The Role of the Renin-Angiotensin-Aldosterone System in Cardiovascular Homeostasis in Normal Human Subjects

JOSE SANCHO, M.D., RICHARD RE, M.D., JAMES BURTON, PH.D., A. CLIFFORD BARGER, M.D., AND EDGAR HABER, M.D.

SUMMARY To examine the role of angiotensin II in the maintenance of blood pressure and the control of aldosterone secretion in man, eight normal subjects were studied on a tilt table in sodium replete and sodium depleted states prior to and subsequent to the intravenous infusion of an angiotensin converting enzyme inhibitor (CEI). In both the sodium replete or sodium depleted state, upright tilting resulted in an increase in heart rate and a narrowing of pulse pressure. None of the sodium replete or depleted subjects fainted. Tilting was accompanied by a rise in plasma renin activity with an associated rise in plasma aldosterone concentration. When converting enzyme inhibitor was administered, which blocked the generation of angiotensin II, sodium replete subjects were able to compensate for an upright tilt, despite the absence of angiotensin II, without significant hemodynamic change when compared to control state. In sodium depleted subjects, after the administration of converting enzyme inhibitor, there was a sharp and significant decrease in systolic and diastolic blood pressure associated with a significant rise in heart rate. All but one sodium depleted subject fainted within seven minutes. Both plasma aldosterone concentration and plasma renin activity rose on tilting in both sodium replete and sodium depleted subjects. After the administration of converting enzyme inhibitor, plasma aldosterone failed to rise in association with a rise in plasma renin activity. In supine subjects, after the administration of converting enzyme inhibitor, plasma renin activity rose but plasma aldosterone concentration fell. In sodium depleted subjects, after the administration of CEI, aldosterone fell to a level significantly lower than that in supine controls and to a level no different from the supine sodium replete subject. These results indicate that angiotensin II is essential for blood pressure maintenance in sodium depleted individuals, that angiotensin II exerts a direct feedback control on renin secretion, and that angiotensin II is the primary stimulus to aldosterone secretion in response to both sodium depletion and to posture.

THE PRECISE ROLE of the renin-angiotensin system in the maintenance of blood pressure in normal human subjects remains unclear. The recent availability of effective competitive inhibitors of the angiotensin converting enzyme has permitted a critical examination of this question. Recent experiments in rats, rabbits, and trained conscious dogs have demonstrated the importance of angiotensin II in the maintenance of blood pressure in the sodium depleted animal. In the experiments described here, the role of the renin-angiotensin system in the maintenance of blood pressure and in the regulation of aldosterone in relation to sodium depletion, diuretic administration, and posture was examined in normal human subjects.

Methods

Subjects

Eight normal subjects (seven men, one woman), ranging in age from 19 to 27 years, were studied. All subjects were in good health by history, had a normal physical examination, and showed unremarkable results on screening laboratory tests; there was no prior ingestion of drugs. The protocol of the investigation was approved by the institution's Human Studies Committee and written, informed consent was obtained from subjects. Subjects were studied under three different protocols.

Group I

Four subjects were maintained for three days on a 110 mEq Na+, 100 mEq K+ diet. At the end of this period, there was a mean weight loss from the ambulatory value of 0.83 ± 0.9 (sd) kg, indicating that modest sodium depletion had occurred. On the subsequent day, subjects remained supine on a tilt table for 30 min, were then tilted upright to 70° for 30 min, and subsequently were permitted to remain supine for an additional 30 min. Heart rate and blood pressure were monitored, and blood samples were obtained after 30 min in the supine position; 5, 15, and 30 min after assuming the upright position; and again at 30 min of the second supine period (fig. 1). A single injection of 0.25 mg/kg of converting enzyme inhibitor (CEI) was administered intravenously 30 min after the end of the tilt; after an additional 30 min in the supine position the subjects were tilted again. Blood samples in this second period were obtained at 5, 15, and 30
min of the supine period after CEI and after 5, 15, and 30 min of tilting.

Subsequently, to reduce total body sodium, the subjects were either maintained on a 10 mEq Na⁺, 100 mEq K⁺ diet for three days (two subjects) or were administered 80 mg of furosemide orally 12 hours prior to the study. The mean weight loss was 2.67 ± 1.18 (so) kg. The same experimental protocol described above was then repeated. The total volume of blood samples taken in each session was 120 ml.

