Gender Affects Renal Vasoconstrictor Response to Ang I and Ang II

Sanjay K. Gandhi, Jay Gainer, Debbie King, Nancy J. Brown

Abstract—This study tested the hypothesis that gender affects the pressor and renal vasoconstrictor responses to angiotensin (Ang) I and Ang II in salt-replete normotensive subjects. Ang I and Ang II were infused in graded doses into 9 men and 8 women in a randomized, single-blind, crossover study. There were no differences between genders in baseline blood pressure, heart rate, sodium excretion, renal plasma flow, angiotensin-converting enzyme (ACE) genotype, ACE activity, plasma renin activity, aldosterone, or Ang II levels. Although pressor responses to Ang I and Ang II were similar in men and women, there was a negative relationship between the change in mean arterial pressure and the change in heart rate during Ang I and II infusion in women only. The half-time of the pressor response after discontinuation of Ang I but not Ang II infusion was greater in men than in women (9.5±2.2 versus 4.3±2.1 minutes, P<.05). This difference in duration did not result from gender differences in the metabolism of Ang I because Ang II levels measured during Ang I infusion were identical in men and women. In contrast, the renal vasoconstrictor response to Ang I and Ang II was significantly increased in women compared with that in men (Ang I, −243±31 versus −138±13 U/1.73 m²; Ang II, −233±25 versus −175±18 U/1.73 m²; P<.03). These data suggest an effect of gender on baroreflex reactivity during angiotensin infusion. Moreover, in the setting of similar Ang II concentrations, the dramatic difference in the renal vasoconstrictor responses to Ang I and Ang II between salt-replete men and salt-replete women suggests gender differences at a pharmacodynamic level. (Hypertension. 1998;31[part 1]:90-96.)

Key Words: angiotensin I angiotensin-converting enzyme renal plasma flow gender

Sensitivity to exogenous Ang II has been used to define an intermediate phenotype of essential hypertension termed nonmodulation.1 The nonmodulator phenotype is characterized by defective adaptation to changes in sodium intake.1 In nonmodulators, aldosterone and RPF responses to exogenous Ang II in the salt-depleted and salt-replete states, respectively, are blunted.1 Recently, Hopkins et al10 reported that gender affected the RPF response to Ang II in hypertensive and normotensive control subjects who had been genotyped at the angiotensinogen M235T locus. Fisher et al11 reported that the frequency of the nonmodulator phenotype, as measured by the aldosterone response to Ang II, is decreased in premenopausal but not postmenopausal normal- to high-renin hypertensive women compared with men.1 This finding suggests that gender and hormone status may influence sensitivity to exogenous Ang II. The pressor response to Ang II has been studied extensively in both pregnant and nonpregnant premenopausal women1-4; however, there are few studies comparing men and women.

Gender differences in response to Ang II could result from gender differences in ACE activity, AT1 receptor sensitivity, or both. Recently, measurement of the relative responses to exogenous Ang I and Ang II has been used to explore the differences in functional ACE activity in subjects of different ACE I/D genotypes.5,6 With this model, if ACE activity is increased in one group, then infusion of exogenous Ang I would be expected to result in enhanced conversion of Ang I to Ang II and, therefore, an increased response to Ang I. Responses to exogenous Ang II might be similar in both groups. For example, Ueda et al7 reported an increased pressor response to Ang but not Ang II in normotensive men who were homozygous for the ACE D allele compared with men who were homozygous for the I allele. Alternatively, chronically increased endogenous Ang II levels might be expected to alter sensitivity of its own receptor,9 resulting in altered responses to both exogenous Ang I and Ang II. The effect of Ang II on its receptor is complicated and depends on the receptor subtype and the tissue.10 Ang II downregulates AT1 receptors in vascular smooth muscle.11 On the other hand, Ang II upregulates the AT1 receptor in the rat adrenal gland,12 and low sodium intake is associated with enhanced adrenal responsiveness to Ang II in humans.13

The present study tested the hypothesis that gender affects the pressor and renal vasoconstrictor responses to exogenous Ang I and Ang II in salt-replete, normotensive subjects, independent of ACE I/D genotype, and examined whether gender differences in response to angiotensin reflect differences in ACE activity as measured by conversion of Ang I to Ang II.

