Responses to Converting Enzyme and Renin Inhibition
Role of Angiotensin II in Humans

Naomi D.L. Fisher, Donald Allan, Imre Kifor, Cynthia L. Gaboury, Gordon H. Williams, Thomas J. Moore, Norman K. Hollenberg

Abstract We compared the renal vascular responses to angiotensin converting enzyme inhibition and renin inhibition to assess the influence of angiotensin II (Ang II). We examined the renal and endocrine responses to the renin inhibitor enalkiren, to captopril, and to placebo in nine healthy and nine hypertensive men on a 10-mmol sodium diet. Ang II was infused to assess effects of the agents on renal and adrenal responsiveness to Ang II. Plasma Ang II concentration was suppressed similarly with enalkiren and captopril—an identical level of blockade was achieved. Although renal plasma flow was stable during placebo, a substantial rise was seen with both agents (+133±26 mL/min per 1.73 m²) and captopril (+99.4±22.6). There was remarkable intrasubject concordance between the renal plasma flow responses to renin inhibition and converting enzyme inhibition (r= .90, P<.004). The vasodilator response to both agents correlated inversely with the fall in renal plasma flow induced by Ang II alone (r= .66, P<.05). Both agents significantly enhanced the renal vascular response to Ang II (P=.01), and, furthermore, the renal vasodilator response to captopril predicted the potentiation of the renal plasma flow response to Ang II after either agent (enalkiren: r=.91, P<.001; captopril: r=.56, P<.05). Concordance of the maximal renal plasma flow response to the two agents appeared in the hypertensive men as well. Our results indicate that the acute renal response to captopril largely reflects a reduction in Ang II formation. In healthy subjects, individual responses reflect differences in the extent to which Ang II contributes to renal vascular tone. Because differences in neither plasma Ang II concentration nor renal or adrenal responsiveness to Ang II explain the individual variation, the data suggest a crucial variation in intrarenal Ang II concentration. Hypertension. 1994;33:44-51.

Key Words • angiotensin II • renal circulation • sodium • aldosterone

Our understanding of the physiology and pathophysiology of the renin-angiotensin system in humans has come largely from examination of responses to pharmacologic blockade with angiotensin converting enzyme (ACE) inhibitors. These agents, although used extensively, may be limited as scientific tools by their lack of specificity; in addition to inhibiting the formation of angiotensin II (Ang II), they also inhibit the degradation of kinin and affect the formation of prostaglandins.1-5 Renin inhibitors have great substrate specificity,6-8 which gives them the potential to be more reliable tools for identifying the contribution of Ang II. In the case of the renal blood supply, one might anticipate that ACE inhibition, with its concomitant accumulation of potent renal vasodilators, would lead to a larger renal vascular response than does renin inhibition. On the other hand, several investigations have suggested a particularly prominent renal vascular response to renin inhibition.9-13

In this study, we undertook a systematic comparison of the renal and endocrine effects of the renin inhibitor enalkiren with those of the ACE inhibitor captopril in healthy normotensive and hypertensive men. The studies were performed with subjects on a restricted salt intake to maximize activity of the renin-angiotensin system. We also examined the influence of both classes of agent on the renal vascular and adrenal responses to infusion of Ang II, on the premise that accumulation of prostaglandins or bradykinin in the kidney would blunt the renal vascular response during ACE inhibition9-13 relative to the response during renin inhibition.

Methods

Subjects and Protocols

Subjects were nine healthy men (ages 20 to 49 years; mean±SEM, 35±3.1) and nine patients with essential hypertension (27 to 61 years, 47±7). All were free of cardiovascular, renal, and endocrine disease, except for blood pressure elevation in the hypertensive subjects, whose untreated blood pressures were between 90 and 110 mm Hg diastolic and less than 170 mm Hg systolic. All were within 20% of ideal body weight. After an outpatient evaluation, during which secondary forms of hypertension were excluded by history, physical examination, and appropriate laboratory studies, hypertensive patients were taken off all antihypertensive agents for at least 3 weeks before study. All subjects were studied during a 10-day admission to a metabolic ward at the Clinical Research Center of The Brigham and Women's Hospital. Written informed consent was obtained from each patient, and the protocol was approved by the Human Subjects Committee of the institution.

