Renal and Endocrine Responses to a Renin Inhibitor, Enalkiren, in Normal Humans

Paul Cordero, Naomi D. Fisher, Thomas J. Moore, Ray Gleason, Gordon H. Williams, and Norman K. Hollenberg

Interpretation of renin-angiotensin blockade with angiotensin converting enzyme inhibitors is potentially confounded by their multiple effects. We used a selective renin inhibitor (enalkiren, A-64662) to explore the renal and endocrine effects of angiotensin II in healthy men. Each received 90-minute enalkiren infusions at 2-day intervals, on a low (10 mmol, 16 subjects) and high (200 mmol, 12 subjects) salt diet. Plasma renin activity, immunoreactive plasma angiotensin II and aldosterone concentrations, inulin, and p-aminohippurate clearance were measured by standard methods. Plasma renin activity fell at 0.1 μg/kg, but the threshold for biologic effect was 256 μg/kg, where plasma immunoreactive angiotensin II and aldosterone concentration fell, and renal plasma flow rose (p<0.01). The maximal renal vascular response (+152±23 ml/min/1.73 m²) occurred at 512 μg/kg (p<0.01). Diastolic and mean blood pressure fell modestly but significantly (p<0.05). Responses were limited on a high salt diet. We confirm that conventional plasma renin activity measurement is misleading in humans receiving a renin inhibitor. The renal vascular response to renin inhibition in this study appeared to substantially exceed reported responses to angiotensin converting enzyme inhibition, perhaps reflecting a crucial and relatively inaccessible intrarenal locus. (Hypertension 1991;17:510–516)

Pharmacological interruption of the renin-angiotensin system has played a special role in attempts to define its role in normal physiology and in the pathogenesis of disease. The reason is fundamental. Ablation of the source of a hormone followed by replacement has been crucial to defining the hormone's contribution.1 In the case of the renin-angiotensin system, where the kidney is not only the source of the hormone but also a determinant of sodium homeostasis, the value of the ablation experiment has been limited. Pharmacological interruption of this system, therefore, has essentially replaced ablation as a pivotal step.

The most widely used agents, the angiotensin converting enzyme (ACE) inhibitors, block an enzyme that has multiple functions, including degradation of kinins and consequent prostaglandin forma-

tion.2–6 Thus, the specificity of responses to ACE inhibition is suspect. The angiotensin antagonists available until recently were partial agonists with substantial angiotensinlike activity, which limited their usefulness.7,8 Although a new family of angiotensin antagonists has been developed,9 little is known about their action in humans.

Agents that block renin activity specifically are very attractive, because of renin's great substrate specificity.10,11 Angiotensinogen, in all species, is the only known substrate for renin. In this study, we have assessed the renal and endocrine response to a renin inhibitor in normal men who were studied when in balance on both a highly restricted and a liberal sodium intake. The results raise questions in two important areas: the measurement of plasma renin activity in the presence of a renin inhibitor and the magnitude of the response of the renal blood supply to a reduction in angiotensin II formation.

Methods

Subjects and Protocols

The subjects were 16 healthy men ranging in age from 22 to 48 years (35±2 years). All were free of cardiovascular, renal, and endocrine disease and were within 20% of ideal body weight, with an average weight (±SEM) of 70.6±2.3 kg. After an outpatient evaluation, all were studied during a 14-
day admission to a metabolic ward at the Clinical Research Center of 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 constant isocaloric diets throughout the entire hospitalization, including a 10 meq daily sodium intake for the first 11 days. In 12 of the subjects, dietary sodium intake was then increased to 200 meq/day for 2–3 days before the last protocol day. Daily dietary potassium (100 meq) and fluid (2,500 ml) intake were constant on both diets. Twenty-four-hour urine samples were collected daily and were analyzed for sodium, potassium, and creatinine. When 24-hour urine sodium matched intake (usually on day 5 for the 10 meq sodium diet and day 3 for the 200 meq sodium diet), responses to renin inhibitor infusions were assessed, as described below.

