Assessment of Renin Dependency of Hypertension With a Dipeptide Renin Inhibitor

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To evaluate the participation of the renin-angiotensin system in sustaining hypertension, we administered the specific dipeptide renin inhibitor enalkiren (A-64662) to 18 patients with essential hypertension. Ascending intravenous bolus doses (0.03, 0.1, 0.3, and 1.0 mg/kg) of the inhibitor were each given at 45-minute intervals to patients maintained on an ad libitum sodium diet who were studied while in bed in the semirecumbent posture. Enalkiren produced marked decreases in plasma renin activity (PRA) that were still evident 8 hours after completion of dosing. Systolic and diastolic blood pressures were decreased in a dose-dependent fashion without an effect on heart rate. Repetition of this procedure after patients were subjected to sodium depletion by 1 week of thiazide treatment produced amplified decreases in blood pressure. Despite the short plasma half-life of the inhibitor, these blood pressure-lowering effects were sustained for 4–8 hours when compared with parallel placebo administration in the same patients. Both the baseline PRA and the inhibitor-induced changes in PRA correlated significantly with blood pressure changes during the unstimulated and the sodium-depleted studies. However, effects of the inhibitor on diastolic blood pressure in the latter study correlated most closely with actual increases in renin produced by diuretic pretreatment. Thus, this specific renin inhibitor has demonstrated the dependency of blood pressure on the renin-angiotensin system even during basal conditions in hypertensive patients. Moreover, renin response to sodium depletion appears to be an attribute that additionally characterizes individual hypertensive patients. (Circulation 1990;81:1768–1774)

The renin-angiotensin-aldosterone system participates in the physiological regulation of blood pressure, renal function, and fluid volume balance. The role of the renin axis in mediating essential hypertension in humans has been elaborated by a series of studies with selective probes of this system’s components. Early evidence that vasoconstrictor effects of the renin-angiotensin system might sustain hypertension was provided by studies with the β-adrenergic blocker propranolol.1 This agent, which inhibits renal release of renin, produces blood pressure decrements that correlate with its inhibitory effects on plasma renin activity (PRA).2 When hypertensive patients were classified into differing renin subgroups by a renin-sodium nomogram,3 the greatest antihypertensive effects occurred in the high-renin patients and the least in the low-renin patients. These findings were confirmed by observations with the competitive angiotensin II analogue saralasin. This agent selectively decreased blood pressure in patients with increased PRA.4 The intravenously administered nonapeptide angiotensin converting enzyme–inhibitor teprotide5 and the oral agent captopril6 were also shown, at least in the short term, to produce their greatest blood pressure decrements in high-renin patients. Moreover, the response to captopril was enhanced when diuretic pretreatment was used to convert sodium-dependent hypertension to renin-dependent hypertension.7

The agents used in these studies, however, are not entirely specific: β-Blockers have pharmacological actions additional to their renin-lowering effects, and the partial-agonist properties of saralasin do not allow the participation of angiotensin II mechanisms to be accurately quantified.8 Similarly, actions of the
converting enzyme inhibitors, which might increase concentrations of vasodilatory kinins and possibly of prostaglandins,9 influence renal function,10 and inhibit sympathetic mechanisms,11 can obscure the role of the renin system. Direct inhibitors of renin's action on its substrate offer an alternative for studying this system in hypertension. Differing types of inhibitors, including monoclonal renin antibodies,12 pepstatin, and renin inhibitor peptides,13 have decreased renin activity and blood pressure.

Most recently, dipeptides derived from the amino acid sequence of human angiotensinogen in the vicinity of its scissile bond have displayed high potency as renin inhibitors. Although not fully evaluated, the apparent specificity of these agents makes it unlikely that they would directly affect kinins or other hormonal systems. The agent enalkiren (A-64662) has produced marked and prolonged blood pressure decreases in sodium-depleted monkeys.14 Preliminary clinical studies have demonstrated suppression of PRA and angiotensin II in normal volunteers15 and decreases in blood pressure in hypertensive patients.16 In the present study, we have administered this agent intravenously to patients with low-, normal-, and high-renin essential hypertension during unstimulated conditions and during diuretic treatment. Our findings indicate that even under basal conditions, the renin-angiotensin system participates in sustaining hypertension; moreover, the reactive responses of renin to sodium depletion are of key importance for blood pressure regulation in the clinical setting.

