Hypertension After Neonatal Uninephrectomy in Rats Precedes Glomerular Damage

Lori L. Woods, Douglas A. Weeks, Ruth Rasch

Abstract—The present study was designed to determine whether adult hypertension caused by a reduced number of nephrons from birth is due to preceding glomerular damage. Newborn male Sprague-Dawley rat pups were uninephrectomized during the first 24 hours after birth (UNX rats). At 20 weeks of age, chronically instrumented UNX animals were hypertensive on a normal-sodium (0.20%) diet compared with sham-operated controls (142±2 versus 124±2 mm Hg in controls). Body weights and the total kidney-to–body weight ratio were not significantly different in adult UNX animals compared with controls. Glomerular filtration rate (GFR) was reduced by 49% in UNX rats (1.85±0.24 versus 3.65±0.22 mL/min). Urine protein excretions were higher in UNX rats (20±2 versus 7±1 mg/d in controls). On a high-sodium (3.15%) diet, arterial pressure increased more in UNX than in controls (28±9 versus 3±1 mm Hg). In contrast, in animals studied at 8 weeks of age, GFR was only reduced by 26% in UNX animals (2.02±0.06 versus 2.73±0.07 mL/min). Their hypertension (125±2 versus 117±2 mm Hg) was also salt sensitive (increase on high-sodium diet of 35±11 versus 8±2 mm Hg in controls) but was not associated with proteinuria or histological signs of glomerular disease. Number of glomeruli per kidney in UNX animals was not different from controls, but individual glomerular volume increased by 41%. Thus, surgical removal of 50% of the nephrons, when done during development, causes reduced renal function and salt-sensitive hypertension in adulthood. Hypertension is present earlier in life than signs of glomerular disease, which suggests that hypertension is a major contributor to rather than primarily resulting from onset of renal disease. (Hypertension. 2001;38:337-342.)

Key Words: kidney ■ glomerular filtration rate ■ renal blood flow ■ sodium ■ development ■ gender

Research has established that the kidney plays a dominant role in long-term regulation of arterial pressure and, therefore, in the development of hypertension.1 Brenner and colleagues2 have postulated that the risk of developing essential hypertension in adulthood is inversely related to nephron endowment at birth. We have recently shown in female rats that surgical reduction in the number of nephrons (uninephrectomy) when done during development, results in hypertension in adulthood;3 these results support the postulate of Brenner et al. However, other investigators have shown in male rats that uninephrectomy at 10 days postnatal age results in signs of glomerular damage relatively early in life.4 Because hypertension can be either a cause or a result of glomerular disease (or both), it is important to establish the time course of the occurrence of these 2 phenomena. The present study was designed to test the hypothesis that uninephrectomy during development results in hypertension before glomerular damage occurs.

Methods
Female Sprague-Dawley rats (Simonsen) weighing 250 to 300 g each were bred at Oregon Health Sciences University and maintained on a normal-sodium (0.20%) diet (Purina 5755) ad libitum throughout pregnancy and lactation. Within the first 24 hours after birth, newborn male pups (n=7, 8 weeks, and n=5, 20 weeks) were uninephrectomized (UNX rats) as previously described. Sham-operated (n=6, 8 weeks) or unoperated littermates (n=3, 8 weeks, and n=6, 20 weeks) were used as controls. No differences occurred between data from sham and unoperated controls, so the 2 groups were pooled. Pups were weaned to the above diet at 22 days of age and instrumented at either 7 or 19 weeks of age. Immediately before surgery, 24-hour urine collections were performed for determination of protein excretion. All procedures on animals were conducted in accordance with institutional guidelines.

