Male Gender and Not the Severity of Hypertension Is Associated With End-Organ Damage in Aged Stroke-Prone Spontaneously Hypertensive Rats

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Background: It is well-known that gender affects the progression of kidney failure. Male patients exhibit faster development of age-dependent renal disease than do women. In the present study, we examined arterial blood pressure (BP), proteinuria, and end-organ damage in male and female retired breeders from our colony of stroke-prone spontaneously hypertensive rats (SHRSP).

Methods: Male (n = 7) and female (n = 11) SHRSP littermates maintained on Purina Laboratory Chow 5008 and water were studied starting at 53 weeks of age. Systolic BP was measured by tail-cuff plethysmography and 24-h urinary protein excretion was quantified while animals were housed in metabolic cages. Blood was obtained by retro-orbital bleeding. Mean arterial pressure (MAP) was then monitored by radiotelemetry. Organs were preserved for histopathologic assessment.

Results: Tail-cuff systolic BP did not differ between the sexes. Male SHRSP exhibited greater proteinuria (128 ± 7 mg/d) than females (21 ± 5 mg/d, P < .001). Blood urea nitrogen was higher in males (22 ± 2 mg%) than females (15 ± 1 mg%, P < .005). The MAP by radiotelemetry did not differ between the sexes (179 ± 3 mm Hg in males vs 192 ± 6 mm Hg in females, 2 weeks after probe implantation). Stroke-related mortality was greater in males (83%) than females (10%). Renal vascular disease including thrombotic microangiopathy affecting glomeruli and microvessels and cardiac damage were more prominent in male SHRSP.

Conclusions: These findings demonstrate that male gender is a major risk factor for multisystem end-organ damage associated with aging and hypertension in SHRSP, despite comparable degrees of hypertension among males and females. Am J Hypertens 2005;18:878–884 © 2005 American Journal of Hypertension, Ltd.

Key Words: Hypertension, gender, proteinuria, stroke-prone SHR, thrombotic microangiopathy.
been maintained on a regular sodium diet (Purina Laboratory Chow 5008, Ralston-Purina, St. Louis, MO) and tap water starting at 4 weeks of age. Because SHRSP siblings are used for mating (inbred), this provided us with the opportunity to examine gender differences in pathology in male and female animals that were not only matched for age but in most cases were also from the same litters.

Methods

Animals

Studies were conducted using 7 male and 11 female SHRSP/A3N (generation F5) retired breeders from our colony at New York Medical College. Animals were housed in a room at an ambient temperature of 22°C ± 1°C with a 12-h light/12-h dark cycle and allowed free access to Purina Laboratory Chow 5008 (Ralston-Purina) and tap water ad libitum. The Institutional Animal Care and Use Committee at New York Medical College approved all procedures.

Protocol

Animals were weighed and examined for signs of stroke on alternate days starting at 53 weeks of age. Systolic blood pressure (BP) and heart rate were measured by tail-cuff plethysmography at 54 weeks of age. Animals were housed individually in metabolic cages (Nalgene, Rochester, NY) for quantitative collection of urine and measurement of food and water intake during 24-h periods. At 57 weeks of age, blood was collected by retro-orbital bleeding within 1 min after induction of anesthesia with AErrane (isoflurane; Anaquest, Madison, WI). One week later all animals were surgically instrumented with radiotelemetry BP probes as described below. After allowing 2 weeks for recovery from surgery, systolic BP, mean arterial pressure (MAP), diastolic BP, and heart rate were measured at 15-min intervals and the average of the readings was taken during a 24-h period. Animals were placed in metabolic cages for collection of 24-h urine output. Upon death or sacrifice, hearts and kidneys were rapidly removed, blotted dry, and weighed. Transverse sections of the organs were then cut at a thickness of 3 to 4 mm and preserved in 10% neutral-buffered formalin for histologic examination.

Radiotelemetry

Radiotelemetry probes (TA11PA-C40; Data Sciences International, St. Paul, MN) that measure and transmit arterial BP and heart rate were implanted using sterile surgical techniques as described by our laboratory. One male and one female died during surgery and, therefore, telemetry data could not be obtained from these animals. Transmitted data were harvested and analyzed using the Dataquest Art Silver data-acquisition system (version 1.10, Data Sciences International).

