Noradrenergic content and turnover rate in kidney and heart shows gender and strain differences

GAIL DUNPHY, AND DANIEL ELY
Department of Biology, The University of Akron, Akron, Ohio 44325-3908

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NURSING STUDIES HAVE SHOWN that increased activity of the sympathetic nervous system (SNS) plays a role in the pathogenesis of essential hypertension (10, 20, 26, 29). SNS activity can be estimated by measurement of plasma catecholamine levels (13), norepinephrine (NE) turnover (9) or spillover rate (8), microneurography (33), and heart rate variability (28).

Our laboratory has shown that the Y chromosome of a spontaneously hypertensive rat (SHR) father when backcrossed to a normotensive Wistar-Kyoto rat (WKY) mother increased SNS indexes (4) and maintained an increase in blood pressure (BP) of ∼15–20 mmHg even after 11 generations (5). The Y chromosome effect accelerated the pubertal rise of androgen levels (6) and required the androgen receptor for full effect (7). In addition, testosterone and the SHR Y chromosome increased the storage and release of NE in the isolated kidney (19).

Additionally, gender differences in hypertension have been demonstrated. The incidence and severity of hypertension have been shown to be lower in women than men (30). Because gender differences in SNS regulation have been implicated in many models of hypertension (2, 14–17, 24), it is possible that one mechanism of protection in females may be reduced activation or enhanced inhibition of the SNS. For instance, Li and Duckles (25) have shown that sensitivity to adrenergic nerve stimulation in rat tail arteries was greater in males compared with females. The amount of NE available to modulate an end-organ response can be affected by neurotransmitter synthesis, release, and reuptake.

NE stores are maintained in equilibrium by balanced processes of synthesis and removal. Brodie et al. (1) have shown that catecholamine levels decline exponentially after pharmacological blockade of tyrosine hydroxylase (TH), the rate-limiting enzyme in the NE synthesis pathway. TH activity is one technique to assess catecholamine synthesis. In the work of Kohler et al. (21), no gender differences were observed in TH activity in vascular tissue, and its activity was not affected by gonadectomy. However, more recently Kuma and co-workers demonstrated that castration lowered vascular (23) and adrenal medullary (22) TH activities in SHR. TH activities in testosterone-replaced SHR recovered to the levels obtained in intact SHR. Gender differences in presynaptic α2-adrenoceptor number have been observed (18, 27), and, when blocked, the increased release of NE to nerve stimulation was greater in isolated hearts from females compared with males and was abolished by ovarectomy (3).

Therefore the objective of this study was to compare gender differences in NE content and turnover rate in normotensive and hypertensive rats. We hypothesized that if the Y chromosome was responsible for SNS hyperactivity, then the strains with the SHR Y chromosome would have the highest kidney and heart NE...
content and/or turnover compared with the strains with the Y chromosome for the normotensive WKY. In addition, we predicted that the females will have lower kidney and heart NE content and turnover rate than the strain-matched males.

**METHODS**

*Rat strains.* The parental WKY/hsd and SHR/hsd strains were originally obtained from Harlan Sprague Dawley (Indianapolis, IN) and have been inbred in our laboratory since 1981. In the following studies, we also used the consomic strain (SHR/y usa), developed in our laboratory, which has the SHR Y chromosome backcrossed into the WKY background for 17 generations (32). Briefly, a WKY female was mated with a SHR male. The males of the F1 generation were mated with a WKY female. This protocol was continued for 17 generations. As a result, 99.9% of the autosomes of the SHR/y strain are from the WKY strain and only the Y chromosome is from the SHR strain. A second congenic was developed concurrently from a SHR × WKY cross, and sons were backcrossed to a SHR. This strain is designated SHR/a, because it has the SHR autosomal, X chromosomal, and pseudoautosomal regions of SHR with a WKY Y chromosome. The SHR/y and SHR/a male strains are hypertensive. Females in all strains have lower BP than the strain-matched males. The SHR/y and SHR/a females are borderline hypertensive (32).

