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ALTERATIONS IN ALDOSTERONE BIOSYNTHESIS IN ESSENTIAL HYPERTENSIVES

BESS F. DAWSON-HUGHES, THOMAS J. MOORE, ROBERT G. DLUHY, STEPHEN PODOLSKY, AND GORDON H. WILLIAMS

SUMMARY We studied hypertensives with decreased adrenal responsiveness to infused angiotensin II (AI) to assess their responsiveness to other aldosterone secretagogues, ACTH and potassium, which are thought to stimulate aldosterone synthesis at sites different from one another and from AI. All subjects, following sodium restriction, received an infusion of AI in increasing doses (0.1-3 ng/kg per min). The increment in aldosterone between control and the highest infusion dose divided by the increment in plasma AI was used as the index of adrenal responsiveness. All normotensive controls (NC) had a ratio greater than 0.5. Hypertensives with a normal ratio were designated normal responders (NR) and those with a lower ratio were abnormal responders (AbR). The slope of the regression line between aldosterone and AI was significantly less for the AbR (0.02 ± 0.04) than for the NR (1.20 ± 0.02, P < 0.001) and the NC (1.00 ± 0.03, P < 0.001) groups. During infusion of cosyntropin in increasing doses (0.05-1.5 mIU/kg per 30 min), the aldosterone response of the AbR was significantly less than that of the NR (P < 0.018) or the NC (P < 0.05) groups. Similarly, after infusion of potassium (0.33 mEq/min), the increment in aldosterone in the AbR group (7.6 ± 2.2 ng/dl) was significantly less than that in the NR (14.2 ± 2.5 ng/dl, P < 0.05) and the NC (18 ± 5 ng/dl, P < 0.05) groups. Thus hypertensives with decreased aldosterone responsiveness to infused AI also had decreased responsiveness to infused ACTH and potassium, suggesting that their defect lies in the intracellular aldosterone biosynthetic pathway. Circ Res 49: 627-632, 1981

ABNORMALITIES in the renin-angiotensin-aldosterone axis in subjects with essential hypertension have been documented (Streeten et al., 1969; Collins et al., 1970; Moore et al., 1977; Dluhy et al., 1979). Included in these is the observation that some hypertensive subjects, when sodium restricted, have decreased aldosterone responsiveness during infusion of angiotensin II (AI) (Moore et al., 1977; Dluhy et al., 1979). The defect resulting in this decreased responsiveness has not been defined. In order to localize the defect, we studied the adrenal sensitivity of this subgroup to other aldosterone secretagogues, ACTH and potassium, which are thought to stimulate the adrenal zona glomerulosa at sites other than the AI receptor. If the hypertensives with decreased responsiveness to AI also have decreased responsiveness to ACTH and potassium, then the most likely site of the defect is intracellular. Alternatively, if their aldosterone responsiveness to ACTH and potassium is normal, then the defect probably resides at the AI receptor.

In this study, sodium-restricted subjects with decreased adrenal sensitivity to infused AI received infusions of ACTH and potassium. Their aldosterone responsiveness was then compared with that of both normotensive controls and hypertensive subjects with normal adrenal responsiveness to infused AI.