Methods

This study was performed in 18 patients with mild-to-moderate essential hypertension (seated supine diastolic blood pressures between 95 and 110 mm Hg) in whom secondary forms of hypertension or other complicating conditions had been excluded by standard clinical methods. Of these patients, 15 were white, and three were black; 16 were men, and two were women. Their ages ranged from 32 to 67 years (mean, 57 years). Before entering the study, all patients signed an informed consent document approved by the Human Studies Committee of the Veterans Administration Medical Center, Long Beach, California.

Throughout the study, patients consumed essentially an ad libitum diet with minimal restraints on sodium intake. As indicated by 24-hour urine collections, such hypertensive patients have sodium intakes of 80–160 meq/day, with a mean sodium intake of approximately 120 meq/day. The multiple brief hospital admissions required by the study protocol made 24-hour urine collection on study days too unreliable for analysis. The patients were instructed not to take any medications, including nonsteroidal anti-inflammatory agents, throughout the study (other than for diuretic treatment described below). They had all discontinued any previous antihypertensive therapy at least 3 weeks before the study.

Each patient was studied on each of 3 separate in-hospital days. On each study day, the patient was transferred to a special study bed to facilitate continuous electrocardiographic monitoring throughout the procedures (instituted as a safety measure because of limited experience with the drug A-64662 in hypertensive humans). The formal study day was divided into an initial stabilizing period of 45 minutes to 1 hour to allow patients to adapt to the semirecumbent posture (lying on the back, with the head raised sufficiently to provide comfort) in the study bed; an initial baseline (placebo) period of 45 minutes, during which only the drug diluent (1 M saline) was administered intravenously; four consecutive 45-minute periods, during which the active drug (A-64662) was administered intravenously in ascending doses of 0.03, 0.1, 0.3, and 1.0 mg/kg; and a final period lasting until 8 hours after the final drug dose had been administered to allow observations of duration of action. The administrations of the drug (vehicle) were by direct intravenous push (administered over 5 minutes) at the start of each 45-minute dosing period. Blood pressures and heart rates were measured automatically every 5 minutes throughout the first 4 hours of study and again every 5 minutes during 30-minute periods thereafter by an automated oscillometric device (Dinamap, Critikon, Tampa, Florida) that was calibrated for accuracy against a conventional mercury sphygmomanometer. Blood samples for measurement of PRA were obtained at the end of the initial equilibration period and at the end of the baseline placebo period on each study day. The average of these two values was used to provide the baseline PRA for each patient. Plasma aldosterone concentrations were measured at baseline and at the end of the study day (approximately 8 hours after the final drug dose).

The 3 separate study days for each patient were comprised as follows. On one day, the above protocol was performed with the vehicle having been substituted for the active drug during all dosing periods (referred to as the “Placebo day”). On another day, the patients were studied as described above while the patients continued to eat their regular diets and without any additional treatment or stimulation (referred to as “active study day 1”). The third day of study also employed the same protocol of treatment but occurred after 1 week of pretreatment with the diuretic drug hydrochlorothiazide, 25 mg twice daily (referred to as “active study day 2”). The active agent used in these studies was the dipeptide renin inhibitor enalkiren (A-64662), supplied by Abbott Laboratories, Chicago, Illinois. Characteristics of this agent have been reviewed previously.17

PRA was measured by radioimmunoassay,18 with the incubation step performed at pH 7.4. Plasma aldosterone concentration was also measured by radioimmunoassay.19 Statistical analysis was performed by paired t tests and by analysis of variance (ANOVA) for repeated measures. Further details are given, as appropriate, in the “Results” section. Pear-
son correlation coefficients (r) were calculated for changes in blood pressure versus log-transformed PRA measurements. Values are given as mean±SEM.

Results

Blood Pressure

The effects on systolic and diastolic blood pressures and on heart rate of an initial vehicle (placebo) administration followed by four ascending doses of enalkiren are shown in Figure 1. The data are shown for the 3 separate study days: placebo, unstimulated, and sodium depleted. ANOVA for repeated measures, in which each of the 3 study days was treated as a separate factor, indicated a highly significant difference between the days (\( F=28.74, p<10^{-6} \) for systolic blood pressures; \( F=29.04, p<10^{-6} \) for diastolic blood pressures). Moreover, Tukey’s range test indicated that each day (A, B, or C in Figure 1) was significantly different from each of the other 2 days (\( p<0.001 \)) for both systolic and diastolic blood pressures. For heart rate, the values during active-treatment days (A and B) were not different from each other but were different from the placebo day (\( p<0.05 \) for each). All 18 patients entering the protocol completed the first day of testing with enalkiren, but one patient discontinued the study before active study day 2 because of clinically adverse effects of the diuretic. Moreover, five patients did not receive the highest dose of enalkiren on active study day 2 because of excessive blood pressure changes or such symptoms as dizziness while receiving the third dose (0.3 mg/kg).

