Arterial and Venous Angiotensin II in Normal Subjects

Relation to Plasma Renin Activity and Plasma Aldosterone Concentration, and Response to Posture and Volume Changes

W. GORDON WALKER, M.D., MICHAEL A. MOORE, M.D.,* JOHN S. HORVATH, M.D.,† and PAUL K. WHELTON, M.D.

SUMMARY Plasma renin activity, arterial and venous angiotensin II (A II) concentrations, and plasma aldosterone concentration were measured in 16 normal subjects (mean age = 34 years) after 8 hours of recumbency, following 2 hours of ambulation, and again 30 minutes after administration of furosemide intravenously. Measurements were obtained during periods of sodium restriction and again during sodium surplus. Both arterial and venous A II exhibited a 3-fold increase from low values of 8.8 ± 2.5 and 8.6 ± 2.5 pg/ml of plasma, respectively, during recumbency on high sodium intake to values of 23.9 ± 4.1 and 26.5 ± 6.2 pg/ml, respectively, following intravenous furosemide during sodium restriction. Corresponding values for aldosterone exhibited a 5-fold rise from 5.6 ng/100 ml to 32.0 ng/100 ml, whereas plasma renin activity (PRA) measured by an in vitro assay exhibited a 20-fold rise from 0.6 ± 0.2 ng of angiotensin I (A I) generated per ml per hour to 13.1 ng/ml per hour. Despite the disparity in the magnitude of these increases, significant correlations were identified between all four of the measured parameters, indicating a major role of the renin-angiotensin system in regulating aldosterone output in response to volume and posture-related stimuli. Values of arterial and venous immunoreactive A II were closely correlated (r = +0.72, P < 0.005), but significant differences were demonstrated between low and high salt periods, suggesting that changes in metabolism of A II in the peripheral circulation may occur during sodium restriction.

THE DEMONSTRATION of an increase in aldosterone secretion and excretion in response to infusion of angiotensin II (A II) by Laragh et al.1 and Genest et al.2 identified the role of the renin-angiotensin system in aldosterone output. Subsequent studies characterized the response of this system to sodium intake, posture, blood volume and extracellular fluid volume, and related stimuli.3-4 From these data it was inferred that a change in plasma renin activity (PRA) was associated with a concordant change in plasma concentration of A II, thereby controlling the rate of aldosterone production.7

Attempts to confirm the existence of this sequence directly by measurement of plasma A II under similar conditions have produced conflicting information. Nielsen and Moller* used bioassay techniques but failed to detect changes in A II in response to salt depletion alone, although the addition of diuretics produced increased levels of angiotensin II in five of seven normal subjects. Radioimmunoassay has increased the sensitivity of measurement of A II but use of this method has not consistently produced data in accord with earlier studies in which only renin or renin plus aldosterone were measured. Page et al.9 Gocke et al.10 and Brown et al.11 noted significant increases in A II in response to severe sodium restriction in normal subjects. Brown et al. also identified a significant correlation between levels of A II and plasma aldosterone. Catt and associates12 failed to find significant changes in plasma A II levels in response to posture or sodium restriction but demonstrated an increase following chlorothiazide administration. Mendelsohn et al.13 also failed to note an increase in A II with sodium deprivation, although plasma aldosterone values rose. On the basis of similar data, Best and associates14 concluded that the dissociation implied an alternate mechanism of control of aldosterone.

We have used an assay developed in our laboratory15 to compare responses in arterial and venous A II, renin, and aldosterone in normal subjects exposed to variations in salt intake and to administration of diuretics. Significant correlations were demonstrated between these four variables as they changed in response to the imposed stimuli.

Methods

Sixteen normal volunteers (average age, 34 years; range, 24-47) were admitted to the clinical research ward of the Johns Hopkins Hospital after the purpose of the study had been explained and their consent obtained. Each was maintained on a regular diet permitting salt ad libitum plus the additional administration of 2 g of sodium chloride in tomato juice three times daily (salt supplement to diet = 102
probably is a significant fraction of the immunoreactive
dose of 20 mg of furosemide (Lasix) was given intrave-
cumbency and before the subject had risen from the supine
the .3-8 hexapeptide was available. This influences the
high enough to provide significant interference with A II
cross-reactivity with A I. Levels of A I in plasma measured
samples measured in duplicate (within assay) is 14%. All
were drawn in plastic syringes containing 1,000 U of purified
Nielsen et al. 18 and used in the assay. Under the standard
conditions outlined by Haber and associates' 6 Antibody to angioten-
sin I (A I) was raised in rabbits after immunization with A I
conjugated to albumin and administered with Freund's
adjuvant. 17 125 I-labeled A I was prepared by the method of
radioimmunoassay procedure developed in this laboratory
under the standard 1-g salt diet from the hospital dietary depart-
ment for an additional 5 days. At the end of the 5-day
period of sodium restriction, blood samples were again
obtained. Daily urinary sodium and creatinine were deter-
mained to monitor sodium excretion. Blood pressure and
body weight were monitored during both periods.

