Role of Angiotensin II in the Hormonal, Renal, and Electrolyte Response to Sodium Restriction

Suzanne Rogacz, Norman K. Hollenberg, and Gordon H. Williams

SUMMARY Adrenal responses to angiotensin II (ANG II) are enhanced with restriction of sodium intake. To determine whether increased circulating ANG II levels are responsible for the enhanced responsiveness, the adrenal and blood pressure responses to ANG II in human subjects were assessed four times: in balance on a high and a low salt diet and before and after the administration of a converting enzyme inhibitor (enalapril). Before enalapril administration, sodium restriction significantly increased (p<0.02) plasma renin activity, ANG II, and aldosterone levels; the aldosterone response to ANG II was enhanced twofold (p<0.01); and the blood pressure response to ANG II infusion was reduced significantly (p<0.05). Despite a fixed and low plasma ANG II concentration when enalapril was employed, the adrenal response to ANG II on the low salt diet was enhanced to the same degree as that observed before administration of the converting enzyme inhibitor. Conversely, enalapril substantially altered the blood pressure response to ANG II with sodium restriction, completely preventing the reduction in responsiveness. If the subjects were first given enalapril and then sodium intake was restricted, ANG II levels did not change significantly but renal excretion of both sodium and potassium was substantially modified. The rate at which renal excretion of sodium fell to match intake was retarded strikingly (p<0.001); conversely, renal retention of potassium increased significantly (p<0.03) as low salt balance was attained. Possibly because of the potassium retention, aldosterone levels rose, but significantly less than when enalapril was absent. These results indicate that in humans, an increase in circulating ANG II concentration is not involved in the induction or maintenance of an enhanced adrenal response to ANG II with sodium restriction. However, ANG II does appear to be important, at least over the short term, in the maintenance of sodium and potassium homeostasis when sodium intake is restricted. (Hypertension 9: 289-294, 1987)

KEY WORDS • aldosterone • sodium • potassium • angiotensin II • kidney

Changes in sodium intake modify both the response of vascular smooth muscle and the adrenal to angiotensin II (ANG II). The mechanism underlying the sensitization of the vascular smooth muscle to ANG II on a high sodium intake appears to be clear: all of the studies on smooth muscle agree that the increase in sensitivity reflects an increase in the number of available ANG II receptors. On the other hand, the mechanism underlying the enhanced sensitivity of the adrenal when sodium intake is restricted is unclear. Some studies have suggested that a receptor mechanism is involved. Other studies have denied a central role for angiotensin-mediated receptor changes. Studies in humans are necessarily more indirect but are important because of the observation that a major subgroup of patients with essential hypertension, the “nonmodulators,” show an abnormality in the modulation of adrenal responsiveness with shifts in sodium intake. One approach to assess the role of angiotensin-mediated shifts in responsiveness has exploited the ability of converting enzyme inhibitors to reverse the increase in the ANG II concentration that occurs when sodium intake is reduced. In normal subjects, converting enzyme inhibition has not been shown to modify adrenal responsiveness, whereas it increases vascular smooth muscle responsiveness to ANG II with sodium restriction. On the other hand, in nonmodulators with essential hypertension, converting enzyme inhibition has resulted in an increase in adrenal responsiveness to ANG II.
It is not yet clear whether normal subjects and this subgroup of patients with essential hypertension differ qualitatively or quantitatively. One shortcoming of all previous studies addressing this issue in humans has been that the endocrine responses to the restriction of sodium were allowed to evolve before the converting enzyme inhibitor was administered. One possibility, supported by studies in rats\(^1\) and not yet explored in humans, is that converting enzyme inhibition fails to modify the normal adrenal response because of a prolonged “memory.” It is possible that reducing plasma ANG II concentration after the renin-angiotensin-aldosterone system has been activated by restriction of sodium intake is too late; the adrenal response has already occurred and is not reversed quickly by reducing plasma ANG II concentration.

This study was designed with two goals. First, to determine whether adrenal memory is involved in the enhanced state of responsiveness, the adrenal response to ANG II was determined on two different occasions before and after sodium intake was restricted. These two studies were repeated after the angiotensin converting enzyme (ACE) inhibitor enalapril was given in doses adequate to prevent any increase in plasma ANG II concentration from the moment at which a low sodium diet was instituted. Our second goal, reflecting this experimental approach, was to quantitate more precisely the overall importance of activation of the renin-angiotensin system in the renal response to sodium restriction.