Group II

Five subjects were studied during four experimental sessions on separate days on an unrestricted diet with and without CEI. They were also studied subsequent to the oral administration of 80 mg of furosemide (weight loss, 1.54 ± 0.37 kg) with and without CEI. In each experimental session the subjects were maintained in the supine position for 30 min and then diluent (placebo) or 0.125 mg/kg of CEI was administered followed by a continuous infusion of diluent or 0.125 mg/min of CEI. Two and one-half hours after beginning the infusion the subjects were tilted to 70° for seven min or until they fainted. Heart rate and blood pressure were monitored and blood samples were obtained at intervals as indicated in figure 2. Total volume of blood samples taken in each session was 60 ml.

Group III

To reduce total body sodium without use of diuretics, four subjects were maintained first on a 110 mEq Na⁺, 100 mEq K⁺ diet for five days and then on a 10 mEq Na⁺, 100 mEq K⁺ diet for another five days. During days 4 and 5 of each diet, the 24-hour urine sodium excretion was 138 ± 24 mEq on the high sodium diet and 15 ± 11 mEq on the low sodium diet. The average weight loss was 2.16 ± 0.39 kg on the sodium restricted diet. Each subject was studied on four separate experimental days corresponding to days 4 and 5 of each diet. In two subjects the CEI was administered during the first experimental session while the placebo was administered during the second experimental session of each diet. The order was reversed for the other two subjects.

In each study, after a supine equilibration period of 30 min, an injection of either 0.25 mg/kg of CEI or diluent (placebo) was given. Fifteen minutes after the injection the subjects were tilted to 70° for seven minutes or until they felt faint.

**FIGURE 1.** Representative example of a subject (J.C.) in group I examined in the supine posture and during tilting while sodium replete and sodium depleted prior to and subsequent to the administration of converting enzyme inhibitor (C.E.I.).

HR = heart rate; PRA = plasma renin activity; PA = plasma aldosterone.

**FIGURE 2.** Mean data of five sodium replete subjects (group II) studied in the supine position and during tilting, with and without converting enzyme inhibitor.
Plasma Bradykinin

Plasma bradykinin levels were measured in two subjects in group I prior to, and 5, 30, and 60 min after the administration of CEI, and in one subject in group II prior to, and 15 and 150 min after the administration of CEI. The limit of detection of the assay was 0.1 ng/ml.

Laboratory Procedures

All blood samples were chilled and centrifuged immediately; the plasma was separated and frozen until assays were begun. Plasma samples for bradykinin were treated with trichloroacetic acid immediately after centrifugation. Plasma renin activity and plasma bradykinin were measured by radioimmunoassays previously described. Plasma aldosterone was measured by a direct radioimmunoassay on plasma extract. The limit of detection of the assay was 9 pg/ml.

Converting Enzyme Inhibitor

Converting enzyme inhibitor, <Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro>,7 was initially obtained from Squibb Drug Company (SQ 20,881). Later experiments were performed with peptide synthesized in our laboratory. Solid phase synthetic techniques as previously employed in our laboratory were used. The inhibitor was purified to homogeneity and fully characterized. It was dissolved in isotonic saline, sterilized by Millipore filtration, and placed in 1-ml ampules. Samples were tested for sterility by culture in thioglycolate medium incubated for seven days at 37°C and for pyrogenicity (LaWall and Harrison, Philadelphia). Bioassay showed the peptide, prepared in this laboratory, was as equipotent as that made by the Squibb Drug Company.

Statistical Evaluation

Statistical computations were done on the logarithmic transforms of the data. In all cases the variance was assessed as being homogeneous on the logarithm transformed data by Bartlett's Test. The paired t-test was used to compute the significance. The results are expressed as mean ± standard error of the mean. Nonsignificant differences were those with P > 0.05.

Results

Group I

A representative example of the data obtained in group I is presented in figure 1. The data are summarized in tables 1 and 2. Figure 1 shows that on tilting in the sodium replete state, there is a rise in heart rate associated with a narrow pulse pressure. The subject did not faint during the 30 min period of tilting. Immediately on return to the supine position, pulse and systolic and diastolic pressure returned to control values. The administration of CEI did not cause any significant hemodynamic effect. Thirty minutes later upright tilting resulted in hemodynamic changes that were not significantly different from the control tilting period. Prior to the administration of CEI, tilting resulted in a modest rise in plasma renin activity and a doubling in plasma

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<th>TABLE 1. Effect of Tilting, CEI Administration, and Sodium Depletion on Blood Pressure and Heart Rate in Group I Subjects*</th>
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*Values given are means ± standard error.
†P < 0.05.
§Hemodynamic parameters listed in the table at 2 minutes after tilting.
Abbreviation: CEI = converting enzyme inhibitor.

