>
</tr>
<tr>
<td>Plasma volume, mL</td>
<td>13±0.2</td>
<td>14±0.2</td>
</tr>
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Values represent the mean±SEM. Initial weight refers to weight at day 0 of pregnancy. Differences between control and 5/6 Nx; †differences between nonpregnant and pregnant rats; P<0.05, ANOVA.
drome.\textsuperscript{18} Although the transcapillary escape rate may be increased in uremia, accounting for renal albumin loss, the resulting error has been estimated within the range of <5%.\textsuperscript{18} In addition, the lower hematocrit observed in both groups of pregnant rats is consistent with a similar degree of hemodilution and is consistent with previous reports.\textsuperscript{19}

We can speculate that the normally elevated aldosterone levels in pregnant rats with 5/6 Nx were enough stimuli for renal water and sodium retention, even in the presence of this significant reduction in functional renal mass. However, it is worth noting that 5/6 Nx pregnant rats had significant polyuria, which is in keeping with the urinary concentrating defect observed in this model, most probably caused by a marked reduction in the expression levels of several aquaporins in the kidney.\textsuperscript{20} Therefore, other water-retaining mechanisms, probably acting at extrarenal sites, might be involved.

Pregnant rats with 5/6 Nx had a nonsignificant rise in PRA but normally elevated aldosterone levels. Lower PRA can be partly attributed to the reduced renal mass and to the negative feedback caused by increased intrarenal angiotensin II levels\textsuperscript{21} and by hypertension. In addition, the reduced neuronal nitric oxide synthase activity described in this model may have a negative impact on renin synthesis by juxtaglomerular cells.\textsuperscript{22,23} The apparent dissociation between PRA and aldosterone levels has been reported previously in nonpregnant rats, and hyperaldosteronism appears to be an additional factor instrumental in sustaining the hypertension and fibroproliferative destruction of the residual kidney.\textsuperscript{11,24,25} The high aldosterone-to-PRA ratio can be explained by several mechanisms. First, in 5/6 Nx rats, it has been described adrenal hypertrophy, particularly of the glomerulosa zone.\textsuperscript{11} In addition, the fixed dietary potassium load in the face of reduced filtration has been shown to be a stimulus for aldosterone secretion.\textsuperscript{26} and, finally, renal metabolism of aldosterone might be reduced in the 5/6 Nx rats, secondary to gross renal structural abnormalities.

Urinary kallikrein activity was severely reduced in both nonpregnant and pregnant rats with 5/6 Nx, either when expressed in absolute values or when corrected per creatinine excretion. Several studies indicate that the renal kallikrein-kinin system is involved in the control of blood flow and participates in the regulation of systemic blood pressure in different conditions, including pregnancy.\textsuperscript{27} Renal kallikrein is elevated in normal pregnancy\textsuperscript{6,28} and reduced in pregnancies complicated with preeclampsia or idiopathic fetal growth restriction,\textsuperscript{6,29} conditions characterized by a reduced volume expansion. Moreover, administration of a nitric oxide synthase inhibitor to pregnant rats produces a limited plasma volume expansion associated with reduced renal kallikrein.\textsuperscript{14} This is the first time that we have observed normal volume expansion in the face of marked reductions in kallikrein activity, suggesting that volume expansion in pregnant rats with chronic renal failure probably is dependent on additional mechanisms other than kallikrein activity.

It has been reported that male rats with 5/6 Nx have a significant reduction in urinary kallikrein activity and in renal kallikrein expression,\textsuperscript{13} which is compatible with the renal structural abnormalities and the severe reduction in functional renal mass described in this model. The possible role of reduced kallikrein in some of these alterations is under discussion. In this respect, it has been shown that human tissue kallikrein gene delivery to 5/6 Nx rats attenuates hypertension, renal injury, and cardiac remodeling, suggesting a possible role for kallikrein.\textsuperscript{30} Despite lower urinary kallikrein activity, near-term 5/6 Nx pregnant rats had a significant reduction in blood pressure, and it has been described that the remnant kidney is still capable of an additional gestational renal vasodilatation.\textsuperscript{19}

To perform our experiments, we choose a model of renal failure that had moderate elevations in plasma creatinine and reductions in creatinine clearance rather than end stage. There was no apparent interference with normal cycling, ovulation, and implantation processes, as can be deduced by the fact that litter size was not affected. Similar reproductive outcomes were reported with another model of 5/6 Nx,\textsuperscript{31} although they described reduced number of ova per oviduct. However, when partial nephrectomy was performed at day 14 of pregnancy, a significant reduction in fetal weight was observed.\textsuperscript{32} It interesting to note that none of the 5/6 Nx pregnant rats had deterioration of renal function when compared with nonpregnant rats. In this model, no increase in glomerular capillary pressure with pregnancy was reported,\textsuperscript{19} suggesting an absence of a hemodynamic mechanism by which pregnancy could exacerbate the renal damage. In addition, the finding that 5/6 Nx did not modify maternal weight or weight gain throughout the study period indicates a lack of any significant nutritional alteration produced by this degree of renal failure. Considering that maternal malnutrition affects fetal growth,\textsuperscript{33} the relatively normal maternal nutritional status most probably contributed to the adequate fetal weight observed in 5/6 Nx rats.

Perspectives

In the present study, we demonstrate that pregnant rats with mild renal failure caused by 5/6 Nx have a normal plasma volume expansion and an adequate fetal growth, despite lower PRA and kallikrein activity. The apparent dissociation between PRA and aldosterone levels was an unexpected finding that most probably contributed to the normal volume expansion. These rats also had a late fall in blood pressure, suggesting that the characteristic reduction in total peripheral vascular resistance still occurs in this model. Hence, it is reasonable to speculate that other vasodepressor systems, such as prostaglandins or nitric oxide, are activated to compensate the reduced kallikrein activity, thus allowing vasodilatation and secondary volume expansion.

Acknowledgments

This study was partially supported by grant 1.000.553 from the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT), Chile.

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_Hypertension._ 2003;42:744-748; originally published online August 4, 2003;
doi: 10.1161/01.HYP.0000085783.96609.0C

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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