All subjects were placed on low salt, constant isocaloric diets throughout the entire hospitalization, with 10 mmol daily sodium intake. Daily dietary potassium (100 mmol) and fluid intake (2500 mL) were constant. Twenty-four-hour urine samples were collected daily and analyzed for sodium, potas-
sium, and creatinine. When sodium urinary matched sodium intake (usually on day 5), the first study was initiated.

Each subject underwent a double-blind washout period following each of 3 treatment days. Each study day began with a 60-minute baseline infusion of p-aminohippurate (PAH) and inulin, which continued after the administration of a study drug. On each study day subjects received one study pill and a 90-minute study infusion. On two of the study days, enalikiren or placebo infusion was administered after oral placebo in a double-blind fashion. Enalikiren was delivered intravenously over 90 minutes at a dose of 0.5 mg/kg, shown to be at the top of the dose-response curve for renal vasodilation.

On a third study day, captopril was delivered as a 25-mg pill, also known to be at the top of the renal vasodilator dose-response curve, followed by placebo infusion, both single-blind.

For assessment of the effects of the agents on tissue sensitivity to Ang II, a 45-minute intravenous infusion of Ang II (Hypertensin, CIBA-GEIGY) at 3 ng/kg per minute was begun 135 minutes after each study drug or placebo was given. Blood pressure and heart rate were monitored during the treatments and 2-minute intervals during the Ang II infusions. The electrocardiogram was monitored continuously.

Blood samples were drawn for measurement of PAH, inulin, and hormone levels at baseline, 45, and 90 minutes and at 135 and 180 minutes for assessment of the effects of the Ang II infusion.

On the first rest day, while subjects were in low-salt balance, the hormonal and hemodynamic responses to a postural stimulus were assessed in each. Blood was drawn from an intravenous catheter after overnight recumbency and again after 2 hours in the standing position for the measurement of plasma renin activity (PRA), plasma aldosterone, and cortisol concentrations. Blood pressure and heart rate were monitored at the same time. Low-renin hypertension, defined by a PRA level in the upright position of less than 0.69 ng angiotensin I (Ang I)/L per second (<2.5 ng Ang I/mL per hour), was identified in five of the nine hypertensive patients studied. One non-modulator was identified in this study by the criterion of a blunted Ang II–induced rise in aldosterone (<555 pmol/L [<20 ng/dL]) on a low salt diet, which rises after administration of an ACE inhibitor.

One subject (J.B.) responded exuberantly to renin inhibition while not increasing renal plasma flow (RPF) at all with captopril and experienced twice the fall in aldosterone with enalikiren as with captopril. A clear rise in PRA and fall in Ang II concentration indicated that he indeed received captopril. An explanation may lie in his state of salt balance; urinary Na⁺ calculated from his captopril study day contained 2211 fraction collector. Solution A contained 30% methanol, 0.15 mol/L Na₂HPO₄, 10 mL assay buffer per 500 µL solution. Fused-salt columns (Pharmacia LKB, Piscataway, NJ). The HPLC apparatus included an LKB 2150 pump, 2152 controller, and 2211 fraction collector. Solution A contained 30% methanol, 10 mM TEA, and 10 mM sodium acetate titrated to a pH of 6.2. Solution B was similar but contained 80% methanol. The elution times of the angiotensin peptides were assessed by injecting 4-nmol aliquots of each peptide and reading the absorbance with an LKB 2151 variable-wavelength monitor tuned to 214 nm and connected to an IBM XT computer loaded with CHROMATOCHART software (IMI, State College, Pa). After the proper gradient was set and the SD of the elution times of each peptide was determined, the system was washed for several days until baseline peptide levels, deter-
mined by radioimmunoassay, were low. Fractions were collected every minute over 72 minutes in polypropylene test tubes containing 50 μL of 10% glycerol and 150 μL of 50% assay buffer (0.05 mol/L K, HPO4, 0.003 mol/L EDTA, 0.02% sodium azide, 0.01% Triton X-100 [Serva, New York, NY]) and then dried overnight in a SpeedVac concentrator.