Methods

Subjects
Nine normotensive women and 9 normotensive men who had been previously genotyped at the ACE I/D locus were initially enrolled in...
the study. Subjects completed an initial medical history and underwent a physical examination, electrocardiogram, and laboratory screening. None had any history or evidence of cardiovascular, endocrine, or renal disease. All were within 30% of ideal body weight as defined by the Metropolitan Life Insurance weight tables. All subjects gave written, informed consent; the study protocol was approved by the Institutional Review Board of Vanderbilt University and conducted in accordance with institutional guidelines.

Because ACE genotype has been reported to influence conversion of Ang I to Ang II, only subjects who were homozygous (II or DD) at this locus were studied, and the number of subjects of each genotype enrolled in each gender group was the same (3 II and 6 DD). Data from 1 subject, a woman who was homozygous for the ACE D allele, were subsequently excluded when it was discovered that she had inadvertently been given vehicle alone instead of Ang I during one of her study days.

Protocol

Fig 1 illustrates the study protocol. Subjects were provided a xanthine-free, 200 mmol sodium, 100 mmol potassium, 2564-ml fluid diet for 8 days and collected their urine for measurement of sodium, potassium, and creatinine. On the morning of the 6th and 8th days of the diet, each subject reported to the Vanderbilt General Clinical Research Center. Subjects were studied in the supine position. At 7 AM, an intravenous catheter was placed in each arm. One catheter was used for drug infusion and the other was used for venous blood sampling. BP and HR were measured every 1 to 2 minutes throughout the study with an automated BP cuff (Dinamap, Critikon). After placement of the intravenous catheters, each subject was given an 8 mg/kg loading dose of PAH followed by a constant infusion at 12 mg/min. After 1 hour of constant PAH infusion, steady-state PAH concentrations were achieved and at the end of each dose. PRA and aldosterone were measured at baseline and after each 10 ng·kg⁻¹·min⁻¹ angiotensin dose. Blood was drawn for measurement of Ang II and Ang-(1–7) at baseline and after the 10 and 20 ng·kg⁻¹·min⁻¹ doses.

Analytical Methods

ACE I/D genotype was determined by polymerase chain reaction using previously published primers. All DD homozygotes were confirmed by using I-specific primers. Urine electrolyte levels were measured by flame photometry. All blood samples were collected on ice, spun, and frozen at −70°C until the time of assay. PRA was measured by RIA for Ang I at pH 7.4 and 37°C. Aldosterone was measured by RIA with the Coatacount aldosterone assay kit from Diagnostic Products Corp.

Blood for angiotensin measurements was collected in chilled tubes containing a cocktail of protease inhibitors. Ang II measurements were evaluated by RIA, with techniques as previously described. Plasma was extracted on Sep-Pak columns (Waters/Millipore) activated with 5-nl sequential washes of a mixture of ethanol/water/4% acetic acid (83:13.4, vol/vol/vol), methanol, ultrapure water, and 4% acetic acid. The sample was eluted and reconstituted in assay buffer. Recovery of radiolabeled angiotensin added to the sample and followed through the extraction was 92%. Samples were corrected for recovery. Ang II was measured by RIA with the Nichols Institute RIA (San Juan Capistrano, Calif). This antibody shows 67% cross-reactivity with Ang III, 70% with Ang-(3–8), and 91% with Ang-(4–8) but <0.1% with Ang I. The minimum detectable level of the assays was 4 pg per tube for Ang II. The intra-assay coefficient of variation was 12% for Ang II.

