Primary Aldosteronism Caused by Adrenal Cortical Carcinoma

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Abstract

Adrenocortical carcinoma rarely produces pure primary aldosteronism. We document the occurrence of such a case. Through tumor steroid analysis, we show that the content of aldosterone per gram of tumor tissue is diminished compared to aldosterone producing adenomas. We also demonstrate the occurrence of angiotensin II receptors on the tumor, a finding hitherto noted only on aldosterone producing adenomas.

Primary aldosteronism ordinarily signifies autonomous production of aldosterone by an adrenal adenoma or bilateral hyperplasia of the adrenal glands. Rarely, it may be caused by a carcinoma. When produced by a tumor, the neoplasm is usually benign, less than four centimeters in diameter and associated with the classical features of mineralocorticoid excess, hypokalemia, hyporeninemia and hyperaldosteronism (Weinberger et al., 1979; Bennett et al., 1971). In contrast, carcinomas producing primary aldosteronism are larger and exhibit increased secretion of cortisol and/or androgens (Alterman et al., 1969; Brooks et al., 1972; Filipecki et al., 1972). Nonetheless, a smaller group of eight patients (Crane et al., 1965; Revach et al., 1977; Salassa et al., 1974) has been reported in which primary aldosteronism appears to have occurred as the only endocrine manifestation of an adrenocortical carcinoma. We report a similar patient with chemical and clinical primary aldosteronism, due to adrenocortical carcinoma. Results of the tumor analysis of steroid content indicate diminished aldosterone content per gram of tumor tissue compared to benign aldosterone producing adenomas (APA). We demonstrate the presence of angiotensin II receptors on the tumor, a finding hitherto noted only on APA (Brown et al., 1980).

Methods

Tumor tissue aldosterone determination

Tissue was prepared in the following manner: eight grams of frozen tumor tissue were pulverized with a tissue pulverizer. Eight ml of phosphate saline buffer was then added, and the tissue was homogenized with a polytron. Methanol (32 ml) containing 0.1% triethylamine was added to the homogenate. The mixture was shaken for 30 minutes and then centrifuged for 15 minutes at 3000 r.p.m. Multiple aliquots of the supernatant were transferred to extraction tubes, and
the methanol was evaporated with a stream of nitrogen in a water bath. Recovery tracers, approximately 20,000 dpm, were added for the steroid measured. Final tissue concentrations are adjusted for the percentage recovery. Distilled water was added to each tube to adjust the volume to the amount used in each assay. Control cadaver adrenal tissue was processed in a similar manner at a later date. Only the tail portion of the adrenal glands was used, thereby eminently most of the medulla. Three of the four glands were frozen within 6 hours of death and the fourth within 12 hours. All cadavers were stored in a cooler at a temperature of 3.5–6.8°C prior to autopsy. Previous study (Kaplan, 1967) shows less than 15% loss of steroid activity in adrenal tissues processed in this fashion.

Aldosterone was measured by RIA using a procedure previously described (Mayes et al., 1970). Aliquots of tumor supernatant were extracted with methylene chloride, evaporated under nitrogen and purified by paper chromatography using a Bush B-5 system. The aldosterone content of the purified samples was determined by RIA using an antisem developed against an aldosterone 3-oxime conjugate.

Angiotensin II receptor binding procedure.

Frozen adrenal tumors (aldosterone-producing carcinoma and cortisol producing adenoma) were thawed, minced and dispersed using a teflon-glass homogenizer in 20 mM sodium bicarbonate solution as previously described in detail for the rat adrenal (Douglas and Catt, 1976). The homogenates were filtered through nylon fabric and the 10,000–100,000 × g fractions were used for studies of angiotensin II binding. The pellets were resuspended in 50 mM Tris buffer pH 7.4 to a final concentration of 2.1 mg/ml with the aldosterone producing carcinoma and 5 mg/ml with the cortisol producing adenoma. Adrenal tissue was incubated with 50 mM Tris-HCl (pH 7.4) with 120 mM NaCl, 5 mM dithiothreitol, 0.8% BSA, 20 fmole of 125I-angiotensin II and 100–250 μg of protein. Binding studies were performed in triplicate at 20°C for 2 to 20 minutes. All binding was corrected for non-specific binding in the presence of excess unlabeled angiotensin II (1 μg). Non-specific binding represented less than 25% of total binding with the aldosterone-producing carcinoma and approximately 50% of total binding with the cortisol-producing adenoma. Separation of bound and free hormone was achieved by Millipore filtration using HAWP (0.45 μ) nitrocellulose filters. Filters were counted in a Packard gamma spectrometer. Comparisons are made to binding by fresh and frozen human and rat adrenal glomerulosa. A Statie Riggo microtome was used to obtain the outer 0.1 mm cortical slices from a patient who underwent bilateral adrenalectomy for Cushing’s Disease. This procedure yields tissue with significant increments in aldosterone production to low concentrations of angiotensin II in vitro (Brown et al., 1980). Rat tissue was the decapsulated portion of adrenals from female Sprague Dawley rats (225–250 g) as previously described (Douglas and Catt, 1976). Identical procedures were utilized for preparation of all tissues. Binding constants were determined by Scatchard analyses with the aid of a computer for linear regression analysis. All human tissue used for these studies were obtained with the approval of the Human Experimentation Committee, University Hospitals of Cleveland.