Methods

Fifteen patients with normal-renin essential hypertension were studied at the Clinical Research Center of the Peter Bent Brigham Hospital. Their responses were compared with those of 14 normal controls studied under identical conditions. The criteria for inclusion of hypertensive subjects in the study were as follows: plasma renin activity (PRA) during a posture study was normal, defined in our laboratory as 2.4-15 ng/ml per hr, on upright posture and a 10-mEq sodium diet (Tuck et al., 1975). Each subject had an outpatient supine diastolic blood pressure greater than 90 mm Hg determined on three different occasions and documented evidence of hypertension for at least 6 months and an average of 6 years before the study. Patients with renal disease, primary aldosteronism, pheochromocytoma, and Cushing’s syndrome were excluded by urinalysis, serum creatinine, plasma aldosterone, and 24-hour urine vanillylmandelic acid, metanephrine, and 17-hydroxycorticosteroid levels. Whereas all subjects studied had normal rapid-sequence intravenous pyelograms and normal 211H-labeled Hippuran renograms, renal arteriography—to absolutely exclude the possibility of renal arterial obstruction—was not performed. All hypertensive
medications were discontinued for at least 2 weeks prior to admission. The subjects were fed a constant, isocaloric diet of 10 mEq sodium and 100 mEq potassium throughout their hospitalization. Daily 24-hour urine samples were analyzed for sodium, potassium, and creatinine. Each study was started at 8 a.m. after the subject had been fasted and supine for 8 hours. Infusions were carried out in a randomized order. When the 15 hypertensive subjects had achieved metabolic balance, usually on the 5th day of sodium restriction, two supine control samples were drawn at least 30 minutes apart and the subjects then walked for 2 hours. A blood sample was obtained after 120 minutes of upright posture; all samples were analyzed for PRA, aldosterone, cortisol, sodium, and potassium.

Angiotensin II Infusion

The 15 hypertensives and 14 normal controls received an infusion of AII. Responses of nine of these normal controls have been previously reported (Moore et al., 1980). An intravenous catheter was placed in each of the subject's arms, one for infusion and one for blood sampling. Blood pressure was monitored by using an indirect recording sphygmomanometer (Arteriosonde, Roche) with the cuff placed on the arm above the sampling catheter. After the basal period of 30 minutes, during which time 5% dextrose in water was infused at 10 ml/hr into each arm, angiotensin amide (Hypertensin, Ciba) was infused consecutively at the rates of 0.1, 0.3, 1.0, and 3.0 ng/kg per min for 30 minutes each. A blood sample was drawn at the end of the control period and each incremental infusion dose and analyzed for aldosterone, AII, PRA, cortisol, sodium, and potassium.

ACTH Infusion

Thirteen hypertensive patients and 5 normal controls received an infusion of cosyntropin, a synthetic alpha 1-24-ACTH preparation, in 5% dextrose in water. An intravenous catheter was placed in each of the subject's arms, one for infusion and the other for sampling. After a basal period of 30 minutes, during which time 5% dextrose in water was infused at 10 ml/hr into each arm, cosyntropin was infused consecutively at the following rates: 0.05, 0.15, 0.5, and 1.5 mIU/kg per 30 min for 30 minutes each. The doses of cosyntropin were chosen because they are in the physiological range and have previously been shown to provide measurable but submaximal stimulation of aldosterone and cortisol in normal subjects (Rayfield et al., 1973; Kem et al., 1975). One blood sample was drawn at the end of the control period and each incremental infusion dose and analyzed for aldosterone, ACTH, cortisol, sodium, and potassium; PRA was measured at the beginning and end of the infusion.

Potassium Infusion

Fifteen hypertensive patients and seven normal controls received an infusion of potassium chloride. Responses in four of the normal controls have been reported previously (Himathongkam et al., 1975). Each subject had a 30-cm intravenous catheter inserted into the antecubital vein of one arm for infusion and a catheter into a vein in the opposite arm for sampling. After a 30-min control period, isosmotic potassium chloride in water was infused at a constant rate of 0.33 mEq/min (20 mEq/hr) for 2 hours. This dose was chosen to assess adrenal responsiveness to small changes in serum potassium levels within the physiological range. It has been shown previously to be in the most sensitive area of the dose response curve in normal volunteers (Himathongkam et al. 1975). Blood samples were drawn at the end of the control period and every 30 minutes throughout the infusion and analyzed for aldosterone, PRA, cortisol, sodium, and potassium.

Laboratory Procedures

All blood samples were collected on ice, spun immediately, and the plasma separated and frozen until the time of assay. Serum and urine sodium and potassium levels were measured by flame photometry using lithium as an internal standard. Angiotensin II, aldosterone, PRA, and cortisol were measured by radioimmunoassay techniques as previously described (Emanuel et al., 1973; Underwood and Williams, 1972).