COLLECTION OF BLOOD SAMPLES

All blood samples (venous and arterial) were obtained at
approximately 8 a.m. after a minimum of 8 hours of re-
cumbency and before the subject had risen from the supine
position after the night's sleep. After 2 hours of ambulation,
arterial and venous blood were again obtained and then a
dose of 20 mg of furosemide (Lasix) was given intrave-
ously. Thirty minutes after furosemide had been adminis-
tered, arterial and venous blood samples were drawn for a
third time. The procedure was identical during high and
low sodium intake periods and provided six samples from
each subject.

Blood samples were drawn into chilled plastic syringes
and immediately transferred to chilled centrifuge tubes,
centrifuged at 2,000 rpm at 4°C, separated, frozen, and
stored at -20°C until assayed.15 ' 6 All blood samples for
measurement of renin and A II were drawn into ethylenedi-
aminetetraacetic acid (EDTA) but samples for aldosterone
were drawn in plastic syringes containing 1,000 U of purified
beef lung heparin; otherwise all blood samples were handled
in an identical fashion.

MEASUREMENT OF PLASMA RENIN ACTIVITY

PRA was measured by the radioimmunoassay technique
described by Haber and associates14 Antibody to angioten-
sin I (A I) was raised in rabbits after immunization with A I
conjugated to albumin and administered with Freund's
adjuvant.17 125 I-labeled A I was prepared by the method of
Haber et al.18 and used in the assay. Under the standard
variables in the supine and erect positions also varied inversely with the total 24-hour
sodium excretion when measured for the recumbent patient (r = 0.471, P < 0.05)
and after 2 hours of ambulation (r = 0.529, P < 0.02). The magnitude of change in PRA between the supine and erect positions also varied inversely with the total 24-hour sodium excretion (r = 0.529, P < 0.02).
Table 1  Mean Values for Plasma Renin Activity (PRA), Arterial and Venous Angiotensin II (A II), and Plasma Aldosterone Concentrations in 16 Normal Individuals (14 Male, 2 Female)

<table>
<thead>
<tr>
<th></th>
<th>Supine</th>
<th>Erect</th>
<th>Furosemide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High sodium intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>0.6 (±0.2)</td>
<td>1.7 (±0.3)</td>
<td>2.7 (±0.6)</td>
</tr>
<tr>
<td>Venous A II (pg/ml)</td>
<td>8.6 (±2.5)</td>
<td>9.5 (±2.5)</td>
<td>14.6 (±3.0)</td>
</tr>
<tr>
<td>Arterial A II (pg/ml)</td>
<td>8.8 (±2.5)</td>
<td>12.2 (±4.1)</td>
<td>16.6 (±6.6)</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/100 ml)</td>
<td>5.6 (±1.1)</td>
<td>8.8 (±1.8)</td>
<td>11.5 (±2.2)</td>
</tr>
<tr>
<td><strong>Low sodium intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>4.4 (±1.5)</td>
<td>9.9 (±2.3)</td>
<td>13.1 (±2.1)</td>
</tr>
<tr>
<td>Venous A II (pg/ml)</td>
<td>18.5 (±4.8)</td>
<td>23.8 (±5.7)</td>
<td>26.5 (±6.2)</td>
</tr>
<tr>
<td>Arterial A II (pg/ml)</td>
<td>16.1 (±4.6)</td>
<td>18.8 (±4.9)</td>
<td>23.9 (±4.1)</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/100 ml)</td>
<td>16.8 (±4.3)</td>
<td>21.8 (±3.6)</td>
<td>32.0 (±5.2)</td>
</tr>
</tbody>
</table>

Measurements were made (1) after a minimum of 8 hours of recumbency (supine); (2) after 2 hours of ambulation (erect); and (3) 30 minutes after 20 mg of intravenous furosemide. Results are given as mean values (±SEM).

* Average sodium excretion on day of measurement = 298 mEq.
† Average sodium excretion on day of measurement = 22.7 mEq.