Materials and Methods

Subjects and Protocol

Ten normal men ranging in age from 30 to 56 years and in weight from 57 to 81 kg were admitted to the Clinical Research Center of the Brigham and Women’s Hospital. During their entire stay, all subjects were maintained on isocaloric constant diets containing 100 mEq of potassium with sodium intake varying from 200 to 10 mEq. When balance had been achieved on a high sodium intake, an ANG II infusion was administered and the subjects were then switched to a 10-mEq sodium intake. A second ANG II infusion was administered between the 6th and 8th days on the sodium-restricted diet. Following this infusion, the 200-mEq sodium diet was resumed for 3 days: high salt balance was achieved routinely within 48 to 72 hours. On the 3rd day of high salt intake, a 10-ng dose of enalapril was given at 2000. Enalapril at this dose was administered between the 6th and 8th days on the sodium-restricted diet. Following this infusion, the 200-mEq sodium diet was resumed for 3 days: high salt balance was achieved routinely within 48 to 72 hours. On the 3rd day of high salt intake, a 10-ng dose of enalapril was given at 2000. Enalapril at this dose was administered daily at 0630 for the remainder of the study. The morning following the first dose of enalapril, an ANG II infusion was administered (high salt converting enzyme inhibitor infusion). The subjects were then begun on a 10-mEq sodium intake, and a repeat ANG II infusion was given 6 to 8 days later.

ANG II Infusion

Each ANG II infusion was begun between 0830 and 0900. On days that enalapril was also administered, the infusion was begun 2 to 2.5 hours after the last dose. The subjects remained supine and received an infusion of ANG II amide (Hypertensin; CIBA-Geigy, Pharmaceuticals Division, Summit, NJ, USA) using an electronic infusion pump (Harvard Apparatus, Millis, MA, USA), at doses of 3 and 10 ng/kg/min for 45 minutes at each dose. Blood pressure and heart rate were measured every 2 minutes with an electronic digital recording sphygmomanometer (Dyna-Mapp; Critikon, Tampa, FL, USA), and blood samples were obtained for potassium, aldosterone, cortisol, and ANG II concentration at the start of the study and at the completion of each dose of ANG II.

Laboratory Procedures

All blood samples were collected on ice and immediately cold-centrifuged, and the plasma was separated and frozen until the time of assay. Sodium and potassium were measured using flame photometry with lithium as an internal standard. Serum creatinine was measured by an autoanalyzer technique. Plasma renin activity (PRA), ANG II, aldosterone, and cortisol were analyzed by previously described radioimmunoassay techniques.\(^16,17\)

Group means are presented with the SEM as the index of dispersion. Statistical probability was assessed using the paired \(t\) test for normally distributed data, linear regression, and the Fisher exact test for nonhomogeneously distributed data. The null hypothesis was rejected when \(p\) achieved a level of 0.05 or less. The protocol was approved by the Committee for the Protection of Human Subjects from Research Risks at the Brigham and Women’s Hospital, and written informed consent was obtained from each subject.

Results

As anticipated, sodium excretion fell rapidly on the low salt diet, with equilibrium being established in 5 to 7 days. There was no difference in serum sodium, potassium, or creatinine concentrations during restriction of sodium intake (Table 1). After 5 to 7 days of sodium restriction, PRA, ANG II, and aldosterone had all increased significantly \((p<0.01)\), with the ANG II levels increasing by 50% and the aldosterone levels by 300% (see Table 1). Neither diastolic blood pressure nor cortisol levels differed during the pre-enalapril studies.

The increase in plasma ANG II concentration during the course of the ANG II infusion did not differ significantly during the study periods (Figure 1). In contrast, the increment in blood pressure and aldosterone varied with the level of sodium intake (see Figure 1). When the subjects achieved balance on the low sodium diet, the rise in aldosterone with the 10-ng ANG II infusion was more than twofold greater than what it had been on the high salt diet \((p<0.01)\). Diastolic blood pressure was also affected by the level of sodium intake, with a significant \((p<0.05)\) reduction in vascular response to ANG when in balance on a 10-mEq sodium intake \((16.8 ± 2.5 \text{ vs } 9.6 ± 2.0 \text{ mm Hg})\).
The effects of converting enzyme inhibition on basal hormonal and renal responses to sodium restriction

When sodium intake was reduced abruptly from 200 to 10 mEq/day, renal sodium excretion showed the anticipated exponential fall, with a rate constant of 0.24 ± 0.009, reflecting a half-time of 27.5 hours. During enalapril administration the rate at which external sodium balance was achieved was retarded significantly (p < 0.02), with a rate constant of 0.16 ± 0.01, reflecting a half-time of 37.8 hours (Figure 2). Even after 6 days on a low salt diet the amount of sodium excreted in the presence of enalapril was 2.5 times greater than in its absence (see Table 1). However, there were no significant changes in serum sodium or creatinine concentration.