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<th>TABLE 2. Effect of Tilting, CEI Administration, and Sodium Depletion on Plasma Renin Activity (PRA) and Plasma Aldosterone (PA) in Group I Subjects*</th>
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*Values given are means ± standard error.
†P < 0.05.
‡P < 0.01.
\*Supine values were immediately prior to tilting. Upright values were maximal values recorded. Post-CEI tilt values in Na depleted subjects were at 2-5 minutes, immediately prior to fainting.
aldosterone concentration. A rapid return of plasma renin activity and plasma aldosterone toward control values occurred when the supine position was assumed. No significant change in either plasma renin activity or plasma aldosterone was observed when CEI was administered. However, on tilting there was a much greater rise in plasma renin activity than occurred during the control period, but no rise in plasma aldosterone concentration was observed.

During the sodium depleted state, hemodynamic changes on tilting were similar to those observed in the sodium replete stage during the control period. The administration of CEI resulted in a slight decrease in diastolic pressure which rapidly returned to control values. However, on tilting, there was a rapid fall in both systolic and diastolic pressure to hypotensive levels associated with a significant rise in heart rate. As soon as the subject was returned to the horizontal position because of fainting, both systolic and diastolic pressure and pulse rate returned to control levels. The control values for both plasma renin activity and plasma aldosterone were higher in the sodium depleted state than in the sodium replete control. On upright tilting, there was a considerable rise in plasma renin activity accompanied by a more marked rise in plasma aldosterone than was observed during the sodium replete state. These values returned toward normal when the supine position was resumed. After administration of CEI in the supine position, plasma renin activity doubled without a concomitant rise in plasma aldosterone. Seven minutes after upright tilting, there was a further striking rise in plasma renin activity associated with a slight fall in plasma aldosterone concentration.

Since all but one of the subjects studied fainted between two and seven minutes after upright tilting in the sodium depleted state, after the administration of CEI, all comparisons of upright hemodynamic values were made at two minutes. Under these circumstances, in both sodium replete and sodium depleted subjects, blood pressure did not change significantly within two minutes. The only hemodynamic alteration observed was a rise in heart rate in both sodium replete and sodium depleted subjects. When CEI was administered, no significant blood pressure change in sodium replete subjects was observed, although a rise in heart rate was seen five minutes after the administration of the drug. This change was transient and did not persist at 30 minutes.

In the sodium depleted subjects, there was a transient fall in diastolic pressure associated with a rise in heart rate. Neither of these changes is seen at 30 minutes. On upright tilting after the administration of CEI, no significant change in either pulse or blood pressure was seen in sodium replete subjects. However, in sodium depleted subjects, there was a marked fall in systolic and diastolic pressure accompanied by a significant rise in heart rate.

Table 2 shows that both plasma renin activity and plasma aldosterone concentration rose in response to tilting in sodium replete and sodium depleted subjects. Because of the large standard error of the mean in the aldosterone values for sodium depleted subjects, the rise did not approach significance. In the supine position, in response to CEI in sodium replete subjects, plasma renin activity did not rise significantly, although plasma aldosterone levels fell. In sodium depleted subjects, CEI administration was associated with a striking rise in plasma renin activity accompanied by a significant fall in plasma aldosterone concentration. In sodium replete subjects, after the administration of CEI, there was a significant rise in plasma renin activity in comparison to the upright control. On the other hand, plasma aldosterone values fell markedly. In sodium depleted subjects, probably because plasma renin was already markedly elevated, the further rise in plasma renin was not significant. However, in spite of these very high plasma renin activities, after the administration of CEI, plasma aldosterone concentration was significantly lower than in the control period.

Group II

In group II subjects sodium depletion was achieved by the administration of diuretics, and particular attention was directed to measuring blood pressure, heart rate, and hormonal changes in the supine position.