Plasma PAH levels were measured by spectrophotometric autoanalyzer techniques. The intraassay coefficient of variation for PAH clearance in our laboratory is 3.91±3.53% (mean±SD). Baseline RPF was measured by determining the clearance of PAH from the plasma by using the formula: 

\[
\text{C}_{\text{PAH}} = \frac{\text{dose of PAH}}{\text{PAH area}} \times \frac{1.73}{\text{body surface area}}
\]

When corrected for body surface area, PAH clearance reflects renal perfusion. At the end of each dose of angiotensin; however, this equation assumes a steady-state concentration of PAH has been achieved. A prior study using radioxenon suggested that RPF reaches a new steady state within 10 minutes of initiation of intravenous Ang II. A pilot study conducted in one of the investigators suggested that PAH concentrations had also reached a new steady state within this time. Nevertheless, because we could not be certain that PAH had achieved a new steady state in each subject within the angiotensin infusion time, we expressed renal perfusion after angiotensin infusion as a “renal perfusion index,” defined by arbitrary units (U) per 1.73 m² of body surface area, rather than as a clearance, defined in milliliters per minute.

Statistical Analysis

Results are presented as meanSEM. Statistical analysis was performed using the SPSS Advanced Statistical Program (SPSS Inc). Angiotensin dose-response curves in men and women were compared by ANOVA with repeated measures, in which the initial between-subject variables were gender, ACE genotype, and race, and the within-subject variables were angiotensin (I versus II) and dose. There was no significant effect of race on MAP (F=0.22, P=0.65), RPF (F=0.21, P=0.66) or aldosterone (F=0.27, P=0.62) responses to Ang I and Ang II, and for this reason, race was excluded from the final analyses. Comparisons between groups at specific Ang I or Ang II doses were made with two-tailed, unpaired Student’s t test, whereas within-group comparisons were made with paired Student’s t test. Data that did not follow the normal distribution (Ang II levels) were logarithmically transformed before analysis. Probability values reported are from the ANOVA unless otherwise indicated. The criterion for significance was a value of P<0.05.
Results

The Table shows the characteristics of the subjects studied. Seven of the 8 women were premenopausal and were studied during the late luteal or early follicular phase of their menstrual cycle. The eighth woman was perimenopausal and taking conjugated estrogens. There were no differences between men and women studied with respect to age, race, body mass index, family history of hypertension, ACE I/D genotype frequencies, baseline 24-hour urine sodium excretion, RPF, PRA, ACE activity, Ang II levels, or aldosterone levels. Two of the 6 subjects who were homozygous for the ACE I allele and 4 of the 11 who were homozygous for the ACE D allele were African Americans.

Pressor Response

Fig 2 shows the pressor response to Ang I and Ang II. MAP increased significantly in a dose-dependent manner in response to both Ang I and Ang II (F=60.1, P<.01 for dose effect). The pressor responses to Ang I and Ang II were similar (F=0.04, P= .85). The pressor response to Ang II was similar in men and women (F=0.54, P=.47). Men tended to have a greater pressor response to Ang I than did women, although this difference was not statistically significant (F=3.5, P=.086).

Although there were no significant gender differences in the pressor response to Ang I and Ang II, the duration of the pressor response to Ang I was significantly different (F=6.05, P=.027) between men and women (Fig 3). After discontinuation of Ang I, MAP returned to baseline with a half-time of 4.3±2.1 minutes in women. However, the half-time of return of MAP to baseline was 9.5±2.2 minutes in men (P=.04 versus women) after discontinuation of Ang I. To some extent, this prolonged half-time of MAP return in men reflected attainment of a new steady state after Ang I infusion (Fig 3). By contrast, there was no gender difference in the half-time of resolution of the pressor response after discontinuation of Ang II. Thus, the half-time of resolution of the pressor response was significantly greater for Ang I than for Ang II in men (9.5±2.2 versus 2.8±0.7, P=.026).

Pharmacokinetics of Ang I

Ang I is metabolized to Ang II via ACE and to Ang-(1–7) via metalloendopeptidase 24.15, endopeptidase 24.11, and prolylendopeptidase 24.26. To determine whether gender differences in the duration of the pressor response to Ang I could be attributed to differences in the metabolism of Ang I, Ang II and Ang-(1–7) levels were measured at the end of the 10 and 20 ng·kg⁻¹·min⁻¹ doses of Ang I. Ang-(1–7) was below detectable levels during Ang I infusion in 4 of 5 subjects (2 male, 3 female) in whom it was measured and, therefore, was not measured in the remaining subjects.

