A Delayed Suppression of the Renin-Aldosterone Axis following Saline Infusion in Human Hypertension

MICHAEL L. TUCK, M.D., GORDON H. WILLIAMS, M.D., ROBERT G. DLUHY, M.D., MARTIN GREENFIELD, M.D., AND THOMAS J. MOORE, M.D.

SUMMARY  The purpose of this study was to compare the acute suppressibility of the renin-angiotensin-aldosterone (RAA) axis in normotensive (n = 23) and essential hypertensive (n = 62) subjects. Only those hypertensive subjects with normal plasma renin activity (PRA) levels (sodium restricted, upright) were included in the study. Acute suppression of the RAA axis was determined by measuring PRA, plasma angiotensin II (AII), and plasma aldosterone (PA) at frequent intervals during the infusion of isotonic saline (500 ml/hour for 6 hours). Although all parameters fell significantly from control levels by 20-30 minutes in the normotensive subjects, we found that 60% of the hypertensive subjects showed no significant decline in PRA or PA until 120-240 minutes after beginning the infusion. The other hypertensive subjects showed normal RAA suppression. In addition, while there were no significant differences between the three groups in control PRA or PA levels, we found that the PA levels from 30 to 240 minutes during the saline were significantly higher (P < 0.01) in the hypertensive subjects with delayed suppression. That there were two distinct populations in the hypertensive group was suggested by the bimodality of the frequency response curve, with peaks occurring at 30 and 240 minutes. These studies indicate an abnormality in the acute suppression of the RAA axis in a substantial proportion of subjects with normal renin essential hypertension. Since previous studies in normal subjects have reported that the early phase of response to saline infusion is related to the sodium ion per se and not to intravascular volume expansion, we have come to the conclusion that the present data are consistent with the hypothesis that the delayed suppression hypertensive group has a diminished ability to respond to the sodium ion.

PREVIOUS STUDIES of the renin-angiotensin-aldosterone axis in subjects with essential hypertension have occasionally revealed abnormalities in aldosterone secretion, or more commonly hyperresponsive plasma renin activity (PRA) following volume depletion. However, most studies have assessed these responses before and after long-term manipulation of sodium balance or at a single point in time. Therefore, the present study was performed to determine whether there are abnormalities in the short-term regulation of renin, angiotensin, or aldosterone in essential hypertension. The acute response of these parameters was assessed in sodium-restricted subjects following intravenous volume expansion with isotonic saline.

Methods

Sixty-two subjects with essential hypertension (43 male, 19 female) were studied on the Clinical Research Center of the Peter Bent Brigham Hospital and their responses were compared with those of 23 normal controls (18 male, 5 female) studied under identical conditions. The age range of normal subjects was 20-52 years (mean = 34 years). The patients with high and low renin essential hypertension also were excluded, since it was required that all subjects have a plasma renin level in the normal range after 3 hours of upright posture following the ingestion of a 10-mEq sodium diet for 5-6 days. The normal range in our laboratory is 2.4-15.0 ng/ml per hour.

The protocol was approved by the Human Subjects Committee of the Peter Bent Brigham Hospital and written informed consent was obtained from all subjects.

PROTOCOL

All antihypertensive medications were discontinued at least 2 weeks prior to admission. During hospitalization, all subjects were maintained on a constant activity pattern simulating normal daily activity. On the day of admission, the subjects were given an ad lib. diet, and on the next day blood for supine PRA was obtained at 8 a.m. Then the subjects were fed an isocaloric diet containing 10 mEq of sodium and 100 mEq of potassium. Urine specimens were collected and analyzed for sodium, potassium, and creatinine. When subjects had achieved metabolic balance, usually...
on the 5th or 6th day of sodium restriction, after an overnight fast and maintenance of the supine position for at least 12 hours, 0.9% sodium chloride was infused at a constant rate of 500 ml/hour for 6 hours beginning at 8 a.m. Blood samples were obtained after 30 and 60 minutes and then 2, 4, and 6 hours after the beginning of the infusion. In 20 hypertensive subjects and 15 normal controls, additional samples were obtained at 10 and 20 minutes. The following determinations were performed on all samples: PRA, plasma aldosterone (PA), plasma cortisol, serum sodium and potassium, and hematocrit. A 24-hour aldosterone secretory rate was estimated on the day prior to the saline infusion and 24-hour collections of urine for sodium, potassium, and creatinine were obtained on the day of the saline infusion. In 18 hypertensive subjects the protocol was modified. Plasma angiotensin II (A II) levels also were measured, fractional 24-hour collections were performed at 6-hour intervals during the saline infusion, and an aldosterone secretory rate was measured after 2 days of a 200-mEq sodium intake.