Radioimmunoassay

Samples were reconstituted in 50 μL of a 50% assay buffer and 2.5 mg/mL radioimmunoassay grade bovine serum albumin (Sigma Chemical Co, St Louis, Mo) containing 123I-Ang II (Du Pont—New England Nuclear, Boston, Mass) and 100 μL of assay buffer containing Ang II antibody (Arnel Inc, New York, NY) and were incubated for 48 hours at 4°C. Two hundred microliters of donkey anti-rabbit magnetic separation reagent (Amersham International, Arlington Heights, Il) was added, and samples were placed into magnetic test tube holders 15 minutes later. The trays were emptied after 10 minutes, washed with 750 μL of buffer (0.1% gelatin, 0.01% Triton X-100, 0.05 mol/L NaCl, 0.10 mol/L MgCl2, 0.02% sodium azide), and again emptied after 10 minutes. Tracer counts were recorded on a Micromedic 4/20 Automatic gamma counter for 3 minutes per tube. Counts were converted to femtomoles of Ang II using standard curves and plotted with the RIA-AID software package (RMA Inc, Cambridge, Mass). PEARFIT software (Jandel Scientific, Corte Madera, Calif) was used to analyze the area under each peak and to calculate total Ang II per sample. Recovery in the HPLC system was 94±3%. The results have not been corrected for recovery. This method produced a lower limit of detection for angiotensins of 0.2 fmol per tube. Buffer blank sensitivity was 0.1 to 0.2 fmol per tube, and plasma blank sensitivity was similarly 0.1 to 0.2 fmol per tube. During HPLC separation, the Ang II distributed in 5 of the 80 tubes used, with approximately 90% distributed in 3 central tubes. Making correction for the losses associated with processing described in the recovery experiments above, the sensitivity for detection of Ang II in plasma of this assay is 0.6 to 0.7 fmol/mL.

Analyses

Group means are presented with the SEM as the index of dispersion. Three-way comparison analyses among the treatment groups were performed by ANOVA. Regression analyses were used to determine concordance of responses within a group to two different agents. t Tests were used to compare healthy subjects with hypertensive patients and the Wilcoxon rank sum test in the analysis of low- versus normal-renin hypertension. The α-level for significance was less than .05.

Results

Restriction of salt intake led to the anticipated fall in sodium excretion and activation of the renin-angiotensin-aldosterone system (Table 1). PRA levels remained unchanged during placebo, in both healthy subjects and hypertensive patients, were suppressed to very low levels after enalkiren, and rose after captopril administration, again as anticipated (Fig 1, left; Table 2). Peak PRA values 90 minutes after captopril were largely determined by baseline values (r=.77). Plasma Ang II concentration was essentially unchanged between 0 and 90 minutes during placebo infusion (6.9±0.6 to 7.8±0.7 fmol/mL [7.2±0.6 to 8.1±0.7 pg/mL]), whereas the levels were suppressed to a similar extent by both enalkiren (7.2±0.8 to 2.6±0.6 fmol/mL [7.5±0.8 to 2.7±0.6 pg/mL]) and captopril (8.5±1.6 to 2.3±0.2 fmol/mL [8.9±1.7 to 2.4±0.2 pg/mL]). The nadir Ang II concentration achieved with either antagonist was unrelated to baseline values (Fig 1, right).

Table 1. Baseline Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Index</th>
<th>Normotensive (n=9)</th>
<th>Hypertensive (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35.3±3.1</td>
<td>47.1±3.7</td>
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<tr>
<td>Admission blood pressure, mm Hg</td>
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<tr>
<td>Systolic</td>
<td>114±2</td>
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<tr>
<td>Diastolic</td>
<td>72±2</td>
<td>96±1.9</td>
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<tr>
<td>Body weight, kg</td>
<td>72.9±2.3</td>
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<td>Serum creatinine, μmol/L</td>
<td>106±6</td>
<td>106±6</td>
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<tr>
<td>24-hour urinary Na, mmol²</td>
<td>11±1.9</td>
<td>13±2.8</td>
</tr>
<tr>
<td>Plasma renin activity, (ng Ang I/L)/t²</td>
<td>1.17±0.19</td>
<td>0.36±0.11</td>
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<tr>
<td>Plasma aldosterone, pmol/L t²</td>
<td>718±133</td>
<td>682±97</td>
</tr>
<tr>
<td>PAH clearance, (mL/min)/1.73 m²</td>
<td>603±25</td>
<td>546±34</td>
</tr>
<tr>
<td>Inulin clearance, (mL/min)/1.73 m²</td>
<td>110±3.5</td>
<td>107.1±3.7</td>
</tr>
<tr>
<td>Cortisol, nmol/L t</td>
<td>259±19.3</td>
<td>251±28</td>
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</table>