Ang II levels increased significantly after Ang I infusion in both men and women; however, there was no gender difference in plasma concentrations of Ang II after Ang I infusion (F<0.01, P=.99). This finding contrasts a dramatic effect of ACE genotype on Ang II concentrations after Ang I infusion (Fig 4).

HR Response

Gender differences in the interaction between Ang II and the autonomic nervous system could contribute to gender differ-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (n=9)</th>
<th>Women (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>26.4±1.64</td>
<td>34.4±3.8</td>
</tr>
<tr>
<td>Race, WAA*</td>
<td>5.4</td>
<td>6.2</td>
</tr>
<tr>
<td>Family history of hypertension, +/−</td>
<td>2.7</td>
<td>1.7</td>
</tr>
<tr>
<td>Body mass index kg/m²</td>
<td>27.0±0.95</td>
<td>25.4±1.85</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>81.6±1.98</td>
<td>81.5±2.76</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>60.0±3.23</td>
<td>67.0±2.02</td>
</tr>
<tr>
<td>ACE genotype, DD:II</td>
<td>6.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Urine Na, mmol/L</td>
<td>158±30</td>
<td>149±18</td>
</tr>
<tr>
<td>PRA, ng Ang I per mL/h</td>
<td>0.954±0.127</td>
<td>0.675±0.279</td>
</tr>
<tr>
<td>ACE activity IU/L</td>
<td>40.7±7.6</td>
<td>32.5±6.2</td>
</tr>
<tr>
<td>Ang II, pg/mL</td>
<td>9.5±3.2</td>
<td>23.2±7.1</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>223±41</td>
<td>177±44</td>
</tr>
<tr>
<td>RPF, mL · min⁻¹ · (1.73 m²)⁻¹</td>
<td>556±22</td>
<td>649±54</td>
</tr>
</tbody>
</table>

*W indicates white American; AA, African American.

Figure 2. Effect of male (squares) and female (circles) gender on the change in MAP in response to Ang I (A) and Ang II (B). *P<.005 vs baseline.
ences in the duration of the pressor response after Ang I. Ang II has been reported to alter baroreflex function in men. In the present study, we assessed baroreflex reactivity during Ang I and Ang II infusions by determining the relationship between the increase in BP and the change in HR (Fig 5). There was a difference in the relationship between the change in HR and the change in BP after Ang I and Ang II in men and women. In women, there was a significant, negative, linear relation between the change in BP and the change in HR after Ang I ($R = -0.95, P = .046$) and Ang II ($R = -0.97, P = .026$). In men, there was no relationship between HR and BP after either Ang I ($R = 0.64, P = .35$) or Ang II ($R = .26, P = .74$).

Renal Perfusion

Fig 6 shows the change in renal perfusion index in response to Ang I and Ang II. Renal perfusion decreased significantly and in a dose-dependent manner in response to both Ang I and Ang II in both groups ($F = 89.34, P < .001$ for dose effect). The renal vasoconstrictor responses were similar after Ang I and Ang II. The decrease in renal perfusion was significantly greater in women than in men after either Ang I or Ang II ($F = 6.59, P = .025$ for gender effect). There was no significant effect of ACE genotype on the renal vasoconstrictor response to Ang I or Ang II ($F = 0.80, P = .39$).

Renin and Aldosterone

There was no difference in baseline ($P = .44$, unpaired Student’s $t$ test) aldosterone levels or between Ang I ($P = .44$, $t$ test) and Ang II ($P = .18$, $t$ test) stimulated aldosterone levels in men and women. The change in aldosterone in response to Ang II was significantly greater in women than in men (558±61 versus 325±74 pmol/L, $P = .031$, unpaired Student’s $t$ test); the change in aldosterone in response to Ang I tended to be greater in women, but this difference was not significant (514±98 versus 346±57 pmol/L, $P = .15$, $t$ test). The effect of gender on Ang II–stimulated aldosterone was seen in the setting of a significant effect of ACE genotype on both baseline and stimulated aldosterone levels ($F = 8.6, P = .012$).

