Role of Androgens in Mediating Renal Injury in Aging SHR

Lourdes A. Fortepiani, Licy Yanes, Huimin Zhang, Lorraine C. Racusen, Jane F. Reckelhoff

Abstract—Men have an increased risk of cardiovascular and renal diseases and develop greater renal injury despite similar levels of blood pressure when compared with women. The mechanisms responsible for this predisposition are unknown. Using the spontaneously hypertensive rat (SHR), we have found that androgens play an important role in the development of hypertension in young male SHR. However, the role that androgens play in age-related renal injury and dysfunction in SHR is unknown. Our hypothesis was that despite reductions in serum testosterone with age, androgens mediate renal injury and dysfunction in male SHR. Male SHR were castrated at 8 months of age, studied at 18 months of age, and compared with age-matched, intact males and young intact males (4 months). Serum testosterone was reduced by 30% in aging males compared with young SHR. With castration, blood pressure (mean arterial pressure [MAP]) was decreased by >20 mm Hg compared with old males, glomerular filtration rate (GFR) was increased by >35%, and renal vascular resistance (RVR) was reduced by >40%. MAP, GFR, and RVR in castrated, old males were similar to values in young males. With castration, glomerular sclerosis was reversed and proteinuria was also decreased by >80% when compared with old intact males. In addition, in castrated old males, plasma renin activity was decreased by 30% compared with old males and by 60% compared with young rats. The data support the hypothesis that despite a reduction in testosterone with age, androgens play an important role in age-related renal injury and dysfunction in SHR. (Hypertension. 2003;42:11022–11026.)

Key Words: age • aging • androgens • hypertension, renal • renal disease

Comparison performed in age-matched groups show that men have higher blood pressures than women until old age, when blood pressures become similar.1,2 Men also have a greater incidence of renal disease and proceed to end-stage renal failure faster than do women, even when exhibiting similar levels of blood pressure.3 The kidneys of men also undergo greater decline in renal function with age than do the kidneys of women.4,5 This suggests that male sex hormones might play a role in mediating cardiovascular disease in men. However, the role that androgens play in the control of blood pressure and renal injury in men, especially with age, is not clear.

We have previously found that androgens play a role in the development of hypertension in young, spontaneously hypertensive rats (SHR), because castration at 5 to 7 weeks of age reduces blood pressure when measured at 4 months of age, and testosterone treatment of ovariectomized females increases blood pressure.6,7 This difference in blood pressure between intact and castrated males is still present at 8 months of age, the oldest age we have studied thus far.8 We also found that the renin-angiotensin system (RAS) plays an important role in the development of hypertension in young SHR, because blockade of the RAS with converting-enzyme inhibitors removed the sex difference in blood pressure, and testosterone treatment of ovariectomized females was not capable of increasing their blood pressure.7 However, because androgen levels presumably decrease with age, the role of androgens in age-related renal injury and dysfunction in SHR has not been investigated. The present study then was performed to determine whether, despite reductions in androgen levels with age, androgens mediate the age-related renal injury and dysfunction in male SHR.

Methods

Rats

Male SHR, aged 8 months or 12 weeks (n = 7 to 8 per group), were obtained from Taconic Farms, Germantown, NY. SHR were maintained on standard rat chow (Teklad) and tap water in an environment with a 12-h/12-h light/dark cycle as they aged. The rats were divided into 3 groups: group 1, young males (4 months, n = 8); group 2, old males, aged 18 months (n = 8); and group 3, males that were castrated at 8 months of age (n = 7) and studied at 18 months of age. The protocols were developed according to the Guide for the Care and Use of Laboratory Animals, Animal Resources Program, Division of Research Resources, National Institutes of Health, and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