* Ang I indicates angiotensin I; PAH, p-aminohippurate.
† In balance on 10 mmol Na+ diet.
‡ Baseline of first study day.

Studies in Healthy Subjects

In the healthy subjects, plasma aldosterone concentration fell continuously during placebo infusion in accordance with normal diurnal secretion of corticotropin; the maximum decline was 225±72 pmol/L (8.1±2.6 ng/dL) at 135 minutes (Fig 2, Table 2). Enalkiren and captopril led to a larger fall in plasma aldosterone concentration, as expected (P=.025). Infusion of Ang II from 135 to 180 minutes caused a similar rise in plasma aldosterone concentration in the three treatment groups (Table 3).

During placebo infusion, RPF was very stable, with a minor tendency to fall over time (Fig 2, Table 2). A substantial rise in RPF was seen with both captopril and enalkiren. The maximal rise in RPF with enalkiren was 153±26 mL/min per 1.73 m² and with captopril was 99±23 mL/min per 1.73 m² (P=.001, ANOVA). Inulin clearance did not change significantly with any treatment.

There was remarkable concordance in each subject between the RPF response to renin inhibition and ACE inhibition (r=.88, P<.004) (Fig 3, Table 4). Responses to enalkiren were larger in seven of the nine subjects, although this difference did not reach statistical significance. The slope did not differ from 1.0 (1.27±0.25 [SD]), and the intercept did not differ from zero (−5±32 [SD] mL/min per 1.73 m²).

Ang II infusion led to the anticipated rise in plasma aldosterone concentration, and the response was not influenced by either captopril or enalkiren in healthy subjects (Fig 2). Renal vascular responses to Ang II, on the other hand, were more complex. The fall in RPF induced by Ang II during placebo treatment correlated inversely with the vasodilator response to captopril and enalkiren (r=−.66, P<.05, Table 4).
Treatment with both captopril ($P<.01$) and enalkiren ($P<.01$), as anticipated, enhanced the renal vascular response to Ang II (Table 3). Not anticipated was the finding that the renal vasodilator response to captopril (Table 4) predicted the enhancement of the renal vascular responsiveness to Ang II not only after captopril ($r=.56$, $P<.05$) but also after enalkiren ($r=.91$, $P<.001$, Fig 4). On the other hand, there was no correlation between either basal Ang II concentration or concentration of Ang II after pharmacologic intervention and any measure of renal vascular responsiveness. Neither was there any significant correlation between either basal or feedback-stimulated PRA and renal vascular responsiveness.

Studies in Essential Hypertension

Hypertensive patients were older than the normotensive subjects ($P=.0001$) and heavier and had higher mean systolic and diastolic admission blood pressures ($P<.001$) (Table 1). Plasma aldosterone concentration was similar in the healthy subjects and hypertensive patients, as were serum creatinine and 24-hour urinary sodium. PAH clearance tended to be lower in the hypertensive patients, but the difference was not statistically significant. Baseline PRA on the first study day (Table 1) was significantly lower among the hypertensive patients than the healthy subjects ($P<.003$), reflecting the presence of five low-renin hypertensive patients in our study group.

Among the hypertensive patients, mean plasma aldosterone concentration fell only slightly during placebo, whereas enalkiren and captopril each caused larger decrements at 90 and 135 minutes ($P=.03$, $P<.02$, ANOVA, Table 2). As in the healthy subjects, Ang II infusion caused a similar rise in aldosterone regardless of pretreatment.

