Gender modulates activation of renin-angiotensin and endothelin systems in hypertension and heart failure

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Hypertension is an important risk factor for the development of congestive heart failure (CHF). Development of hypertension and CHF is associated with activation of multiple neuroendocrine systems, including the renin-angiotensin (RAS), endothelin, and atrial natriuretic peptide (ANP) systems. Variations of these neurohumoral systems have been proposed to mediate the progression of hypertension and CHF. However, gender modulates activation of renin-angiotensin and endothelin systems in hypertension and heart failure. 

Gender modulates activation of renin-angiotensin and endothelin systems in hypertension and heart failure. J Appl Physiol 92: 935–940, 2002. First published October 19, 2001; 10.1152/japplphysiol.00558.2001.—Sexual dimorphism may occur during the development of hypertension and congestive heart failure (CHF). Male and female spontaneous hypertension heart failure (SHHF) rats with established hypertension, but before CHF (age 5–8 mo) and during cardiac decompensation leading to CHF (age 18–20 mo in male rats and 22–24 mo in female rats), were studied. At 5–8 mo, male SHHF rats showed early activation of the renin-angiotensin system (RAS), as indicated by increased plasma renin activity (PRA) and higher serum angiotensin-converting enzyme activity compared with female rats. The increase in PRA in female rats was delayed compared with male rats, but it reached comparable levels just before CHF. Urinary endothelin excretion was significantly greater in 5- to 8-mo-old female rats compared with age-matched male rats. Urinary endothelin excretion increased in both male and female rats as CHF developed. Plasma atrial natriuretic peptide (ANP) was comparable at both time points, and both genders showed similar, marked increases as CHF developed. In conclusion, male rats show early activation of the RAS, whereas female rats show early activation of the endothelin vasopressor system. During cardiac decompensation, generalized activation of the RAS, endothelin, and ANP systems occurs and is similar in male and female SHHF rats.

plasma renin activity; atrial natriuretic peptide; spontaneous hypertension heart failure

Many studies have implicated sex hormones as modulators of risk factors for cardiovascular disease (7). In humans, men tend to develop hypertension and subsequent heart failure at an earlier age compared with women (1, 16). Onset of cardiovascular disease in women is frequently delayed until after menopause (7, 23). Still, the role of sex hormones in the development and control of hypertension and subsequent heart failure remains controversial. Sexual dimorphism in the pattern of neurohumoral activation may have consequences on the rate of cardiac remodeling and decompensation, as well as on response to therapy (6, 26, 27, 30) and survival (1).

The spontaneous hypertensive heart failure rat (SHHF/Mcc-fa-o or SHHF) is a multifactorial, genetic model of hypertension and CHF. Despite a similar genetic predisposition to the development of hypertension and heart failure, male SHHF rats develop and die from overt CHF at an earlier age compared with female rats (12, 19, 20). Significant decline in cardiac function occurs in female SHHF rats after they cease to cycle, and serum estrogen concentrations begin to fall (31). Furthermore, female SHHF rats respond differently to various classes of antihypertensive drugs compared with male rats, suggesting mechanistic differences in control of hypertension (6, 26, 27, 30).

We examined the influence of gender on plasma renin activity (PRA), serum angiotensin-converting enzyme (ACE) activity, urinary endothelin excretion, and plasma ANP concentrations in lean male and female SHHF rats at a time when hypertension was established but before cardiac decompensation. Additionally, these same parameters were investigated during the period of cardiac decompensation and onset of CHF. We show that gender modulates activation of the RAS and endothelin systems during the period of hypertension before cardiac functional decompensation in this model. This may contribute to the different rates at which cardiac hypertrophy and CHF develop in male vs. female SHHF rats. However, once cardiac...
decompensation begins, generalized neurohumoral activation occurs, and gender differences are no longer apparent.

**MATERIALS AND METHODS**

Lean male and female SHHF rats were obtained from the breeding colony at The Ohio State University. These animals were housed under standard conditions (12:12-h light-dark cycle) with ad libitum access to water and food (Prolab rat/mouse/hamster diet, Agway, Syracuse, NY). This animal facility is American Association for Accreditation of Laboratory Animal Care accredited, and the animal protocol procedures were approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee.

