Age- and Sodium-Sensitive Hypertension and Sex-Dependent Renal Changes in Rats With a Reduced Nephron Number

Francisco Salazar, Virginia Reverte, Fara Saez, Analia Loria, M. Teresa Llinas, F. Javier Salazar

Abstract—We have demonstrated that the reduction of angiotensin II effects during the nephrogenic period reduces the nephron number and induces the development of hypertension. The hypotheses examined are that this reduction of angiotensin II effects leads to the development of an age-dependent sodium sensitive hypertension and that the hypertension is angiotensin II dependent. Newborn rats were treated with an angiotensin II type 1 receptor antagonist during the first 2 weeks of age. At 3 to 4 and 11 to 12 months of age, changes in systolic blood pressure, proteinuria, and renal function in response to a prolonged high sodium intake were examined. The basal blood pressure response to the administration of the angiotensin II receptor antagonist was also evaluated at both ages. Basal blood pressure was similarly elevated (P<0.05) in male and female treated rats, and the increment was age dependent. High sodium intake only elicited a blood pressure elevation (136±1 to 154±3 mm Hg; P<0.05) and a decrease in glomerular filtration rate (28%; P<0.05) at 11 to 12 months in treated rats. Blockade of angiotensin II receptors during renal development induced an increase (P<0.05) in proteinuria that was age and sex dependent, but high sodium intake only induced an elevation in proteinuria in the younger rats (50%; P<0.05). Hypertension was maintained by angiotensin II at both ages because blood pressure decreased to normal levels after treatment with an angiotensin II type 1 receptor antagonist. This study shows that the reduction of angiotensin II effects during the nephrogenic period modifies renal function and induces the development of an angiotensin II–dependent hypertension that becomes sodium sensitive during aging.

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Key Words: angiotensin II • renal function • nephrogenesis • proteinuria

The importance of nephron number in the development of hypertension and renal dysfunction is supported by experimental and clinical studies1–9 demonstrating that the alteration of nephrogenesis regulation leads to significant changes in arterial pressure and renal function during the adulthood. These effects of the reduction in nephron number during renal development seem to be more significant than those elicited by a decrease in nephron number later in life.3 One mechanism that is involved in the regulation of nephrogenesis is the renin-angiotensin system (RAS). The role of RAS has been confirmed in previous studies of our group demonstrating that the blockade of the angiotensin II (Ang II) type 1 (AT1) receptors during the late nephrogenic period reduces nephron number by 37%, induces the development of hypertension, and elicits important renal changes that are greater in male than in female rats.7,8 An age- and sex-dependent increment in proteinuria is observed in rats when the effects of Ang II via the AT1 receptor are reduced during the nephrogenic period.7,8 These rats also have a decrease in renal functional reserve, because the response to an increment in plasma amino acid levels is deteriorated, and their renal excretory ability to eliminate an acute sodium load is impaired.9 Based on these previous studies, we hypothesized that the blockade of AT1 receptors during nephrogenic period leads to the development of sodium-sensitive hypertension. Therefore, our first objective was to evaluate whether rats with a reduction in nephron number, induced by treatment with an AT1 receptor antagonist (ARA),7 develop a sodium-sensitive hypertension and whether this hypertension is age and/or sex dependent.

The second objective of this study was to examine whether the hypertension in adult rats treated with an ARA during nephrogenic period (ARAnp) is maintained by Ang II. The possible involvement of Ang II in this experimental model of hypertension is supported by studies showing that other stimuli that also reduced Ang II levels during renal development, such as a low protein intake or a high sodium diet (HSD), lead to a significant activation of the RAS during adulthood.10–12

Materials and Methods

Sprague-Dawley rats were purchased from the University of Murcia Animal Research Laboratory. The study was approved by the University of Murcia review committee, and experimental protocols were designed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All of the rats were
housed in rooms with controlled temperature (23°C to 24°C) and 12:12-hours dark-light cycles. Food with a normal sodium content (NSD; 0.4%, Panlab) and water were supplied ad libitum. Female Sprague-Dawley rats (>230 g body weight) were placed with a male, taking day 0 of pregnancy the morning that sperm was found in the vaginal smear. On postnatal day 0, litter size was fixed between 8 and 10 to assure similar nourishment during the suckling period. Litters with <8 pups were excluded. Newborn rats were treated from postnatal day 1 to postnatal day 14 with vehicle (isotonic saline) or an ARA (L-158.809, Merck Sharp & Dohme) at an oral dose of 7 mg/kg per day. Twenty-eight pregnant rats gave rise to the 103 offspring used in this study at 3 to 4 or 11 to 12 months of age.