PRA decreased significantly in response to Ang I (from 0.96±.12 to 0.42±.06 ng Ang I per mL/h, $P = .003$) and Ang II (0.95±0.16 to 0.38±0.08 ng Ang I per mL/h, $P = .002$ by paired Student’s $t$ test) in men. PRA also tended to decrease in response to Ang I (0.79±.29 to 0.43±.11 ng Ang I per mL/h) and Ang II (0.56±.28 to 0.37±.09 ng Ang I per mL/h) in women. Although the decrease was not statistically significant in women, there was no difference between baseline PRA ($P = .57$ for Ang I, $P = .24$ for Ang II, unpaired Student’s $t$ test) or change in PRA ($P = .32$ for Ang I, $P = .12$ for Ang II, $t$ test) between the two groups.
Sensitivity to Ang II

To characterize the gender differences in sensitivity to Ang II, we examined the relationships between pressor, renal, and adrenal responses as a function of the change in Ang II concentration after Ang I in men and women. There was a significant correlation between the increase in MAP and change in Ang II concentrations in both men \(\Delta MAP = 0.128(\Delta \text{Ang}) + 4.00, R = .72, P < .0001\) and women \(\Delta MAP = 0.0716(\Delta \text{Ang}) + 6.72, R = .63, P = .001\). Similarly, there was a significant correlation between the decrease in renal perfusion index and Ang II concentration in both men \(-(\Delta \text{renal perfusion}) = 0.783(\Delta \text{Ang}) + 22.9, R = .75, P < .00001\) and women \(-(\Delta \text{renal perfusion}) = 1.12(\Delta \text{Ang}) + 59.1, R = .82, P < .000001\). Although women tended to be less sensitive to the pressor effect and more sensitive to the renal vasoconstrictive effect of Ang II, these differences were not significant \((P = .06\) and \(P = .14\), respectively). In contrast, there was a marked gender effect on aldosterone sensitivity to Ang II. Thus, the slope of the relationship between the change in aldosterone and the change in Ang II concentration was significantly greater \((t = 3.1, P < .01)\) in women \(\Delta \text{aldosterone} = 5.37(\Delta \text{Ang}) + 32.3, R = .93, P < .000001\) than in men \(\Delta \text{aldosterone} = 2.77(\Delta \text{Ang}) + 26.6, R = .75, P < .001\).

Discussion

Although the pressor response to Ang II has been studied extensively in pregnant and nonpregnant women, this is the first study to compare the pressor and renal vasoconstrictor responses to Ang I and Ang II in salt-replete normotensive men and women.

The acute pressor response to Ang II reflects direct vasoconstriction, a direct effect on cardiac contractility, facilitation of sympathetic tone, enhanced noradrenergic neurotransmission, and adrenal catecholamine release. There was no effect of gender on the pressor response to Ang I or Ang II in this study. However, although the magnitude of the pressor response to Ang I and Ang II was similar in men and women, the duration of the pressor response to Ang I was prolonged in men compared with that in women. It is unlikely that differences in the systemic clearance of Ang I or Ang II contributed to this effect because plasma Ang II levels were identical in men and women during steady-state infusion of Ang I. This finding contrasted a dramatic effect of ACE genotype on Ang II levels after Ang I, as has been observed previously by Ueda et al. However, it is not possible to exclude an effect of gender on the conversion of Ang I to Ang II in specific tissues. An alternative explanation for the difference in the duration of the pressor response in men versus that in women is that there may be gender differences in activation of the sympathetic nervous system or counterregulatory mechanisms. In this regard, it is noteworthy that the men appeared to have achieved a new, higher, steady-state MAP immediately after discontinuation of Ang I infusion (Fig 3).