Group means have been presented with the standard error of the mean as the index of dispersion. Statistical probability was evaluated with the Fisher Exact Test (FET). Slopes of regression lines were determined using analysis of least squares. Differences between slopes were determined using standard techniques (Armitage, 1971). The protocol was approved by the Human Subjects Committee of the Peter Bent Brigham Hospital. Written permission for the procedures was obtained after a complete description of the protocol.

Results

Hypertensives were grouped according to their adrenal responsiveness to infused AII. The increment in plasma aldosterone (Aldo) between control and the highest infusion dose (3 ng/kg per min) divided by the increment in plasma AII (ΔAldo/ΔAII) was determined for each subject. As shown in Figure 1, all normal controls (NC) had a ratio greater than 0.5. Seven of the 15 hypertensives had a ratio greater than 0.5 and were designated normal responders (NR). The hypertensives with a lower ratio, indicating decreased adrenal responsiveness to infused AII, were designated abnormal responders (AbR). Previously, we have used the increment in plasma aldosterone between supine and upright posture divided by the increment in PRA as an estimate of adrenal sensitivity to AII (Moore et al., 1977). By this definition, the NR subjects in the current study also had a significantly higher ratio than did the AbR subjects (8.4 ± 1.8 vs. 3.8 ± 0.9; P < 0.03). During the infusion of 3 ng/kg per min of AII, all three groups had a similar increase in
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mean blood pressure above control (NR, 9 ± 2 mm Hg; AbR, 13 ± 2 mm Hg; and NC, 8 ± 1 mm Hg).

The hypertensives were not randomly selected for this study. Three patients who had decreased adrenal responsiveness to infused AII 5 years ago were recalled and on repeat infusion, again had decreased aldosterone responsiveness.

There was no significant difference in the mean age of the two groups of hypertensives undergoing infusion of AII (NR range, 26-47 years; AbR, 38-60 years). Although the hypertensives tended to be older than the normal controls, there was no correlation between age and our index of adrenal responsiveness, the ΔAldo/ΔAII ratio (r = 0.18).

Weight loss on the low sodium diet was similar for the two groups (NR, 1.7 ± 0.4 kg; AbR, 2.3 ± 0.3 kg) and represented 2-2.7% of their total body weight. Mean serum sodium values for all subjects ranged from 137-144 mEq/liter on each infusion day. There were no significant differences in either serum or urine potassium and sodium levels at the start of any infusion in the two groups (Table 1). Serum potassium levels rose similarly during the potassium infusion in each group but did not change significantly during infusions of AII or ACTH. Also, there were no significant differences in either basal PRA or aldosterone levels between NR and AbR hypertensives or between the NR hypertensives and the normal subjects. Plasma cortisol levels rose significantly during the cosyntropin infusion in each group of hypertensives (P < 0.002) but were normal and did not change significantly during infusions of AII and potassium.

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**TABLE 1 Basal Data on Infusion Days**

<table>
<thead>
<tr>
<th>Study condition</th>
<th>NR</th>
<th>AbR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Na (mEq/liter)</td>
<td>11 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Urine K (mEq/liter)</td>
<td>83 ± 3</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>Serum K (mEq/liter)</td>
<td>4.3 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>AII (pg/ml)</td>
<td>24 ± 4</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>16 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>PRA (ng/ml per hr)</td>
<td>39 ± 0.7</td>
<td>5.6 ± 1.0</td>
</tr>
<tr>
<td>ACTH infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Na (mEq/liter)</td>
<td>12 ± 3</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Urine K (mEq/liter)</td>
<td>86 ± 2</td>
<td>79 ± 9</td>
</tr>
<tr>
<td>Serum K (mEq/liter)</td>
<td>4.5 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>73 ± 5</td>
<td>69 ± 15</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>20 ± 5</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Cortisol (μg/dl)</td>
<td>16 ± 3</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>PRA (ng/ml per hr)</td>
<td>4.1 ± 0.6</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>KCl infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Na (mEq/liter)</td>
<td>7 ± 2</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Urine K (mEq/liter)</td>
<td>86 ± 4</td>
<td>81 ± 7</td>
</tr>
<tr>
<td>Serum K (mEq/liter)</td>
<td>4.3 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>18 ± 2</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>PRA (ng/ml per hr)</td>
<td>3.8 ± 0.5</td>
<td>5.6 ± 0.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. Number of subjects (NR and AbR, respectively): AII infusion, 7 and 8; ACTH infusion, 7 and 8; KCl infusion, 9 and 10.