Converting enzyme inhibition also modified potassium balance, resulting in a significant decrease in the renal excretion of potassium. Only the initial 4 balance days were used in the analysis to avoid confounding of the data by the changes in aldosterone level that occur with ANG II infusion. Assuming that bowel loss of potassium was 12 mEq/day across all sodium intakes (a figure derived from the unaccounted-for potassium loss in both high salt states as the low salt diet was begun), renal retention of potassium increased in the presence of enalapril (p < 0.05; Figure 3). Serum potassium was not significantly altered by treatment with enalapril.

Converting enzyme inhibition enhanced the PRA response to sodium restriction: the levels achieved following enalapril were three times greater than preinfusion values (p < 0.02). Following enalapril administration, ANG II levels were suppressed slightly on the high salt diet, and they did not change significantly with sodium restriction. Thus, neither of the basal ANG II levels during enalapril administration differed significantly from pre-enalapril levels during high salt intake.

As anticipated, enalapril administration produced a fall in blood pressure even on the high salt diet. However, the fall was substantially enhanced with sodium restriction (p < 0.02; see Table 1). Plasma aldosterone levels, however, were approximately doubled (p < 0.02) during the course of sodium restriction despite administration of doses of converting enzyme inhibitor sufficient to block any significant increase in basal ANG II levels (see Table 1).

The effect of converting enzyme inhibition on the response to ANG II

Following enalapril administration, there was no change in the response of the adrenal to ANG II. The aldosterone rise with ANG II was essentially identical to that observed before enalapril administration (see Figure 1). In contrast to the absence of an effect on adrenal responses to ANG II, the pressor response was substantially different after enalapril administration. With sodium restriction, no significant change inpressor response to ANG II occurred, in contrast to the decreased responsiveness observed before enalapril administration (see Figure 1).

Discussion

We have known for a decade that sodium intake modulates adrenal and vascular responsiveness to ANG II. Restriction of intake enhances the adrenal and reduces vascular (particularly renal vascular) responsiveness, whereas sodium loading has the opposite effect. One purpose of this study was to assess whether the circulating level of ANG II plays a role in the enhanced adrenal responsiveness. Since the circulating ANG II levels parallel both the duration of sodium restriction and the degree of enhanced adrenal responsiveness, ANG II could be the mediator of the enhanced responsiveness, as suggested from several animal studies. Restriction of sodium intake in rats

Table 1. Basal Data on ANG II Infusion Days for 10 Normal Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before enalapril</th>
<th>After enalapril</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High Na diet</td>
<td>Low Na diet</td>
</tr>
<tr>
<td></td>
<td>(200 mEq)</td>
<td>(10 mEq)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.9 ± 2.2</td>
<td>70.4 ± 2.2</td>
</tr>
<tr>
<td>Urinary K (mEq/L)</td>
<td>228 ± 14</td>
<td>11.5 ± 2.2*</td>
</tr>
<tr>
<td>Urinary Na (mEq/L)</td>
<td>86.7 ± 5</td>
<td>96.4 ± 4.3</td>
</tr>
<tr>
<td>Serum Na (mEq/L)</td>
<td>139 ± 0.5</td>
<td>137 ± 0.6</td>
</tr>
<tr>
<td>Serum K (mEq/L)</td>
<td>4.16 ± 0.06</td>
<td>4.22 ± 0.1</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.82 ± 0.04</td>
<td>0.87 ± 0.04</td>
</tr>
<tr>
<td>PRA (ng ANG I/ml/hr)</td>
<td>1.07 ± 0.26†</td>
<td>3.72 ± 0.81*</td>
</tr>
<tr>
<td>ANG II (pg/ml)</td>
<td>24.8 ± 1.8*†</td>
<td>33.8 ± 2.1*</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>9.8 ± 1.4*†</td>
<td>30.5 ± 5.5*</td>
</tr>
<tr>
<td>Cortisol (µg/dl)</td>
<td>15.4 ± 1.2</td>
<td>11.8 ± 1.0</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>68.3 ± 2.5</td>
<td>68.9 ± 0.9*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. DBP = diastolic blood pressure. *P < 0.02, compared with respective low Na diet values. †P < 0.02, compared with respective postenalapril values.
increases their circulating ANG II levels, increases the number of ANG II receptors on the cells of the zona glomerulosa, and enhances their adrenal responsiveness. A similar pattern of enhanced response is observed when ANG II is infused continuously for several days. Other studies, however, suggest that the circulating ANG II levels do not mediate the enhanced adrenal responsiveness to ANG II.

