Adrenal Response to Angiotensin II in Black Hypertension
Lack of Sexual Dimorphism

Naomi D.L. Fisher, Shelley Hurwitz, Xavier Jeunemaitre, Deborah A. Price, Gordon H. Williams, Norman K. Hollenberg

Abstract—Adrenal responsiveness to angiotensin (Ang) II is markedly blunted in black hypertensive patients compared with white hypertensive patients. One characteristic of this blunted adrenal response in whites is a powerful sexual dimorphism: premenopausal white women rarely show blunted responses. This abnormality, most evident when the system is activated by a low-salt diet, is a cardinal feature of the syndrome of nonmodulation, affecting a large percentage of white hypertensive patients. Nonmodulation is also marked by an increase in cardiovascular risk beyond that from hypertension itself. This study investigated whether young black women are likewise spared its expression or whether the adrenal unresponsiveness common among black hypertensive patients is unaccompanied by a gender bias. We compared the adrenal response to Ang II in 382 hypertensive patients (313 white, 69 black; 238 male, 144 female). Ang II was infused when subjects were in balance on a 10-mmol Na+ intake. As anticipated, white hypertensive patients showed a very strong sexual dimorphism, with women having twice the aldosterone response of men (P=0.0001). Blacks, on the other hand, showed no gender difference (P=0.9). Increasing age had the dramatic effect of reducing responsiveness in white women but not in blacks. Young black women demonstrated the same blunting of adrenal responsiveness as older black women and black men of all ages. Mechanisms protecting against a blunted adrenal response to Ang II in young white women are absent in blacks. These differences may contribute to the markedly increased prevalence of hypertension in young black women. (Hypertension. 2001;38:373-378.)

Key Words: angiotensin II ■ race ■ gender ■ aldosterone ■ potassium ■ hypertension, renal

We have reported a striking frequency of blunted adrenal responses to angiotensin (Ang) II infusion in black hypertensive patients.1 Blunting of the adrenal response to Ang II is a cardinal feature of the syndrome of nonmodulation (NM). One additional characteristic of this response in NM is a powerful sexual dimorphism: premenopausal white women rarely show a blunted adrenal response.2 NM is also marked by an increase in cardiovascular risk factors and cardiovascular risk itself.3,4 Young black women have up to 3 times the risk for coronary heart disease and death of white women.5,6 A large degree of this excess risk is attributable to differences in coronary risk factors, including elevated blood pressure.7,8 This striking racial difference in risk has not been observed among men.

Prompted by these observations, we undertook a systematic analysis of adrenal responsiveness in a large cohort of black and white hypertensive patients to test the hypothesis that the sexual dimorphism protecting young white women from phenotypic expression of NM is not present among blacks. The finding that black women have a significantly greater frequency of a blunted adrenal response to Ang II than do young white women may help explain their dramatic increase in cardiovascular risk.

Methods

The data reported in the present study were collected from the Human Reverse Functional Genomic Studies (HERMES) group. The overall characteristics of this population, and some of the data on some subjects, have been reported previously.9,10 However, the analyses reported herein are original.

We studied 382 hypertensive patients (69 black and 313 white) from 298 families. Subjects for this report were studied at the Brigham and Women’s Hospital in Boston and the Hôpital Broussais in Paris. Sixty-eight families provided 152 siblings; 230 subjects were the sole members of their families. Race was determined by self-identification and supported by physical appearance. Human Subjects Committees approved similar protocols at each site. All subjects were free of overt cardiovascular or renal disease and of known or suspected secondary hypertension or renal insufficiency. Hypertension was defined by seated blood pressures >90 mm Hg diastolic and ≥140 mm Hg systolic. Antihypertensive medications were discontinued at least 2 weeks before study. Diabetes mellitus type 2 was diagnosed in 28 patients: only 3 were black, and only 1 of these was female. No woman was taking oral contraceptive agents; 5 women (4 white, 1 black) were taking postmenopausal hormone replacement therapy.
Protocols
Each subject was placed on a constant 10-mmol sodium and 100-mmol potassium isocaloric diet. Studies were performed after 5 to 7 days, when external sodium balance had been achieved (urinary sodium <30 mmol/d). Hormonal responses to a postural stimulus were assessed in each subject after 1 or 2 hours in the standing position for the measurement of plasma renin activity (PRA). Low-renin hypertension was defined by a PRA of <0.69 ng Ang I/L per second (<2.5 ng Ang I/mL per hour) at the end of the posture study. On a separate study morning, each patient received an infusion of Ang II amide at 3 ng/kg per minute for 40 minutes, delivered by an infusion pump.