The mean baseline blood pressure values (mean of all readings obtained during the initial vehicle administration) were 154±4/95±2 mm Hg on active study day 1 and 143±4/92±2 mm Hg on active study day 2. On active study day 1, five of 18 (28%) patients experienced a decrease in diastolic blood pressure of more than 10 mm Hg, and 12 of 18 (67%) had a decrease of more than 5 mm Hg. On active study day 2, eight of 17 (47%) and 15 of 17 (88%) patients had blood pressure decreases of more than 10 mm Hg and 5 mm Hg, respectively. Thus, the blood pressure–lowering effects of the renin inhibitor generally were greater on study day 2 than on study day 1. The duration of action of intravenous enalkiren is shown in Figure 2. Significant decreases in systolic and diastolic blood pressures were sustained for at least 4 hours after the highest dose was administered on study day 1, and for at least 8 hours afterward on study day 2.

Based on pretreatment PRA values obtained at the beginning of active study day 1, we classified four patients as having high-renin, nine as having normal-renin, and five as having low-renin hypertension. In this study, the low-renin patients had PRA values of less than 0.5 ng/ml/hr, the normal-renin patients had between 0.5 and 3.5 ng/ml/hr, and the high-renin patients had more than 3.5 ng/ml/hr. The effects of the highest administered dose of the renin inhibitor on blood pressures in the high-, normal-, and low-renin subgroups are shown in Figure 3 for study days 1 and 2. Possibly because of the variable effects within these patient subgroups and the small sample size within each subgroup, there were no differences between the responses in high- and normal-renin patients for systolic or diastolic blood pressure on either active study day. Also, as displayed in Figure 3, the systolic response in the low-renin group was significantly lower than in the normal-renin but not in the high-renin group on active study day 1 (\( p<0.05 \),

![Figure 1](http://circ.ahajournals.org/Download/1770-Circulation-Vol81-No6-June1990-Figure1.jpg)

**Figure 1.** Line plots of changes in systolic (upper panel) and diastolic (middle panel) blood pressures (mm Hg) and in heart rate (bpm) (lower panel) in hypertensive patients in the semirecumbent posture during administration of ascending bolus doses (mg/kg) of placebo or the renin inhibitor enalkiren (A-64662) given at 45-minute intervals. ANOVA for repeated measures and Tukey’s range test indicated that pairwise differences between each line (A, B, and C) were significant for both systolic and diastolic blood pressures and that A and B were each different from C, although not different from each other, for heart rate. Fuller details are given in text. Values are means of readings obtained at 5-minute intervals throughout the study. For simplicity, typical SEM values (which ranged between 1 and 3 mm Hg throughout the studies) are shown only for the final values of each line.
but this difference was not significant on active study day 2; the diastolic response in the low-renin group was less than in the high-renin group on active study day 1 (p<0.05) and active study day 2 (p<0.05), but this difference was not significantly different from the responses in the normal-renin group.

**Renin and Aldosterone**

PRA values during the 3 study days are shown in Figure 4. The baseline value on study day 2 (after diuretic stimulation) was significantly higher (p<0.05) than on either of the other 2 days. The range of baseline values in individual patients is shown in Figure 5. PRA was decreased significantly by the renin inhibitor throughout each study day regardless of whether the diuretic was used. No change in PRA occurred during the placebo day. The relations between baseline PRA values and the treatment-induced decreases in systolic and diastolic blood pressures are shown in Figure 5. The correlations were significant for both systolic and diastolic blood pressures on active study day 1 and active study day 2. Because PRA declined to nearly zero (as shown in Figure 4) with the renin inhibitor, the maximum treatment-induced changes in PRA were almost identical in magnitude to the actual baseline values. Not surprisingly, the correlations between changes in log PRA and changes in blood pressure were similar to those observed with the baseline log PRA values: r=0.46 (p<0.05) for systolic and r=0.62 (p<0.01) for diastolic on study day 1, and r=0.59 (p<0.01) and

**FIGURE 2.** Line plots of changes in systolic (upper graph) and diastolic (lower graph) blood pressures (mm Hg) in hypertensive patients during 8 hours after bolus (1 mg/kg) administration of enalkiren (A-64662).