Assays and Analyses

Initial measurements of systolic BP were obtained by tail-cuff plethysmography using a Natsume KN-210 manometer and tachometer (Peninsula Laboratories, Belmont, CA). Urinary protein concentration was determined using the sulfosalicylic acid turbidity method. Sodium and potassium concentrations in urine and plasma were measured using an IL 943 flame-photometer (Instrumentation Laboratory, Lexington, MA). Plasma aldosterone concentration was determined by standard radioimmunoassay (Diagnostic Products Co., Los Angeles, CA). Blood urea nitrogen (BUN) levels were measured colorimetrically using reagent kit 535-B purchased from Sigma (St. Louis, MO).

Histology

Transverse sections from at least two different regions of each kidney and heart were embedded in paraffin. Histologic sections (2 to 3 μm) were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and trichrome stains and examined by light microscopy at ×20 and ×40 in a blinded fashion for lesions as previously described. The PAS stained midtransverse sections were used for quantitative analysis. As in our previous studies, we assessed renal microvascular damage by counting the total number of arterial and arteriolar profiles showing thrombocytocritic lesions or proliferative vasculopathy. Thrombocytocritic lesions in this study in the aged SHRSP on regular salt intake consisted of focal mural fibrinoid necrosis that was only occasionally associated with fragmented and extravasated erythrocytes and oblitative thrombosis. Proliferative vasculopathy consisting of microvascular proliferation of markedly swollen myointimal cells with or without thrombocytocritic lesions were also counted and the total microangiopathic vascular damage was expressed in aggregate as the number of lesioned arteries and arterioles per 100 glomeruli. This was calculated by dividing the total number of lesioned vascular profiles by the total number of glomeruli in the same transverse section and multiplying by 100. In addition to acute or organizing lesions of TMA, several vessels were variably thickened due to medial hypertrophy and myointimal fibroplasia. These were counted separately and also expressed per 100 glomeruli.

Gluomerular lesions of TMA consisted of dilated capillaries containing eosinophilic fibrinoid material with mesangiolysis associated often with intra- and extra-capsular swelling. Damaged glomeruli also showed variable degrees of capillary tuft retraction with mild-to-complete obliteration of the capillary space suggesting ischemic collapse. These were considered a consequence of vascular obliteration or of resolved thrombocytocritic glomerular lesions. The number of glomeruli exhibiting lesions in either category was enumerated from each kidney and expressed together as a percentage of the total number of glomeruli present per transverse section. Similarly, seg-
mentally and globally sclerotic glomeruli were counted per transverse section and expressed per 100 glomeruli. Hearts were semiquantitatively assessed for the degree of acute or organizing infarcts. The cardiac damage index was expressed from 0 to 3 as follows: 0 = no damage; 0.5 = <5%; 1 = 5% to 10%; 2 = 11% to 15%, and 3 = >15% of the sampled myocardium per transverse section.

Statistical Analysis
Data were analyzed for significant differences using a one-way analysis of variance followed by post-hoc analysis using the method of Bonferroni. Fisher’s exact test was used to compare the incidence of mortality between males and females during the study. Data were analyzed using the BMDP software package (BMDP Statistical Software, Los Angeles, CA). Differences between means were considered statistically significant at \( P < .05 \). Data are expressed as mean ± SEM.

Results
Pretelemetry Measurements
Male and female SHRSP had similar levels of systolic BP as measured by tail-cuff plethysmography at 54 weeks of age (Fig. 1A). Heart rates also showed no difference between the groups and averaged 367 ± 11 beats/min in males and 360 ± 14 beats/min in females. In contrast, male SHRSP showed substantial proteinuria (128 ± 7 mg/d) as compared with female SHRSP (21 ± 5 mg/d) (Fig. 1B). Likewise, BUN was significantly higher in males than females (Fig. 1C). Plasma levels of sodium and potassium were not significantly different between male and female SHRSP (Table 1). Plasma aldosterone levels tended to be higher in males than in females, but this difference was not statistically significant (\( P > .07 \)).