Potassium supplementation was undertaken in a subset of patients in an immediately dispersing extended-release oral dosage form (20 mmol) 3 times daily over a period of 1 month, and the Ang II infusion was then repeated.

Laboratory Procedures and Statistical Analyses
PRA, cortisol, and aldosterone were assayed by radioimmunoassay techniques previously described. Active renin was performed by using an immunoradiometric assay. Plasma angiotensinogen (AGT) was measured by the generation of Ang I after the addition of human renin.

The index of dispersion was the standard error of the mean. Analyses of mixed models were performed to account for the correlation structure in the data set that consisted of families with 1 to 4 siblings. The logarithm transform was applied to PRA and to active renin to achieve normality. Race and gender were fixed effects, and family membership was random. A significant interaction between race and gender indicated that the race effect depended on the whether the subjects were black or white. Significant interactions were followed by the assessment of simple main effects. Age was analyzed as a covariate in the mixed-models design.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results
Within each gender, the racial groups were well matched for age, body mass index (BMI), and admission blood pressure (Table 1). Serum creatinine was higher in black men than in whites (P=0.004). Black men (but not black women in this subset) had a greater frequency of low-renin hypertension than did whites (P=0.04), and they also had higher urinary sodium on the low-sodium diet (P=0.02).

As anticipated, adrenal responses to Ang II among females overall were significantly larger than they were among males (900±66 versus 496±22 pmol/L, P=0.006). However, racial analysis revealed that this relationship holds only among whites (Figure 1); the mean rise in aldosterone among women was twice that of men (1080±83 versus 516±25 pmol/L, P=0.0001). Among blacks, the gender difference was not seen; the aldosterone responses were 416±41 pmol/L for females and 352±48 pmol/L for males (P=0.9).

The effect of age on aldosterone response was examined for both genders (Figure 2). In women, the relationship between aldosterone responsiveness and age depended significantly on race (P=0.004). Among white women, increasing age had a dramatic effect on reducing aldosterone response (mean loss of 332 pmol/L per decade, P=0.0001). In contrast, there was no significant effect of age among black women (loss of response, 23 pmol aldosterone/L per decade, P=0.8).

Among white men, age had a modest effect of decreasing aldosterone response (loss of 61 pmol/L per decade, P=0.05). Among black men, no effect of age was seen (loss of 23 pmol/L per decade, P=0.8) (Figure 2).

Table 1. Demographic Information

<table>
<thead>
<tr>
<th>Variable</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>105</td>
<td>39</td>
</tr>
<tr>
<td>Black</td>
<td>208</td>
<td>30</td>
</tr>
<tr>
<td>Age, y</td>
<td>45.8±10.7</td>
<td>45.4±8.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.3±5.0</td>
<td>28.6±5.5</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>150±19</td>
<td>156±19</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>90±13</td>
<td>93±10</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>75±2.4</td>
<td>84±4.9</td>
</tr>
<tr>
<td>Serum potassium, mmol/L</td>
<td>4.1±0.3</td>
<td>4.2±0.3</td>
</tr>
<tr>
<td>Urinary sodium, mmol/24 h</td>
<td>11.0±7</td>
<td>11.7±6</td>
</tr>
<tr>
<td>Urinary potassium, mmol/24 h</td>
<td>70.3±20</td>
<td>64.7±20</td>
</tr>
<tr>
<td>Low renin, %</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

BP indicates blood pressure. Values are mean±SEM.
Given our previous report that low-renin hypertension is marked by low aldosterone responsiveness on a low-salt diet, we examined the effect of low-renin status on the interaction between gender and race. First, the overall frequency of low-renin hypertension was greater in blacks than in whites, as anticipated (27% versus 16%, respectively; \( P = 0.014 \)). It was highly unlikely that the blunted adrenal responsiveness among young black women was due to a greater incidence of low-renin hypertension, because the frequencies in white and black women did not differ (Table 1, \( P = 0.47 \)). Nonetheless, to pursue this possibility, we analyzed the normal-renin hypertensive population separately. Both in terms of the interaction between race and gender and the effects of age on aldosterone response, the results did not differ from those of the overall hypertensive group.

Cortisol concentration was much higher in men than in women (\( P = 0.0004 \)). In addition, basal cortisol concentration was marked by a significant racial difference on the low-salt diet, with values lower in blacks than in whites (\( P = 0.03 \)); plasma concentrations were lowest in black females (Table 2).