Arterial samples were available, a significant correlation likewise was demonstrated between arterial A II and renin ($r = +0.33; n = 75; P < 0.005$). The values are included in Table 2. In similar fashion, comparison of arterial and venous values for A II for all samples yielded a highly significant correlation ($r = +0.72; n = 76; P < 0.005$). The relation between venous and arterial A II, defined from these data, (Fig. 2) was: venous A II = 4.45 (±3.66) + 0.69 (±0.16) arterial A II, where the values in parentheses represent the 95% confidence limits of the intercept and slope, respectively. Thus, under the conditions of the present study, venous A II concentration was 70% of the simultaneously obtained arterial value for the entire data set, a finding in keeping with the observations reported by Catt et al. It does appear from the present study, however, that there are differences that depend on the level of salt intake (see below).

Arterial A II and PRA also correlated significantly with plasma aldosterone concentration. For the ungrouped data, arterial A II vs. aldosterone yielded an $r = +0.28; n = 81; P < 0.02$. For PRA vs. aldosterone the corresponding values were $r = +0.28; n = 91; P < 0.02$. Thus for the total group of observations, significant correlations were identified between PRA, arterial and venous A II, and plasma aldosterone. Further examination of the data included evaluation of the influence of sodium intake, posture, and intravenous administration of diuretics on renin-angiotensin aldosterone system.

Responses of PRA, A II, and Aldosterone to Sodium Intake Posture and Diuretics. The influence of sodium intake, posture and administration of diuretics was evaluated from the data as grouped in Table 1. Correlations between each of the four variables (PRA, arterial A II, venous A II, and aldosterone) were tested using the mean values of the six sets of data obtained from the subjects in the supine and upright positions and following intravenous furosemide on both low and high sodium intake. Results are shown in Figures 3 and 4 and values for all the correlation coefficients are presented in Table 2. All correlation coefficients are highly significant ($P < 0.005$ for 4 degrees of freedom), indicating concordant responses in PRA, arterial and venous A II, and plasma aldosterone in response to the physiological stimuli of postural change and alterations in sodium intake as well as to administration of diuretic.

**RELATION BETWEEN ARTERIAL AND VENOUS A II**

In addition to the high correlations between arterial and venous A II described above, comparison of arterial and venous A II from the high and low salt periods revealed a difference in behavior that was significant. Separate calculation of the relation between venous and arterial A II for the high and low salt periods yielded the following regression equations:

$$
A_{\text{upright}} = 0.33 \cdot A_{\text{supine}} + 1.08 \\
A_{\text{upright}} = 0.32 \cdot A_{\text{supine}} + 0.89
$$
TABLE 2  Correlations between Plasma Renin Activity (PRA), Arterial and Venous Immunoreactive Angiotensin II (A II) and Plasma Aldosterone in Normal Subjects

<table>
<thead>
<tr>
<th></th>
<th>Arterial A II</th>
<th>Venous A II</th>
<th>Plasma Aldosterone</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group means</td>
<td>Individual</td>
<td>Group means</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>PRA</td>
<td>+0.92</td>
<td>&lt;0.005</td>
<td>+0.96</td>
</tr>
<tr>
<td>Arterial A II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+0.72</td>
<td>&lt;0.005</td>
<td>+0.95</td>
</tr>
<tr>
<td>Venous A II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+0.42</td>
<td>&lt;0.005</td>
<td>+0.94</td>
</tr>
</tbody>
</table>

r = correlation coefficient; P = probability that correlation this great or greater could have occurred by chance alone.

For high salt: venous A II = 4.4 (±1.5) + 0.53 (±0.07) arterial A II.

For low salt: venous A II = 3.8 (±3.5) + 0.82 (±0.14) arterial A II.

The intercepts of these two equations are nearly identical and probably not different from zero. There does appear to be a significant disparity between the two slopes (regression coefficients). A test of this disparity (null hypothesis; \( t = \text{difference between slopes} / \text{SE of difference} = 0.29 / 0.118 = 2.45; P < 0.025; n = 70 \) establishes the difference as significant.20 Thus during the high salt period the relative arteriovenous extraction, or conversion to metabolites that were not immunologically reactive, appeared to be significantly greater than that observed during the low salt period.

Inspection of the data from which these equations are calculated (fig. 5A and B), however, reveals this difference is not a uniform one that characterizes all individuals subjected to sodium restriction. Instead, the different slope for the low sodium group appears to result from nine observations in which venous A II concentration exceeded the corresponding arterial value by a substantial amount. In the remainder, the relation between arterial and venous A II differs only moderately, if at all, from that seen in the high salt group. It thus appears that the major part of the difference may be attributed to production of A II in the peripheral capillary beds in some individuals on restricted sodium intake.