* Normal responder hypertensives.

† Abnormal responder hypertensives.

**FIGURE 2 Regression relationships between aldosterone and AII responses to infused AII. The slope of the regression line of the abnormal responder (AbR) group (n = 8) was significantly less than that of both the normal responder (NR) group (n = 7, P < 0.001) and the normal control (NC) group (n = 14, P < 0.001).**
Figure 3 Incremental responses of plasma aldosterone, ACTH, and cortisol to graded infusions of cosyntropin. The aldosterone response of the AbR group (n = 8) at the two highest doses was significantly less than it was in either the NR (n = 7, P < 0.016) or the NC (n = 15, P < 0.05) groups. The slope line for normotensive subjects was also significantly steeper than that of the abnormal responding hypertensive group (slope 1.00 ± 0.03 vs. 0.20 ± 0.04; P < 0.001) but was not significantly different from that of the NR hypertensive group.

ACTH Infusion

Figure 3 shows the mean increment in aldosterone, ACTH, and cortisol levels in response to graded infusions of cosyntropin in the two subgroups of hypertensives and the normal controls. ACTH and cortisol rose similarly in all groups. However, the aldosterone response at the two highest infusion rates in the abnormal responder subgroup was significantly less than it was in either the normally responsive hypertensives (P < 0.016, FET) or the normotensive controls (P < 0.05, FET).

KCI Infusion

After 1.5 hours of a constant infusion of 0.33 mEq KCl/min, the subjects' serum potassium levels reached a steady state. Thus, aldosterone and potassium levels at 1.5- and 2-hour intervals were measured and compared with basal values (Fig. 4). The aldosterone increment in the AbR group (7.6 ± 2.2 ng/dl) was significantly less than that in the NR group (14.2 ± 2.5 ng/dl; P < 0.05) and the normotensive controls (18 ± 5 ng/dl; P < 0.05). There was no significant difference between the normally responsive hypertensive and normal control groups. Serum potassium increments during the infusion were similar in three groups (NR, 0.5 ± 0.1; AbR, 0.6 ± 0.2; and NC, 0.7 ± 0.1 mEq/liter).

Discussion

Some hypertensive subjects, when sodium restricted, have a decreased adrenal responsiveness to infused AII, compared with normal volunteers (Moore et al. 1977; Dluhy et al., 1979). The nature of the defect causing this altered adrenal sensitivity has not been defined. Possible sites of the defect include the AII receptor and the intracellular aldosterone biosynthetic pathway. To localize the defect, we have studied adrenal responsiveness of this subgroup of hypertensive subjects to other aldosterone secretagogues, ACTH and K⁺, because these substances presumably stimulate aldosterone synthesis at sites different from one another and from AII. For example, ACTH has been shown to...
bind to glomerulosa cells at a cell surface receptor and to activate adenylyl cyclase (Leffkowitz et al., 1970a) in a calcium-dependent manner (Leffkowitz et al., 1970b; Fakunding et al., 1979). AII also has been shown to stimulate the adrenal glomerulosa at a specific cell surface receptor site, but one distinct from the ACTH receptor (Lin and Goodfriend, 1970). On the other hand, potassium is generally thought to affect aldosterone production at an intracellular site early in aldosterone biosynthetic pathway (pregnenolone to corticosterone) (Brown et al., 1972; Kaplan and Bartter 1962) and/or late in the pathway (corticosterone to aldosterone) (Williams et al., 1972; McKenna et al., 1978).