Four black hypertensive patients, 2 men and 2 women, whose initial adrenal responsiveness to Ang II was blunted were administered oral potassium supplementation (60 mmol KCl daily) for at least 1 month before a repeat Ang II infusion. Potassium excretion, as measured in 24-hour urine collections, rose in all 4 patients (from 62 ± 10 to 84 ± 11 mmol/d). In contrast, the aldosterone response to Ang II did not rise in any of the 4 patients after potassium replacement (Table 3).

The sexual dimorphism in adrenal responsiveness was not related to either basal PRA or to active renin. Indeed, basal PRA was lower among females than males overall (\( P = 0.02 \)), whereas adrenal responses were more brisk among females (Table 2). Active renin, likewise, was significantly higher in men compared with women at baseline (\( P = 0.03 \)). There was no difference in basal aldosterone secretion on the low-salt diet in terms of race or gender. The significantly lower PRA in women was associated with a 20% higher plasma AGT concentration in both black and white females than in men (\( P = 0.01 \)). This gender effect did not depend on race (\( P = 0.87 \)). As opposed to the stimulated aldosterone concentration after Ang II, basal aldosterone concentrations did not differ between the races or the genders.

**Discussion**

The present report confirms our earlier findings of a prominent sexual dimorphism in essential hypertension that is marked by a very low frequency of adrenal unresponsiveness among young females. However, when examined separately by race, the data reveal an interesting dichotomy. Although, on average, white women have twice the adrenal responsiveness of white men, black women have responses no different from those of black men. Blunted adrenal responsiveness is a cardinal feature of a common intermediate phenotype of hypertension referred to as NM. Young white women seem to benefit from a protection against the expression of this phenotype, a protection that is lost with increasing age. In contrast, black women seem never to benefit from augmented responsiveness, even in youth.

NM characterizes a distinct subset of up to 50% of the essential hypertensive population, marked by adrenal and renal vascular responses to Ang II that are not modified by changes in sodium intake. Specific abnormalities defined in this subset include failure of sodium loading to enhance the renal vascular response to Ang II, salt-sensitive high blood pressure, insulin resistance, and increased cardiovascular risk. In a previous report, we postulated that young women are protected against the expression of NM through the action

### TABLE 2. Baseline Hormonal Values

<table>
<thead>
<tr>
<th>Variable</th>
<th>Female</th>
<th>Male</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White</td>
<td>Black</td>
<td>White</td>
</tr>
<tr>
<td>Patients, n</td>
<td>105</td>
<td>39</td>
<td>208</td>
</tr>
<tr>
<td>PRA, ng Ang I \cdot L(^{-1}) \cdot s(^{-1})</td>
<td>0.81 ± 0.07</td>
<td>0.61 ± 0.09</td>
<td>0.9 ± 0.05</td>
</tr>
<tr>
<td>Active renin, ( \mu )U/mL</td>
<td>39.0 ± 4</td>
<td>28.2 ± 3</td>
<td>54.3 ± 5</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>313 ± 11</td>
<td>256 ± 13</td>
<td>364 ± 10</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>555 ± 523</td>
<td>497 ± 54</td>
<td>599 ± 22</td>
</tr>
<tr>
<td>Aldosterone after Ang II, pmol/L</td>
<td>1635 ± 115</td>
<td>914 ± 73</td>
<td>1116 ± 35</td>
</tr>
<tr>
<td>AGT, ng Ang l/mL</td>
<td>1192 ± 113</td>
<td>1134 ± 60</td>
<td>998 ± 33</td>
</tr>
</tbody>
</table>

*Values are mean ± SEM.*
of female sex hormones, especially because young white women do not show the trait, whereas older women do.\(^2\)

Although we did not measure estradiol and progesterone in the present study, it is unlikely that any differences in sex steroids exist to account for the differing responses, because there are no data to suggest any such racial effects on sex steroid production. Is it also unlikely that differences in PRA or active renin underlie the findings presented above. Lower PRA and active renin concentrations in women compared with men, in light of an overall greater female adrenal responsiveness, are rather more compatible with higher AGT concentrations in women suppressing renin production and activity via the short or long feedback loop.

A more likely explanation lies in the realm of gene transcription, with AGT as a possible candidate. Estrogen is 1 of the main stimuli for the production of AGT\(^17\); we report plasma concentrations of AGT 20% higher in women than in men. A significant association of AGT and estradiol responsiveness, are rather more compatible with higher AGT concentrations in women suppressing renin production and activity via the short or long feedback loop.