Systolic blood pressure (SBP) was measured in conscious rats by the tail-cuff method as described previously (7.8) using an LE 5002 Storage Pressure Meter. A heater lamp was on the tail to improve signal quality. To obtain an accurate SBP reading, rats were first habituated to the measurement device. Definitive measurements began when rats remained unperturbed in the chamber throughout the inflation-deflation cycles. The SBP values in each rat and day are the mean values of ≥6 measurements. It was found in preliminary experiments that the SBP values obtained using the tail-cuff method are correlated (P < 0.01) with those obtained in conscious freely moving rats through a femoral artery catheter that was exteriorized at the nape of the neck.

**Experimental Protocols**

**Changes in Sodium Intake**

Rats were kept in individual metabolic cages to evaluate the changes in urine flow rate (UV), glomerular filtration rate (GFR), and proteinuria during 24-hour periods. After 2 days of adaptation, rats were maintained with an NSD during 3 days. Then, sodium diet was increased (8%, Panlab) for 7 consecutive days and decreased again to normal levels (0.4%) during 4 days (recovery period). Blood samples from the tail were obtained during the basal period, last day of HSD, and last day of the recovery period. Blood pressure, GFR, proteinuria, and UV were measured during NSD, day 7 of HSD, and the last day of the recovery period. Blood pressure was also measured on day 3 of HSD.

Prolonged changes in sodium intake were performed in 4 groups of rats treated with vehicle during the nephrogenic period (control) and in 4 groups of ARAnp-treated rats. The number of rats in each group at 3 to 4 months of age was 9 for control males, 9 for control females, 9 for ARAnp-treated males, and 9 for ARAnp-treated females. The number of 11- to 12-month-old rats in each group was 8 for control males, 7 for control females, 6 for ARAnp-treated males, and 6 for ARAnp-treated females.

**Prolonged Blockade of Ang II Effects at 3 to 4 and 11 to 12 Months of Age**

To evaluate whether hypertension was Ang II dependent, an ARA (L-158.809, Merck Sharp & Dohme) was administered by gavage during 3 consecutive days at a single dose of 7 mg/kg per day (dissolved in 0.60 mL of isotonic saline). SBP levels were measured by tail cuff during the basal period and each day of ARA administration. The given dose of ARA reduced by >80% the blood pressure increment (27 ± 2 mm Hg) induced by the IV Ang II infusion (30 ng/kg). The number of rats in each group at 3 to 4 months of age was 10 for control rats and 10 for ARAnp-treated rats. The number of 11- to 12-month-old rats in each group was 10 for control rats and 10 for ARAnp-treated rats. Similar numbers of males and females were included in each group.

**Analytic Methods**

Filtration rate was determined by the endogenous creatinine clearance. This method has been used previously by our group in conscious animals.6,11 and the values of GFR found were similar to those obtained in anesthetized rats using the [14C] inulin clearance.6,9 Urine flow rate was determined gravimetrically. Proteinuria was measured by micro Lowry method. This procedure is based on the micro Lowry method. This procedure is based on Peterson’s modification.14 An alkaline cupric tartrate reagent complexes with the peptides chains and forms a purple color, in which absorbance is read at 543 nm. Standard and reagent of Lowry and Folin were from Sigma.

**Statistical Analysis**

Data in text, tables, and figures are expressed as means ± SEs. Significant differences between experimental periods within 1 group were evaluated using ANOVA for repeated measures and the Fisher’s test. Significant differences between groups were examined with the use of ANOVA and Fisher’s test.

**Results**

**Changes in Sodium Intake**

Table 1 shows the response to a 7-day HSD in 3- to 4-month-old rats treated with vehicle or an ARAnp. It can be observed that basal SBP was elevated (P < 0.05), and basal GFR was unchanged in ARAnp-treated rats. Arterial pressure and renal hemodynamics did not change throughout the study in these groups. Proteinuria was greater in males than in females (P < 0.05), and basal proteinuria levels were enhanced only in ARAnp-treated males (P < 0.05). Proteinuria increased (P < 0.05) in male and female ARAnp-treated rats during HSD and decreased to basal levels during the recovery period.