Discussion

The present study reveals a highly significant positive correlation between PRA, arterial and venous A II, and aldosterone when the mean value for each of these parameters is compared for the six sets of conditions defined by variations in posture, sodium intake, and responses to diuretics (Tables 1 and 2). When similar comparisons were made for the individual observations from each subject in

![Figure 2](plasma-renin-activity-mpg/ml/hr.png)

**Figure 2**  Relationship between mean values of plasma renin activity, in ng (mg)/ml per hour, and the corresponding mean values for arterial (left) and venous (right) immunoreactive A II. A highly significant correlation is evident in plasma renin activity and both arterial and venous values. Bars represent ±1 SEM. Significance of correlation coefficient was evaluated for 4 (n - 2) degrees of freedom and data are included in Table 2.

![Figure 3](plasma-renin-activity-mpg/ml/hr.png)

**Figure 3**  Relationship between mean values for plasma renin activity [ng (mg)/ml per hour], arterial and venous immunoreactive A II [ng (mg)/ml], and aldosterone [ng (mg)/100 ml] for each set of observations. Symbols have same significance as those in Figure 2. Bars represent ±1 SEM. Correlation coefficients (r) and significance levels (P) are shown in lower right corner of each graph.
Figure 4. Relationship between simultaneously measured arterial and venous values of immunoreactive A II, in pg (μg)/ml. A highly significant correlation exists (r = +0.72; P < 0.005) between these two measurements although it is apparent that substantial disparities were occasionally encountered (see Fig. 5A and B).

The values for the arterial and venous concentrations of immunoreactive A II reported here are comparable to the values in the two previous reports in which simultaneous arterial and venous measurements were made.12-25 The supine and erect venous values for high salt intake in this study agree well with the values of Brown et al.11 for five subjects on high sodium intake, but are slightly less than the values reported by Catt and associates12 for a larger group in whom salt intake was not always specified. They are also somewhat below the values reported by Page et al.9 and Best et al.14 although these differences may reflect differences in salt intake and the smaller number of subjects studied.11-24

The values for arterial and venous measurements of immunoreactive A II reported here are comparable to the values in the two previous reports in which simultaneous arterial and venous measurements were made. The data are the same as shown in Figure 4, but Figure 5A (left) shows only those values obtained during sodium restriction and SB (right) presents corresponding data from the period of sodium surfeit. The heavy line in each graph is constructed from the regression line calculated from the data depicted therein. For sodium restriction this equation is Y = 3.8 (±3.5) + 0.53 (±0.07) X. The 95% confidence limits for the regression equations are indicated by the broken lines. The regression slopes are significantly different for the two graphs but it is likely that the difference is mainly due to a tendency for venous values of A II to exceed the simultaneous arterial values for subjects on low salt intake. This did not occur during a high sodium intake.

The present observations confirm and extend the studies of Page et al.9 and Brown et al.11 which tend to show consistent responses for renin, A II, and aldosterone to alterations in sodium intake. Catt et al.22 and Best et al.14 reported a tendency for A II to rise on assumption of the upright posture or on ambulation, although the difference did not achieve statistical significance, possibly owing to the relatively small numbers of subjects in each study. It seems likely that the failure to demonstrate a correlation between circulating A II and salt intake in other studies also may be attributable in part to the small numbers of subjects studied.12-24
The relationship between immunoreactive A II in arterial and venous plasma in the present study suggests that some change may occur in the metabolism of A II when there is a marked change in sodium intake. For the complete study (75 paired observations) there is a highly significant correlation between arterial and venous values (r = +0.72; P < 0.005). The regression coefficient (slope) of the relation defining venous A II as a function of arterial A II indicates that the venous value averages approximately 70% of the simultaneous arterial value. This figure agrees well with the value reported by Catt et al.22 from data that included observations on both normal and disease states. When the data from the periods of high and low sodium intake were examined separately in the present study, the significantly greater slope for the sodium-restricted group appeared to be related to the frequent occurrence of venous values for A II that substantially exceeded simultaneous arterial values. Possible explanations for this difference include an increased concentration of A II in the arterial plasma delivered to peripheral capillary beds in some individuals on sodium-restricted diets, increased levels of peripheral converting enzyme27-28 in some individuals, or a combination of the two. A comparatively sensitive assay for measurement of A I in arterial and venous plasma is necessary to decide which of these two possibilities is more likely.