A second major stimulus to aldosterone release is adrenocorticotropic hormone (ACTH). Although they were not measured directly, there were striking gender and racial differences in plasma cortisol. We and others have previously reported higher plasma cortisol concentrations and urinary cortisol excretion in men than in women at baseline, in response to psychological stress, and on both high- and low-sodium diets.\(^2,32\) In addition, corticotropin secretion was not seen in blacks.\(^31\) Enzymatic studies demonstrated that these racial differences were quantitative.

A related phenomenon governed by a differential gender effect between the races is renal kallikrein excretion. Urinary kallikrein is higher among ovulating females, but despite the confirmation of ovulatory cycle phases, this midcycle elevation was not seen in blacks.\(^5\) Enzymatic studies demonstrated that these racial differences were quantitative.

The racial difference in adrenal unresponsiveness could not be attributed to dissimilarities in age, BP, or BMI. It is conceivable that the lower aldosterone response in blacks is caused by greater sodium retention, even on a low-sodium diet. Data to prove this hypothesis would best be gathered daily during the transition from a low to high salt intake. However, it is clear from PRA that this explanation could not apply equally to both men and women.

Potassium is an important stimulus for aldosterone secretion. Neither serum nor urinary potassium concentration differed between the 4 groups of black and white men and women. However, arguments can be made that a total body deficit of potassium that exists among blacks cannot be reversed with short-term supplementation. For that reason, we administered prolonged potassium supplementation to black hypertensive patients, and although the sample size precludes definitive statements, there was absolutely no augmentation of response.

A second major stimulus to aldosterone release is adrenocorticotropic hormone (ACTH). Although they were not measured directly, there were striking gender and racial differences in plasma cortisol. We and others have previously reported higher plasma cortisol concentrations and urinary cortisol excretion in men than in women at baseline, in response to psychological stress, and on both high- and low-sodium diets.\(^2,32\) In addition, corticotropin secretion was greater in males than females in a small group of healthy subjects.\(^33\) However, studies by Yanovski et al\(^34\) have demonstrated that black women have cortisol responses to infused ACTH that are no different from those of whites, despite greater concentrations of immunoreactive ACTH in blacks. Similarly, in a large study of adolescents, Pratt et al\(^35\) failed to find any racial difference in plasma cortisol. The present study differs by its low salt content. The finding in the present study that black females have the lowest plasma cortisol concentration must be replicated but raises intriguing possibilities about a racially and sexually differentiated influence of stress on cortisol production and perhaps on cardiovascular reactivity more generally. There is need to pursue the puzzling possibility that blacks showed blunted responsiveness to 2 adrenal cortical hormones that are the byproducts of differing synthetic pathways.

### TABLE 3. Responses to Potassium Supplementation

<table>
<thead>
<tr>
<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>36</td>
<td>32</td>
<td>31</td>
<td>38</td>
<td>42±7</td>
</tr>
<tr>
<td>Urinary potassium, mmol/24 h</td>
<td>Basal</td>
<td>66</td>
<td>78</td>
<td>32</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>94</td>
<td>93</td>
<td>52</td>
<td>99</td>
</tr>
<tr>
<td>Aldosterone response, ng/dL</td>
<td>Basal</td>
<td>10</td>
<td>6</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>12</td>
<td>8</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>
Despite an overall reduction in the death rate due to cardiovascular disease in the United States over the last several decades, the rate of decline is less for women than men and lowest for African American women.6,36 The death rate due to cardiovascular disease has been assessed as 34% to 69% higher in black women than in white women, compared with a 5% higher rate for black men than for white men. Many reasons are postulated for the increased cardiovascular disease rates seen among blacks compared with whites. Lesser access to medical care has long been cited, together with lower socioeconomic status.37,38 Much of the risk can be attributed to increased in cardiac risk factors, most notably including diabetes mellitus and obesity.8 Other possibilities include an increase in smoking habits, dyslipidemias,39 hypercoagulability, and genetic factors.

On the basis of studies performed in humans and experimental animals, a defect in the regulation of the tissue renin–Ang II system has been proposed to explain the abnormalities associated with NM.40 The known higher frequency of these abnormalities in blacks compared with whites has now been refined, and we have determined that black women are even more severely affected than are black men. The possibility of a genetically determined increase in the activity of the renin-angiotensin-aldosterone system as a predisposing factor for hypertension, especially in black women, remains an important future avenue of investigation.

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