Surgical Preparation of Juvenile and Adult Animals
Under ketamine, xylazine, and acepromazine anesthesia, juvenile and adult animals were chronically instrumented with femoral arterial and venous catheters and a bladder catheter, allowed to recover, and trained as described previously.3

Experimental Protocol
For physiological measurements, each rat was placed in a wire restrainer and urine was allowed to drain continuously through the bladder catheter. Mean arterial pressure was measured directly as previously described.3 An arterial blood sample was taken for measurement of microhematocrit and plasma protein. Inulin (Sigma

Received November 30, 2000; first decision January 16, 2001; revision accepted January 28, 2001.
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Chemical Co) and para-immunohippuric acid (PAH; Sigma) in 5% dextrose were given intravenously as a bolus (0.7 mL containing 88 mg of inulin and 8.8 mg of PAH) followed by continuous infusion (0.024 mL/min of 55 mg/mL of inulin and 5.5 mg/mL of PAH). An additional infusion of 0.024 mL/min of 5% dextrose was begun 40 minutes later to maintain adequate urine flow. At least 60 minutes after inulin/PAH infusion was begun, 4 successive 20-minute urine collections (clearance periods) were performed. A blood sample was taken at the midpoint of each urine collection. Urine volume was determined gravimetrically. After the blood was centrifuged, plasma was frozen at −20°C, and red cells were resuspended in an equivalent volume of saline and returned to the animal.

Some animals were placed on a high-sodium (3.15%) diet (Purina diet 5883), and arterial pressure measurements were repeated daily or every other day beginning 7 to 8 days later. Equilibration on the diet was verified by body weight measurement and constancy of arterial pressures between 8 and 10 to 14 days, and the “high salt” blood pressure value was taken to be the plateau value (when pressure had been the same for 2 to 3 consecutive days).

When all experiments were completed, each rat was killed with a commercial euthanasia solution. Kidneys and adrenals were removed immediately and weighed. All left adrenals were intact, and left and right adrenal weights were not different from each other in any of the groups. Kidneys were fixed in formalin and embedded in paraffin or fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L phosphate and embedded in Technovit embedding medium. Sections (3-μm, 3 to 4 per kidney) of paraffin-embedded tissue were stained with hematoxylin and eosin and were examined for renal pathology. Technovit-embedded tissue was used for determination of glomerular number and volume by use of stereologic techniques as described previously.

### Analytical Measurements

Inulin and PAH in plasma and urine were assayed after deproteinization of the plasma with zinc sulfate. Glomerular filtration rate (GFR) and effective renal plasma flow were calculated as renal clearance of inulin and PAH. Values obtained for the 4 clearance periods were averaged to give a single value for each animal. Plasma protein was measured by refractometry (National Instrument). Urine protein was determined gravimetrically. After the blood was centrifuged, plasma was frozen at −20°C, and red cells were resuspended in an equivalent volume of saline and returned to the animal.

Total kidney weight represents combined weight of both kidneys in control animals and weight of the single remaining kidney in uninephrectomized animals.

### Results

Body weights at weaning were not significantly different between control and UNX animals (control, 65±2 g, versus UNX, 59±1 g; P=0.06). Body weights at time of study also were not different (Table 1). Thus, neonatal uninephrectomy did not appear to affect growth. Total kidney weights, and thus the kidney-to-body weight ratio, were reduced in UNX at 8 weeks (although this did not reach significance) but were not significantly different in control and UNX animals at 20 weeks. Hematocrits tended to be slightly higher in UNX animals compared with controls of the same age, but plasma protein levels were not different between control and UNX animals at either age.

Mean arterial pressures and renal hemodynamic variables are shown in Figure 1. Mean arterial pressure was signifi-

### Statistical Analysis

Data are expressed as means±SE. Data for groups were compared by use of a 2×2 (treatment×age) ANOVA. Within-animal changes in response to a high-sodium diet were compared with a paired t test. Statistical significance was indicated by a value of P<0.05 or better.

