Neonatal uninephrectomy causes hypertension in adult rats

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Woods, Lori L. Neonatal uninephrectomy causes hypertension in adult rats. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R974–R978, 1999.—This study was designed to test the hypothesis that a reduced number of nephrons from birth leads to increased arterial pressure in adulthood. Newborn Sprague-Dawley rat pups were uninephrectomized during the first 24 h after birth. In chronically instrumented adult animals (22 wk), mean arterial pressure on a normal (0.20%)-Na⁺ diet was higher in uninephrectomized rats (133 ± 2 mmHg vs. 121 ± 2 mmHg in controls, P < 0.0001). Body weights were not significantly different, but the total kidney-to-body weight ratio was significantly reduced by 14% in adult uninephrectomized animals (P < 0.05). Glomerular filtration rate was reduced by ~30% in uninephrectomized rats (1.84 ± 0.09 vs. 2.63 ± 0.14 ml/min, P < 0.0002), and effective renal plasma flow was reduced to a lesser degree (6.37 ± 0.38 vs. 7.87 ± 0.51 ml/min, P < 0.03), such that the filtration fraction was also reduced (0.291 ± 0.007 vs. 0.338 ± 0.014, P < 0.01). After 7–10 days on a high (3.15%)-Na⁺ diet, arterial pressure increased more in uninephrectomized animals than in controls (20 ± 3 vs. 1 ± 1 mmHg, P < 0.003). Thus surgical removal of 50% of the nephrons, when done during development, caused reduced renal function and a salt-sensitive hypertension in adulthood. These data suggest that a reduced nephron endowment from birth, caused by genetic and/or perinatal environmental factors, could contribute to essential hypertension in adulthood.

the kidney; glomerular filtration rate; renal plasma flow; salt sensitivity; development

Many years ago, Guyton and colleagues (11) advanced the idea that the kidney plays a dominant role in long-term regulation of arterial pressure and thus in the development of hypertension, an idea that has been supported by numerous experimental studies (14, 15). Over the past several years, Brenner and colleagues (3) have postulated that the risk of developing essential hypertension in adulthood is inversely related to the nephron endowment at birth. Evidence in support of this hypothesis is that hypertension is more prevalent in human populations that have smaller kidneys (20, 26–28) and that rats in inbred models of hypertension have fewer nephrons than their respective controls (3).

Although a severe surgical reduction in nephron number (% nephrectomy) in adult animals is known to cause hypertension (1, 12), reports are conflicting as to whether a less severe reduction in renal mass, i.e., uninephrectomy, results in an increased blood pressure (13, 25, 30, 32). However, nephrectomy in human patients and animals has generally not been done very early in life. It is possible that reductions in nephron number that occur early, while the nephrons are still developing, may have a greater effect on long-term control of blood pressure. The present study was designed to test the hypothesis that uninephrectomy during the immediate postnatal period in rats results in an increased blood pressure in adulthood.

METHODS

Female Sprague-Dawley rats (Simonsen) weighing ~250–300 g were bred at Oregon Health Sciences University and maintained on a normal-protein (19%), normal-Na⁺ (0.20%) diet (Purina 5755) ad libitum throughout pregnancy and lactation. Within the first 24 h of life, newborn female pups (n = 10) were anesthetized with isoflurane (~2% in 0.5–1.0 l/min oxygen, by mask), placed on their sides on a heating pad at 37°C, and swabbed with betadine. Under aseptic conditions, an incision was made in the left flank and the left kidney was gently lifted. A single ligature was placed around the renal vessels and ureter and tied tightly. The distal portions of the renal vessels and ureter were then cut, the kidney was removed, and the incision was sewn closed. The pup was given 0.1 ml of 0.45% saline and 2.5% dextrose subcutaneously and allowed to remain on the heating pad until it was moving, at which time it was replaced in the litter with its mother. The total time away from the litter generally did not exceed 30 min. Sham-anesthetized littermates (n = 9) were used as controls. Pups were weaned to the above diet at 22 days of age and maintained on that diet until instrumentation at ~135 days. Before surgery, some animals [n = 7 controls, n = 6 uninephrectomized (UNX)] were housed overnight in metabolic cages for 24-h urine collections.