**FIGURE 3.** Bar graphs of effects on systolic (upper graph) and diastolic (lower graph) blood pressures (mm Hg) of a bolus administration of enalkiren (A-64662, 1 mg/kg) in semirecumbent hypertensive patients divided into high- (n=4), normal- (n=9), and low- (n=5) renin subgroups. Values are mean±SEM. *Indicates significant change from baseline.

r=0.69 (p<0.01) for systolic and diastolic, respectively, on study day 2. The baseline PRA values also correlated with the changes (mostly decreases) in
heart rate observed on active study day 1 ($r = -0.53$, $p < 0.05$). Moreover, the correlation between changes in heart rate and those in blood pressure did not reach significance. The diuretic treatment between study day 1 and study day 2 produced changes in blood pressure and in PRA as measured by differences between the baseline values obtained at the start of the 2 study days. The untreated renin values (baseline on day 1) were predictive of the subsequent diuretic effects on PRA, as there was a significant correlation ($r = 0.74$, $p < 0.01$) between the original baseline PRA and the diuretic-induced increase in PRA. Figure 6 shows the relations between the diuretic-induced effects on PRA and blood pressure and the blood pressure–lowering effect of the highest administered dose of enalkiren during active study day 2. Additionally, it was noted that the unstimulated baseline PRA on active study day 1 was predictive of the effects of the renin inhibitor on diastolic blood pressure during the diuretic-treated day 2 study ($r = -0.65$, $p < 0.01$).

Plasma-aldosterone concentrations were measured at baseline on each study day and again 8 hours after administration of the final dose of enalkiren or placebo. On the placebo day, the baseline value of aldosterone was $185 \pm 33$ pg/ml, and it decreased by $40 \pm 16$ pg/ml ($p = \text{NS}$) by the end of the study; on active study day 1, baseline aldosterone level was $191 \pm 38$ pg/ml, and it decreased by $70 \pm 30$ pg/ml ($p < 0.05$); on active study day 2, baseline aldosterone level was $273 \pm 49$ pg/ml, and it decreased by $166 \pm 37$ pg/ml ($p < 0.01$).

**Discussion**

Studies with differing inhibitors of components of the renin-angiotensin system, including $\beta$-blockers, competitive angiotensin II analogues, and converting enzyme inhibitors, have shown previously that the renin system has a role in sustaining certain forms of hypertension.\(^{1,4-6}\) We have now administered the specific dipeptide renin inhibitor enalkiren (A-64662) to 18 patients with essential hypertension. This agent produced significant and sustained decrements in PRA and in blood pressure even under relatively basal conditions; the patients were eating essentially an ad libitum sodium diet and were studied while in the semirecumbent posture.

Baseline PRA measured immediately before drug administration correlated significantly with the drug-induced changes in blood pressure, the largest blood pressure decrements tending to occur in patients with the highest renin values. Predictably, the changes in blood pressure also correlated with the treatment-induced changes in renin: Because the renin inhibitor consistently decreased PRA levels to nearly zero, the changes in these values thus were virtually identical to baseline measurements.

Similar correlations between baseline renin values and blood pressure changes have been observed previously in patients treated with saralasin,\(^8\) and the intravenously administered converting enzyme inhibitor teprotide,\(^5\) and also during the acute response to oral treatment with the converting enzyme inhibitor captopril.\(^6\) It is interesting, however, that the correlations between baseline renin levels and blood pressure changes in the present study, although significant, have only moderate predictability ($R^2 = 26\text{-}42\%$), despite the care taken in obtaining the plasma renin values and in measuring the blood pressure responses. Moreover, the differences in blood pres-
sure responses between the high-, normal-, and low-renin subgroups were relatively modest and actually were not significant during the sodium-depletion study. This finding is slightly at variance with the lack of response reported recently in low-renin patients treated with the same agent, but it is possible that the minimal responses reported by the other investigators may have been due to a more profoundly low-renin status of their patients or to other factors predisposing to therapeutic resistance. The ability of our lower-renin patients to respond could possibly be explained, as reported recently, by effects of the renin inhibitor on noncirculating renin-angiotensin systems; the participation of these systems in sustaining vasoconstriction may not always be accurately reflected by plasma renin measurements.