Measurement of Mean Arterial Blood Pressure (MAP) and Renal Hemodynamics

SHR were anesthetized with a thiobuturitate (110 mg/kg Inactin, RBI), and renal clearance was performed with [3H]inulin (15 to 20
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<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>BW, g</th>
<th>KW, g</th>
<th>MAP, mm Hg</th>
<th>GFR, ml/min/g</th>
<th>RPF, ml/min/g</th>
<th>RVR, mm Hg/ml</th>
<th>Protein Excretion, mg/24 h</th>
<th>Serum Testosterone, ng/dL</th>
<th>PRA, ngA1/ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males at 4 mos (n=8)</td>
<td>331.6±6.8</td>
<td>2.92±0.10</td>
<td>169±6</td>
<td>0.82±0.05</td>
<td>3.84±0.50</td>
<td>19.42±3.09</td>
<td>9.90±2.19</td>
<td>177.0±28.0</td>
<td>24.70±2.75</td>
</tr>
<tr>
<td>Males at 18 mos (n=8)</td>
<td>390.0±6.9</td>
<td>3.60±0.10</td>
<td>183±5</td>
<td>0.61±0.07</td>
<td>3.42±0.78</td>
<td>45.14±13.93</td>
<td>32.34±7.78</td>
<td>119.8±16.9</td>
<td>14.5±2.00</td>
</tr>
<tr>
<td>Castrated males at 18 mos (n=7)</td>
<td>360.3±9.1†</td>
<td>2.10±0.01†</td>
<td>162±5†</td>
<td>0.83±0.12†</td>
<td>5.38±1.01</td>
<td>25.17±7.66†</td>
<td>5.16±0.84†</td>
<td>1.2±0.1†</td>
<td>9.6±1.7†</td>
</tr>
</tbody>
</table>

BW indicates body weight; KW, kidney weight; GFR, glomerular filtration rate; MAP, mean arterial pressure; RPF, renal plasma flow; RVR, renal vascular resistance; and PRA, plasma renin activity.

*P<0.01, compared with young males; †P<0.01, compared with old males.

μCi/mL, 0.9% NaCl at 1.5 mL/h; New England Nuclear), as previously described. After the final period but before the [3 H]inulin was stopped, a sample of femoral arterial blood was taken, and the left renal vein was cannulated for renal venous blood sampling to determine extraction of [3 H]inulin by the kidney and for calculation of renal plasma flow.10

Morphological Studies
Kidney sections from old intact and old castrated male SHR were numbered and examined by an observer who was not aware of the identity of the groups. Morphological study was not performed on young males, because Tolbert and colleagues11 found that SHR kidneys were not damaged as late as 9 months of age. Kidneys were embedded in paraffin and cut into 5-μm sections. The sections were stained with methenamine silver and periodic acid–Schiff reagent. Two hundred glomeruli from each kidney were examined, and each was graded for injury: <25% of the glomerulus damaged; 25% to 50% damaged; 50% to 75% damaged; >75% damaged; and global sclerosis. The data from all rats in a group were averaged and expressed as a percentage of glomeruli from each kidney exhibiting the 5 levels of injury.

Serum Testosterone
Testosterone was measured with a commercially available radiolmmunoassay kit (Coat-A-Count total testosterone, Diagnostic Products), as previously described.6

Urinary Protein Excretion
Protein in the urine, collected for 24 hours, was measured as previously described with the Coomassie method (Bio-Rad).12

Plasma Renin Activity (PRA)
PRA was measured with a radioimmunoassay kit, as previously described.13

Statistical Analyses
Statistical differences for all data were determined by ANOVA with Stat-View 512 software and the Dunnett test. Data are expressed as mean±SEM.

Results
Effect of Aging and Long-Term Castration on Blood Pressure, Renal Function, and Injury
With the exception of renal morphology and hematocrit values, all of the data are shown in the Table. In old male SHR, body and kidney weights were higher than in young males or in age-matched, castrated males. Hematocrit was similar in young (47.5±0.68) and old (49.1±1.0) rats but was reduced in old castrated rats (45.8±1.0; P<0.01 for old males vs castrated males).

Systemic and Renal Hemodynamics and Renal Morphology
When compared with old intact males, blood pressure in old castrated males was reduced by >20 mm Hg and was similar to the blood pressure in young males (Table). Glomerular filtration rate (GFR) was increased by 30% in old castrated males compared with old intact males, but renal plasma flow (RPF) was not significantly changed. GFR and RPF were also not different when old castrated males were compared with young males. The reduction in blood pressure in castrated males also resulted in a reduction in renal vascular resistance (RVR) compared with intact, old rats. In castrated males, RVR was similar to RVR in young males. These data suggest that chronic castration either protects against renal injury, results in vasoconstriction, or both.

To test this hypothesis and as an index of renal damage, urinary protein excretion was measured and was 3- to 4-fold higher in old males than in young rats and was 7-fold higher in old, intact males compared with castrated rats (Table). To further address renal damage, kidneys of old castrated rats and old intact males were sectioned, and glomeruli (200 per kidney) were graded by a blinded observer. The old intact males exhibited glomerular sclerosis, glomeruli with mesangiolysis, tubular atrophy, and dilated tubules with casts. Castration completely prevented the glomerular sclerosis found in intact male rats (Figure). Thus, the data suggest that androgens play an important role in the age-related renal injury in male SHR.