Surgical preparation of adult animals. The animals were anesthetized with a mixture of 55% ketamine (100 mg/ml), 28% xylazine (20 mg/ml), 11% acepromazine (10 mg/ml), and 6% sterile water, administered at 1.0 ml/kg intraperitoneally. The sites of the incisions were shaved and swabbed with betadine, and all catheters, suture, instruments, and gloves used were sterile. Through a midline abdominal incision, a stainless steel Silastic-covered catheter was inserted through a puncture hole within a purse-string suture in the bladder, and the purse-string was drawn up tightly and tied. The catheter was allowed to exit through a puncture hole in the skin on the ventral surface of the abdomen, and the muscle and skin were sutured closed. The bladder was flushed with chloramphenicol sodium succinate (30 mg/ml), and the catheter was plugged. Tygon catheters were implanted into the left femoral artery and vein, tunneled under the skin to exit on top of the head, filled with heparin (500 U/ml), and plugged with stainless steel pins. A mixture of rat chow and 5% dextrose was provided in a bowl for the first 24 h after surgery to encourage eating. Animals were allowed to recover in individual cages for at least 7 days before any experiments were conducted and were maintained on the normal diet. Vascular catheters were flushed every 2 or 3 days to maintain patency. During the recovery period, the animals were placed in a wire restrainer in the study room for at least 2 h on at

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least three occasions to acclimatize them to the study conditions.

Experimental protocol. On the days on which physiological measurements were made, the rat was placed in a wire restrainer and urine was allowed to drain continuously through the bladder catheter into a tube. Mean arterial pressure was measured through the arterial catheter using a pressure transducer (Statham, Oxiod, CA) connected to a polygraph (Grass Instruments, Quincy, MA), and a reading was taken after at least 30 min, once the pressure had stabilized. All blood pressure measurements were made between 6:00 and 9:00 AM. An arterial blood sample was taken for measurement of microhematocrit, plasma protein, and plasma renin activity (PRA). The sample for PRA was placed on ice in a tube containing sodium EDTA and centrifuged at 4°C, and the plasma was frozen at −20°C. Inulin (Sigma, St. Louis, MO) and p-aminophenolurate (PAH) (Sigma) in 5% dextrose were given intravenously as a bolus (0.45 ml containing 56 mg inulin and 5.6 mg PAH) followed by a continuous infusion (0.024 ml/min of 74 mg/ml inulin and 7.4 mg/ml PAH) throughout the rest of the experiment. At least 60 min after the beginning of the inulin/PAH infusion, three or four successive 20-min urine collections (clearance periods) were done, with a blood sample taken at the midpoint of each. Blood was collected in sterile urokinase-sodium heparinized syringes. Urine volume was determined gravimetrically. After centrifugation of the blood, the plasma was frozen at −20°C and the red blood cells were resuspended in an equivalent volume of saline and returned to the animal.

Five animals from each group were then placed on a high (3.15%)-Na+ diet (Purina diet 5883, modified from diet 5755), and the arterial pressure measurements were repeated at 7 and 10–11 days after the diet was changed. Body weight was sometimes reduced for the first few days after beginning the high-Na+ diet, but the animals then regained the lost weight, and body weights plateaued by 7–10 days. Equilibration on the diet was verified by body weight measurement and constancy of arterial pressures between 7 and 10–11 days.

When all experiments were completed or when the instrumentation was no longer functional, the rats were killed with a commercial euthanasia solution. Kidneys were removed immediately and weighed. The right kidney was step sectioned (3 or 4 per kidney) was stained with hematoxylin and eosin and used for evaluation of renal pathology. Slides of tissue from control and UNX animals were examined in a blinded fashion.

Analytic measurements. Inulin (31) and PAH (4) in plasma and urine were assayed after deproteinization with zinc sulfate. Glomerular filtration rate (GFR) was calculated as the renal clearance of inulin \[ \text{GFR} = \frac{(U_{in}P_{in})}{V}, \] where \( U_{in} \) and \( P_{in} \) are the urine and arterial plasma inulin concentrations, respectively, and \( V \) is the urine flow rate. Effective renal plasma flow (ERPF) was calculated as the renal clearance of PAH. The values obtained for the three or four clearance periods were averaged to give a single value for each animal. Plasma protein was measured by refractometry (National Instrument, Baltimore, MD). Urine protein was determined on 24-h samples by precipitation with sulfosalicylic acid using albumin standards. PRA was measured by radioimmunoassay using a kit from New England Nuclear.

Statistical analysis. The data are expressed as means ± SE. Data for the groups were compared using an unpaired t-test. Within-animal changes in response to a high-Na+ diet were compared using a paired t-test. Statistical significance was indicated by a value of \( P < 0.05 \) or better.

### RESULTS

Body weights at weaning and at the time of study were not significantly different between control and UNX animals (Table 1). Thus neonatal uninephrectomy did not appear to affect growth. Total kidney weights were also not significantly different in control and UNX animals; however, the kidney-to-body weight ratio was slightly reduced in UNX rats. Hematocrits (0.33 ± 0.02, control vs. 0.37 ± 0.02, UNX) and plasma protein levels (6.3 ± 0.1 g/dl, control vs. 6.3 ± 0.1 g/dl, UNX) were not different between the two groups. Urinary protein excretion was not significantly different between the two groups (5 ± 1 mg/day, control vs. 8 ± 2 mg/day, UNX).