Colony records of siblings were used to determine differences in time of death from CHF between lean male \(n = 20\) and female SHHF rats \(n = 15\). Onset of CHF was recognized by development of dyspnea, piloerection, cyanosis, ascites, pleural effusion, cold tail and extremities, and necropsy examination of the heart. Age at which spontaneous cessation of estrus cycling occurred was determined by vaginal cytology. Conscious, systolic blood pressure was measured by tail-cuff method (model ICT-2H, Gison Duograph, Middleton, WI). M-mode echocardiographic measurements were taken of systolic and diastolic left ventricular internal dimensions (LVVIDS and LVIDD, respectively) by using a Sonos echocardiograph machine equipped with a 7.5-MHz pediatric transducer (Hewlett-Packard, Waltham, MA). Restraint for echocardiography was provided by intraperitoneal injection of 7.0 mg ketamine (Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) and 0.7 mg xylazine (Rompun, Bayer, Shawnee Mission, KS) per 100 g body wt. The left ventricular fractional shortening (FS) was calculated as \[ \text{FS} = \left( \frac{\text{LVIDD} - \text{LVIDD}}{\text{LVIDD}} \right) \times 100\% \].

A subset of male and female SHHF rats was euthanized by an overdose of pentobarbital sodium at a time when hypertension was established but before the cardiac functional decompensation (age 5–8 mo). Additionally, male and female SHHF rats were compared at the time of initial cardiac functional decline (age 18–20 mo in male rats and age 20–24 mo in female rats). By this latter age, the female rats have spontaneously ceased estrus cycling. For comparison with lean male SHHF rats, similar samples were obtained from additional, conscious SHHF rats by tail clip bleeding. For comparison, similar samples were obtained from age-matched male WF rats. ANP was measured by using a modification of a commercially available radioimmunoassay kit (Peninsula Laboratories, Belmont, CA) (27).

Data are expressed as means \pm SE. Comparisons were done using ANOVA, and differences were reported as significant when \(P < 0.05\). Tukey-Kramer multiple-comparisons post hoc test was used for mean separation. The log rank test was used to compare the survival of the lean male and female SHHF rats.

**RESULTS**

Hypertension developed at a similar age in male and female SHHF rats (Fig. 1). Before CHF, systolic blood pressure was similar between male and female SHHF rats at each age. As the rats showed cardiac decompensation, blood pressures fell to within the normotensive or hypotensive range (data not shown). Left ventricular FS was similar in male and female rats until 12–13 mo of age (Fig. 2). After that time, male rats showed a decline in FS, terminating in CHF. A similar decline in FS in female SHHF rats was delayed until after they spontaneously ceased estrus cycling (Fig. 2). Consequently, lean female SHHF rats developed overt CHF at a significantly later age compared with lean male rats. Male SHHF rats \(n = 20\) developed CHF at a mean age of 21.5 \pm 0.6 mo (range 20.1–23.0 mo) vs. female SHHF rats \(n = 15\) at age 26.9 \pm 0.5 mo (range 24.2–29.0 mo) \(P < 0.001\).

![Fig. 1. Tail cuff systolic blood pressure in lean male (○; \(n = 4\) at each time point) and female spontaneous hypertensive heart failure rats (●; \(n = 4\) at each time point). Values are means ± SE. There were no significant differences between genders at any age.](http://jap.physiology.org/ by [GammaCoat PRA, Incestar, Stillwater, MN] as previously described (31). Serum ACE activity was measured by detecting generation of hippuric acid from hippuryl-L-histidyl-L-leucine (17).

To obtain 24-h urinary endothelin excretion, additional rats were placed in metabolic cages. During the 24-h collection period, the rats were fasted but allowed water ad libitum. Urine volumes were measured at the end of the 24-h collection period and samples frozen and stored at \(-70^\circ C\) until assayed. Endothelin concentrations were determined by using a commercially available radioimmunoassay kit (Amersham, Arlington Heights, IL). Samples were extracted according to manufacturer’s protocol before assay and then corrected for extraction efficiency. Plasma ANP concentrations were obtained from additional, conscious SHHF rats by tail clip bleeding. For comparison, similar samples were obtained from age-matched male WF rats. ANP was measured by using a modification of a commercially available radioimmunoassay kit (Peninsula Laboratories, Belmont, CA) (27).
activity declined in older lean male SHHF rats and was similar to that observed in older female SHHF rats. Young female SHHF rats showed a significantly greater 24-h urinary excretion of endothelin compared with age-matched male rats (Table 2). Both male and female rats showed significant increases in urinary endothelin excretion during cardiac decompensation, and a gender difference was no longer apparent at that time.