RPF in the hypertensive patients did not change significantly during placebo infusion (Table 2). Enalkiren and captopril each caused a significant increase in RPF at 90 ($P=.005$) and 135 ($P<.002$, ANOVA) minutes. There was concordance among the hypertensive patients, as in the healthy subjects, of the maximal RPF response to enalkiren and captopril ($r=.64$, $P=.06$).

The RPF response to Ang II infusion in the hypertensive patients was potentiated after both enalkiren

<table>
<thead>
<tr>
<th>Table 2. Hormonal and Renal Vascular Responses</th>
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<td>Aldosterone, pmol/L</td>
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<tr>
<td>Normotensive subjects (n=9)</td>
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<tr>
<td>Placebo</td>
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<tr>
<td>Enalkiren</td>
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<tr>
<td>Captopril</td>
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<tr>
<td>Hypertensive patients (n=9)</td>
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<tr>
<td>Placebo</td>
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<tr>
<td>Enalkiren</td>
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<tr>
<td>Captopril</td>
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</table>

Ang I indicates angiotensin I. Values are mean±SEM.

* $P<.004$ compared with placebo.

† $P<.002$. 

Fig 1. Left: Plot shows plasma renin activity (PRA) values in healthy subjects and hypertensive patients at 0 and 90 minutes of each treatment. Placebo PRA values scattered near the line of equality, whereas enalkiren treatment suppressed PRA uniformly to very low levels. PRA after captopril administration varied with basal PRA. (Conversion factor for PRA: nanograms angiotensin I [Al] per milliliter per hour to nanograms angiotensin I per liter per second, 0.2778.) Right: Plot shows plasma angiotensin II (All) concentrations at baseline and 90 minutes of each treatment. Note the similar suppression of plasma angiotensin II at 90 minutes (the end of enalkiren infusion) with both enalkiren and captopril, regardless of basal angiotensin II levels. (Conversion factor for angiotensin II: picograms per milliliter to femtomoles per milliliter, 0.96.)
FIG 2. Line graphs show time course of changes in plasma aldosterone concentration and renal plasma flow in healthy subjects. Intravenous placebo and enalikiren were administered over 90 minutes; angiotensin II (All) was infused at time 135 on each treatment day. Aldosterone levels rose similarly after angiotensin II infusion with all three treatments. Renal plasma flow, constant during placebo, showed a similar, substantial rise after enalikiren and captopril. The fall induced by angiotensin II was enhanced similarly by both antagonists. (Conversion factor for aldosterone: nanograms per deciliter to picomoles per liter, 27.74.)

and captopril compared with placebo (P=.025, ANOVA, Table 3). Aldosterone concentration rose to a similar extent with all three treatments.

Although on admission mean diastolic blood pressure had been 96±1.9 mm Hg (Table 1), in the interval between admission and study, diastolic blood pressure in the hypertensive patients fell to a range of 83.2±3.8 to 85.0±4.4 mm Hg (Table 2), approximately 12 mm Hg lower. Both enalikiren and captopril caused a small but significant further fall in diastolic blood pressure (Table 2). In the hypertensive patients, the fall in diastolic blood pressure after either agent was statistically significant compared with placebo (P=.002); in the healthy subjects, both agents decreased diastolic blood pressure, but significance was reached only with captopril (P<.004). There was no difference in the Ang II-induced blood pressure changes with either agent.

RPF rose less in the five low-renin hypertensive patients after renin inhibition than in the healthy subjects (40.6±20 versus 132.9±26 mL/min per 1.73 m², P=.009) and also less than in the normal-renin hypertensive patients (90±12, P=.03). A similar pattern emerged with the RPF response to captopril. Low-renin hypertensive patients also demonstrated a smaller blood pressure response to both agents compared with the normal-renin hypertensive patients, although the difference reached significance only with captopril (P=.005).

![Graph showing maximal rise in renal plasma flow](http://hyper.ahajournals.org/)

**Fig 3.** Plot shows comparison of maximal renal plasma flow (RPF) responses to enalikiren and captopril in healthy subjects. Note the concordance of the responses.