Mean arterial pressures and renal hemodynamic variables are shown in Fig. 1. Mean arterial pressure was significantly increased after neonatal uninephrectomy (\( P < 0.0001 \)). Absolute GFR (\( P < 0.0002 \)) and ERPF (\( P < 0.03 \)) were reduced in UNX animals, as were GFR and ERPF normalized to body weight. GFR normalized to kidney weight was significantly reduced; however, the reduction in ERPF normalized to kidney weight did not reach statistical significance. Filtration fraction was also significantly reduced (Table 1).

There was no significant difference in body weights of control or UNX rats on the normal diet compared with weights after 7–10 days on the high-Na+ diet (change of 0 ± 3 g). In control animals, arterial pressure did not increase significantly (a change of 1 ± 1 mmHg) on the high-Na+ diet (Fig. 2). By comparison, in UNX animals, arterial pressure increased by 20 ± 3 mmHg during the high-Na+ diet (\( P < 0.005 \)), a response that was significantly greater than that in the control animals (\( P < 0.003 \)). Thus the hypertension in UNX animals was salt sensitive.

PRA in control animals was 4.1 ± 0.8 ng ANG 1·ml−1·h−1 on the normal-Na+ diet and was suppressed to 1.6 ± 0.5 ng ANG 1·ml−1·h−1 on the high-Na+ diet (\( P < 0.02 \)). Similarly, in UNX animals, PRA was 4.2 ± 0.9 ng ANG 1·ml−1·h−1 on the normal-Na+ diet and was suppressed to 0.3 ± 0.2 ng ANG 1·ml−1·h−1 on the high-Na+ diet (\( P < 0.02 \)). The baseline values and the

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<th>Table 1. Body weights and renal hemodynamic variables in control rats and rats uninephrectomized during the first 24 h of postnatal life</th>
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<td>ERPF/body wt, ml·min−1·100 g−1</td>
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Values are means ± SE. Total kidney weight represents combined weight of both kidneys in control animals and weight of single remaining kidney in uninephrectomized animals. GFR, glomerular filtration rate; ERPF, effective renal plasma flow. *\( P < 0.05 \) or better compared with controls.
response to the high-Na\(^+\) diet were not significantly different in the two groups of animals.

Histopathological analysis showed no significant glomerular lesions in either group, although the average glomerular size appeared larger in the UNX animals. UNX rats also showed focal areas of cortical tubular collapse, associated chronic inflammation, and local tubular dilatation. This was only a minor finding, affecting only small, widely spaced zones in an otherwise normal tubulointerstitial compartment.

**DISCUSSION**

The most important finding of this study is that a surgical reduction in the number of nephrons by 50% in early postnatal development results in an increased arterial blood pressure in adulthood. These data provide the first direct evidence that a reduced endowment of nephrons from birth can lead to adult hypertension.

The effect on blood pressure of a 50% reduction in nephron number (uninephrectomy) in adult animals or humans remains controversial. In humans, some investigators have reported an increased prevalence of hypertension (13), whereas others found no significant change in blood pressures 10 or more years after uninephrectomy (25, 30, 32). Unfortunately, most human studies looking at this issue are retrospective, some lack appropriate controls, and often subjects were taking antihypertensive medication, making interpretation difficult. Thus it remains possible that the blood pressures of adult humans increase after nephrectomy without necessarily reaching the "hypertensive" range. Uninephrectomy in adult rats of several strains has been reported to have no significant effect on either mean arterial pressure or systolic pressure (6, 8, 22). Indeed, some (10) but not all (29) investigators have found that even a more extreme reduction in renal mass (5/6 nephrectomy) fails to cause hypertension if it does not involve infarction.

Somewhat in contrast to uninephrectomy in adulthood, there are at least suggestions in the literature that uninephrectomy relatively early in life may increase blood pressure (9, 21), although this is also controversial (16, 24). In the guinea pig, uninephrectomy within the first 36 h after birth resulted in an increased arterial pressure at 10 days of age compared with sham-operated animals (5).

In the present study, it was found that adult mean arterial pressures were significantly increased in rats UNX within the first 24 h of postnatal life. An important distinction between the present study and previous work is the stage of development at which renal mass was reduced. In the present work, uninephrectomy was performed midway through the period of nephrogenesis, which occurs from midgestation until 7–10 days after birth in the rat (18). In contrast, in humans and guinea pigs, nephrogenesis is completed before birth (7). Thus it seems likely that reducing the number of nephrons during the developmental period, when physiological control mechanisms are still plastic, may reprogram the future blood pressure set point in a way that does not occur if the reduction takes place later in life.