Male and female rats showed no significant difference in plasma ANP concentrations during the maintenance phase of hypertension (5–8 mo of age; Table 2) and were similar to age-matched male WF rats (83.2 ± 20.3 pg/ml; n = 5). Plasma ANP greatly increased in both male and female rats during cardiac decompensation. However, no increase in plasma ANP was observed in age-matched male WF rats (86.7 ± 17.3 pg/ml; n = 4).

**DISCUSSION**

In summary, both male and female SHHF rats developed hypertension at a similar age and reached similar levels of hypertension (Fig. 1). Systolic blood pressure progressively increased from 2 to 4 mo of age, after which rats remained hypertensive until the onset of cardiac decompensation and CHF. The age of development of hypertension in male and female SHHF rats is similar to that observed for spontaneously hypertensive rats (SHR) (15) and is consistent in that SHHF rats were originally derived from a cross between SHR and Koletsky strains (19, 20). However, during the maintenance phase of hypertension (5–8 mo of age), male and female SHHF rats show sexual dimorphism in the activation of the RAS and the renal endothelin system.

Male SHHF rats showed marked activation of the RAS in association with the maintenance phase of hypertension (Table 2), which is consistent with previous studies (11). PRA was increased in male SHHF compared with female SHHF rats at 5–8 mo of age, despite similar levels of hypertension. Male SHHF rats also had greater serum ACE activity compared with

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**Table 1. Heart weight and left ventricular weight from male and female SHHF rats at the time of hypertension (5–8 mo of age) and during cardiac decompensation leading to congestive heart failure (18–20 mo in male rats and 22–24 mo in female rats)**

<table>
<thead>
<tr>
<th></th>
<th>Male SHHF</th>
<th>Female SHHF</th>
<th>Male WF</th>
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<tbody>
<tr>
<td></td>
<td>5–8 mo (n = 9)</td>
<td>18–20 mo (n = 5)</td>
<td>5–8 mo (n = 9)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>379 ± 11*</td>
<td>424 ± 25*</td>
<td>219 ± 3‡</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.62 ± 0.10*</td>
<td>2.78 ± 0.13†</td>
<td>1.10 ± 0.03*</td>
</tr>
<tr>
<td>LVS, g</td>
<td>1.19 ± 0.06*</td>
<td>1.57 ± 0.06†</td>
<td>0.77 ± 0.02*</td>
</tr>
<tr>
<td>Heart/body weight</td>
<td>0.43 ± 0.02*</td>
<td>0.66 ± 0.04†</td>
<td>0.50 ± 0.01†</td>
</tr>
<tr>
<td>LVS/body weight</td>
<td>0.31 ± 0.01*</td>
<td>0.37 ± 0.02*</td>
<td>0.35 ± 0.01*</td>
</tr>
<tr>
<td>Heart/brain weight</td>
<td>0.84 ± 0.06*</td>
<td>1.30 ± 0.06†</td>
<td>0.60 ± 0.02*</td>
</tr>
<tr>
<td>LVS/brain weight</td>
<td>0.62 ± 0.04*</td>
<td>0.73 ± 0.03*</td>
<td>0.42 ± 0.01†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. SHHF, spontaneous hypertension heart failure; WF, Wistar-Furth; LVS, left ventricle plus interventricular septum. Heart/body weight and LVS/body weight indexes = (organ weight/body weight) × 100. *†‡§Within rows, groups marked with different symbols are significantly different, P < 0.05.
female SHHF rats (Table 2). Estrogen has been demonstrated to suppress ACE activity in rats and primates (5, 25, 32). In previous studies, modulation of ACE activity was observed in response to ovariectomy and estrogen replacement therapy in SHHF rats (32). ACE activity in both male and female SHHF rats was significantly lower than that observed in normotensive WF rats. This is similar to findings reported for the stroke-prone spontaneously hypertensive (SHRSP) and Wistar-Kyoto rat strains (8, 14). In the SHRSP rats, the mechanism contributing to this difference between normotensive rats and hypertensive strains may relate to differential genetic regulation of an ACE allele (14).

By the time cardiac decompensation occurred in the present study, gender differences in PRA and ACE activity were no longer evident. PRA was markedly elevated in both male and female SHHF rats. Male SHHF rats showed a decline in ACE activity at 18–20 mo of age, whereas older female SHHF rats showed a mild but nonsignificant increase in ACE activity, similar to that observed after cessation of estrus cycling (32).

