Gender-Differences in Myocardial Adaptation to Afterload in Normotensive and Hypertensive Rats

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Abstract—Echocardiographic studies suggest that women appear to exhibit a greater degree of myocardial hypertrophy in response to increased afterload than men. Therefore, gender differences and the role of estrogen and testosterone in the development of myocardial hypertrophy were studied in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. Male and female rats were either surgically neutered or underwent a sham operation at 21 days of age. A subgroup of neutered females of each strain received 17β-estradiol replacement. At 6 months, the heart weight-to-body weight ratio was assessed and correlated with systemic blood pressure. Compared with males, females had significantly smaller body and heart weights in both normotensive and hypertensive strains. Despite this, females consistently had significantly greater heart weight-to-body weight ratios. In females, neutering significantly lowered the heart weight-to-body weight ratio in WKY rats, which was returned to intact levels with estrogen replacement. Female SHR showed similar, but not statistically significant, responses. In males, neutering appeared to result in a higher heart weight-to-body weight ratio in WKY rats, but the opposite was seen in SHR. In addition, there was a significant correlation between arterial blood pressure and heart weight-to-body weight ratio (systolic $r=0.45$, $P=0.0015$; diastolic $r=0.52$, $P=0.0002$) in intact males and females of both strains, and for a given diastolic pressure, females always exhibited a greater heart weight-to-body weight ratio than males. Thus, a greater degree of myocardial hypertrophy in females appears to be related to the presence of estrogen in both normotensive and hypertensive rats. Females show a stronger relationship between heart/body weight and blood pressure than males, which occurred independent of the presence of estrogen. (Hypertension. 2000;36:774-779.)

Key Words: gender ■ myocardium ■ hypertrophy ■ rats, inbred SHR ■ rats, inbred WKY

Myocardial hypertrophy is prevalent in a substantial portion of individuals with essential hypertension,1,2 and it is recognized as an independent risk factor for congestive heart failure and sudden cardiac death.3 Significant gender differences in cardiovascular disease have been observed.4 However, the impact of gender on the development of myocardial hypertrophy is less clear. Recently, alterations in systemic blood pressure were identified as a predictor of myocardial hypertrophy in women but not in men.5,6 Significant gender differences in the response to aortic stenosis have been observed in patients over age 60, with women having significantly smaller left ventricular (LV) chamber sizes and marked concentric hypertrophy, whereas men had lower wall thicknesses and higher wall stress, which is more consistent with eccentric hypertrophy.7 In addition, women had a greater prevalence of LV hypertrophy in response to systemic hypertension than men,1,8 although women had significantly less LV mass than men when corrected for height.5 Levy et al9 showed that increased LV mass was associated with a higher relative risk for death from cardiovascular disease in women (relative risk 2.12) compared with men (relative risk 1.73), although the 95% CIs overlap to a large extent. Ambulatory blood pressure monitoring of men and women aged 18 to 45 years with borderline to mild hypertension showed that LV mass and wall thickness increased with increasing systolic blood pressure (SBP) in women only.5 In patients >50 years old with uncontrolled hypertension, LV hypertrophy had a significantly higher prevalence in women (80%) than in men (55%).8 However, an examination of normotensive males and females from 14 to 70 years old showed that males had a consistently higher ratio of LV mass to body surface area than did females.10 Therefore, although some controversy exists, gender differences appear to be present in patients with myocardial hypertrophy.

To clarify these conflicting reports, the present study was conducted to assess the effect of gender and the sex hormones estrogen and testosterone on heart size and body weight in both normotensive and hypertensive rats. The degree of hypertrophy was correlated with systemic SBP and diastolic blood pressure (DBP) as an index of afterload.

Methods

Age-matched male and female spontaneously hypertensive rats (SHR) were used as a model of essential hypertension with associ-
ated myocardial hypertrophy, whereas Wistar-Kyoto (WKY) rats were used as the control group. At 21 days of age, female rats of each strain were randomly allocated either to be surgically neutered via bilateral ovariectomy (SHR 11, WKY 9), leaving the uterus intact, or to undergo a sham operation (SHR 10, WKY 10). A subgroup of neutered females of each strain underwent hormone replacement with 17β-estradiol (ERT) with a slow-release pellet (60 days, 1.7 mg/pellet) that was implanted subcutaneously beginning at 30 days of age (SHR 18, WKY 10), for a total of 120 days of ERT. This dosage is reported to achieve constant physiological levels of plasma hormones. Overall, there were 3 groups of females for each strain; intact, neutered, and neutered plus ERT. Age-matched males were either surgically neutered via castration (SHR 18, WKY 10) or sham operated (SHR 15, WKY 15), for a total of 2 groups of males in each strain. Animals were treated in accordance with the guidelines of the Canadian Council on Animal Care and the National Institutes of Health.