To define the relationship between inhibitor dose and response, each subject received a series of ascending enalikiren (A-64662) doses, with a single dose being administered on each day. A rest interval of 48–72 hours was allowed before the next higher dose was administered. Studies began at about 7:00 AM. All subjects had been recumbent and fasting overnight. After a 60-minute control period to establish basal effective renal plasma flow, glomerular filtration rate, plasma renin activity (PRA), immunoreactive angiotensin II (irAng II), aldosterone, cortisol, and electrolyte concentrations, an infusion of enalikiren was superimposed. Each dose was administered over a 90-minute period, and observation was continued while the subjects remained recumbent for an additional 90 minutes thereafter. Each of 16 subjects received two or three doses on a low salt diet followed by a single dose, the highest used during the studies on a low salt diet, after balance had been achieved on the high salt intake in 12 of the subjects.

An ascending log dose schedule was followed. The first subject received 1, 2, and 4 µg/kg over a 90-minute period on a low salt diet, followed by a repeat of the 4 µg/kg dose on a high salt diet. Thereafter, subjects received 2, 4, and 8; 4, 8, and 16; 8, 16, and 32, and so forth, to a maximum dose range of 1,000 µg/kg over a 90-minute period. At least four subjects received each dose of 16 µg/kg or higher on a low salt diet.

Blood pressure during each infusion was recorded by an automatic recording device (Dinamap, Critikon Inc., Tampa, Fla.) at 5-minute intervals, and the electrocardiogram was monitored continuously. Blood samples were drawn for measurement of p-aminohippurate (PAH), inulin, and hormone levels at the times indicated in Figure 2.

Renal Clearance Studies

PAH (Merck Sharp & Dohme, West Point, Pa.) and inulin (Taylor Pharmacal Co., Decatur, Ill.) clearances were assessed after metabolic balance was achieved on each diet. An intravenous catheter was placed in each of the subject’s arms, one for infusion and the other for blood sampling. The subjects were supine and had been in a fasting condition for at least 8 hours. A control blood sample was obtained and then loading doses of PAH (8 mg/kg) and inulin (50 mg/kg) were given. A constant infusion of PAH and inulin was initiated immediately at a rate of 12 mg/min for PAH and 30 mg/min for inulin with an IMED pump (IMED Corp., San Diego, Calif.). This infusion rate achieved plasma PAH concentration in the middle of the range in which tubular secretion dominates excretion. At this plasma level of PAH, clearance is independent of plasma concentration and, when corrected for individual body surface area, represents about 90% of renal plasma flow. Likewise, at the level of plasma inulin achieved, inulin clearance reflects glomerular filtration. Basal PAH and inulin clearances were calculated from their plasma levels and infusion rates for each substance. Plasma samples reflecting the control clearances were obtained 60 minutes after the start of the PAH/inulin infusion when a steady state had been achieved, and at 45-minute intervals thereafter.

Laboratory Procedures

Blood samples were collected on ice, spun immediately, and the plasma was frozen until the time of assay. Serum and urine sodium and potassium levels were measured by flame photometry, with lithium as an internal standard. Serum creatinine, PAH, and inulin were measured by an autoanalyzer technique. irAng II, PRA, aldosterone, and cortisol were assayed by radioimmunoassay techniques that have been described in detail.

Group means have been presented with the standard error of the mean as the index of dispersion. One-way analysis of variance (ANOVA) followed by pairwise comparisons among dose levels using the Newman-Keuls test was used to determine the dose at which a significant change from baseline had occurred. Two-way, repeated-measures ANOVA followed by Neuman-Keuls test was used to determine significant dose, time, and dose×time interaction responses. The α level for significance was 0.05 or less.