Sodium Replete Subjects. Examination of figure 2 shows that the only statistically significant hemodynamic effect of CEI in the supine subject was a transitory fall in diastolic pressure associated with an equally transient rise in heart rate, both of which occurred five minutes after the administration of CEI (P < 0.01). Even though CEI levels were maintained by continuous infusion, the changes in heart rate and blood pressure on tilting were similar to those of the control tilt.

The administration of CEI was associated with an elevation of plasma renin activity which, because of a large standard error, achieved significance only in the 30 minute sample (P < 0.05). No significant changes (compared to control levels) were observed in plasma aldosterone concentrations after the administration of CEI.

Sodium Depleted Subjects. The administration of CEI produced hemodynamic changes in the supine position similar to those observed in sodium replete subjects (fig. 3); both systolic (P < 0.01) and diastolic pressures (P < 0.05) fell and heart rate (P < 0.01) rose five minutes after the administration of CEI but returned to baseline within 30 min. As in the depleted subjects in group I, upright tilting after the administration of CEI produced a striking fall in blood pressure. Four of the five subjects fainted in association with this blood pressure drop.

The administration of CEI produced a significant increase in plasma renin activity at 30 (P < 0.05), 60 (P < 0.05), 90 (P < 0.01), 120 (P < 0.05), and 150 (P < 0.05) min supine, and during tilting (P < 0.01). These values were significantly higher than those obtained during the control study. This contrasts markedly with the transient renin elevation observed after CEI administration in the sodium replete subjects. Plasma aldosterone was significantly depressed in relation to control values at 90 (P < 0.01), 120 (P < 0.01), and 150 (P < 0.05) min supine and during tilting (P < 0.05). These results point to sustained plasma aldosterone depression in the supine position after CEI administration despite marked elevation of plasma renin activity. The elevation of renin and the depression of aldosterone in the upright state is similar to data seen in group I.

Plasma bradykinin concentrations were determined in one subject after sodium depletion and CEI administration. At
EFFECT OF CEI
(80 mg Furosemide)

FIGURE 3. Mean data of five sodium depleted subjects (group II) studied in the supine position and during tilting, with and without converting enzyme inhibitor.

5, 15, and 150 min supine and 2 min subsequent to tilting, bradykinin concentrations were detectable and did not differ from control values.

Group III

The purpose of this study was to determine whether the hemodynamic results seen with dietary sodium depletion would duplicate the results seen with diuretic agents (groups I and II, respectively). After dietary sodium depletion, CEI administration was followed by a transient fall in diastolic pressure in the supine position. On upright tilting, a profound fall in both systolic and diastolic pressures was again seen (control, 101 ± 12/83 ± 7; post-CEI, 81 ± 11/35 ± 9; P < 0.05).

Discussion

Angiotensin converting enzyme is a dipeptidylcarboxypeptidase that generates angiotensin II from the decapeptide angiotensin I.11-13 There is considerable evidence that this enzyme also inactivates bradykinin.14, 18 Angiotensin converting enzyme inhibitor (CEI) is a nonapeptide originally isolated from the venom of Bothrops jararaca which has been shown to inhibit angiotensin converting enzyme in lung extract19 and in intact lungs in vivo.19, 20 Collier, Robinson, and Vane have demonstrated that CEI inhibits the pressor effect of infused angiotensin I in man with an estimated half-life of blockade of about three hours. Unlike the angiotensin II blocker sarcosyl-alanyl-angiotensin II (P113), CEI has no angiotensin II agonist activity, and therefore it represents an ideal agent for the study of the renin-angiotensin system. Because of the short half-life of angiotensin II, the administration of CEI undoubtedly results in negligible circulating angiotensin II levels.

The data presented in this study indicate that angiotensin II is required for the hemodynamic adjustment to upright tilting in sodium depleted man; in its absence the sympathetic nervous system and other homeostatic mechanisms are inadequate to maintain blood pressure. Normal functioning of the baroreceptor reflex is indicated by the elevation in heart rate generally observed when blood pressure falls. In the normal sodium replete subject, homeostatic mechanisms are adequate for the maintenance of blood pressure during tilting even in the absence of circulating angiotensin II.