Measurements After Implantation of Telemetry Probes
Fig. 2 shows the average 24-h systolic BP, MAP, and diastolic BP measured 2 weeks after implantation of radiotelemetry probes in male and female SHRSP. Arterial pressures tended to be higher in females than in males; however, these differences were not statistically significant. Likewise, heart rate showed no difference between the groups and averaged 348 ± 7 beats/min in males and 365 ± 5 beats/min in females. The gender of SHRSP had no effect on the urinary sodium-to-potassium ratio (0.56 ± 0.05 in males and 0.60 ± 0.03 in females). There was also no difference between the groups in urine output (31 ± 3 mL/d in males and 30 ± 5 mL/d in females), food intake (18 ± 2 g/d in males and 16 ± 2 g/d in females), and water intake (53 ± 6 mL/d in males and 49 ± 5 mL/d in females). Urinary protein excretion was measured concurrently with the radiotelemetric monitoring of BP and was significantly greater in male versus female SHRSP (Fig. 3). This difference was the same as previously observed before the implantation of radiotelemetry probes (Fig. 1B).

Mortality
Survival was not an anticipated end point in this study, as the animals were not maintained on a 1% NaCl drinking solution and Stroke-Prone Rodent Diet. However, five of six male SHRSP displayed neurologic signs of stroke and died within 6 weeks of implantation of radiotelemetry probes (Table 2). In contrast, only 1 of 10 female SHRSP showed signs of stroke and died during this period. The female SHRSP that survived remained severely hypertensive and were sacrificed at various times until 78 weeks of age.
Terminal Organ Weights

Absolute kidney and heart weights were significantly greater in male than in female SHRSP (Table 2). Terminal body weight, however, was not greater in male SHRSP, and therefore the heart-to-body weight and kidney-to-body weight ratios were not significantly different between the sexes.

Histology

Kidney (Table 2) shows the results for histologic evaluation of renal lesions. Kidneys from female SHRSP (average age = 60.1 ± 1.5 weeks) showed a significantly lower incidence of ischemic glomeruli (2.4 ± 1.2 vs 8.5 ± 1.4 in males) and those with mostly organizing thrombocytopenic lesions of TMA (1.1 ± 0.8 vs 3.8 ± 1.1 in males) compared with male SHRSP (average age = 59.5 ± 1.0 weeks). Likewise, renal microvascular damage consisting of TMA was less in female than male SHRSP (1.1 ± 0.7 vs 4.4 ± 1.2 in males) (Table 2). Thus, although matched for age, female SHRSP had significantly less total glomerular and vascular damage than male SHRSP. Of note, the glomerular and microvascular lesions of TMA in the aged male SHRSP on regular salt intake in the present study were considerably less compared with the results from our previous studies using younger saline-drinking male SHRSP.17,19 Instead, the occurrence of chronic lesions inclusive of focal segmental and global glomerulosclerosis (11.1 ± 0.7 vs 1.6 ± 1.0 per 100 glomeruli, P < .001) and concentric hypertrophy and intimal fibroplasia of arteries of all sizes (25.0 ± 0.8 vs 11.7 ± 3.1 per 100 glomeruli, P < .005) were more pronounced in male versus female SHRSP, respectively. These lesions were suggestive of chronic but severe vascular injury similar to those seen in the so-called benign nephrosclerosis in humans. Vascular obliteration in aggregate, tubular casts and tubulointerstitial scarring were also less prominent in female SHRSP. Fig. 4A,B illustrates the representative renal histologic changes in male and female SHRSP littermates.

Heart Semiquantitative analysis of the hearts for acute and fibrosing or organizing infarcts (cardiac damage index) revealed significantly greater damage in male than in female SHRSP (Table 2). Fig. 4C,D illustrates the representative cardiac histologic changes in male and in female SHRSP.