Results

Restriction of salt intake led to the anticipated fall in sodium excretion and activation of the renin-angiotensin-aldosterone system (Table 1). Blood pressure did not change with changes in salt intake. Administration of the renin inhibitor in the subjects in balance on a low salt diet led to dose-related falls in plasma aldosterone and irAng II concentration, and an increase in renal plasma flow. When the mean responses of irAng II, aldosterone, and renal plasma flow from baseline to the end of the 90-minute enalikiren infusion were compared over doses of enalikiren administered, the response reached statistical significance for each of the three variables at the 256 µg/kg/90 min dose (Figure 1). When the data were examined over time at each enalikiren dose, however, the threshold for a significant response was
TABLE 1. Baseline Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Index</th>
<th>Low salt</th>
<th>High salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>33.9±2.2</td>
<td>33.9±2.2</td>
</tr>
<tr>
<td>Subjects (n)</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>113±2.6/</td>
<td>111±2.6/</td>
</tr>
<tr>
<td></td>
<td>68±4.1</td>
<td>67±1.5</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>70.6±2.3</td>
<td>70.8±2.5</td>
</tr>
<tr>
<td>24-hour urine Na (meq)</td>
<td>3.4±0.5</td>
<td>0.6±0.11</td>
</tr>
<tr>
<td>Plasma renin activity (ng Ang I/ml/hr)</td>
<td>28.0±3.3</td>
<td>7.0±1.5</td>
</tr>
<tr>
<td>Plasma Ang II concentration (pg/ml)</td>
<td>646±22</td>
<td>640±39</td>
</tr>
<tr>
<td>Plasma aldosterone concentration (ng/dl)</td>
<td>122±4.2</td>
<td>115±6.7</td>
</tr>
<tr>
<td>PAH clearance (ml/min/1.73 m²)</td>
<td>646±22</td>
<td>640±39</td>
</tr>
<tr>
<td>Inulin clearance (ml/min/1.73 m³)</td>
<td>122±4.2</td>
<td>115±6.7</td>
</tr>
</tbody>
</table>

lower for all three variables. A significant increase in renal plasma flow \( (p=0.042) \) occurred at the 64 \( \mu g/kg/90 \) min dose, and significant decreases in plasma irAng II \( (p=0.03) \) and aldosterone \( (p<0.01) \) concentrations occurred at the 128 \( \mu g/kg/90 \) min dose. Increased enalkiren doses led to a further increase in renal plasma flow and decrease in plasma irAng II concentration, with a maximum response for each variable observed at 256–512 \( \mu g/kg/90 \) min. No further increase in the response occurred at 1,000 \( \mu g/kg. \) Inulin clearance did not change significantly. The time course of the response is shown in Figure 2. For all of the indexes, evidence of renin inhibition was sustained to 180 minutes, 90 minutes after cessation of the inhibitor infusion.

The magnitude of the increase in renal plasma flow was of some interest. At a dose of 256 \( \mu g/kg/90 \) min, renal plasma flow rose from 622±13 to 707±16 ml/1.73 m² \( (p<0.001) \). At 512 \( \mu g/kg/90 \) min renal plasma flow rose from 576±15 to 728±33 ml/kg/min/1.73 m² \( (p<0.001) \), an increase of 152±23 ml/min/1.73 m².

Blood pressure changes were small, but a statistically significant fall in both diastolic and mean blood pressure \( (p<0.01) \) did occur (Figure 3). The largest fall in mean and diastolic blood pressure, 83±1 to 78±2 mm Hg and 67±1 to 60±1 mm Hg, respectively, occurred at the 256 \( \mu g/kg/90 \) min dose. Systolic blood pressure did not change. In one individual, systolic blood pressure fell to about 70 mm Hg during the first 20 minutes of the infusion of a 256 \( \mu g/kg/90 \) min dose, with a vasovagal reaction (data not included). The infusion was discontinued and there were no untoward effects. Rechallenge was not attempted.

The pattern of changes in PRA in relation to enalkiren dose differed strikingly from the response of plasma irAng II and aldosterone concentration, and renal plasma flow. The lowest dose used, 1 \( \mu g/kg/90 \) min, resulted in a rapid fall in PRA, to about 50% of baseline in 20 minutes (Figure 4). The next dose dropped PRA by 50% in 10 minutes, but it remained in the measurable range, at 2–3 ng angiotensin I/hr throughout the remainder of the infusion. The 4 \( \mu g/kg/90 \) min dosages dropped PRA by over two thirds in 10 minutes, and the levels fell to the threshold for the method in 30 minutes (Figure 4). All doses in all subjects thereafter rapidly led to unmeasurable PRA levels, the time to nadir decreasing with increasing dose.