Basal UV was enhanced (P < 0.05) in ARAnp-treated rats at 3 months of age (Table 1). No significant differences in UV between groups were found during changes in sodium intake. Food intake was similar in vehicle and ARAnp-treated male rats during NSD (17 ± 1 and 18 ± 1 g/d, respectively), HSD (13 ± 1 and 15 ± 1 g/d, respectively), and the recovery period (21 ± 1 and 23 ± 1 g/d, respectively). Food intake was also

**Table 1. Changes in SBP, GFR, Urinary Protein Excretion Rate, and UV in Response to a 7-Day Increment of Sodium Intake at 3 to 4 Months of Age in Rats Treated With Vehicle or an ARAnp**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>ARAnp</td>
<td>Vehicle</td>
<td>ARAnp</td>
</tr>
<tr>
<td></td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>116±2*</td>
<td>127±1*</td>
<td>113±3</td>
<td>128±1*</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>118±2</td>
<td>130±2*</td>
<td>116±1</td>
<td>126±2*</td>
</tr>
<tr>
<td>Recovery</td>
<td>115±2</td>
<td>129±2*</td>
<td>114±2</td>
<td>127±1*</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.93±0.04</td>
<td>0.98±0.06</td>
<td>0.84±0.02</td>
<td>0.81±0.02</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>0.92±0.04</td>
<td>0.95±0.04</td>
<td>0.80±0.03</td>
<td>0.80±0.02</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.92±0.03</td>
<td>0.96±0.05</td>
<td>0.82±0.01</td>
<td>0.83±0.03</td>
</tr>
<tr>
<td>Uprot, µg/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>46±2</td>
<td>61±5*</td>
<td>30±2</td>
<td>34±1</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>55±5</td>
<td>87±9†</td>
<td>42±3</td>
<td>56±5†</td>
</tr>
<tr>
<td>Recovery</td>
<td>53±2</td>
<td>62±3</td>
<td>31±2</td>
<td>42±2</td>
</tr>
<tr>
<td>UV, µL/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>6±1</td>
<td>16±2*</td>
<td>7±1</td>
<td>12±1*</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>59±6†</td>
<td>99±7†</td>
<td>59±5+</td>
<td>76±6†</td>
</tr>
<tr>
<td>Recovery</td>
<td>12±2†</td>
<td>27±5†</td>
<td>14±2+</td>
<td>22±3†</td>
</tr>
</tbody>
</table>

Data are means ± SEs. *P<0.05 vs vehicle; †P<0.05 vs basal.
similar in vehicle and ARAnp-treated female rats during NSD (14±1 and 14±1 g/d, respectively), HSD (12±1 and 13±1 g/d, respectively), and the recovery period (17±1 and 18±1 g/d, respectively).

The effects of prolonged changes in sodium intake at 11 to 12 months of age are shown in Table 2. Basal SBP was elevated in ARAnp-treated rats, being the increment greater than that found at 3 to 4 months. Contrary to what was observed in the younger rats, HSD elicited an SBP increment greater than that found at 3 to 4 months. The increment of SBP in both groups of rats treated with vehicle during the nephrogenic period and in the 3-month–old ARAnp-treated rats. However, HSD elicited an SBP increment (15±1 to 15±3 mm Hg; P<0.05) in the 11- to 12-month–old ARAnp-treated rats.

**Table 2. Changes in SBP, GFR, Urinary Protein Excretion Rate, and UV in Response to a 7-Day Increment of Sodium Intake at 11 to 12 Months of Age in Rats Treated With Vehicle or an ARAnp**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n=8)</td>
<td>ARAnp (n=6)</td>
</tr>
<tr>
<td>Basal SBP, mm Hg</td>
<td>119±4</td>
<td>137±2*</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>120±3</td>
<td>151±4†</td>
</tr>
<tr>
<td>Recovery</td>
<td>115±3</td>
<td>140±2*</td>
</tr>
<tr>
<td>GFR, mL/min</td>
<td>1.10±0.04</td>
<td>0.82±0.03*</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>0.97±0.08</td>
<td>0.60±0.08†</td>
</tr>
<tr>
<td>Recovery</td>
<td>1.09±0.07</td>
<td>0.81±0.06*</td>
</tr>
<tr>
<td>Uprot, µg/min</td>
<td>71±6</td>
<td>197±22*</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>65±9</td>
<td>202±35*</td>
</tr>
<tr>
<td>Recovery</td>
<td>76±6</td>
<td>190±35*</td>
</tr>
<tr>
<td>UV, µL/min</td>
<td>6±1</td>
<td>22±4*</td>
</tr>
<tr>
<td>High Na⁺ intake</td>
<td>42±7†</td>
<td>72±17†</td>
</tr>
<tr>
<td>Recovery</td>
<td>12±2†</td>
<td>39±10†</td>
</tr>
</tbody>
</table>