### Table 1. Body Weights and Renal Hemodynamic Variables in Control and UNX Rats. Means±SE.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>UNX (n=7)</th>
<th>Control (n=6)</th>
<th>UNX (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight at study, g</td>
<td>274±7</td>
<td>273±3</td>
<td>438±32†</td>
<td>413±13†</td>
</tr>
<tr>
<td>Total kidney weight, g</td>
<td>2.78±0.14</td>
<td>2.09±0.11</td>
<td>2.73±0.28</td>
<td>2.71±0.29</td>
</tr>
<tr>
<td>Kidney/body weight ratio, %</td>
<td>0.958±0.050</td>
<td>0.783±0.035†</td>
<td>0.649±0.029†</td>
<td>0.678±0.070</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>44±1</td>
<td>46±1*</td>
<td>40±2†</td>
<td>44±2</td>
</tr>
<tr>
<td>Plasma protein, g/dL</td>
<td>6.3±0.1</td>
<td>6.3±0.1</td>
<td>6.7±0.1†</td>
<td>6.7±0.1†</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.359±0.013</td>
<td>0.274±0.015*</td>
<td>0.353±0.012</td>
<td>0.245±0.015*</td>
</tr>
<tr>
<td>GFR/kidney weight, mL·min⁻¹·g⁻¹</td>
<td>0.98±0.06</td>
<td>0.98±0.04</td>
<td>1.34±0.02†</td>
<td>0.75±0.19*</td>
</tr>
<tr>
<td>ERPF/kidney weight, mL·min⁻¹·g⁻¹</td>
<td>2.81±0.13</td>
<td>3.54±0.23</td>
<td>4.04±0.20†</td>
<td>2.97±0.59</td>
</tr>
<tr>
<td>Final urine protein excretion, mg/dl</td>
<td>4±1</td>
<td>5±1</td>
<td>7±1†</td>
<td>20±2†</td>
</tr>
</tbody>
</table>

*P<0.05 or better vs controls, †P<0.05 or better vs 8-wk-old animals.
significantly increased at both 8 and 20 weeks after neonatal uninephrectomy, and hypertension was greater in older animals. Urine protein excretions (Table 1) were significantly higher in UNX rats than in controls in the 20-week-old groups but were not different between 8-week-old UNX and control groups. Thus, hypertension was present in UNX animals before proteinuria appeared. Absolute GFR was reduced by 26% at 8 weeks and by 49% at 20 weeks in UNX animals, although GFR normalized to kidney weight was reduced only in the older animals. Effective renal plasma flow was not different in control and UNX animals at 8 weeks of age but was significantly reduced in UNX compared with controls by 20 weeks. Filtration fraction was reduced in UNX at both ages (Table 1). Body weights of young rats increased after 8 to 14 days on the high-sodium diet, consistent with their rapid growth at this age, but no differences existed between the 2 groups (change, 29±5 g in controls and 27±6 g in UNX animals). In 8-week-old control animals, arterial pressure increased significantly (but by only 8±2 mm Hg) on the high-sodium diet (Figure 2). In comparison, in 8-week-old UNX animals, mean arterial pressure increased by 35±11 mm Hg during the high-sodium diet, a response that was significantly greater than that in the control animals. In the older groups, body weights were unchanged in control rats after 8 to 14 days on the high-sodium diet, but arterial pressure responses to a high-sodium diet in 20-week-old control animals were salt sensitive. In contrast, arterial pressure responses to high-sodium diet in 20-week-old UNX animals were similar to those in the younger rats: pressure increased by 3±1 mm Hg in controls and by 28±9 mm Hg in UNX. Thus, hypertension in the UNX animals was salt sensitive.

Histopathological analysis showed that all except 1 of the 20-week-old UNX animals had severe chronic renal parenchymal injury, with extensive interstitial fibrosis, tubular atrophy, and focal glomerulosclerosis (Figure 3). These abnormalities were not present in the kidneys of 20-week-old control animals. No histopathologic differences were seen between kidneys of control and UNX animals at 8 weeks of age.

Stereologic data are shown in Table 2. Glomerular number per kidney was not significantly different between control and UNX animals. However, average individual glomerular volume and total volume of all glomeruli (per kidney) were increased in the UNX animals.

