Rapid Modulation of Renal and Adrenal Responsiveness to Angiotensin II

Paul R. Conlin, Thomas J. Moore, Gordon H. Williams, Norman K. Hollenberg

Angiotensin II (Ang II) has profound effects on the adrenal zona glomerulosa and vascular smooth muscle, producing stimulation of aldosterone secretion and vasoconstriction in a dose-dependent manner. The magnitude of these vascular and adrenal responses to Ang II is regulated also by shifts in salt intake. When dietary salt intake is abruptly reduced from high to low, the enhanced adrenal response to Ang II becomes apparent only after approximately 2 days and reaches its maximum at 4 to 5 days. No data are available on how quickly these response patterns, once established by restriction of salt intake, can be reversed with a salt load.

Rapid suppression of the circulating renin-angiotensin-aldosterone system can be induced by intravascular volume expansion with colloid and crystalloid infusions or head-out water immersion. Both saline infusion and immersion have been shown to suppress plasma renin activity (PRA) and aldosterone in parallel. Tuck et al compared the effects of saline- and dextran-induced volume expansion in salt-restricted normotensive subjects. Both maneuvers resulted in a similar nadir for plasma renin activity (PRA) and aldosterone 6 hours after the start of the infusions; however, a temporal difference in the suppression pattern was seen, with dextran producing a slower suppression than saline.

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We have used the protocol of Tuck et al to ascertain how quickly changes in adrenal and renal vascular Ang II responsiveness can be induced by short-term salt and/or volume expansion after sodium restriction. Our first goal was to establish the rapidity with which the renal and adrenal response patterns, once sensitized by sodium restriction, could be reverted to that seen when the individual is salt- and/or volume-replete. Our second goal was to identify the relative contributions of salt and volume expansion to the regulation of vascular and adrenal responsiveness to Ang II.

Methods

Subjects

We studied 15 normotensive subjects. Each was admitted to the Clinical Research Center of the Brigham and Women's Hospital for study. The study was approved by the Human Subjects Committee of the Brigham and Women's Hospital, and written, informed consent was obtained from each subject before participation.

All subjects consumed an isocaloric constant diet during the study. Five subjects initially consumed a diet of 200 mmol sodium and 100 mmol potassium for the first 2 days of study. Subsequently, their diet was changed to one that contained 10 mmol sodium and 100 mmol potassium, which was continued for the duration of the study. Ten additional subjects were studied only on the diet containing 10 mmol sodium and 100 mmol potassium daily, which was continued for the duration of the study. Daily 24-hour urine collections were assessed for sodium and creatinine excretion to document external sodium balance.

Acute Volume Expansion

After a low-sodium balance was achieved, 5 subjects received an infusion of normal saline or dextran-40 (Rheomacrodex, Pharmacia LKB Biotechnology, Piscataway, NJ) to expand intravascular volume rapidly. After a 5- to 6-day reequilibration on the low-salt diet, the alternate infusion was administered. Each of the 5 subjects received both agents in random order. The first infusion was performed after an overnight fast and with subjects in the supine position at 8 AM on the day after low-sodium balance had been achieved. Saline was administered at 500 mL/h for 4 hours; dextran was infused at 250 mL/h and was accompanied by 250 mL/h of 5% dextrose in water (D5W) for a total of 500 mL/h over 4 hours. The doses of both the salt-containing and non–salt-containing solutions were designed to achieve similar volume expansion. The smaller amount of dextran infused was chosen because dextran-induced intravascular volume expansion occurs in part by attracting fluid from extracellular and intracellular compartments and results in an approximate doubling of the infused volume. The additional D5W was coadministered to avoid this potential interstitial and intracellular space volume contraction. During the infusion studies, blood samples for measurement of plasma aldosterone, PRA, cortisol, hematocrit, and sodium and potassium concentrations were drawn at baseline and 15, 30, 60, 120, and 240 minutes after the start of the infusions. A total of 150 mL of blood was removed over the course of each of the study days and was replaced by D5W provided through the intravenous tube used for blood drawing.