At 150 days of age, rats were weighed, anesthetized (100 mg/kg IP Inactin, sodium thiobutabarbital; Research Biochemicals International), intubated, and ventilated to ensure normal blood gas and acid-base status. The right carotid artery was cannulated to allow measurement of arterial blood pressures and heart rate and to allow blood gas assessment. Once baseline hemodynamic measurements were made, the heart was exposed, rapidly excised, and blotted dry; the great vessels were removed; and the heart weight, composed of atria, ventricles, and septum, was recorded. Total heart weight–to–body weight ratio was used to express the degree of myocardial hypertrophy.

Data are expressed as mean±SEM. Intact male versus female data and intact male versus neutered males were compared by Student’s t test. One-way ANOVA with Scheffé’s post hoc test was used to compare parameters (eg, hemodynamics) in response to treatment within a strain of females. Pearson’s correlation coefficient was used to assess the relationship between blood pressure and the degree of myocardial hypertrophy. Statistical significance was accepted at P<0.05.

Results

Hemodynamics
The Table shows systemic arterial pressures and body and heart weight data for intact males and females, both normotensive and hypertensive. In both males and females, SHR showed dramatically higher SBP and DBP pressures than WKY rats, confirming the presence of significantly increased afterload in the SHR. There were no statistically significant gender differences in either SBP or DBP within WKY and SHR strains, although female SHR did show 4% to 6% higher values than male SHR. To determine whether sex steroids affected these parameters, male and female rats of each strain were surgically neutered to reduce levels of circulating sex hormone. Although neutering reduced plasma testosterone and estradiol concentrations below detectable levels, systemic blood pressures in male SHR rats did not differ compared with intact rats, whereas neutered male WKY rats had a 9% lower SBP and an 8% lower DBP than intact males (P=NS). Neutered WKY and SHR females did not have significantly different SBPs or DBPs than intact females of the same strain (Table). Neutered WKY females that received ERT had an SBP that was 11% higher than that of the intact females, whereas the DBP was 12% higher than that of the neutered females and 15% higher than that of intact females, although these differences did not achieve statistical significance. Neutered SHR females that received ERT had SBPs and DBPs that were comparable to those of intact and neutered females.

Body Weight
Body weights were always higher in intact males than in intact females: by 62% in WKY rats and 66% in SHR (P<0.001, Table). Neutered males had a significantly lower body weight than intact males: by 14% in WKY rats and 19% in SHR. In WKY rats, neutered females had body weights that were 14% greater than those of intact females (P<0.05), whereas neutered females that received ERT had body weights 21% below those of neutered females and 10% below those of intact females (P<0.05) (Table). In SHR, neutered females had 15% greater body weights than intact females (P<0.05). SHR females that received ERT had body weights that were 30% lower than those of neutered females and 19% lower than those of intact females (P<0.05).

Heart Weight
In WKY rats, total heart weights were 28% lighter in intact females than in intact males. Similarly, in SHR, intact
females had 33% lighter heart weights than intact males \((P<0.001)\). Neutered WKY males did not have significantly different total heart weights compared with intact males. However, neutered SHR males had 25% lower heart weights compared with intact SHR males. Neutered WKY and SHR females did not have total heart weights that were different than those of intact females (Table). Neutered WKY females that received ERT had heart weights that were 9% lower than those of intact females and 8% lower than those of neutered females \((P=NS)\), whereas neutered SHR females that received ERT had heart weights that were 17% lower than those of intact females and 21% lower than those of neutered female SHR \((P<0.05)\).

Heart Weight–to–Body Weight Ratio
The heart weight–to–body weight ratio in females was significantly higher than that in males: by 16% in WKY rats \((P<0.01)\) and by 11% in SHR \((P<0.05)\) (Figure 1). Neutered WKY males had heart weight–to–body weight ratios similar to those of intact males (Figure 2), whereas neutered SHR males showed a trend toward 8% lower heart weight–to–body weight ratios compared with intact SHR males \((P=0.052)\). Heart weight–to–body weight ratios in females (Figure 3) showed that in WKY rats, neutered females had a 13% lower heart weight–to–body weight ratio than intact females \((P<0.05)\). In contrast, neutered SHR females had only a modest 8% lower heart weight–to–body weight ratio compared with intact females (NS). Neutered WKY and SHR females that received ERT had heart weight–to–body weight ratios similar to those of intact females, and in the WKY group, this was 18% higher than those of neutered females \((P<0.05)\).