**Discussion**

The most important findings of this study are that surgically reducing the number of nephrons by 50% in male rats during

**Table 2. Glomerular Number and Volume in 8-Week-Old Male Control and UNX Rats**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>UNX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular No./kidney</td>
<td>28 850±3615</td>
<td>25 500±2802</td>
</tr>
<tr>
<td>Average individual glomerular volume, 10² μm³</td>
<td>1.43±0.12</td>
<td>2.01±0.19*</td>
</tr>
<tr>
<td>Total glomerular volume, mm³/kidney</td>
<td>39.3±2.5</td>
<td>49.9±2.9*</td>
</tr>
</tbody>
</table>

Values are mean±SE; control, n=6; and UNX, n=4. *P<0.05.
early postnatal development results in increased arterial blood pressure later in life and that this hypertension precedes the development of proteinuria and histological evidence of renal disease. This latter finding indicates that hypertension is likely to be an initiating factor that contributes to eventual glomerular damage. These data suggest that in humans, reduced nephron endowment from birth could lead to essential hypertension in adulthood and predispose an individual to development of renal disease.

The kidney is well known to be the major long-term regulator of arterial blood pressure. Although textbooks commonly give the number of nephrons in a human kidney to be 1 million, considerable variation actually occurs among apparently normal individuals. Brenner and colleagues have postulated that an individual’s risk of developing hypertension is inversely related to his or her endowment of nephrons, i.e., the number of nephrons present at birth. (In humans, nephrogenesis is normally completed by 36 weeks of gestation, so that the number of nephrons present at birth is the most an individual will ever have.) Indeed, the prevalence of hypertension is higher in human populations with smaller kidneys, Moreover, rat strains that exhibit hypertension also are known to have fewer nephrons than nonhypertensive strains. Although uninephrectomy in adulthood is variably reported to increase or have no effect on blood pressure in humans and does not appear to affect blood pressure in rats, the literature suggests that uninephrectomy earlier in life may increase blood pressure over the long term. However, the effect of a reduced number of nephrons from birth on arterial pressure and the association between reduced nephron endowment, hypertension, and renal disease are not well understood.

We found in the present study that male rats with a nephron number reduced by 50% from birth are hypertensive in adulthood (20 weeks). These animals also demonstrate moderate proteinuria. To our knowledge, our studies are the first in which nephrectomy was performed as early as the day of birth. However, our results are generally consistent with findings of Nagata et al, who reported moderate proteinuria by 12 to 16 weeks of age and heavy proteinuria by 24 weeks of age in male rats of the same strain (Sprague-Dawley) that were UNX at 10 days of age. In the present studies, uninephrectomy was performed approximately halfway through the period of nephrogenesis in the rat, whereas in the other study, uninephrectomy was performed just after nephrogenesis was completed. Thus, the possibility existed for new nephrons to be formed in our animals. However, we showed that this did not occur. This is consistent with a report by Nyengaard that uninephrectomy done several days after birth in the (female) rat does not cause formation of new nephrons.

Although the present results are generally consistent with previous reports in male animals uninephrectomized later in the perinatal period, they differ somewhat from our previous findings in female rats. We found that female rats uninephrectomized within the first 24 hours after birth are also hypertensive at ≈20 weeks of age. However, in females we found no histological evidence of glomerular damage at that age and no proteinuria, which suggests that hypertension induced by a reduction in nephron number from birth was not subsequent to glomerular damage. In contrast, we show here that in similarly treated male animals of the same age, moderate proteinuria and renal pathology were already present with the hypertension. Hypertension in males also appeared to be greater than in females of this age (18 mm Hg mean arterial pressure compared with controls in males and 12 mm Hg in females). Thus, males appear to experience renal damage earlier after neonatal uninephrectomy, but the contribution of this damage to the hypertension that is also present at this age was not clear.