Discussion

The results of the present study demonstrate that male gender is a major risk factor for end-organ damage associated with aging and hypertension in SHRSP on normal salt intake. Previous studies have shown that female SHR22 and SHRSP22 possess lower arterial pressure than their male counterparts at a young age. It has been suggested that this difference was due to testosterone, as castration greatly lowered arterial pressure in males, whereas ovariectomy did not result in higher BP than in

Table 1. Plasma electrolyte and aldosterone levels in aged male and female stroke-prone spontaneously hypertensive rats

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma sodium (mEq/L)</td>
<td>143 ± 1</td>
<td>143 ± 1</td>
</tr>
<tr>
<td>Plasma potassium (mEq/L)</td>
<td>3.6 ± 0.1</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>401 ± 5</td>
<td>232 ± 4*</td>
</tr>
<tr>
<td>Plasma aldosterone (pg/mL)</td>
<td>633 ± 72</td>
<td>378 ± 102</td>
</tr>
<tr>
<td>Age of animals (weeks)</td>
<td>56.3 ± 0.4</td>
<td>56.9 ± 0.1</td>
</tr>
<tr>
<td>Number of animals</td>
<td>7</td>
<td>11</td>
</tr>
</tbody>
</table>

Animals were maintained on Purina Laboratory Chow 5008 and tap water ad libitum. Body weight was measured and blood was obtained by orbital bleeding 1 week before implanting radiotelemetry probes. Values are mean ± SEM. * P < .01 for male versus female SHRSP.

FIG. 3. Urinary protein excretion in male (n = 6) and female (n = 10) stroke-prone spontaneously hypertensive rats at 60 weeks of age. All animals were fed Purina Laboratory Chow 5008 and tap water ad libitum. Values are mean ± SEM.

FIG. 2. Systolic blood pressure (SBP), mean arterial pressure (MAP), and diastolic blood pressure (DBP) in male (n = 6) and female (n = 10) stroke-prone spontaneously hypertensive rats. Animals were instrumented with radiotelemetry probes for continuous monitoring of arterial blood pressure from an indwelling aortic cannula. All animals were maintained on Purina Laboratory Chow 5008 and tap water ad libitum. Values are mean ± SEM.
intact females. In normotensive postmenopausal women, estrogen administration in the form of estrogen replacement therapy, has been found to slightly lower ambulatory BP. The results of our measurements of BP by tail-cuff plethysmography in the retired breeders from our colony indicated that there was no gender difference in systolic BP in aged SHRSP. To further substantiate these results, arterial BP was measured from an indwelling aortic cannula using radiotelemetry probes and was also found to be comparable in males and females. The BP in fact tended to be higher in females. We also observed a dissociation between BP lowering and vascular protection in our previous studies as chronic treatment with angiotensin-converting enzyme (ACE) inhibitors, AT1 receptor antagonists, aldosterone receptor antagonists, or a low dose of amiloride failed to reduce systolic BP but provided marked vascular protection in saline-drinking male SHRSP. Similarly, in the present study, greater end-organ damage in males could not be accounted for by differences in hypertension as BP measured by tail-cuff plethysmography or radiotelemetry showed no difference between the sexes. The females may have derived partial cardiovascular protection from having given birth to multiple litters of pups and, although not measured, a lower arterial BP than males at a young age. Nonetheless, the ambient level of arterial pressure monitored during the 6-week period that radiotelemetry probes were implanted was not predictive of end-organ damage in aged SHRSP.