In our previous studies, sodium restriction was achieved first and then an ACE inhibitor, either enalapril or captopril, was given. Adrenal responsiveness to ANG II was not altered, even though circulating ANG II levels were suppressed to those seen in the high salt state. Mueller et al. attempted to provide an explanation for these discrepant results; in rats an enhanced adrenal responsiveness is dependent in part on the circulating ANG II levels. If ANG II levels were allowed to rise with sodium restriction and then reduced by administration of an ACE inhibitor, there was no change in the responsiveness of the adrenal to infused ANG II. Conversely, if an ACE inhibitor was given before the onset of sodium restriction, the enhanced adrenal responsiveness with sodium restriction was abolished. They concluded that there was a built-in memory of the previous high ANG II state that persisted when circulating ANG II levels fell. The present study suggests that this is not the case in humans. Although enalapril was administered before the onset of sodium restriction, the adrenal responsiveness to ANG II was still enhanced when sodium intake was restricted.

What mediates the enhanced adrenal responsiveness to ANG II with sodium restriction? Levels of serum potassium and cortisol — and, by inference, adreno-
corticotropic hormone — were not different before and during ACE inhibition. Thus, alterations in the activity of these aldosterone secretagogues are not likely explanations. In vitro studies have shown that the late pathway of aldosterone biosynthesis (i.e., the conversion of corticosterone to aldosterone) is enhanced with sodium restriction. This enhancement is maintained in the presence of converting enzyme inhibition. Thus, it is likely that the late pathway is responsible for the enhanced responsiveness. What maintains the enhanced enzyme activity of the late pathway? The present study did not address the answer to this question, but two candidates are dopamine and potassium.

Both in vivo and in vitro studies suggest that dopamine is a potent inhibitor of the adrenal's response to ANG II and that dopamine levels increase during sodium loading and are reduced with sodium restriction. Metoclopramide, a dopamine agonist, enhances the adrenal response to ANG II when given to subjects on a high sodium intake but has no effect in subjects on a low sodium intake. Dopamine may be an important mediator of the reduction in adrenal responsiveness to ANG II with dietary sodium loading.

Isolated adrenal cells obtained from humans or animals on a high potassium diet exhibit increased basal aldosterone output and enhanced rates of conversion of corticosterone into aldosterone. Potassium loading also enhances the aldosterone response to ANG II in vivo. In this study, potassium input was held constant and adrenal responsiveness was nevertheless enhanced by sodium restriction in both the presence and absence of the converting enzyme inhibitor enalapril. However, significant renal potassium retention did occur and may have offset the inhibition of aldosterone release produced by enalapril.

An additional purpose of this study was to examine the role of ANG II in the renal handling of potassium and sodium as the transition is made from high salt to low salt intake. Although attempts to assess changes in total body potassium in a study such as this are limited by an inability to quantitate the contribution of gastrointestinal potassium excretion, the change in the renal handling of potassium resulting from treatment with a converting enzyme inhibitor is nevertheless significant. To the degree that aldosterone is active on the colon, the assumption of constant bowel loss across sodium intakes could substantially underestimate gastrointestinal potassium loss. As sodium restriction was initiated, there was a slight positive potassium balance, which was then dissipated as the renin-angiotensin-aldosterone axis was activated. When a converting enzyme inhibitor was present, thereby limiting activation of this system, potassium retention increased.

This study also demonstrated that the renal handling of sodium as the transition was made from a high salt to a low salt state was altered substantially by converting enzyme inhibition. More sodium was excreted on each balance day, and significantly more time was required for low salt balance to be attained. Indeed, most of the subjects never attained 10-mEq sodium balance in the 7 days after ACE inhibition. To the extent that aldosterone determines distal tubular reabsorption of sodium, differences in the basal level of aldosterone may largely account for the observed natriuresis following ACE inhibition.

Quantitative aspects of the relationship between sodium and the renin-angiotensin system are of interest. In normal humans the half-time relating sodium excretion to time after an abrupt reduction of sodium intake is about 24 hours, as confirmed in this study. In patients with essential hypertension, in whom disruption of sodium handling and altered renal and adrenal function are thought to reflect an abnormality in angiotensin-mediated control (nonmodulation), the half-time is prolonged to about 36 hours. The impact of converting enzyme inhibition on renal sodium handling in this study, in which the half-time was extended to 37 hours, is clearly compatible with a major role for altered renin-angiotensin-aldosterone relationships in the disturbed sodium handling characteristic of nonmodulators.

This study strongly supports the hypothesis that circulating ANG II levels are not involved in the enhanced adrenal responsiveness to ANG II with sodium restriction in normal humans. Interruption of the renin-angiotensin-aldosterone axis by converting enzyme inhibition results, at least over the short term, in renal sodium loss and potassium retention. The retention of potassium may partly override the converting enzyme inhibition and maintain enhanced adrenal responsiveness by creating a potassium-loaded system. The importance of potassium, at both the intracellular and the extracellular levels, to the maintenance of the integrity of the renin-angiotensin-aldosterone system and to sodium homeostasis warrants continued investigation.

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