These initial findings in older animals led us to study an additional group of similarly prepared but younger male animals to determine whether glomerular damage preceded hypertension. In fact, we found that hypertension was already present in these animals at 8 weeks of age, before either proteinuria or histopathological signs of glomerular disease were seen. Thus, hypertension appears to be an initiating factor in the onset of glomerular disease in this model. The fact that hypertension was greater in older than in younger males (18 versus 8 mm Hg) suggests that hypertension caused by reduced nephron endowment initiates a vicious cycle of progressive renal disease and worsening hypertension. This finding also is supported by the renal function data. Total GFR was reduced by 26% and effective renal plasma flow not at all compared with controls in the younger UNX males, changes that were comparable to those we reported in UNX females at 22 weeks. Considering that UNX animals had only half as many nephrons as controls, the remaining nephrons had increased their individual GFR by an average of 48% compared with controls at this stage. This compensatory hyperfiltration is a well-known response to loss of nephrons in adulthood. However, male rats normally increase GFR almost in parallel with their body growth and in excess of their kidney growth between 8 and 20 weeks of age. The deficit in total GFR increased from 26% to 49% by the time the male UNX animals reached 20 weeks of age. Thus, on average, the remaining nephrons were no longer hyperfiltering. It is not possible to state for certain whether the relative loss of function was due to complete loss of function in some nephrons or partial loss in most nephrons. However, histopathological findings would suggest the latter to be more likely. Additionally, we counted glomeruli in the remaining kidney from two 20-week-old male UNX, and the glomerular numbers (32 600 and 22 500) suggest that on average glomeruli have not “dropped out.” Thus, a reduced number of nephrons from birth appears to lead to an increased arterial pressure, which in turn causes progressive renal damage and loss of renal function, leading to further increases in arterial pressure.

Renal function curves such as those in Figure 2 classically have been used to analyze arterial pressure regulation in altered functional or pathological states of the kidneys. Salt sensitivity of the hypertension in UNX animals, illustrated by the slopes of the renal function curves, would be consistent with at least 2 mechanisms for hypertension: reduced renal mass and inappropriate activation of the renin-angiotensin-aldosterone system. Given that renal mass was known to be reduced initially in the UNX animals in the present study, this
factor is likely to have contributed to the salt sensitivity of the hypertension in the UNX rats. However, the function of the renin-angiotensin system also could have been altered in these animals. We found that high sodium intake was as effective for suppression of plasma renin activity in female UNX rats as in control animals, which suggests that the salt sensitivity of the hypertension of the UNX rats was not due to inadequate suppression of the renin-angiotensin system.3

Results of the present study may have important implications for origin of human hypertension. Numerous studies by several different investigators in a number of populations around the world now have demonstrated convincingly an inverse relationship between early growth patterns and risk for adult disease, particularly cardiovascular disease and hypertension.23–34 This indicates that factors in the maternal environment during pregnancy and development can “program” the offspring for increased cardiovascular risk. Several animal models of this phenomenon are currently being studied, including maternal dietary protein or global food restriction, impairment of the uterine or placental circulation, perinatal blockade of the renin-angiotensin system, and increased exposure to maternal glucocorticoids, all of which lead to hypertension in the offspring.35–43 We43 and others44,45 have shown that, at least in some of these models, nephron number is reduced. Results of the present study suggest that factors that determine an individual’s size at birth, particularly maternal environmental factors, may also “program” his or her risk for hypertension and related cardiovascular disease, at least in part, through determination of the number of nephrons with which he or she is endowed. Furthermore, this reduced nephron number may also predispose the individual to development of progressive renal disease.

Acknowledgments

The present studies were supported by grant 1PO1HD34430-01A1 from the National Institute of Child Health and Human Development (L.L.W.) and a Grant-in-Aid from the American Heart Association (L.L.W.), and by the Danish Diabetes Foundation (R.R.), NOVO (R.R.), and the Aarhus University Research Foundation (R.R.). The authors thank Kathryn Parent, Joan Phillips, Vicki Feldmann, and Birgitte Grann for technical assistance.

References


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*Hypertension*. 2001;38:337-342
doi: 10.1161/01.HYP.38.3.337

*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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