The blood pressure–lowering effects of the renin inhibitor were amplified during diuretic therapy. Indeed, in addition to the antihypertensive effects of the diuretic drug itself, the renin inhibitor produced blood pressure decrements that were greater than when the diuretic had been given previously as the sole treatment. This synergistic effect appeared to be mediated by increases in PRA produced by diuretic therapy, as these stimulated renin values correlated significantly with subsequent blood pressure responses to the renin inhibitor. Thus, the inhibitor had its greatest effects in those patients whose renin levels had risen most, or whose blood pressures had fallen least, in response to the diuretic.

These observations support earlier reports by other investigators that there is a reciprocal relation between the renin-angiotensin system and sodium balance in sustaining hypertension. Our experience with the renin inhibitor, however, has demonstrated that this relation is neither uniform nor entirely predictable among hypertensive patients. We observed that the renin inhibitor produced its greatest blood pressure decrements in those patients, regardless of their previous renin classification, in whom diuretic pretreatment had been least effective in decreasing blood pressure. The strongest predictor of blood pressure effects of the renin inhibitor was the amplitude of the reactive renin responses evoked by the diuretic. This suggests that the sensitivity of the renin response to sodium depletion may characterize individual hypertensive patients at least as strongly as their unstimulated PRA. These observations are consistent with a dual involvement of renin mechanisms: Under resting or basal conditions, the tissue renin-angiotensin system appears to be a major participant in sustaining blood pressure in hypertensive patients, but during such stimuli as sodium depletion, circulating renin may assume a more important role as a determinant of blood pressure.

Because we wanted to avoid the risk of excessive hypotensive responses in this initial study of hypertensive patients, we administered the dipeptide renin inhibitor in ascending bolus doses. The blood pressure decrements produced by these differing doses appeared to describe a dose-response relation.

Although it is possible that the greater blood pressure decreases with the higher doses might have reflected the effects of time rather than the amount of drug being administered, our finding of plateau responses during the 45-minute periods after each dose level indicates at least some degree of dose dependency. Moreover, our day-long parallel placebo studies showed that blood pressure changes did not occur simply because of the passage of time. These findings potentially suggest a clinical difference between the renin inhibitor and the converting enzyme inhibitors. Maximum blood pressure decrements with the converting enzyme inhibitors often can be achieved with low doses; higher doses of those agents simply prolong their duration of action. It is possible that the renin inhibitor is distributed in a different fashion than the converting enzyme inhibitors and might have differing access to sites where it could have effects on renin mechanisms. The prolonged duration of action of this intravenously administered renin inhibitor, despite its short plasma half-life, supports this possibility.

Together with the decreases in blood pressure produced by the renin inhibitor, there were slight decreases in heart rate when patients were studied during unstimulated conditions. The change in heart rate tended to parallel the change in blood pressure, and, like blood pressure, correlated inversely with baseline PRA. This ability of the inhibitor to prevent the tachycardia that can be associated with peripheral vasodilatory effects suggests that the renin-angiotensin system modifies heart rate responses to changes in blood pressure.

The dipeptide agent enalkiren produces its effects by inhibiting the enzymatic action of renin on its substrate. In this study, we found that enalkiren caused marked suppression of PRA. However, it is not yet certain that assays of renin activity are completely valid while the renin inhibitor is still present in the plasma. In previous observations with this agent, there was some dissociation between changes in plasma renin levels and those in blood pressures; this could have been due to either the actions of the inhibitor on noncirculating renin systems or to inaccuracies in the assay of renin activity produced by the renin inhibitor itself. Thus, as a confirmation of the drug’s effect on the renin-angiotensin system, we measured plasma-aldosterone concentrations, which during conditions such as those in the present study, probably change in parallel with PRA. During the study day when only placebo was administered, plasma-aldosterone values decreased by 20%, but during the basal and sodium-depleted active-treatment days, plasma-aldosterone concentrations declined by 37% and 60%, respectively. It is noteworthy that these treatment-induced decreases were significant even though the plasma samples were drawn 8 hours after administration of the final bolus of renin inhibitor. This indicates a sustained inhibitory effect of this agent on the renin-angiotensin system.
Future studies of long-term administration of renin inhibitors will be valuable in further defining the renin mechanisms involved in clinical hypertension. Such studies could determine whether other endocrine, renal, or neuronal factors are influenced by sustained renin inhibition. The short-term findings described in this report potentially are of clinical and therapeutic interest. They have shown the possible influence of renin inhibition as a single-agent therapy, especially in patients who are sodium depleted. It is yet to be determined whether this pharmacological approach will be efficacious for long-term antihypertensive therapy and for the treatment of heart failure.

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