Rats were acclimated from birth to a 12-h light (0600–1800)-dark (1800–0600) cycle, and this was continued throughout the entire experimental procedure with a controlled temperature (27–29°C) and humidity (50–70%). All animals were treated in a humane manner according to the National Institutes of Health guidelines, and all experiments were approved by the University of Akron Institutional Animal Use and Care Committee.

*Catecholamine content and turnover.* α-Methyl tyrosine inhibits TH and prevents reaccumulation of NE, which is released in response to neural stimuli. The endogenous tissue levels then decline at a rate proportional to the initial NE concentration (31). When the log of NE is plotted vs. time, the slope of the straight line is 0.434 times the fractional turnover rate (k). The NE turnover rate is calculated as the product of k times the endogenous concentration of NE at time 0 (1). The reciprocal of k is the turnover time (1) or the time interval required for the biosynthesis of an amount of NE equal to that stored in the tissue.

The experimental design included adult (15–24 wk old) male and female rats (6–8 animals from each strain and gender were analyzed at each of the time points) of the WKY, SHR, SHR/y, and SHR/a strains as previously described. Rats were injected with the ester of α-methyl-DL-p-tyrosine (Sigma Chemical; an initial dose of 250 mg/kg ip at time 0 and booster injections of 125 mg/kg every 3 h) until termination (12). Control rats were injected with saline at time 0. Zero, 3, and 15 h after saline or α-methyl-DL-p-tyrosine injection, rats were anesthetized with sodium brevital (50 mg/kg ip, Lilly, Indianapolis, IN) and decapitated, and heart and kidney were rapidly removed. Tissues were weighed, frozen in liquid nitrogen, and stored at −80°C until NE assay. Tissues were homogenized in ice-cold mobile phase. After removal of protein by centrifugation, NE was assayed by using an HPLC system with an electrochemical detector (11).

*Statistics.* All values are expressed as means ± SE. The k of NE was calculated by least-square linear regression of the log NE content vs. time. Multiple comparisons were performed by two-way ANOVA and pairwise *t*-tests. Significance was assumed if *P* < 0.05. All statistics were performed using SigmaStat (Jandel Scientific).

**RESULTS**

Male kidney strain comparisons (Fig. 1A) showed a significantly lower NE content in the WKY compared with the other strains with no significant differences between the SHR, SHR/y, and SHR/a strains. Female kidney strain comparisons (Fig. 1A) showed a significantly lower NE content in the WKY and SHR/y compared with the SHR and SHR/a with no significant

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**Fig. 1.** Bar graph of kidney (A) and heart (B) norepinephrine (NE) content. Kidney NE content was significantly higher in females compared with males [2-way ANOVA: *P* < 0.001, DF = 1, *F* = 64.33; Wistar-Kyoto rats (WKY) ***P* < 0.001; spontaneously hypertensive rats (SHR) ***P* < 0.001; SHR × WKY strain with males backcrossed to SHR for 17 generations (SHR/a) ***P* < 0.001] in all strains except WKY. **Male kidney strain comparisons (Fig. 1A)** were performed with paired samples (2-way ANOVA: *P* < 0.001, DF = 5.985) with no significant differences between the SHR, SHR/y, and SHR/a strains. **Female kidney strain comparisons (Fig. 1A)** showed significant heart strain differences, there was a significant interaction between strain and gender (2-way ANOVA: *P* = 0.032, DF = 3, *F* = 3.16).
difference between the WKY and SHR/y or between the SHR and SHR/a. Kidney NE content showed a significant interaction between strain and gender.

The NE content was significantly higher in females compared with males in both kidney (Fig. 1A) and heart (Fig. 1B) in all but the SHR/y strain, in which there was no significant gender difference.

Heart NE content (Fig. 1B) showed a significant interaction between strain and gender, implying an effect of strain that is influenced by gender (and vice versa).

Figure 2 shows a typical example of NE turnover in the kidney. There was a significantly lower kidney NE turnover in the WKY males compared with the SHR/y males (Fig. 2A). There was no significant difference in turnover between the WKY and SHR/y females (Fig. 2B). There was no significant difference in NE turnover between the male SHR and SHR/a or the female SHR and SHR/a.

