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, which result in not only changes in plasma Ang II concentration but also changes in target tissue responsiveness determined by the level of dietary sodium. With a high-sodium diet the vascular response to Ang II, especially the renal vascular response, is enhanced and the adrenal response is reduced. Conversely, when an individual consumes a low-salt diet, adrenal responsiveness is increased, and the vascular response is suppressed.

The mechanisms responsible for normal modulation of adrenal and vascular smooth muscle responsiveness to Ang II may differ. In the case of vascular smooth muscle, multiple studies have revealed a change in Ang II receptor number that appears to be adequate to account for the change in response with shifts in salt intake. The change in receptor number also appears to reflect ambient Ang II concentrations. In the case of the adrenal glomerulosa, there has been substantial controversy, with ambient Ang II concentrations appearing to have less of an influence. In humans the studies have been necessarily more indirect, but the observations are in accord with the more direct animal studies: angiotensin converting enzyme inhibition reverses the blunted renal vascular sensitivity to Ang II induced by restriction of salt intake but does not influence the response of the normal adrenal to Ang II.

Dietary sodium restriction-induced changes in adrenal responsiveness to Ang II require a period of time for their appearance. When salt-replete humans begin to restrict salt intake, 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 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.
We have used the protocol of Tuck et al.⁹ 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 beginning 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 infusion 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.¹⁰ 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-aminomipirinate (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.⁷ 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 sphygmomanometer (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.¹²

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>68±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).

Plasma aldosterone and Ang II concentrations correlated both during a high-salt (r=.68, P<.01) and low-salt (r=.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 and aldosterone concentrations during a high-salt

TABLE 2. Baseline Measurements During High- and Low-Salt Balance

<table>
<thead>
<tr>
<th>Parameter</th>
<th>High-Salt (n=5)</th>
<th>Low-Salt (n=5)</th>
<th>Low-Salt (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-Hour urinary sodium, mmol/d</td>
<td>225±22</td>
<td>15±4*</td>
<td>12±2*</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>220±80</td>
<td>970±420*</td>
<td>920±110*</td>
</tr>
<tr>
<td>Plasma renin activity, ng·L⁻¹·s⁻¹</td>
<td>0.50±0.08</td>
<td>1.84±0.34*</td>
<td>1.12±0.20*</td>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>14±3</td>
<td>34±3*</td>
<td>30±3*</td>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>190±30</td>
<td>190±30</td>
<td>250±30</td>
</tr>
<tr>
<td>Serum Na⁺, mmol/L</td>
<td>139±1</td>
<td>138±1</td>
<td>138±1</td>
</tr>
<tr>
<td>Serum K⁺, mmol/L</td>
<td>4.2±0.1</td>
<td>4.3±0.1</td>
<td>4.3±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM.  
*P<.05 compared with high-salt.
TABLE 3. Biochemical and Hematocrit Responses to Volume Expansion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>After</th>
<th>Before</th>
<th>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>141±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...
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 (○). 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).

Discussion

In this study, rapid saline and/or volume expansion had the anticipated effect on the circulating renin-angiotensin-aldosterone system. PRA and aldosterone levels that were raised by a low-salt diet were reduced by both saline- and dextran-induced volume expansion to levels appropriate to a high-salt intake. Three hours after completion of the acute volume expansion maneuvers, which induced similar falls in hematocrit, basal renal plasma flow and its response to Ang II infusion shifted to levels seen during high-salt intake. The adrenal glomerulosa response was more complex. Saline treatment returned aldosterone responsiveness to a level appropriate to a high-salt intake, whereas dextran infusion did not have the same effect, despite an identical influence on basal concentration.

As demonstrated in Fig 4A, the slopes of the relations between Ang II and aldosterone levels were markedly different when individuals consumed a low-salt versus a high-salt diet. Over the range of Ang II levels achieved, aldosterone responsiveness on a high-salt diet was shallow, whereas with salt restriction the relation became more steep. To clearly define this relation under salt-restricted conditions required that a full dose response to Ang II be obtained. Therefore, a subset of subjects was studied only during low-salt balance and with a range of doses of Ang II. The practicality of performing this multiple dose study in the individuals after saline and dextran infusions was precluded by our desire to identify, within the shortest time possible, whether changes in adrenal and renal responsiveness to Ang II had occurred. Additionally, the limitations on blood drawing imposed by the rigorousness of the study days prevented us from testing more than one Ang II infusion rate. Clearly, the renal blood supply and peripheral vascular responses had shifted during the 7-hour time interval separating the start of the volume expansion and the Ang II infusions. The observation that saline likewise shifted adrenal responsiveness to Ang II during that same time period, whereas dextran administration did not, does not imply that a new steady state had been reached.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline Before Ang II</th>
<th>Saline After Ang II</th>
<th>Dextran Before Ang II</th>
<th>Dextran After Ang II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Na⁺, mmol/L</td>
<td>140±1</td>
<td>140±1</td>
<td>137±1*</td>
<td>137±1*</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>
</tr>
<tr>
<td>Cortisol, nmol/L</td>
<td>190±30</td>
<td>140±30</td>
<td>170±30</td>
<td>140±30</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>
</tr>
<tr>
<td>Angiotensin II, pmol/L</td>
<td>21±2</td>
<td>44±4</td>
<td>25±4</td>
<td>39±7</td>
</tr>
</tbody>
</table>

Ang II indicates angiotensin II. Values are mean±SEM. *P<.05 vs saline infusion.
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 expres-
sion 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 aldoste-
ronesecretagogues acutely enhance secretion by in-
creasing the rate of the early pathway (synthesis of
pregnenolone from cholesterol), it is the increased
activity of aldosterone synthetase that appears to ac-
count for most, if not all, of the effect of salt restriction
on adrenal sensitivity. The slow onset of adrenal sensi-
tization is compatible with the induction of this biosyn-
thetic 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.20
Likewise, ANH has potent inhibitory effects on both
renin and aldosterone secretion, particularly in sodium-
restricted subjects. Shenker21 has shown that ANH
infusion into sodium-restricted normotensive subjects
promptly reduced both PRA and aldosterone to levels
similar to high-salt balance. However, Tuchelt et al,23
using similar maneuvers, showed that adrenal respon-
siveness 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 metoclopramide increases basal aldoste-
rone 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 endog-
enous 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 aldoste-
ronereception, 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 angio-
tensin I concentrations. Thus, the physiological deter-
mintants of sodium-regulated changes in adrenal respon-
siveness 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 aldoste-
ronereception thus might contribute to a delay in
natriuresis, as has been observed acutely after saline
infusion or chronically when dietary sodium is al-
terred. 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, de-
termine 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
clearance 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 glomeru-
losa 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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References


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