Renal Vascular and Adrenal Responses to Angiotensin II

For the measurement of renal plasma flow, infusion of para-aminobipurrate (PAH; Merck, Rahway, NJ) was begun (8 mg/kg loading dose; 12 mg/min continuous infusion) 60 minutes before initiation of the Ang II infusion and was continued during the Ang II infusion. The subjects were supine from 12:30 PM, and the infusions were begun at 3 PM. For measurement of the vascular, renal blood flow, and adrenal responses to Ang II, the 5 subjects completing the high-salt and low-salt diets received Ang II infusions (3 ng/kg per minute for 45 minutes) on four separate occasions during the study: during high-salt balance, on achieving low-salt balance, 3 hours after the saline infusion, and 3 hours after the dextran infusion. In the other 10 subjects, the infusion study (1, 3, and 10 ng/kg per minute for 45 minutes at each dose level) was performed only with subjects on the low-salt diet. These subjects were studied concurrent with the 5 subjects completing the high-salt and low-salt diet studies and served to anchor the steep correlation between Ang II and plasma aldosterone observed in subjects consuming a low-salt diet. This relation was to serve as a major point for comparison after each of the volume expansion maneuvers.

Blood samples were obtained for measurement of plasma aldosterone, PAH, cortisol, sodium, potassium, Ang II, and PRA at the beginning (-10 and 0 minutes) and end of each dose of Ang II. Blood pressure was measured every 2 minutes using an indirect recording sphygmonanometer (Dynamap, Critikon, Tampa, Fla).

Laboratory Procedures

All blood samples were collected on ice and centrifuged at 4°C; plasma was stored at -20°C until the time of assay. Aldosterone and cortisol were measured by radioimmunoassay (Diagnostic Products, Los Angeles, Calif), as were PRA and Ang II.11 Plasma and urinary electrolytes were measured with an ion-selective electrode system and creatinine by autoanalyzer. Plasma and infused PAH concentration was measured by a Technicon autoanalyzer spectrophotometer. Clearance of PAH corrected for body surface area was calculated as previously described.13

Statistical Analysis

Mean values are presented with SEM as the index of dispersion. Differences between means of parameters within groups were tested for significance using the paired t test or analysis of variance; between-group differences were assessed using the unpaired t test. Time-dependent variables were evaluated using two-way repeated-measures analysis of variance. Correlations of plasma Ang II and aldosterone levels from individuals in balance on high-salt and low-salt diets were assessed using linear regression. The Fisher Exact Test was used to compare the distribution of data points representing the relation between Ang II and aldosterone levels after dextran and saline infusions, because the nonhomogeneous distribution of these data precluded the use of linear regression.13 The null hypothesis was rejected at a level of P<.05.
TABLE 1. Clinical Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>High- and Low-Salt Diets</th>
<th>Low-Salt Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Age, y</td>
<td>35±6*</td>
<td>34±3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75±3</td>
<td>72±2</td>
</tr>
<tr>
<td>Male/female</td>
<td>3/2</td>
<td>10/0</td>
</tr>
<tr>
<td>Admission BP (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>109±3</td>
<td>111±3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>66±4</td>
<td>66±2</td>
</tr>
</tbody>
</table>

BP indicates blood pressure. Values are mean±SEM.

Results

The baseline characteristics of the study subjects are shown in Table 1. The 5 subjects completing the high-salt and low-salt protocols were similar in characteristics to the 10 subjects completing the low-salt diet alone. As expected, low-salt intake led to activation of the renin-angiotensin system, with increases in PRA and Ang II levels (Table 2). Likewise, salt restriction increased plasma aldosterone levels when compared with high-salt balance.

Both saline and dextran infusions administered during low-salt balance resulted in a rapid fall in PRA and aldosterone levels. With saline infusion, aldosterone and PRA differed significantly (P<.05) from baseline levels after 60 and 120 minutes, respectively (Fig 1A and 1B). In contrast, dextran infusion caused a slower reduction in both PRA and aldosterone, with significant differences from baseline observed only after 240 minutes (P<.05). Despite this difference in suppression pattern, both agents resulted in similar values for PRA and aldosterone 240 minutes after the start of the infusion studies (Table 3).