LABORATORY PROCEDURE

All blood samples were immediately centrifuged and the plasma was separated and frozen until time for assay. Samples for PRA and A II levels were drawn with ethylenediaminetetraacetic acid (EDTA) as the anticoagulant; heparin was used as the anticoagulant in blood obtained for cortisol and aldosterone determinations. Serum and urine sodium and potassium levels were measured by flame photometry with lithium as an internal standard. PA and cortisol were measured by radioimmunoassay techniques, as previously described. PRA and A II values were measured by a double antibody radioimmunoassay method. The values are reported in reference to the World Health Organization Standards 71-328 and 70-302, respectively. Therefore, the absolute values may differ somewhat from those reported previously from this laboratory. Aldosterone secretion rates were estimated by a radioimmunoassay technique. Statistical analyses were performed as described by Snedecor and Cochran, and the results are expressed as mean ± SEM. The statistical analyses were either by a two-way analysis of variance and P values found in Dunnett’s tables or by Student’s t-test for group data. Chi-square analysis was used for comparison of descriptive parameters in Table 2. Significant differences are P < 0.01 unless otherwise indicated.

Results

NORMAL SUBJECTS

On the day prior to the saline infusion, normal subjects excreted 9 ± 2 (st) mEq of sodium/24 hours, and 88 ± 7 mEq of potassium/24 hours. On the day of saline infusion (460-mEq sodium load) normal subjects gained an average of 1.1 ± 0.1 kg and excreted an average of 168 ± 16 mEq of sodium/24 hours (Fig. 1). On the day of sodium loading, the control supine PRA was 3.8 ± 0.1 (range, 1.2-7.3) ng/ml per hour, and the PA level was 32 ± 2 (range, 15-65) ng/100 ml (Table 1 and Fig. 2). At 60 minutes, after the infusion of 500 ml of isotonic saline, the PA levels had fallen to 52%, while PRA had fallen to 50% (Fig. 3). The decrements were significant at both 30 and 60 minutes, with a P value of less than 0.001 (Dunnett tables). The steep decline in the levels of these two parameters persisted until between 60 and 90 minutes, when a plateau was reached, with a more gradual decline until the end of the infusion at 360 minutes (Table 1). At that time the mean PA levels had fallen to 6 ± 1 (range, 2-12) ng/100 ml and PRA to 0.5 ± 0.1 (range, 0.1-1.5) ng/ml per hour. Plasma A II levels declined in a fashion parallel to that of renin and aldosterone in the 10 subjects in whom it was measured, while plasma cortisol levels showed only a normal diurnal decline (Table 1).

HYPERTENSIVE SUBJECTS

After saline infusion the responses of the subjects with essential hypertension fell into two distinct subgroups. The first group showed responses indistinguishable from the normotensive subjects and is termed the “normal suppres-
Responses to Saline Infusion in Normotensive Subjects and Subjects with Normal Renin Essential Hypertension, Grouped According to the Response of Their Renin-Aldosterone Axis to Saline Infusion.

### TABLE 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Normotensive subjects</th>
<th>Normal suppression</th>
<th>Delayed suppression</th>
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<tr>
<td></td>
<td>Plasma renin activity (ng/ml per hr)</td>
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<tr>
<td>0</td>
<td>3.8 ± 0.4</td>
<td>3.7 ± 0.4</td>
<td>3.6 ± 0.3</td>
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<td>1.9 ± 0.2*</td>
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<td>3.3 ± 0.4</td>
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<tr>
<td>90</td>
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<td>1.8 ± 0.2*</td>
<td>2.8 ± 0.2</td>
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<td>1.6 ± 0.2*</td>
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<td>240</td>
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<tr>
<td>360</td>
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<td>Plasma aldosterone (ng/dl)</td>
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<tr>
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<td>33 ± 3</td>
<td>30 ± 5</td>
<td>27 ± 2</td>
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<tr>
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<tr>
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<td>Plasma cortisol (µg/dl)</td>
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<td>13 ± 1</td>
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<td>5 ± 1</td>
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</table>

Results are expressed as mean ± SEM.
* P < 0.01, significantly less than control.