Figure 3A compares the kidney NE turnover rate in both genders of all four strains. WKY males and SHR/y males were significantly higher than strain-matched females. By contrast, SHR females and SHR/a females were significantly higher than strain-matched males. There was a significantly lower NE turnover rate in the WKY males compared with the other male strains (2-way ANOVA: *P < 0.001, DF = 3, F = 11.582; ***P < 0.001) kidney gender difference in all 4 strains. There was a significantly lower kidney turnover rate in the WKY males compared with the other male strains (2-way ANOVA: *P < 0.001, DF = 3, F = 4.96; SHR/y +P = 0.025; SHR +P = 0.05; SHR/a ++P < 0.001). There was no significant kidney NE turnover difference between the male SHR and SHR/a or between the female SHR and SHR/a and the female WKY and SHR/y. There was a significant (2-way ANOVA ***P < 0.001, DF = 1, F = 40.35) heart gender difference in the WKY strain only. The only significant strain difference was observed between the SHR/y and WKY females (2-way ANOVA: ++P < 0.001, DF = 1, F = 35.38).

Figure 3B shows no significant difference in heart NE turnover between the WKY and SHR/y males. However, there was a significantly higher NE turnover in the SHR/y females compared with the WKY females. Figure 3B shows no significant heart NE turnover difference between the male SHR and SHR/a or female SHR and SHR/a. Although there is a trend toward
gender differences in the heart NE turnover (Fig. 3B), there is only significance in the WKY strain.

**DISCUSSION**

Because of the breeding protocol that produced the SHR/y and SHR/a strains, the female SHR/y has a similar genotype to the WKY female. Similarly, the SHR/a female should be genotypically similar to the SHR female. Any male strain differences between the WKY and SHR/y would be due to the Y chromosome. Kidney NE content and turnover showed a similarity in SHR and SHR/y males. This suggests that there is a locus on the Y chromosome that either directly or indirectly influences the SNS through catecholamine content and turnover rates. In addition, the similarity observed in the WKY and SHR/y females supports this hypothesis. However, there were no strain differences in the heart NE content or turnover, suggesting that there may be NE gender differences that are tissue specific.

An interesting observation is the higher kidney NE content in the females compared with the males in all strains except the SHR/y. This finding was not predicted because in all four strains females have lower BP than strain-matched males (32). If we compare the turnover rates in Table 1 (ng·g⁻¹·h⁻¹) of all the groups, even though the WKY and SHR/y females have a higher NE baseline content than the WKY and SHR/y males, the rate of synthesis (if we accept the premise that NE levels are maintained in a steady state then the rate of decline should equal the rate of synthesis) in the females is actually significantly lower than in the males. In addition, the two normotensive groups (male and female WKY) have the lowest turnover rates of all the groups. The heart follows a similar pattern in NE content and turnover rate, although significance was not observed. A comparison of heart NE content (WKY male = 330 ng; SHR/y male = 403 ng) and turnover rate (WKY male = 3.43 ng·g⁻¹·h⁻¹; SHR/y = 24.56 ng·g⁻¹·h⁻¹) clearly shows the same trend as was observed in the kidney NE content and turnover rates. Evidence for the Y chromosome effect is supported by the observation that the SHR/y males have a significantly higher turnover rate compared with the WKY males, with whom the SHR/y are genotypically similar except for the Y chromosome. In fact, there was no significant difference in turnover rates among the SHR/y, SHR, and SHR/a males, all of which are phenotypically hypertensive.

In conclusion, the hypertensive rats had higher kidney NE turnover rates compared with the normotensive rats. Females had higher baseline NE content in both the kidney and heart compared with strain-matched males. However, only the hypertensive females had higher turnover rates than strain-matched males. In addition, the strain with the Y chromosome from the SHR had higher kidney NE content and turnover rates compared with the WKY strain. This study suggests both a strain and gender difference in SNS activity through noradrenergic neurotransmission.