Hematocrit fell significantly and in parallel during both the saline and dextran infusions, suggesting that similar amounts of volume expansion had occurred (Table 3). Dextran infusion resulted in a slightly greater decrement in hematocrit than saline infusion (P<.05).

Likewise, serum sodium and potassium concentrations showed the anticipated fall (due to hemodilution) during dextran infusion and differed significantly from postsaline values.

Three hours after completion of the saline and dextran administrations, basal levels of both plasma aldosterone and renal plasma flow differed from those seen during low-salt intake but did not differ from levels obtained during high-salt balance (Fig 2). Ang II was then infused (3 ng/kg per minute for 45 minutes) to determine whether changes in target tissue responsiveness had occurred as a result of the rapid extracellular fluid and/or plasma volume expansion. There was a rapid shift in renal vascular and pressor responsiveness to Ang II. The fall in renal plasma flow and rise in mean blood pressure induced by Ang II became equivalent to the responsiveness seen during a high-salt diet. Likewise, in the 3 hours after dextran infusion, the shift in renal vascular and pressor responses to Ang II infusion were essentially identical to those seen after saline (Fig 3).

Plasma aldosterone and Ang II concentrations correlated both during a high-salt (r=0.68, P<.01) and low-salt (r=0.52, P<.001) diet. As anticipated, the relation between plasma Ang II and plasma aldosterone showed substantial enhancement during the low-salt diet (Fig 4A). The influence of the two volume expansion infusions on adrenal responsiveness was complex.Saline infusion rapidly suppressed adrenal responsiveness to Ang II (Fig 4B). The individual points were symmetrically distributed about the regression line relating Ang II and aldosterone concentrations during a high-salt diet.
Table 3. Biochemical and Hematocrit Responses to Volume Expansion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline Before</th>
<th>Saline After</th>
<th>Dextran Before</th>
<th>Dextran After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td>0.42±0.01</td>
<td>0.38±0.01*</td>
<td>0.40±0.01</td>
<td>0.35±0.01*</td>
</tr>
<tr>
<td>Serum Na⁺, mmol/L</td>
<td>140±1</td>
<td>141±1</td>
<td>140±1</td>
<td>136±1*†</td>
</tr>
<tr>
<td>Serum K⁺, mmol/L</td>
<td>4.2±0.1</td>
<td>4.3±0.1</td>
<td>4.2±0.2</td>
<td>4.0±0.1†</td>
</tr>
<tr>
<td>Plasma renin activity, ng·L⁻¹·s⁻¹</td>
<td>1.36±0.20</td>
<td>0.50±0.08*</td>
<td>1.16±0.20</td>
<td>0.74±0.14*</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>32±2</td>
<td>18±2*</td>
<td>27±4</td>
<td>20±4*</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>1330±500</td>
<td>280±60*</td>
<td>1030±470</td>
<td>360±110*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<.05 vs before. †P<.05 vs after.

Diet (8 above and 7 below). All but one of the data points were below the line defined by steady-state low-salt balance relations. Dextran infusion, on the other hand, did not influence the aldosterone response to Ang II despite suppression of the basal levels: indeed, 6 of the 14 data points were on or above the line relating Ang II and aldosterone relations during a low-salt diet. As the range of plasma Ang II concentrations achieved was relatively narrow, the post-saline Ang II-aldosterone relation was particularly sensitive to one data point, and the post-dextran data was more widely scattered and nonhomogeneously distributed. Thus, we compared the responses to saline and dextran by non-parametric analysis. The distributions of data represent-

![Figure 2](http://hyper.ahajournals.org/)

**Fig 2.** Bar graphs show para-aminohippurate (PAH) clearance (A) and aldosterone levels (B) obtained 3 hours after completion of saline and dextran infusions (n=5). High-salt diet (HS) significantly increased renal blood flow and decreased aldosterone levels compared with low-salt intake (LS). Both saline (SAL) and dextran (DEX) infusions administered during low-salt balance led to similarly enhanced renal blood flow and reduced aldosterone levels compared with levels seen during low-salt balance. *P<.05 vs LS.