Ang II has been reported to alter baroreflex reactivity such that the decrease in HR in response to pressor doses of Ang II is attenuated or abolished compared with that produced by other vasoconstrictors. However, prior human studies of the effect of Ang II on baroreflex control of HR have been conducted primarily in men. The findings of the present...
study suggest that gender influences the effect of Ang II on baroreflex response. HR decreased as BP increased in response to Ang I and Ang II in women but did not change in men. Gender differences in the baroreflex response to Ang II could also in part underlie gender differences in the duration of the pressor response to Ang I.

The renal vasoconstrictor response to both Ang I and Ang II was greater in women than in men. This parallel greater decrease in renal perfusion in response to both Ang I and Ang II could suggest an increase in sensitivity at the receptor level in women, due to either decreased endogenous Ang II with consequent upregulation of the AT1 receptor in the renal vasculature or intrinsic differences in AT1 receptor expression or sensitivity. However, the lack of a significant effect of gender on the renal vasoconstrictor/Ang II concentration relationship in the present study, although it may be related to the small sample size (P = .14), does not support the hypothesis that there are gender differences in AT1 receptor sensitivity.

This study suggests a difference between the effect of gender on the pressor response to angiotensin and the effect of gender on the renal vasoconstrictor response. A discrepancy between the pressor and renal vasoconstrictor responses to angiotensin has previously been observed in other studies. For example, while the RPF response to exogenous Ang II is diminished in nonmodulators compared with that in modulators, the pressor response is identical in the two groups.34 Similarly, Chesley and Tepper35 found that progesterone administration blunted the RPF response to exogenous Ang II but did not alter the pressor response. This disassociation of the pressor response from the renal vasoconstrictor response with respect to angiotensin may reflect differences in sensitivity to Ang II between the peripheral and renal vasculature or differences in the relative contributions of direct vasoconstriction by Ang II and the effects of Ang II on the heart, sympathetic nervous system, and other vasoactive substances to the pressor and renal vasoconstrictor responses.

The present study was not designed to assess gender differences in the aldosterone response to Ang I and Ang II, because the subjects were studied under salt-replete conditions. Numerous prior studies have demonstrated that the aldosterone response to Ang II is blunted under conditions of salt repletion and enhanced during salt depletion.13,36,37 Nevertheless, the present study suggests that the aldosterone response to Ang II (and probably to Ang I) is enhanced in normotensive women compared with that in normotensive men. This notion is consistent with data from Fisher et al3 in hypertensive subjects. In addition, women exhibited a greater increase in aldosterone for a given change in plasma Ang II concentration. Further studies in salt-deplete subjects are needed to answer this question definitively.

One limitation of the present study was the large number of variables present. This study was designed to assess the effect of gender only on the pressor and renal vasoconstrictor responses to Ang I and Ang II. This study was not sufficiently robust to assess the effects of ACE genotype, race, or the interaction of these variables with gender. Nevertheless, because some investigators have reported an effect of ACE genotype on the conversion of Ang I to Ang II,34 this study was designed to control for this variable. Similarly, it would have been unethical to exclude any racial group from the study. Gender groups were comparable in the frequency of ACE genotypes, and race and initial analyses controlled for both variables.

In summary, in the present study the pressor response to Ang I and Ang II was similar in men and women. The duration of the pressor response to Ang I was prolonged in men, and gender differences in the effect of Ang II on the autonomic nervous system could contribute to this difference. The renal vasoconstrictor response to both Ang I and Ang II was enhanced in women compared with that in men. This finding in the setting of similar plasma Ang II levels in the two groups suggests that the mechanism involves pharmacodynamic differences. In vitro and ex vivo studies are needed to assess gender differences in receptor sensitivity in specific tissue beds. In addition, all of the women in the current study were estrogen-replete. Estrogen has been shown to alter sensitivity to a number of vasoactive substances.38 Estrogen has been reported to lower serum ACE levels in postmenopausal women.39 Estrogen increases angiotensinogen levels40 but appears to decrease PRA, as quantified immunoradiometrically.41 Thus, further studies are needed to determine whether the gender differences observed in the present study were due to estrogen or other female hormones.

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