Serum Testosterone and PRA
To determine whether androgen levels changed with age, serum testosterone was measured in intact males and compared with that in young intact males (Table). Serum testosterone was decreased by 30% in the aged, male SHR compared with young SHR. Serum testosterone measured in castrated males was considerably reduced, to 1.2±0.1 ng/dL.

Because our previous studies in young SHR supported a role for the RAS in mediating hypertension and to determine whether age and castration affected PRA, PRA was measured and found to be lower in old intact males than in young males but was further reduced by 30% in castrated rats compared with old intact males (Table).

Discussion
In this study, we investigated the role of androgens in renal injury in elderly SHR. We found that castration of SHR not only reduced blood pressure but also improved renal hemo-
dynamics and completely prevented age-related glomerular sclerosis. This is particularly interesting, because the rats were gonadectomized at 8 months of age, rather than at 1 to 2 months of age (as we usually do), yet gonadectomy was still able to protect against glomerular injury and dysfunction with age. These data support our hypothesis that androgens might mediate age-related renal injury, and to our knowledge, this is the first study to show data that suggest specifically that in male SHR, androgens do play an important role in age-related renal injury.

Our studies do not directly address the possible mechanisms by which androgens promote age-related renal injury. The effect of a long-term reduction in blood pressure that occurred with castration cannot be ruled out as a possible mechanism of renal protection. However, it is difficult to imagine that a reduction in blood pressure alone from \(180 \text{ mm Hg} \) to \(160 \text{ mm Hg} \) was capable of such a dramatic reversal of renal injury. Previously, Tolbert et al.\(^{11}\) studied male SHR at 9 months of age and found no glomerular injury; however, at that age, close to the time our rats were castrated, glomerular capillary pressure was already higher than in age-matched Wistar-Kyoto rats. It is already known that removal of the androgens by castration not only resulted in a reduction of systemic blood pressure but also of glomerular capillary pressure, thus protecting against renal injury.

It is also possible that a reduction in PRA aided in the protection against renal injury. Studies by 2 independent laboratories have shown that androgens increase and castration decreases renal mRNA expression of angiotensinogen, the substrate for renin.\(^{15,16}\) In our studies, PRA was found to be significantly reduced in castrated males when compared with either young males or old intact males. A reduction in PRA and thus angiotensin II is one mechanism by which glomerular capillary pressure could be reduced and renal function and morphology protected. Furthermore, if androgens do play a role in controlling PRA, then one might expect that PRA would be higher in young rats than in old ones, because serum testosterone levels are higher in young males, and this was the case in the present studies. Obviously, the 30% reduction in free testosterone that we found in the old males was not sufficient to protect against renal injury. However, it is also possible that, had serum testosterone not decreased with age, more renal injury may have been found in the old males.

Aging is associated with progressive reductions in kidney function in both men and women.\(^4\) However, men have a greater reduction in GFR with age than do women. Neugarten and colleagues\(^3\) performed a meta-analysis of 68 studies and a total of 11,345 patients and evaluated the role that sex played in the progression of renal disease. They found that men with chronic renal disease of various etiologies had a more rapid decline in kidney function with time than did women. However, evaluation of autopsy samples of glomeruli from aging individuals did not show a correlation between sex and glomerular sclerosis.

**Perspectives**

Male sex is a known risk factor for cardiovascular diseases. However, the role that androgens might play in mediating hypertension in men has not been elucidated as yet. There is some evidence that androgens are lower in hypertensive men than in normotensives. Phillips and colleagues\(^1\) reported that middle-aged, hypertensive men had lower serum free testosterone levels than did an age-matched normotensive cohort. However, bioavailable testosterone was not different between the hypertensive and normotensive individuals. It is also not clear which measure is most important, serum free or bioavailable testosterone, when considering changes in androgens that could affect blood pressure, nor has the role of other androgens, such as dihydrotestosterone, been investigated to determine whether they might participate in hypertension. Furthermore, just because androgens are lower during the established phase of hypertension in men does not mean that androgens could not play some role in either development or maintenance of hypertension. Therefore, studies in humans should be done to determine whether androgens are playing some role in control of hypertension, even if serum free testosterone levels are reduced.
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