![Figure 3](http://hyper.ahajournals.org/)

**Fig 3.** Bar graphs show decrement in para-aminohippurate (PAH) clearance (A) and increment in mean blood pressure (B) with angiotensin II infusion (3 ng/kg per minute for 45 minutes) (n=5). Low-salt diet (LS) led to a significant reduction in angiotensin II responsiveness of the renal blood supply compared with high-salt balance (HS). Both saline (SAL) and dextran (DEX) infusions administered during low-salt balance rapidly restored this responsiveness to that seen during high-salt balance. These changes in renal blood flow were associated with baseline measurements of 561±41 (HS), 526±48 (LS), 501±42 (SAL), and 653±87 (DEX) mL·(min·1.73 m²)⁻¹, respectively. Vascular responsiveness to angiotensin II (B) paralleled renal blood flow changes. These changes in mean blood pressure occurred from baseline measurements of 76±3 (HS), 82±4 (LS), 77±3 (SAL), and 79±4 (DEX) mm Hg, respectively. *P<.05 vs HS.
FIG 4. Plots show semilog regression relations of plasma angiotensin II (Ang II) and aldosterone levels for individuals in balance on a high-salt diet (HS) and low-salt diet (LS). Ang II infusion for subjects on HS diet (n=5) was 3 ng/kg per minute for 45 minutes; data points from baseline and after Ang II infusion are shown (•). For subjects on LS diet, Ang II was infused at 3 ng/kg per minute for 45 minutes (n=5) and 1, 3, and 10 ng/kg per minute for 45 minutes at each dose level (n=10); data from baseline and during Ang II infusion are shown (o). A: LS diet led to enhancement in aldosterone responsiveness to Ang II compared with HS diet. B: Regression relations between Ang II and aldosterone depicted in A are reproduced as dashed lines. Note that aldosterone responsiveness after saline infusion (•) became similar to that seen during HS balance, whereas after dextran infusion (•) the data representing aldosterone-Ang II relations were more distributed about the line representing LS balance. The difference in this distribution was significant (P=.05).

Table 4. Biochemical Responses to Angiotensin II Infusion After Volume Expansion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>Before Ang II</th>
<th>After Ang II</th>
<th>Dextran</th>
<th>Before Ang II</th>
<th>After Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Na⁺, mmol/L</td>
<td></td>
<td>140±1</td>
<td>140±1</td>
<td>137±1*</td>
<td>137±1*</td>
<td></td>
</tr>
<tr>
<td>Serum K⁺, mmol/L</td>
<td>4.2±0.1</td>
<td>4.3±0.1</td>
<td>3.9±0.1*</td>
<td>4.0±0.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>190±30</td>
<td>160±30</td>
<td>140±30</td>
<td>140±30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma renin activity, ng·L⁻¹·s⁻¹</td>
<td>0.44±0.12</td>
<td>0.38±0.16</td>
<td>0.76±0.12</td>
<td>0.38±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>21±2</td>
<td>44±4</td>
<td>25±4</td>
<td>39±7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<.05 vs saline infusion.

Ang II indicates angiotensin II. Values are mean±SEM.
achieved. It is very possible that further study at a later time point may have revealed similar shifting in adrenal responsiveness by dextran. Indeed, the apparent scatter of the postdextran Ang II–aldosterone relations may reflect the evolution of this process.