Male gender was associated with marked proteinuria in the aged SHRSP. Histologic evaluation of the kidneys in these aged SHRSP on regular salt intake showed focal lesions of TMA and subacute organizing to chronic glomerular and microvascular damage as was seen in our previous studies that used chronic administration of low doses of enalapril and amiloride in young SHRSP given 1% NaCl to drink. The appearance of chronic and subacute renal microvascular lesions in those animals was probably a consequence of prolonged survival, through 20 weeks of age, with only partial renal protection. Consistent with greater proteinuria and BUN, the kidneys from male SHRSP show extensive glomerular, vascular, and tubulointerstitial damage (lesioned vessels/100 gloms) 4.40.06 (Table 2). Male SHRSP displaying markedly less tubulointerstitial damage (tantoive cardiac section from male SHRSP shows extensive cardiac damage consisting mostly of reactive organizing fibrosis (C), whereas the section from a female SHRSP displays significantly less damage (D).
SHRSP showed greater glomerular and vascular lesions of TMA and glomerulosclerosis, as well as vascular hypertrophy, suggestive of malignant nephrosclerosis superimposed on severe benign nephrosclerotic lesions. Interestingly, male SHRSP on standard Purina diet and water begin to develop moderate proteinuria around 36 weeks of age (unpublished data). In contrast, aged female SHRSP exhibited low levels of urinary protein excretion and a lower incidence of acute and chronic glomerular and vascular renal lesions despite markedly elevated BP that was comparable to that seen in their age-matched male counterparts. These observations are in agreement with the observations that a greater degree of proteinuria and glomerulosclerosis occur in male versus female rats undergoing subtotal nephrectomy in spite of similar degrees of systemic arterial hypertension.24

In a previous study, we found that adrenalectomy largely prevented the development of proteinuria and lesions of malignant nephrosclerosis in saline-drinking male SHRSP, despite the persistence of significant hypertension and that this effect could be readily reversed by infusion of aldosterone, but not angiotensin II (Ang II).12 We therefore examined the plasma levels of aldosterone in the present study and found a strong tendency for increased levels in males. Levels of PRA and angiotensinogen can be elevated by testosterone.21 PRA has also been reported to increase with aging in male SHR25 and, although not measured, may also have been increased in aged male SHRSP. An activated renin-angiotensin-aldosterone system has long been linked to increased cardiovascular risk26 and may be responsible for the greater cardiovascular pathology observed in aged male versus female SHRSP in this study.

The present study indicates that aged male SHRSP not only develop greater proteinuria but also show stroke signs and die earlier than their female counterparts. Although these animals died at various times after implantation of the radiotelemetry probes, the mortality was consistent with the natural progression of vascular disease, and did not appear to be specifically related to the surgical procedure. One male SHRSP, a littermate of those reported on here, showed stroke signs and died before having surgery and therefore was excluded from this study. With only one exception, all male SHRSP displayed overt neurologic signs of stroke (ie, stereotypic limb movements) before they died. Although the absolute kidney and heart weights were greater in male SHRSP, the kidney-to-body weight ratio showed no difference between the sexes and was not predictive of the greater renal histologic damage in male SHRSP. Likewise, the heart-to-body weight ratio at autopsy was equally elevated in male and female SHRSP, which is consistent with the observation that BP was severely and comparably elevated in both the sexes. However, histologic examination of the hearts revealed a much greater incidence and severity of acute and organizing infarction and the consequent reactive fibrosis in males.

The results of the present study suggest male gender as a factor for accelerating end-organ damage in aged SHRSP but do not indicate whether the presence of testosterone, the absence of estrogen, or other gonadal or Y-chromosome effects are responsible for this sex difference. It has been reported that the presence of androgens rather than the absence of estrogen promotes glomerular injury in aging male rats.27,28 In our previous studies,15,16 we also found a high incidence of stroke and renal injury at an early age in saline-drinking female SHRSP. Those experiments were conducted in young (virgin) female SHRSP and identified estrogen as a hormonal factor that promotes the onset of stroke and renal injury. However, estrogen has also been reported to exert vascular protective actions,1,29,30 and our long experience with breeding SHRSP suggests that the females are still estrous cycling well beyond 1 year of age as they can continue to procreate. The factors that govern whether estrogen exerts protective or adverse cardiovascular effects in these various situations remain to be elucidated. High sodium chloride intake may be among the factors that promote an adverse cardiovascular effect of estrogen.16 The SHRSP in the present study were not salt-loaded and this may have favored a beneficial effect of estrogen.

In summary, aged male SHRSP on normal salt intake display a hastened onset of proteinuria and stroke and a pronounced increase in renal and cardiac damage as compared with female SHRSP. These effects of male gender on end-organ damage do not appear to be related to the existing level of arterial BP in these rats.

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References