**TABLE 3. Response to Angiotensin II**

<table>
<thead>
<tr>
<th></th>
<th>Renal Plasma Flow, (mL/min)/1.73 m²</th>
<th>Aldosterone, pmol/L</th>
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<tr>
<td><strong>Normotensive subjects</strong></td>
<td></td>
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<tr>
<td>Placebo</td>
<td>−84.7±7.2</td>
<td>918±205</td>
</tr>
<tr>
<td>Enalikiren</td>
<td>−158.7±19.9</td>
<td>943±175</td>
</tr>
<tr>
<td>Captopril</td>
<td>−146.3±19.9</td>
<td>1040±208</td>
</tr>
<tr>
<td><strong>Hypertensive patients</strong></td>
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<tr>
<td>Placebo</td>
<td>−96.4±10.5</td>
<td>782±119</td>
</tr>
<tr>
<td>Enalikiren</td>
<td>−166.7±17</td>
<td>974±122</td>
</tr>
<tr>
<td>Captopril</td>
<td>−175±31.7</td>
<td>874±128</td>
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</table>
In contrast, low-renin hypertensive patients exhibited significantly greater systolic and diastolic blood pressure responses to Ang II than the normal-renin patients on placebo day (28±3.9/14.3±2.8 versus 10.8±1.4/5.8±0.7, P<.006, F=.02). The one non-modulator in this study showed impressive renal vascular responses to both enalkiren and captopril (108 mL/min per 1.73 m²), compared with a change of −31 mL/min per 1.73 m² with placebo. Similarly, his RPF response to Ang II was greatly enhanced after both enalkiren (−189 mL/min per 1.73 m²) and captopril (−199 mL/min per 1.73 m²), from a baseline response of −56 mL/min per 1.73 m² (placebo day).

**Discussion**

ACE inhibitors have been the major probe used to assess the physiology of the renin-angiotensin system in humans, but the substrate specificity of renin inhibitors promises to make them useful counterparts. Through a systematic comparison of the responses to captopril and enalkiren, we aimed at a better understanding of the contribution of Ang II to the renal vascular actions of ACE inhibition.

A range of renal vasodilator responses to captopril among different individuals has been identified. We and others have recognized such a variation in the past but have attributed the differences to technical aspects of the clearance measurement. An unanticipated yet quite striking observation emerging from this study was the remarkable concordance of the renal vascular responses to ACE and renin inhibition. With a value of r=.88, nearly 80% of the variation in the renal vasodilator response to enalkiren is accounted for by variation in the renal vasodilator response to captopril. These results indicate that, despite a protocol designed to avoid variation due to gender, diet, and posture and a similar plasma Ang II concentration, the angiotensin-mediated level of renal vasconstriction is highly variable among individuals. If vasodilator substances were contributing to the effects of ACE inhibition, the renal vasodilator response to enalkiren would have been less than that to captopril. These observations confirm and extend studies in animals which indicate that the renal vasodilator response to captopril is dominated by a reduction in Ang II formation.

The variation in the contribution of Ang II to renal vascular tone in the non-normotensive subjects could not be related to variation in plasma Ang II concentration. An alternative possibility, that the enhanced renal vasodilator response to captopril reflected an increased contribution of circulating Ang II because of supersensitivity of the renal blood supply to angiotensin, was assessed in this study and ruled out. Indeed, the RPF response to captopril was inversely correlated with the baseline RPF response to Ang II, so that those subjects with the largest renal vasodilator response to pharmacologic blockade had the smallest baseline response to Ang II. In addition, both ACE and renin inhibition greatly enhanced the Ang II-induced fall in RPF (Table 3). Again, had vasodilator kinins or prostaglandins made a substantial contribution to the renal vasodilator response to ACE inhibition, one would have expected a blunting of the RPF response to Ang II after captopril instead of the reported potentiation: prostaglandins and bradykinin interfere with the action of Ang II on the renal blood supply.

Furthermore, the strong relation demonstrated in this study between the rise in RPF induced by renin inhibition and the enhancement of the renal vascular response to Ang II (Fig 4) argues for a similar mechanism underlying both phenomena, likely involving Ang II. We hypothesize that the site of this critical Ang II pool is intrarenal.