Data are means±SEs. Uprot indicates urinary protein excretion rate. *P<0.05 vs vehicle; †P<0.05 vs basal.

Changes of SBP in response to HSD are depicted in Figure 1. Results obtained in males and females have been pooled, because no sex differences in SBP were found. It can be observed that HSD did not elicit significant changes in the SBP in both groups of rats treated with vehicle during the nephrogenic period and in the 3-month–old ARAnp-treated rats. However, HSD elicited an SBP increment (15±1 to 15±3 mm Hg; P<0.05) in the 11- to 12-month–old ARAnp-treated rats.

**Figure 1.** SBP during normal sodium intake and after a 7-day increment of sodium intake in rats treated with vehicle (Control) or an AT₁ receptor antagonist (ARAnp) during the nephrogenic period. Upper and lower figures show the results obtained in 3- to 4- and 11- to 12-month–old rats, respectively. Data are expressed as means±SEs. *P<0.05 vs normal sodium. †P<0.05 vs control.

**Prolonged Blockade of Ang II Effects at 3 and 4 to 11 and 12 Months of Age**

The SBP responses to the 3-day administration of an ARA to 3- to 4- and 11- to 12-month–old rats treated with vehicle or an ARAnp are depicted in Figure 2. Results obtained in males and females have also been pooled, because there were not significant sexual differences in the SBP response to the 3-day AT₁ receptor blockade. It can be observed that basal SBP was greater (P<0.05) at 11 to 12 (145±2 mm Hg) than at 3 to 4 (128±2 mm Hg) months of age in ARAnp-treated rats. Basal SBP was similar at both ages in vehicle-treated rats. Figure 2 shows that the ARA administration reduced SBP to similar levels in control rats and in those rats in which Ang II effects were blocked during the nephrogenic period. This SBP response was not significantly different in 3- to 4- and 11- to 12-month–old ARAnp-treated rats.
activity during renal development.\textsuperscript{10,11,17} Low protein intake during pregnancy reduces RAS activity in the newborn offspring, and these animals have hypertension associated with a reduced nephron number and an enhanced expression of the AT\textsubscript{1} receptors later in life.\textsuperscript{11,12,17,18} This AT\textsubscript{1} receptor overexpression was also found in the hypertensive offspring of females on a HSD during pregnancy.\textsuperscript{10,19} Our results confirm that Ang II contributes to the maintenance of hypertension at both ages, because the 3-day ARA administration reduces SBP to similar levels in hypertensive and normotensive rats.

The results showing that proteinuria increases in male but not in female ARAnp-treated rats at the young age and with NSD also confirm those reported by our group.\textsuperscript{7,8} The presence of estrogens may contribute to the greater renal glomerular protection in females when nephron number is diminished.\textsuperscript{20,21} This study extends our previous observations by showing that proteinuria increases in young male and female ARAnp-treated rats when exposed to a HSD. These results suggest that the renal damage produced by the reduction of Ang II effects during renal development increases the sensitivity to stimuli inducing proteinuria even at the young age. It is proposed that the mechanisms protecting the young female kidney with lower nephron number to enhance proteinuria\textsuperscript{7,8} are not effective enough to avoid an increase in proteinuria when sodium intake is elevated.