Figure 4 shows the relationship between DBP and heart weight–to–body weight ratio for intact males and females of both WKY and SHR strains. The overall correlation (males plus females, Figure 4A) showed a highly significant direct relationship between these parameters. This relationship was stronger in females (Figure 4C) than in males (Figure 4B). Neutered females continued to exhibit this relationship \((r=0.61, P<0.01)\), whereas neutered males had no correlation between afterload and the degree of myocardial hypertrophy \((r=0.08, P=0.69)\). Although the slope of the DBP relationship in males (Figure 4B) was steeper than that of the females (Figure 4C), for any given diastolic pressure, the females had a greater heart weight–to–body weight ratio than the males, as shown by the greater intercept for females \((γ=3.3)\) compared with males \((γ=2.5)\). Similar results were seen in the relationship between SBP and heart weight–to–body weight ratio, with females showing a stronger relationship \((r=0.71, P<0.001)\) than males \((r=0.40, P=0.045)\). Neutered females showed a similar relationship \((r=0.56, P<0.01)\) to that seen with DBP, whereas neutered males showed no relationship between SBP and heart weight–to–body weight ratio \((r=0.10, P=0.63)\).

Discussion
The present study had several major new findings. First, although males were larger overall and had larger hearts, females showed a larger cardiac mass adjusted for body weight than males in both normotensive (WKY) and hypertensive (SHR) rats. This occurred despite similar systemic blood pressures for males and females of both strains. Second, this relationship was influenced by the presence of sex steroids, because in the absence of estrogen (neutered
females), the heart weight–to–body weight ratio was lower, and this was restored to intact levels in neutered females that received ERT. Testosterone did not appear to have a similar impact on the degree of myocardial hypertrophy relative to body weight, because only a trend toward a reduced heart weight–to–body weight ratio was seen after neutering in hypertensive males. Third, there was a significant correlation between blood pressure (SBP and DBP) and heart weight–to–body weight ratio in the overall population of males and females such that for any given diastolic pressure, females had a greater heart weight–to–body weight ratio than males.

In humans, LV hypertrophy has a prevalence of 15% to 20% in the adult population and is recognized as an independent risk factor for arrhythmias, sudden cardiac death, myocardial infarction, and congestive heart failure.11,12 M-mode echocardiograms from men and women aged 23 to 35 years showed that LV mass was more strongly correlated with SBP in women than in men.13 Findings from the present study support this and extend the observation to DBP, because correlation analyses showed that at any given afterload, females showed a relatively greater degree of myocardial hypertrophy than males.

Figure 3. Heart weight–to–body weight ratio (mg/g) from female intact (open columns), neutered (Neut; solid columns), and ERT (Est; cross-hatched columns) WKY rats (A) and SHR (B). In WKY females, neutering significantly reduced this ratio compared with intact animals, and the ratio was restored to baseline levels by ERT. Values are mean±SEM. *P<0.05 vs intact. †P<0.01 vs ERT.

Figure 4. Relationship between systemic DBP (mm Hg) and heart weight–to–body weight ratio (mg/g) in (A) intact male and female WKY rats and SHR (y=8.3×10^{-3}x+2.8), (B) males only (y=9.1×10^{-3}x+2.5), and (C) females only (y=6.6×10^{-3}x+3.3). There was a significant relationship between these parameters when all rats were included and in females alone, but this was weakened when males only were considered. Males showed a steeper slope to the relationship with DBP than females, whereas females had a greater intercept than males.
Hemodynamics
An elevation in systemic blood pressure is an important risk for the development of myocardial hypertrophy. Recent analysis of data from the Framingham Heart Study showed that SBP was more important than DBP as a determinant of future complications.\textsuperscript{14} Although there were no significant differences in systemic arterial pressures between intact male and female rats of either strain, both SBP and DBP were 2\% to 6\% higher in females than in males in both normotensive and hypertensive strains. This is similar to the 6\% higher mean arterial pressure found in conscious, unrestrained female Wistar rats by Cabral et al.\textsuperscript{15} Despite somewhat lower DBP in normotensive females compared with males, females had greater heart weight–to–body weight ratios than males, indicating that female rats express a relatively larger heart relative to body weight even in the absence of increased afterload.