There were no significant differences in the basal PRA [3.6 ± 0.3 (range, 1.4–9) ng/ml per hour] or PA levels [27 ± 2 (range, 10–60) ng/100 ml] in the delayed suppression hypertensive subjects compared to the normal suppression or normotensive subjects (Fig. 2). However, there was a significant difference in the response of these subjects during and after the infusion of saline. Significant decrements in the levels of PRA and PA did not occur at either 30 or 60 minutes (Fig. 3 and Table 1). While significant decrements (P < 0.01) in PA and PRA had occurred by 30 minutes in both normotensive subjects and the normal suppression hypertensive subgroup, a significant decline (P < 0.01) in these parameters did not occur in the delayed suppression hypertensive subgroup until 120 minutes. Furthermore, at each time interval the PA levels were significantly higher (P < 0.01) in the delayed subgroup than in either of the other two groups (Table 1 and Fig. 3). At 360 minutes the mean PA level was 9 ± 1 (range, 2–20) ng/100 ml in the delayed suppression subgroup, a value significantly greater (P < 0.01) than that recorded for either of the other two groups. Furthermore, in contrast to the normotensive subjects and the normal suppression hypertensive subjects (where PA levels had declined to 18–20% of control at 360 minutes), PA was only 33% of control in the delayed response subgroup. The decrements in PRA levels were also significantly less in the "normal suppression" subgroup. The second group showed a delayed suppression and is termed the "delayed suppression" subgroup. The two groups were defined on the basis of the time necessary to produce a significant (30%) decrement in the plasma levels of renin activity after the initiation of the infusion of 0.9% sodium chloride. The frequency distribution of their response was bimodal, with peaks occurring at 30 minutes and again at 240 minutes after the initiation of the saline infusion (Fig. 4). A nadir was reached between 60 and 90 minutes.

The mean PRA and PA responses to saline infusion in the normal suppression subgroup were not significantly different from those of normotensive subjects (Table 1). The basal recumbent PRA was 3.7 ± 0.4 (range, 1.2–8.7) ng/ml per hour with a PA of 30 ± 4 (range, 10–72) ng/100 ml (Fig. 2). After a 60-minute infusion of 500 ml of saline, the PRA levels had declined to 59% and PA levels to 53% of control (Fig. 3). As in the normotensive subjects, there was a plateauing of both parameters between 90 and 120 minutes, with a more gradual decline until the termination of the infusion. The mean PA level at the end of the infusion in these subjects was 6 ± 1 (range, 1–13) ng/100 ml with a PRA of 0.8 ± 0.1 (range, 0.1–2) ng/ml per hour (Table 1 and Fig. 3). Subjects in this subgroup gained 0.9 ± 0.1 kg as a result of the saline infusion and excreted 149 ± 12 mEq of sodium/24 hours. These values were indistinguishable from those for the normotensive subjects (Fig. 1).
FIGURE 3 Percent decrement from control of plasma renin activity and aldosterone in response to saline infusion in 23 normal controls and 62 subjects with essential hypertension divided into two groups on the basis of the response of their renin-aldosterone axis to saline infusion (mean ± SEM).

Figure 4 shows the frequency distribution of the time necessary to produce a significant (30%) decrement in plasma renin activity in response to saline infusion (500 ml/hour for 6 hours) in 62 subjects with normal renin essential hypertension.

The serum potassium level declined to a greater extent in the delayed suppression subgroup. Aldosterone secretion rates were not different on either high or low sodium intakes in those subjects in which this parameter was measured.

There were no significant differences in the age, percent female, percent black, or known duration of hypertension between the normal suppression and delayed suppression subgroups (Table 2). Also, there were no significant differences in the secondary effects of the hypertension, such as the mean systolic and diastolic blood pressure, serum sodium, potassium, creatinine, or the percent with left
ventricular hypertrophy. There was no evidence for a difference in state of volume depletion at the time of entrance into the study because the mean sodium and potassium excretion and the mean supine renin activity on the morning after admission were not significantly different (Table 2). Also, there was no indication of a greater degree of volume depletion following sodium restriction; the mean weight loss in the normal suppression subgroup (1.3 ± 0.3 kg) was not different from that in the delayed suppression subgroup (1.2 ± 0.2 kg). Likewise, the preinfusion 24-hour urine sodium and potassium excretion and diastolic and systolic blood pressures were not significantly different (Table 3). However, although systolic blood pressures did not significantly change pre- and post-saline infusion in either group, there was a significant (P < 0.02) increment in diastolic blood pressure in the delayed suppression subgroup (Table 3).