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**REFERENCES**


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**Table 1. Heart and kidney NE baseline content and turnover rates and times after blockade of synthesis in male and female WKY, SHR, SHR/y and SHR/a rats**

<table>
<thead>
<tr>
<th>Rat Strain and Gender</th>
<th>Tissue Type</th>
<th>Initial NE Levels, ng/g</th>
<th>Fractional Turnover Per Hour</th>
<th>Turnover Rate, ng·g⁻¹·h⁻¹</th>
<th>Turnover Time, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY male</td>
<td>Heart</td>
<td>330.81 ± 34.2</td>
<td>0.0103 ± 0.005</td>
<td>3.43 ± 0.35</td>
<td>96.4</td>
</tr>
<tr>
<td>WKY female</td>
<td>Heart</td>
<td>404.59 ± 62.9</td>
<td>0.0089 ± 0.005</td>
<td>4.40 ± 0.56</td>
<td>112.3</td>
</tr>
<tr>
<td>SHR male</td>
<td>Heart</td>
<td>307.15 ± 29.6</td>
<td>0.0295 ± 0.005</td>
<td>8.28 ± 0.90</td>
<td>37.1</td>
</tr>
<tr>
<td>SHR female</td>
<td>Heart</td>
<td>444.52 ± 44.4</td>
<td>0.0437 ± 0.005</td>
<td>19.43 ± 1.94</td>
<td>22.9</td>
</tr>
<tr>
<td>SHR/y male</td>
<td>Heart</td>
<td>403.86 ± 43.5</td>
<td>0.061 ± 0.006</td>
<td>24.56 ± 2.65</td>
<td>16.44</td>
</tr>
<tr>
<td>SHR/y female</td>
<td>Heart</td>
<td>347.4 ± 22.5</td>
<td>0.0387 ± 0.004</td>
<td>13.44 ± 0.87</td>
<td>25.8</td>
</tr>
<tr>
<td>SHR/a male</td>
<td>Heart</td>
<td>327.24 ± 39.2</td>
<td>0.0167 ± 0.005</td>
<td>5.46 ± 1.08</td>
<td>60.0</td>
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<tr>
<td>SHR/a female</td>
<td>Heart</td>
<td>525.71 ± 21.0</td>
<td>0.0276 ± 0.006</td>
<td>14.51 ± 0.58</td>
<td>36.23</td>
</tr>
<tr>
<td>WKY male</td>
<td>Kidney</td>
<td>84.2 ± 5.6</td>
<td>0.138 ± 0.01</td>
<td>11.64 ± 2.89</td>
<td>7.23</td>
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<tr>
<td>WKY female</td>
<td>Kidney</td>
<td>137.6 ± 7.4</td>
<td>0.074 ± 0.01</td>
<td>10.18 ± 0.54</td>
<td>13.5</td>
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<tr>
<td>SHR male</td>
<td>Kidney</td>
<td>109.5 ± 9.0</td>
<td>0.15 ± 0.01</td>
<td>16.43 ± 1.35</td>
<td>6.66</td>
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<tr>
<td>SHR female</td>
<td>Kidney</td>
<td>181.8 ± 9.5</td>
<td>0.148 ± 0.01</td>
<td>26.90 ± 1.42</td>
<td>6.75</td>
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<tr>
<td>SHR/y male</td>
<td>Kidney</td>
<td>113.2 ± 5.0</td>
<td>0.179 ± 0.008</td>
<td>20.24 ± 0.89</td>
<td>5.59</td>
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<tr>
<td>SHR/y female</td>
<td>Kidney</td>
<td>119.2 ± 10.7</td>
<td>0.076 ± 0.01</td>
<td>9.06 ± 0.81</td>
<td>13.16</td>
</tr>
<tr>
<td>SHR/a male</td>
<td>Kidney</td>
<td>122.2 ± 9.7</td>
<td>0.167 ± 0.009</td>
<td>20.43 ± 0.16</td>
<td>5.98</td>
</tr>
<tr>
<td>SHR/a female</td>
<td>Kidney</td>
<td>176.5 ± 6.8</td>
<td>0.184 ± 0.008</td>
<td>32.53 ± 1.25</td>
<td>5.43</td>
</tr>
</tbody>
</table>

Values are means ± SE. NE, norepinephrine; WKY, Wistar-Kyoto rats; SHP, spontaneously hypertensive rats.