The question whether, in addition, there is a more avid uptake of A II in peripheral capillary beds during high sodium intake, as suggested by Brunner et al.,29 cannot be answered from the present data. Inspection of the distribution of the data in Figure 5A and 5B, however, suggests that this also may contribute to the observed differences between the high and low sodium groups. The demonstration that infusion of A II at an increasing rate leads to progressive increase in peripheral uptake (or conversion to a nonimmunoreactive metabolite) of A II during both low and high sodium intake29 does not preclude peripheral production of A II under physiological circumstances of sodium restriction. Proof of this occurrence will require simultaneous measurement of circulating A I levels and the demonstration of a corresponding drop in the concentration of A I across peripheral capillary beds in concert with the increase in A II concentration.

The report of Catt et al.12, and Cain et al.29 that a major constituent of the immunoreactivity in venous blood is the 3-8 hexapeptide of A II also raises the possibility that metabolism or degradation of A II could be quantitatively different from a function of differing salt intake. At present the quantity of blood required for the isolation and quantification of the data in Figure 5A and 5B, however, suggests that metabolism or degradation of A II could be quantitatively different from that of a corresponding drop in the concentration of A I across peripheral capillary beds in concert with the increase in A II concentration.

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The help of E. Barnes and E. Chandler and the nursing and dietary staff of the Clinical Research Center, and the technical and administrative help of Judith Hermann, Carl Geyer, and Sharon Manson are gratefully acknowledged.

References

Plasma Renin Activity during Exercise in the Dog

MEYER D. LISCHITZ, M.D., and LAWRENCE D. HORWITZ, M.D.

SUMMARY Previous workers have suggested that a rise in plasma renin activity (PRA) may mediate some of the hemodynamic changes associated with exercise. To test this hypothesis in nine dogs chronically instrumented for measurement of aortic pressure (catheter) or cardiac output (ascending aorta electromagnetic flow probe) PRA was measured by radioimmunoassay in blood samples drawn before and during running on a level treadmill at 4–8 miles per hour. Exercise caused increases in heart rate from 96 ± 5 (SE) to 186 ± 7 beats/min, cardiac output from 2.8 ± 0.3 to 6.2 ± 0.6 liters/min, and mean aortic pressure from 115 ± 5 to 132 ± 5 mm Hg (P < 0.01). Mean PRA was 6.6 ± 0.7 (SE) ng of angiotensin I/ml per 3 hours before and 7.6 ± 1.2 ng Ang 1 during exercise, values that are not different statistically. Propranolol reduced PRA at rest from 8.6 ± 1.1 to 5.9 ± 1.1 ng Ang 1 (P < 0.05), but there was no significant difference between resting and exercise levels, although the increments in heart rate, cardiac output, and mean aortic pressure were reduced. Standing on hindlimbs for 5 minutes did not cause a change in mean aortic pressure or PRA. However, administration of pentolinium reduced mean aortic pressure, and PRA rose from 6.0 ± 1.1 to 9.8 ± 1.5 ng Ang 1. Exercise, with or without β-adrenergic blockade, does not cause increased PRA in conscious dogs in which the renin-angiotensin system is normally responsive.

To clarify the relationship of PRA to sympathetic tone during exercise, we obtained renin levels by radioimmunoassay of blood samples drawn from dogs performing running exercise of varying speeds and duration. Simultaneous hemodynamic measurements confirmed the severity of the stress. Studies also were made of the effect on PRA of β-adrenergic blockade with propranolol at rest and during running, of postural change, and of hypotension induced by a ganglionic blocking agent, pentolinium.

Methods

Nine dogs, weighing 15.5–22.7 kg, were trained to run on a level treadmill. After training, in seven dogs a sterile thoracotomy was performed under sodium pentobarbital anesthesia (30 mg/kg, iv). An electromagnetic flow probe was implanted around the proximal portion of the ascending aorta, a solid state pressure transducer (Konigsberg P18) was implanted in the left ventricle, and 18-gauge polyvinyl catheters were implanted in the left internal mammary artery, the left atrium, and the left jugular vein.15 In two dogs no thoracotomy was performed and surgery was limited to implantation of catheters in the left jugular vein and the aorta via the left carotid artery. These dogs were prepared in this fashion to ensure that the more extensive instrumentation in the remaining dogs did not influence PRA responses. An interval of at least 3 weeks was allowed for recovery from surgery. At the time of study, each dog could exercise at the same levels as had been attained before surgery.

Exercise was performed with the treadmill at 0° grade. Resting measurements were obtained with the dog standing quietly on the treadmill. All dogs ran for 3 minutes at 6–8 miles per hour (mph). Four dogs continued to run for a total...
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