Other studies have indicated that the RAS contributes to cardiac hypertrophy and that specific blockade of the angiotensin II subtype 1 receptor may decrease or reverse cardiac hypertrophic changes during the transition to CHF (28, 34, 36). It is likely that part of the difference in age of onset of CHF between male and female SHHF rats may be due to the delayed activation of the RAS in the female rats. Chronic activation of the RAS can result in cardiac hypertrophy secondary to volume expansion, hypertension, and increased cardiac afterload. In addition, angiotensin II appears to directly promote myocardial hypertrophy and remodeling independent of pressor effects, resulting in ventricular hypertrophy and stiffness as well as increased end-diastolic left ventricular pressures (4, 13). It is likely that the early activation of the RAS and increased generation of angiotensin II contributed to the left ventricular hypertrophy observed by 5–8 mo of age in the male SHHF rats. Similar hypertrophy was observed in female SHHF rats at a much later age and was also associated with increased activity of the RAS.

Blockade of angiotensin II type 1 receptors will ameliorate cardiac hypertrophy, even when used at subpressor doses (28, 30, 36). In female SHHF rats, angiotensin II-receptor antagonists are still effective in decreasing cardiac hypertrophy in female SHHF, despite a poor depressor response to this class of antihypertensive drug (30, 34).

At 5–8 mo of age, activation of the renal endothelin vasoconstrictor system was observed in female SHHF rats compared with male SHHF rats. Again, both male and female rats showed increased renal endothelin excretion at the time of cardiac decompensation. ACE inhibition did not appear to hasten the onset of cardiac hypertrophy in female SHHF rats, despite a poor depressor response to this class of antihypertensive drug (30, 34).

<table>
<thead>
<tr>
<th>Male SHHF</th>
<th>Female SHHF</th>
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<tbody>
<tr>
<td>5–8 mo</td>
<td>18–20 mo</td>
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<tr>
<td>5–8 mo</td>
<td>22–24 mo</td>
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<tr>
<th>Parameter</th>
<th>Male SHHF</th>
<th>Female SHHF</th>
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<tbody>
<tr>
<td>PRA, ng angiotensin I·ml⁻¹·h⁻¹</td>
<td>8.19 ± 1.19*</td>
<td>13.64 ± 0.68†</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 6)</td>
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<tr>
<td>Serum ACE, nmol·ml⁻¹·min⁻¹</td>
<td>23.2 ± 0.4‡</td>
<td>17.2 ± 0.7†</td>
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<tr>
<td>(n = 4)</td>
<td>(n = 7)</td>
<td></td>
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<tr>
<td>Urinary endothelin excretion, fmol/24 h</td>
<td>70.2 ± 19.1*</td>
<td>429.3 ± 72.0‡</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>(n = 3)</td>
<td></td>
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<tr>
<td>Plasma ANP, pg/ml</td>
<td>96.6 ± 6.0*</td>
<td>2,017.6 ± 85.0†</td>
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<tr>
<td>(n = 4)</td>
<td>(n = 5)</td>
<td></td>
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</tbody>
</table>

Values are means ± SE; n, no. of rats. PRA, plasma renin activity; ACE, angiotensin-converting enzyme activity; ANP, atrial natriuretic peptide. *‡†Within rows, groups marked with different symbols are significantly different, P < 0.05.

There were no gender differences in plasma ANP concentrations at either time point in male and female SHHF rats. As with the RAS and endothelin, marked activation occurred as CHF developed. Increases in plasma ANP concentrations are associated with progressive cardiac hypertrophy in both humans and rats (2, 9, 19). Increased ANP gene expression in the left ventricle is one of several “stable late markers” of cardiac hypertrophy linked with pathological cardiac hypertrophy (31) and may contribute to circulating plasma concentrations (20). It is likely that increases in ANP may serve to antagonize activated vasoconstrictors as well as modulate sodium and water reten-
tion as CHF develops. The lack of gender effect on ANP in consistent with previous studies, which have shown that circulating levels of ANP are not affected by ovariectomy or estrogen replacement in SHHF female rats (31, 32).

Our studies indicate that, despite a similar genetic predisposition to develop hypertension and CHF, gender modulates activation of vasoconstrictor systems and subsequently the course of cardiovascular disease in SHHF rats. Gender differences in the SHHF strain are characterized by early activation of the RAS in male rats, whereas female rats show an early increase in renal endothelin excretion. Development of left ventricular hypertrophy, overt CHF, and death is delayed in female compared with male SHHF rats. Gender differences in vasoconstrictor profiles likely influence the rate at which pathological cardiac hypertrophy develops and may have implications in the development and application of appropriate therapeutic interventions.

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REFERENCES