There are several possible mechanisms that could be responsible for the increased arterial pressure in the UNX animals in this study. In general, hypertension occurs because of the inability of the kidney to maintain sodium and water balance at a normal arterial pressure. This inability can be due to elevated levels of hormonal factors such as angiotensin or vasopressin as well as to a reduced glomerular filtration coefficient.
(Kf), involving a reduced capillary surface area for filtration and/or decreased permeability of the glomerular barrier. In the present studies, baseline PRA was not significantly different in the two groups of animals, suggesting that the hypertension in UNX animals was not due to a chronically increased activity of the renin-angiotensin system. However, an increased arterial pressure per se would be expected to suppress the renin-angiotensin system. The fact that PRA was not suppressed in UNX animals, despite their higher blood pressures, does suggest that the renin-angiotensin system was not entirely normal in these animals.

A reduced glomerular capillary filtration surface area could also have contributed to the need for an increased arterial pressure to maintain fluid and electrolyte balance in the UNX animals in this study. Initially, the total filtration surface area must have been reduced by half, because 50% of the glomeruli were removed by uninephrectomy. However, by the time of study in adulthood, the size of the individual remaining glomeruli appeared to be increased in the UNX animals in this study. The phenomenon of compensatory hypertrophy of remaining nephrons after a reduction in nephron number is well established in animals in which nephrons are lost in adulthood (10). A reduced nephron (glomerular) number would be expected to lead to hypertension unless the capillary surface area and/or the hydraulic permeability of the capillary walls in the remaining glomeruli increased sufficiently to make up for the number of nephrons lost. Although direct measurements of these parameters were not obtained, the fact that total GFR was reduced in UNX animals compared with controls suggests that the compensatory changes in the capillaries of the remaining glomeruli were not sufficient to completely make up for the lost nephrons.

Finally, it was documented that the remaining nephrons have increased their filtration rate by ~35%, assuming that their number remains constant postnephrectomy. This hyperfiltration and a possible accompanying increase in glomerular hydrostatic pressure in the remaining nephrons could have contributed, over the long term, to glomerular injury and progressive nephron loss, thus further increasing the arterial pressure needed to maintain sodium and water balance. Indeed, uninephrectomy in adult rats causes the remaining nephrons to increase their individual filtration rates by ~50% on average (6, 8, 22). If the same was true initially in the animals in this study, this could then have been followed by a gradual decrease associated with progressive glomerular damage due to lifelong hypertension. However, the histopathological evidence does not seem to support this idea, because UNX animals did not appear to have more glomerular damage than controls. The fact that urine protein excretions were not different in UNX animals compared with controls also argues against the presence of major glomerular damage in UNX animals. On the other hand, the possibility cannot be ruled out that a more comprehensive histopathological analysis or study of the animals at an older age could reveal more subtle glomerular damage that was not seen. Of note, male rats UNX at 10 days of age have been reported to show significant glomerular damage and proteinuria by 12 wk after surgery (23). However, the animals in the present study were female, and female rats in general tend to be more resistant to developing renal disease and hypertension than males.

Classically, renal function curves have been used to analyze arterial pressure regulation in altered functional or pathological states of the kidneys (12). The salt sensitivity of the hypertension in the UNX animals in this study, illustrated by the slope of the renal function curve in Fig. 2, would also be consistent with at least two different underlying hypertensive mechanisms: reduced renal mass per se and inappropriately increased levels of ANG II or aldosterone (12). In the adult, surgical reduction of renal mass is known to lead to a salt-sensitive type of hypertension, and because it is known that renal mass was reduced in the UNX animals in this study (at least initially), it is likely that this factor itself contributes to the salt sensitivity of their hypertension. On the other hand, a high Na+ intake reduced PRA in UNX animals to a similar extent as in controls. This suggests that the salt sensitivity of their hypertension is not due to an inadequate suppression of the activity of the renin-angiotensin system and thus physiologically inappropriate high ANG II levels under these conditions.

Perspectives

The results of the present study may have important implications for the etiology of human hypertension. Accumulating evidence in the epidemiological literature indicates that babies that are born smaller or grow more slowly during the first year of life have an increased incidence of hypertension and death from cardiovascular disease when they reach adulthood (2, 19). This suggests that some factor(s) in the perinatal environment, probably related to maternal nutrition, can "program" the individual for increased cardiovascular risk later in life. Recent evidence in the rat indicates that maternal dietary protein restriction during pregnancy leads to hypertension in adult offspring (17), suggesting that the protein content of the maternal diet may provide one important link between the epidemiological findings. Moreover, perinatal exposure to maternal protein restriction is known to result in a reduced number of nephrons in the offspring (33). The results of the present studies, in which the number of nephrons was surgically reduced during the perinatal period, provide strong evidence that a reduced number of nephrons from birth per se could account for the hypertension seen in the offspring of protein-restricted mothers. It is speculated that the protein content of the mothers’ diets and consequent differences in the number of nephrons with which their babies are endowed may provide an important link in the association between early growth rates and later cardiovascular risk reported in human studies.
REFERENCES