An age-dependent increment of proteinuria was found in ARAnp-treated rats, being greater in males than in females. Contrary to what was found at 3 to 4 months of age, proteinuria did not change in the oldest ARAnp-treated rats when sodium intake increased during 7 days. The fall in GFR may explain this absence of changes in proteinuria. Another possible reason is that proteinuria was already elevated to a nearly maximal level. Our results suggest that the reduction of Ang II effects (via AT\textsubscript{1} receptors) during renal development may enhance the susceptibility to develop renal failure, because proteinuria is considered an initial sign of renal damage and potentially a prognostic indicator for the future progression of renal disease.\textsuperscript{22}

As reported previously,\textsuperscript{8} GFR values in conscious 3-month-old ARAnp-treated rats were similar to those found in control rats. However, these results are in disagreement with those also obtained by our group,\textsuperscript{7,9} showing a fall of GFR in anesthetized ARAnp-treated male rats. Because GFR is not altered in the conscious and decreased in the anesthetized male ARAnp-treated rats at 3 to 4 months of age, one possible explanation for the discrepancy is that renal hemodynamic would be more sensitive in ARAnp-treated male rats to the greater endogenous vasoconstrictor levels in anesthetized than in conscious animals.\textsuperscript{13,23} The results of this study showing that only ARAnp-treated males have an age-dependent fall in GFR are consistent with those reported by our group\textsuperscript{8} and in models of prenatally programmed hypertension.\textsuperscript{9} The decrease in GFR in male but not in female ARAnp-treated rats during aging may be a consequence of the greater increment of single-nephron GFR during the young age in males.\textsuperscript{7} These studies suggested that females seem to have a greater protection than their male littermates.
against the long-term detrimental consequences of a decrease in nephron number during renal development.

The reduction in GFR during prolonged HSD in the 11- to 12- but not in the 3- to 4-month-old ARAnp-treated rats could be secondary to a greater activation of the tubuloglomerular feedback mechanism in the oldest rats. Although our results do not allow us to determine the mechanism responsible for the fall in GFR during HSD, it is possible that intrarenal Ang II levels may be involved in this GFR change. The hypothesis is supported by our results showing that arterial pressure decreased to normal levels when an ARA was administered during 3 days and by studies demonstrating that Ang II enhances the response of the tubuloglomerular feedback mechanism.24 The fall in GFR in the oldest ARAnp-treated rats may not be only secondary to the Ang II effects. One possibility is that Ang II induced a greater vasoconstriction in the oldest rats as a consequence of a decrease in NO synthesis. This hypothesis is supported by studies suggesting that endothelial function is altered when arterial pressure is chronically elevated25 and showing that the modulatory effects of the tubuloglomerular feedback mechanism by Ang II are more important when NO production is decreased.26,27

It has also been proposed that the Ang II effects on the afferent arteriole and renal hemodynamics are greater when NO synthesis is reduced.28,29

The development of sodium-sensitive hypertension during aging in rats with reduced Ang II effects during the nephrogenic period was expected because these rats have an impaired ability to eliminate an acute sodium load,9 and it is known that an age-dependent component seems to be necessary for the development of sodium-sensitive hypertension.30 It has also been reported that a reduction in renal mass leads to the development of sodium-sensitive hypertension.31 Although our study did not allow us to define the mechanisms involved in the development of the sodium-sensitive hypertension, our results suggest that endogenous Ang II levels may be involved not only in inducing a fall in GFR but also in stimulating tubular sodium and water reabsorption.26–28,32

As already mentioned, NO synthesis may be reduced during aging in ARA-treated rats. Therefore, because a decrease in NO impairs the renal ability to eliminate a prolonged elevation in sodium intake,33 it is also possible that the sodium-sensitive hypertension in the oldest ARA-treated rats may be secondary to a fall in NO production. Finally, an increase in oxidative stress, as a consequence of the decrease in NO and the increment in Ang II,27 may also be involved in the sodium-sensitive hypertension found in the oldest ARA-treated rats. The role of endogenous NO in the hypertension and renal changes found in this study should be examined in future studies.

In summary, this is the first study providing strong evidence that the decrease of Ang II effects (via AT1 receptors) during the nephrogenic period programs for the development of hypertension that became sodium sensitive later in life and for a progressive impairment of renal function that is more important in males than in females. It has also been shown that this hypertension seems to be Ang II dependent.

**Perspectives**

Considering that human kidney development is completed before birth,2 the results of this study suggest that an insult inducing a reduction of the RAS activity during the third trimester of pregnancy in humans would lead to the development of an age-dependent sodium-sensitive hypertension and sets into motion a cycle of progressive renal injury that would be more important in males than in females. It is proposed that these alterations may be secondary to an activation of the RAS during the adulthood.

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**Disclosures**

None.

**References**


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