These results are consistent with our prior animal experiments,7 which indicated that CEI did not alter the blood pressure of the recumbent sodium replete dog. However, when sodium intake was decreased from 60 mEq/day to 10 mEq/day for one to two weeks, a small but significant fall in blood pressure occurred after the administration of CEI. The more severe the sodium depletion, the greater the degree of hypotension induced by CEI. In a series of adrenalectomized dogs maintained on 25 mg of cortisone and 1 mg of DOCA (desoxycorticosterone) daily and on a low sodium diet (10 mEq/day), a constant infusion of CEI caused an average drop in blood pressure of 29.6 mm Hg. Similar findings were reported by others using either CEI or angiotensin II analogs in the dog20, 21 and in rats.1 The results were also consistent with the data of Gavras et al.,22 who demonstrated that the hypotensive effects of CEI in hypertensive patients are augmented by sodium depletion.

In supine subjects, CEI produced a transient fall in blood pressure at approximately five minutes after its administration. This transient hypotension was greater in sodium depleted subjects. However, in all instances blood pressure and pulse rate returned to control levels by ten minutes, and no differences were observed between subjects administered the placebo or those administered CEI. In the absence of hypotension, the elevation of plasma renin activity seen in group II subjects after CEI administration is best explained as resulting from the release of direct angiotensin II suppression of plasma renin activity. This phenomenon was seen clearly in sodium depleted subjects and to a lesser degree in sodium replete subjects, and thus it suggests that direct angiotensin II suppression of plasma renin activity varies with the underlying level of stimulation of the renin system. Samuels et al.2 examined the question of direct angiotensin II suppression of plasma renin activity with conscious dogs that were markedly sodium depleted. When sufficient phenylephrine, an alpha sympatheticomimetic agent that does not stimulate the release of renin,28 was infused to maintain a normal or elevated blood pressure during the administration of CEI, plasma renin activity rose significantly. This observation indicates that in this preparation, as well as in supine human subjects, when sustained hypotension does not occur as a result of the administration of CEI, renin activity nevertheless rises. The rise in renin was not blunted by the administration of the beta-adrenergic antagonist, propranolol, indicating that the increase in plasma renin activity cannot be explained by stimulation of the beta receptors in the kidney.3 These data support the hypothesis that CEI administration results in elevations of plasma renin activity by removing the inhibitory effect of angiotensin II on renin release. These data are also consistent with previously
reported observations with angiotensin II in man, in the dog, in the sheep, in the isolated kidney, and in renal cell suspensions.

The response of group III subjects to CEI was similar to the response of group I and group II patients. Thus no effects of diuresis other than sodium depletion need be considered in interpreting the data, since group III patients were sodium depleted by diet rather than by furosemide. Also, in group II and group III, each tilt experiment was performed on a separate day, thereby excluding any possible effect of one tilt experiment on a subsequent one.

No elevation in plasma bradykinin concentration was observed in two subjects after CEI administration. Miller et al. were also unable to demonstrate a rise in plasma bradykinin in the dog after CEI administration. Furthermore, the blood pressure effects noted in the dog in response to CEI were duplicated when the action of angiotensin II on its peripheral receptor was blocked by an angiotensin analog, which has no effect on bradykinin degradation. These observations make it unlikely that any effects observed after CEI administration are secondary to increased bradykinin levels.

Oparil and coworkers demonstrated that plasma renin activity rises rapidly on tilting. The data in the present study indicates that plasma aldosterone follows a similar time course. After CEI administration, plasma aldosterone levels no longer follow elevations in plasma renin activity and either remain unchanged or fall during tilting. These observations indicate that the major stimulus to aldosterone secretion in response to tilting is angiotensin II.

Recently, some investigators have suggested that the renin-angiotensin system is not necessarily the primary mechanism inducing the increase in aldosterone secretion during sodium depletion. For example, it has been reported that the elevation of aldosterone secretion produced by dietary sodium restriction in man is not accompanied by a rise in circulating angiotensin II. Our data, however, indicate that the main mediator of the elevation of aldosterone secretion induced by sodium depletion is the renin-angiotensin system, as indicated by the fall of plasma aldosterone levels after CEI administration during salt depletion. Recently it has been suggested that angiotensin I may stimulate aldosterone secretion in vitro. Our data suggest that in vivo circulating angiotensin I levels have little importance in the control of aldosterone secretion since plasma aldosterone falls even in the presence of an increase in plasma renin activity after blockade of the converting enzyme.

Acknowledgments

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