In the studies performed on a high salt diet, sodium excretion rose to match intake by the second to third day (Table 1); PRA, irAng II, and aldosterone fell \( (p<0.01) \); body weight increased by 0.98±0.2 kg \( (p<0.01) \); and renal plasma flow rose by 54±18 ml/min/1.73 m² \( (p>0.05<0.1) \). Blood pressure and renal plasma flow did not show a statistically significant change with the renin inhibitor in the subjects on a high salt diet. Plasma aldosterone concentration showed a change with time, but not with renin inhibitor dose, probably reflecting the normal circadian rhythm. A trend toward a fall in plasma irAng II concentration with dose and time was found \( (p<0.05) \), but the changes were small and the statistical significance was marginal.

Follow-up laboratory evaluation of blood, urine, and electrocardiograms were essentially unremarkable, with one exception. The absolute eosinophil...
count rose in three of the 16 subjects, to a range of 8–13 cell/ml. There were no clinical manifestations of allergy, and in each case the eosinophil count had returned to normal by the time of a 7-day follow-up.

Discussion

Renin inhibition induced an anticipated pattern of response, including a dose-related fall in plasma irAng II concentration. The increase in renal plasma flow and fall in plasma aldosterone concentration occurred over the same dose range effective in reducing Ang II formation, although the threshold for an increase in renal plasma flow was somewhat lower. Also as anticipated, the changes were substantially more striking when the subjects were studied on a low salt diet, which activated the renin-angiotensin-aldosterone system. The blood pressure fall was limited, but ACE inhibitors also induced a minimal blood pressure fall in normotensive recumbent subjects on an identical diet.15 There were two unanticipated observations. First, a striking fall in PRA occurred at inhibitor doses several orders of magnitude below those required to induce the anticipated biological responses, a fall in plasma Ang II and aldosterone concentration, and a rise in renal plasma flow. Second, the increase in renal plasma flow was substantially greater than anticipated, about double the change in essentially identical studies performed earlier with angiotensin converting enzyme inhibitors.12,15,16 Both require discussion.

An unequivocal fall in PRA was evident within 20 minutes of initiating infusion of the inhibitor in doses of 1 and 2 μg/kg/90 min, during which only nanogram quantities had been administered. At 4 μg/kg/90 min, plasma renin activity had fallen within 30 minutes to levels below those typical of a high salt diet. The inhibitor dose required to induce a triad of biological responses suggestive of renin inhibition—very low plasma Ang II and aldosterone concentrations, and a sharp increase in renal blood flow—was two to three orders of magnitude higher. Discrepancies between the biological response to a renin inhibitor and apparent renin inhibition have been described,17–20 but the dose range studied has generally been limited, and the biological responses measured included only blood pressure and angiotensin formation. Perhaps for these reasons the magnitude of the discrepancy has been underestimated. Concordance appeared to be better when a trapping assay was used in place of the conventional PRA assay.21

If one accepts the parallel fall in plasma irAng II and aldosterone concentrations and the rise in renal plasma flow as biological evidence of effective interruption of the renin-angiotensin system in vivo, a probability supported by the fact that these responses occurred at identical inhibitor doses, then the apparent renin inhibition at very much lower doses must be an artifact. One possible mechanism reflects assay conditions for renin measurement in vitro that differ substantially from in vivo conditions. An obvious candidate is pH. Because assay efficiency is increased
at an acid pH, most laboratories perform their renin assays at a pH of 5.5. Enalkiren may bind more effectively to renin under acid conditions. As an alternative, it has been suggested that an acid pH and the use of angiotensinase inhibitors lead to the freeing of highly protein-bound inhibitor from protein in vitro, thus amplifying its effect.20 We used angiotensin inhibitors and an acid pH in our assay. Whether there is sufficient difference in the free and bound enalkiren concentrations in our system to account for a differential of the magnitude shown in this study remains to be determined. Although possible, it seems unlikely that protein binding provides the entire explanation.

Another possible mechanism for the artifact involves prolonged storage of the plasma before the renin assay can be performed. The formation of an irreversible bond between the inhibitor and renin during prolonged exposure is possible, but seems unlikely under the conditions used for storage. In view of the fact that the problem is not unique to this inhibitor,17-20 more investigation is clearly required.