Our data demonstrate that hypertensive subjects with decreased adrenal responsiveness to infused AII also have decreased adrenal responsiveness to infused potassium and ACTH. However, their cortisol response to ACTH is normal. These data suggest that their defect is specific for the adrenal glomerulosa and that it resides in the intracellular aldosterone biosynthetic pathway. Since our infusions were carried out for 2 hours, however, we cannot exclude the possibility that the defined abnormality in adrenal sensitivity could be overcome by more prolonged exposure to agonists of aldosterone secretion.

There are other potential explanations of our findings. It might be argued that our classification of hypertensive subjects into two groups according to their adrenal responsiveness to AII is arbitrary. We agree that the distribution of AAldo/AAII ratios in Figure 1 suggests that there are no distinct subgroups among the hypertensive subjects but that the hypertensive patients constitute a single population with a wider range of adrenal responsiveness to AII than is seen in the normal population. Moore et al. (1977) have reported a similar distribution range of adrenal responsiveness in a larger group of subjects. Although the mean age of our two groups of hypertensives did not differ significantly, the abnormal responders tended to be older. However, it has been documented that adrenal responsiveness to AII is independent of age over the age range of our patients (Takeda et al., 1980). It is well known that the state of sodium and potassium balance can influence adrenal sensitivity to AII (Blair-West et al., 1965; Hollenberg et al., 1975) and ACTH (Venning et al., 1962; Williams et al., 1970). However, no differences in electrolyte balance were found among the three groups during any of the infusions. Another explanation for the (apparently) different aldosterone responsiveness among the hypertensive subjects could be an increase in the metabolic clearance rate (MCR) of aldosterone in the AbR group. This is unlikely, because Wigerhof and Brown have shown no differences in the MCR of aldosterone in normals or in essential hypertensives following AII infusion (Wigerhof and Brown, 1979). Basal PRA levels were normal in our AbR subjects, excluding the diagnosis of hyporeninemic hypoaldosteronism as a cause of their poor aldosterone responsiveness (Schambelan et al., 1972). Their normal cortisol response to ACTH and normal rather than high basal PRA levels exclude generalized adrenal insufficiency in the AbR subjects. Additionally, there were no major differences in the antihypertensive medications taken by the two groups of hypertensives. All of the hypertensive subjects had been on a diuretic until 2 weeks prior to the study, and several in each group had been on a second drug, usually a-methyl dopa.

The finding that three of our AbR subjects had also had decreased adrenal responsiveness to infused AII when studied here 5 years ago on the same diet suggests that adrenal sensitivity for a given subject is constant and that their defect is fixed.

The mechanism of altered adrenal responsiveness to infused AII, ACTH, and potassium during sodium restriction in a subgroup of essential hypertensives is unclear. Our results suggest that the defect is specific for the glomerulosa cell, since our subjects had a normal cortisol response to ACTH and since hypertensives with blunted adrenal responsiveness to AII previously have been shown to have normal vascular responsiveness to AII (Moore et al., 1977). It has been observed that adrenal responsiveness to both AII (Blair-West et al., 1965; Hollenberg et al., 1974) and ACTH (Venning et al., 1962; Tucci et al., 1967) increase during sodium restriction. Even though all of our subjects were on the same low-sodium diet and had similar serum and urine sodium levels, those in the abnormal hypertensive group responded as if they were ingesting a high-sodium diet and had an expanded extracellular space. This abnormal perception of the state of sodium balance provides further support to the thesis that the sodium-dependent change in adrenal responsiveness is attenuated in these patients and that this abnormality in part contributes to their hypertensive state.

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