Neutering did not appear to significantly alter blood pressure in males or females of either strain, although neutered normotensive and hypertensive females had up to 6\% higher SBP and 3\% higher DBP, whereas neutered normotensive males had lower pressures. Interestingly, neutered normotensive females that received ERT had a further elevation of blood pressure, whereas hypertensive females that received ERT had lower SBP and DBP than intact females. Estrogen can induce acute dilatation of the vasculature through endothelium-dependent mechanisms, likely by increasing the local release of NO,\textsuperscript{16} which may have reduced blood pressures in the neutered SHR females that received ERT in the present study. It is known that estrogen oral contraceptives induce hypertension in a small percentage of women,\textsuperscript{17} although the mechanisms for this have not been identified. The normotensive females in the present study also appear to be susceptible to this phenomenon.

Heart Weight–to–Body Weight Ratio
It might be argued that use of the heart weight–to–body weight ratio as a marker of the degree of myocardial hypertrophy is problematic. Because alterations in either measure affect this, then an increase in the ratio may not be indicative of a real increase in cardiac mass. However, data from our laboratory show that the gender difference is specific to the heart, because the liver weight–to–body weight ratio did not show a similar gender difference. Thus, an increase in the heart weight–to–body weight ratio reflects a disproportionate alteration in the size of the heart with respect to the growth of the rest of the body. Although both total heart weight and body weight were lower in females than in males, the heart weight–to–body weight ratio was higher in both WKY and SHR females, suggesting that this disproportionate increase in heart size occurs under both normotensive and hypertensive conditions. This increase appears to be related to estrogen.

The existence of receptors for sex hormones in the cardiovascular system is well documented.\textsuperscript{18–21} Once estrogen binds to the intracellular estrogen receptor, the estrogen-receptor complex can bind to specific estrogen response elements of DNA,\textsuperscript{16} regulating transcriptional activity of specific target genes and potentially having an affect on heart mass. An estrogen-induced increase in the expression of certain genes may explain the greater heart weight–to–body weight ratio in females compared with males that occurred in the present study. This is confirmed by the observation that the removal of estrogen via bilateral ovariectomy lowered the ratio in females, which was restored by ERT. Interestingly, the removal of estrogen did not significantly reduce the degree of hypertrophy in the hypertensive females as it did in the normotensive group, although a similar pattern of response was seen. The present study showed that neutering had no significant effect on heart weight–to–body weight ratio in normotensive (WKY) and hypertensive (SHR) males. Thus, the absence of testosterone, which was associated with lower body weights in both strains of male rats, did not significantly alter the proportional growth of the heart. In contrast to studies in cultured cells, which showed that the presence of testosterone was associated with a 20\% increase in protein synthesis compared with controls,\textsuperscript{20} other factors, that have yet to be determined, are important determinants of the relative growth of the heart in male animals.

Compared with males, females showed a closer relationship between afterload and heart weight–to–body weight ratio. Because this relationship remained significant even in the neutered females, it does not appear to be related to estrogen. Interestingly, heart weight–to–body weight ratio was strongly related to arterial SBP in normotensive and hypertensive female rats but showed a weaker relationship in males. This supports the echocardiographic data for men and women in the HARVEST study by Vriz et al,\textsuperscript{5} which showed a stronger relationship between ambulatory daytime SBP and LV mass in women ($r=0.27$, $P<0.01$) than in men ($r=0.19$, $P<0.001$). Data from the present study showed that in female rats, this relationship was much stronger than in the clinical study and continued to be significant even in neutered animals. Although male rats showed a stronger relationship between SBP and heart weight–to–body weight ratio than was seen in the HARVEST study, there was only a trend toward significance, whereas neutering abolished this relationship in males. In addition, because of the similar observations in normotensive and hypertensive rats in the present study and the HARVEST study, this model may prove useful in future investigations into this phenomenon.

In summary, intact female rats showed significantly greater heart mass relative to body weight than male rats, with a stronger relationship between SBP and heart weight–to–body weight ratio than males, which did not appear to be affected by estrogen. However, the presence of estrogen did appear to modulate the heart weight–to–body weight ratio in both normotensive and hypertensive females, although to a greater degree in normotensive rats. Interestingly, the absence of testosterone in males, however, did not appear to alter the heart weight–to–body weight ratio in either strain.

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