Multiple lines of evidence have suggested that Ang II plays a pivotal role in the control of the renal circulation. Among those lines of evidence, the renal vascular response to angiotensin antagonists and ACE inhibitors has been important. The magnitude of the peak increase in renal plasma flow induced by ACE inhibitors in early studies ranged from 10% to 20%, an increase in the range of 50–100 ml/min/1.73 m².12,15,16 In this study, renin inhibitor doses of 512 μg/kg infused over a 90-minute period induced a striking increase in renal plasma flow that exceeded those levels considerably, with an average increase of 152 ml/min/1.73 m². There are reasons for suspecting that the true response may be even greater than this. In two of the seven subjects who received an enalkiren dose of 512 μg/kg/min, the renal plasma flow response was substantially smaller than the group as a whole, with an increment of 66 and 67 ml/min/1.73 m², respectively. The 95% confidence interval calculated for the seven subjects, including these two, was 96–208 ml/min/1.73 m², a range that excludes both values. Indeed, in these same two subjects...
individuals the decrement in both plasma Ang II and aldosterone concentration was significantly lower than in the other subjects ($p<0.01$). The renal plasma flow change in the other five subjects ranged from 156 to 210 ml/min/1.73 m$^2$, with an average response of $92\pm 9$ ml/min/1.73 m$^2$. Either the ACE inhibitors underestimate the contribution of Ang II to renal vascular tone in this setting or the renin inhibitor used in the present study overestimates that contribution. Clearly, a direct comparison of renin and ACE inhibition is required before this question can be answered. Although other renin inhibitors have not led to such a striking degree of renal vasodilation,$^{22-28}$ none were studied in humans, and the agent used in the present study also appeared to be especially active on the renal blood supply in monkeys.$^{25,26}$

Our premise was that renin inhibition would lead to a smaller renal vascular response than ACE inhibitors, because the latter may lead to the accumulation of bradykinin or prostaglandins.$^{2-6}$ To the extent that these are potent renal vasodilators, one might have anticipated that responses to ACE inhibition would overestimate angiotensin II's contribution. Both prostaglandins and bradykinin are known to interfere with the action of Ang II on the renal blood supply.$^{29}$ There is clear evidence that renal vascular responses to Ang II are enhanced after ACE inhibition.$^{16}$ Thus, it seems unlikely that the kinins or prostaglandins played a major role in the renal vascular response to the ACE inhibitor.

Is it possible that the renin inhibitor has provided a better index of the contribution of Ang II to renal vascular tone, which has been underestimated by ACE inhibitors in the past? The specificity of renin, and therefore of renin inhibitors, would favor that interpretation, although no pharmacological agent is likely to have absolute specificity. One possible interpretation of the data involves an ability of enalapril to reach crucial intrarenal sites not easily reached by ACE inhibitors. That, in turn, would depend on a dominant intrarenal locus for Ang II formation as a determinant of renal vascular tone. Multiple lines of evidence have, indeed, suggested that local Ang II formation makes a substantial contribution to the control of the renal circulation and function.$^{22}$ Another possibility is that blockade of the system is more complete when renin inhibitors are used.

One crucial element to assessment of the completeness of inhibition of the circulating renin pathway obviously involves measurement of circulating Ang II. The Ang II assay in the present study measures intact Ang II, and the antiserum also has cross-reactivity to angiotensin fragments. High-performance liquid chromatography separation improves the specificity of the angiotensin assay$^{18}$ and should be used in future studies in which renin and ACE inhibition are compared.

The results of the present study have provided strong support for the concept that Ang II makes a pivotal contribution to the state of the renal blood supply and to aldosterone release in normal men when the renin system is activated by restriction of salt intake. Renin inhibition is likely to be an important complement to ACE inhibition in dissecting the contribution of this system to the pathogenesis of human disease, although as has generally been the case when new pharmacological agents have been introduced, there are also new questions to address.

References


KEY WORDS • angiotensin • aldosterone • renal circulation • sodium
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