**Discussion**

It is well known that subjects with essential hypertension can be classified into low, normal, or high renin subgroups based on the responses of PRA to sodium restriction and upright posture. To minimize heterogeneity of the hypertensive subjects studied, the present investigation reports the responses to saline infusion only for subjects with normal plasma renin levels while the subjects were upright and on a sodium-restricted diet. Although it has been suggested that subjects with so-called normal renin essential hypertension are homogeneous in their hormonal patterns,14 the present study indicates that they can readily be divided into two groups. Normal suppression of PRA and PA following isotonic infusion was seen in 42%, while 58% showed delayed suppression.

There have been few studies to assess the acute response of the renin-angiotensin-aldosterone axis caused by volume expansion in subjects with essential hypertension. Kem and co-workers17 reported that the response of PRA and aldosterone was normal in 18 subjects with essential hypertension on an ad lib. diet before and after a 4-hour infusion of saline. Since, in the present study, both groups of hypertensive subjects had achieved substantial decrements in renin and aldosterone levels by 240 minutes, it is not surprising that abnormalities similar to those we have found were not observed in Kem’s subjects. Krakoff et al.18 reported the plasma renin responses in subjects with essential hypertension on a 95-mEq sodium intake following infusion of isotonic saline over a 60-minute period. The authors concluded that plasma renin fell to a variable degree. The hypertensive subjects also were classified as renin-responsive or nonresponsive according to the renin response to intravenous diuretic administration. However, examination of their data suggests that the renin-responsive hypertensive group can be divided into two groups. Only three of the nine subjects showed a significant (40–50%) decline in renin activity. In contrast to 46 mEq/3 hours for those individuals who showed a less significant fall in renin activity.
A major consideration is that the data simply reflect an arbitrary division of a homogeneous response pattern which is broader than that which occurs in normotensive subjects. Thus, it is legitimate to subdivide this group of subjects on the basis of closely interrelated variables only if indeed there are two different populations. This is most easily assessed by determining the time necessary to produce a significant decrement (30%) in renin activity (Fig. 4). The data clearly demonstrate a pattern of two populations rather than a continuum, with a peak to peak time interval of 210 minutes and a significant nadir occurring between 60 and 90 minutes. Thus, it is improbable that the two groups reflect the two extremes of the same population; more likely they are distinct populations. The difference in the response of these two groups could be related to different degrees of volume depletion prior to the infusion of saline. If the delayed response group was significantly more volume-depleted prior to saline administration, their response to the administration of sodium chloride could be less rapid. While no direct measurements of volume were made, several indirect parameters make it highly improbable that there were differences in the preinfusion degree of volume depletion. First, basal sodium-restricted PRA and PA levels in the two groups were comparable and were not different from values for normotensive subjects (Table I). Furthermore, the sodium excretion on the day prior to saline infusion and the day of admission to the hospital were not significantly different between the two groups. Finally, there were no significant differences in weight loss between the two groups as they came into sodium balance (Tables 2 and 3). It also is improbable that a greater degree of volume depletion existed in the delayed suppression subgroup on admission to the hospital. For all subjects, antihypertensive medications were discontinued 2 weeks prior to admission. Also, both groups had similar levels of sodium and potassium excretion on the day prior to sodium restriction; moreover, the supine plasma renin levels on the morning after admission were not significantly different between the two subgroups. Thus, while the delayed suppression subgroup responded as if they were more volume-depleted, there is no evidence to support such a thesis.

Assessment of additional factors revealed no significant differences between the two groups. Age, percent black or female, known duration of hypertension, level of blood pressure, evidence for vascular complications, or serum sodium, potassium, or creatinine were similar. However, the delayed suppression group did show a significant increment in diastolic blood pressure in response to the saline infusion. It is possible that the delayed suppression subgroup is less sensitive to a comparable degree of volume expansion. This could occur if there was a difference in the distribution of the saline, a higher receptor threshold for saline or volume expansion, or a sluggish response system. If a sluggish response system were the mechanism, a subnormal response to sodium restriction might be anticipated. Because none was found, either the response system is not altered or the chronicity of the stimulus from sodium restriction is able to overcome the deficiency. Alternatively, the data could be explained by an altered response to the infusion of the sodium ion per se. Recent studies on normal subjects have provided evidence for a sodium-dependent mechanism that can suppress renin and aldosterone secretion independently of changes in intravascular volume. While these studies do not distinguish between renin regulation by a sodium-dependent intrarenal mechanism vs. a change in extrarenal, extracellular fluid volume, they strongly suggest that sodium plays a role in regulating renin release that is independent of its ability to expand intravascular volume. Thus, it is possible that the delayed suppression seen in some subjects with normal renin essential hypertension reflects a loss of sensitivity of this sodium-sensing mechanism.