RPF was constant during placebo infusion, supporting the precision of the clearance measurement. The two active agents demonstrated a somewhat different time course of renal vascular response: captopril reached peak effect on average 90 minutes after the dose, and the effect of enalkiren peaked later, at 135 minutes. Our analyses, therefore, incorporate maximal RPF responses for each treatment day to avoid the potential bias in selecting data from one moment in time. Renal vascular responsiveness to enalkiren and captopril did not depend on basal RPF levels, nor did it vary with plasma Ang II concentration.

The legitimacy of any comparison of ACE and renin inhibition depends on achieving an identical degree of blockade of the renin-angiotensin-aldosterone system. For both enalkiren and captopril, the dose selected had been shown to lie at the top of the dose-response relation. Both agents induced similar falls in plasma
alosterone and plasma Ang II concentrations, indicating that essentially identical suppression of the renin-angiotensin system had been reached, at least as reflected in the plasma compartment.

Plasma Ang II levels were determined in this study using a high-yield extraction followed by HPLC separation and radioimmunoassay. This methodology is superior to standard radioimmunologic measurements of the peptide, because those assays cannot differentiate Ang II from its metabolites. Nussberger et al. reported an assay detection limit in plasma of 0.45 fmol/mL using similar technology, with complete disappearance of plasma Ang II after ACE inhibition. Plasma Ang II levels after pharmacologic blockade in our study were very low but still detectable. We considered a difference in assay sensitivity, but the lower thresholds for both studies were nearly the same. Our study, performed with subjects in low-salt balance, operated with a highly stimulated renin-angiotensin-aldosterone system, possibly explaining both the higher basal and higher inhibited plasma Ang II levels than were encountered during an unrestricted diet. These levels perhaps reflect the difficulty in blocking the tissue renin-angiotensin system during a low salt diet.

The pattern of PRA response was largely anticipated. Captopril has been shown repeatedly to cause a reactive rise in renin release and an increase in PRA, demonstrated here to vary with basal PRA. The very low PRA values measured after enalkiren administration replicate previous reports.

The hypertensive patients demonstrated patterns of adrenal and renal vascular responsiveness similar to the healthy subjects, but the hypertensive patients were much more physiologically heterogeneous. The finding that five of nine hypertensive patients were low-renin was unforeseen and unusual, given the lower overall prevalence of low-renin hypertension in the general hypertensive population. Two of our low-renin patients were black and two were near 60 years of age, factors that increase the likelihood of low-renin hypertension.

Enalkiren and captopril induced modest but significant falls in blood pressure as anticipated. The response to a single dose assessed in subjects who were recumbent and in whom blood pressure had already been sharply reduced by several days' hospitalization is unlikely to predict strongly the therapeutic response.

The low-renin hypertensive patients behaved differently than either the healthy subjects or the normal-renin hypertensive patients. They had smaller renal vascular and blood pressure responses to either ACE or renin inhibition, consistent with a reduced role for Ang II in maintaining renal vascular tone and hypertension.

In that class of hypertensive patients called non-modulators, a blunted renal vascular response to Ang II is associated with an enhanced response to ACE inhibition. Despite our substantial interest in the pathophysiology of non-modulation, only one such patient was involved in this study. Yet the correction of his adrenal and renal responsiveness to Ang II with renin inhibition just as dramatically as with captopril lends further support to the hypothesis that excessive local Ang II is responsible for the non-modulator defect.

These findings must be acknowledged as representing responses to acute, single-dose treatments in individuals in a state of low-salt balance. Effects of chronic treatment remain unknown until studies can be performed after more prolonged administration.

Our findings indicate that, for the renal blood supply and the adrenal, the acute response to ACE inhibition largely reflects a reduction in Ang II formation. The remarkable concordance of the renal vascular response to renin and ACE inhibition suggests that variations in this response actually reflect variation in the degree to which endogenous Ang II contributes to maintaining an individual's renal vascular tone. Because differences in plasma Ang II levels do not account for the variation, it is attractive to speculate that the critical determinant is intrarenal Ang II. A systematic comparison of responses to ACE and renin inhibitors should provide the most definitive insight into the contribution of Ang II to normal and disordered human physiology, especially when the action occurs in relatively inaccessible tissue compartments.

Acknowledgments

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