The mechanisms responsible for sensitization of the adrenal glomerulosa by restriction of salt intake are incompletely understood. A potentially important clue arose from the time course for this activation in humans: an increase in responsiveness first became evident only 48 hours after dietary salt restriction, with full expression present after several days. In rats, this enhanced responsiveness is likewise time dependent, occurring within 48 hours, and can be related to induction of the enzyme involved in the late pathway of aldosterone synthesis (conversion of corticosterone to aldosterone by aldosterone synthetase). Although all aldosterone secretagogues acutely enhance secretion by increasing the rate of the early pathway (synthesis of pregnenolone from cholesterol), it is the increased activity of aldosterone synthetase that appears to account for most, if not all, of the effect of salt restriction on adrenal sensitivity. The slow onset of adrenal sensitization is compatible with the induction of this biosynthetic pathway rather than changes in Ang II receptor density, as occurs in the vasculature. Indeed, adrenal Ang II receptors have been noted to be reduced by sodium restriction in primates. The asymmetry in the onset and offset (hysteresis) of adrenal responsiveness suggests that other regulated steps might be affected by saline infusion: it seems unlikely that the increased aldosterone synthetase levels recruited gradually by a low-salt diet were reversed during the short period of time that followed saline volume expansion.

How does information on the state of sodium balance reach the adrenal glomerulosa? The results of this study support earlier studies which suggest that components of the renin-angiotensin system do not provide crucial information. Other suggested candidates as a source of information to the adrenal have included factors such as atrial natriuretic hormone (ANH), dopamine, or digitalis-like factors. Levels of ANH have been shown to track with the level of sodium intake. However, Tuchelt et al, using similar maneuvers, showed that adrenal responsiveness to Ang II during low-sodium balance was not significantly different in the presence or absence of ANH infusion. Also, appropriate interpretation of the effects of ANH may need to take into account changes in ANH or cyclic GMP metabolism that might occur during shifts in salt intake.

Plasma and urinary dopamine are likewise increased with sodium loading, and dopamine has been shown to have inhibitory effects on aldosterone secretion both in vivo and in vitro. However, short-term dopamine blockade with metaclopramide increases basal aldosterone levels in salt-replete individuals but does not change aldosterone responsiveness to Ang II. Likewise, levels of endogenous digitalis-like factors have been noted to increase in response to short-term volume expansion with saline. Although the identity of endogenous Na+-K+ pump inhibitors remains controversial, it is intriguing that increasing doses of ouabain have been shown to inhibit aldosterone secretion in vitro. Therefore, the contributions of ANH, dopamine, and digitalis-like factors to adrenal modulation are unresolved.

Despite its major role in acutely regulating aldosterone secretion, the state of activation of the renin-angiotensin system is probably not involved in the shifts in responsiveness of the adrenal glomerulosa. Adrenal responsiveness to Ang II during salt restriction does not change when circulating Ang II levels are reduced by either converting enzyme inhibition or β-blocker treatment, which also reduces PRA and plasma angiotensin I concentrations. Thus, the physiological determinants of sodium-regulated changes in adrenal responsiveness remain obscure.

When individuals shift from a very low-salt to a high-salt intake, the kinetics of the natriuretic response are complex. There is a consistent and substantial delay in the natriuretic response so that positive sodium balance and weight gain occur during the first 24 hours. We had anticipated that the very slow onset of adrenal sensitization during salt restriction would be matched by a similar slow offset. This lag in suppression of aldosterone secretion thus might contribute to a delay in natriuresis, as has been observed acutely after saline infusion or chronically when dietary sodium is altered. In our study the adrenal response to Ang II shifted within hours of saline administration, making it unlikely that aldosterone contributes more than a small amount to the previously described delayed natriuresis. In the short period of time after the suppression of PRA and Ang II levels by saline and dextran infusion, the responsiveness of PAH clearance and mean blood pressure to Ang II infusion had reverted to that seen when the subjects were consuming a high-salt diet. This occurred despite the fact that dextran suppressed PRA more slowly and caused a slightly smaller decrement in PRA and Ang II levels than saline. These observations are in keeping with available data from animals and humans that suggest that ambient Ang II levels, acting through a modulation of Ang II receptor density, determine the vascular smooth muscle responsiveness to Ang II. Most intriguing about the present observations is the rapidity of this receptor modulation.

Although we observed similar degrees of volume expansion with the two agents saline and dextran, the present results suggest that the sodium and/or chloride content of the infusion solution provided a specific signal responsible for modulation of adrenal glomerulosa responsiveness. In contrast, the volume signal provided by saline and dextran was sufficient to inhibit renin secretion and modulate the renal and vascular responses to Ang II.

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