In summary, the present study suggests that a large percentage of individuals with normal renin essential hypertension show a delay in the suppression of the renin-angiotensin-aldosterone axis during and after the infusion of saline. These individuals also have a delayed natriuresis, gain more weight with a presumed greater expansion of extracellular fluid volume, and also have a greater increment in blood pressure. Since sodium loading enhances the pressor effect of A II, this sodium-retentive tendency and the failure to normally suppress A II levels in these subjects could combine to elevate arterial pressure. Thus, one mechanism for the maintenance of an elevated blood pressure could be a defect in the sodium-sensitive control of the renin-angiotensin-aldosterone axis which could result in altered sodium homeostasis and pressor activity in this subgroup.

References

13. Rayfield EJ, Rose LI, Dluhy RG, Williams GH: Aldosterone secretory
Electrophysiological Observations on the Digitalis-Potassium Interaction in Canine Purkinje Fibers

GARY J. ANDERSON, M.D., JOHN C. BAILEY, M.D., JOSEPH REISER, PH.D., AND ALAN FREEMAN, PH.D.

SUMMARY We studied the effects of elevating potassium concentration on the membrane potential of Purkinje cells exposed to toxic concentrations of acetylstronathidin or ouabain. Conventional intracellular microelectrode techniques were employed. Rapid elevation of $[K^+]_0$ from 2.7 to 5.4 mEq/liter resulted in an initial increase (more negative) in membrane potential of cells demonstrating ouabain-induced phase 4 depolarization. The increase in maximal diastolic potential occurred initially without suppression of phase 4 depolarization. In cells rendered inexcitable by ouabain or acetylstronathidin, elevation of $[K^+]_0$ consistently increased membrane potential and restored excitability. In four experiments automaticity was initiated within 2 minutes after the increase in $[K^+]_0$. Although automaticity reappeared, as maximal diastolic potential increased, the automatic rate slowed and then pacemaker activity was suppressed. Studies with $^3$H-ouabain showed that the increase in membrane potential paralleled $K^+$-induced release of $^3$H-ouabain from Purkinje cells. These studies suggest that elevation of $[K^+]_0$ reverses digitalis toxic manifestations in canine Purkinje fibers by causing release of cardiac glycosides bound to the membrane. The observed increase in membrane potential of ouabain-treated Purkinje fibers that occurred after $[K^+]_0$ elevation was considered to be mediated in part by restoration of the Na pump and by electrogenic pumping.

SINCE LOEWI first suggested the use of potassium salts to suppress digitalis toxicity, the nature of the potassium-digitalis interaction has been a subject of intense and persistent interest. Observations on man and animals have established that $K^+$ administration suppresses digitalis-induced arrhythmias and permits further administration of digitalis before the reappearance of arrhythmias. One of the mechanisms for this antiarrhythmic effect is suppression of automaticity, a well known effect of potassium. Müller reported in vitro studies demonstrating the electrophysiological consequences of the $K^+$-digitalis interaction with digitalis poisoned Purkinje fibers. After an increase in extracellular potassium concentration ($[K^+]_0$) action potentials appeared more normal and were associated with improved rise times and overshoots, and there was an increase in the maximal diastolic potential. Polimeni and Vassalle suggested that one mechanism for $K^+$ suppression of ouabain-induced arrhythmias was an increase in potassium conductance. This was based, in part, on an earlier study showing the elevation of $[K^+]_0$ increased potassium conductance of pacemaker cells in the Purkinje system.

Our initial studies showed that elevation of $[K^+]_0$ overcame digitalis-induced block and was consistently associated with an increase in membrane potential that was independent of suppression of phase 4 depolarization. The purpose of this report is to describe our observations on the $K^+$-digitalis interaction on canine Purkinje fibers.

Methods

Adult dogs weighing 10-20 kg were anesthetized with sodium secobarbital (30 mg/kg, iv). The heart was removed through a lateral thoracic incision and immediately placed...
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