Urinary aldosterone, plasma aldosterone (PA) and plasma renin activity (PRA) were determined in accordance with previously published procedures (Bravo et al., 1975a; Bravo et al., 1975b; Bravo et al., 1973). Each PA determination is the mean of duplicate determinations at two separate dilutions.

Case Report

A 53 year-old white male was evaluated in July 1978, because of hypertension. The patient was aware of his elevated blood pressure for at least ten years and noted it had always been poorly controlled. Since November 1977, he had experienced muscle weakness in both shoulders and arms. Because of an elevated CPK determination of 626 IU (nl 0–83 IU), his previous physician diagnosed polymyositis and began him on prednisone, 20 mg each day. No other diagnostic procedure was performed. Although the patient believed he experienced some benefit from treatment, his symptoms persisted. His past history revealed chronic alcoholism with a recent exacerbation manifested by the consumption of a half gallon of gin every week for the preceding four months.

On physical examination, the blood pressure was 180/100 mm Hg while on hydrochlorothiazide, 50 mg each day, and propranolol, 40 mg BID. Grade II vascular changes were noted in the fundi. Examination of the abdomen revealed a large mass in the area of the left kidney. The remainder of the physical examination was normal except for generalized muscle weakness. In particular, there were no stigmata of Cushing’s syndrome. The complete blood count was within normal limits except for a WBC of 13,100, with a normal differential. Serum potassium was 1.9 mEq/L. The urinalysis showed a trace of protein but
Vol. 29, No. 6  ALDOSTERONISM CAUSED BY ADRENAL CORTICAL CARCINOMA  703

no other abnormality. The chest X-ray was within normal limits. A previous ECG from April 1978 revealed T-U fusion characteristic of hypokalemia when the serum potassium was 1.8 mEq/L. Neurological consultation failed to substantiate a diagnosis of polymyositis. The prednisone was gradually tapered and the hydrochlorothiazide discontinued. Oral potassium supplementation was begun and on August 4th, the blood pressure was 200/100 mm Hg, the serum potassium 3.0 mEq/L, sodium 148 mEq/L and CO₂ 29 mEq/L. Serum creatinine was 1.5 mg/dl. A twenty four hour urine protein was 607mg. An IVP showed a large mass above the left kidney. The patient developed alcoholic hepatitis and was hospitalized beginning August 18th. During this period, KCl supplementation initially 40mEq TID and later 40mEq BID was given. Following resolution of the alcoholic hepatitis, evaluation of his adrenal function was undertaken. Table 1 shows the diurnal variation of serum cortisol, urinary excretion of glucocorticoid metabolites and the response of serum and urinary glucocorticoids to low dose dexamethasone suppression. Table 2 shows the baseline level of PA, PRA, postural changes and the 24-hour aldosterone excretion rate (AER). The effect on these values of an abortive attempt to salt load the patient is likewise noted. Unfortunately, left ventricular failure followed the initial 2000 ml saline load and precluded further attempts to suppress aldosterone production. A 24-hour urine for metanephrines was 0.8 mg/24 h (nl 0.3–0.9 mg/24 h). Following a left renal angiogram, the patient was operated on September 19th, 1978. A 320 gram encapsulated tumor measuring 9 × 9 × 8 cm was removed. There was no evidence of local invasion or lymph node metastases. Limited exploration of the liver failed to reveal any gross metastases. No liver biopsy was performed. Pathological interpretation of the tumor revealed a pleomorphic neoplasm of the adrenal cortex with mitoses, capsular invasion, broad fibrous bands and areas of vascular invasion (Fig. 1). In addition, several of the slides showed a pattern of anastomosing vascular sinusoids with parallel arrays of necrosis following the pattern of these sinusoids in the tumor. On October 12, 1978, off potassium supplementation, the serum potassium was 5.5 mEq/L and the blood pressure 190/110 mm Hg. Hydrochlorothiazide was begun and on October 24th, while taking 50 mg BID, the serum potassium was 4.7 mEq/L. On the same day, an afternoon ACTH stimulation test using Cortrosyn® 0.25 mg I.M. was performed. The patient had been up ad lib during the day. The baseline serum cortisol of 4.4 ug/dl and aldosterone of 10.6 ng/dl increased to 9.1 ug/dl and 10.8 ng/dl at 30 minutes, 10.9 ug/dl and 16.9 ng/dl at 60 minutes and 11.3 ug/dl and 16.2 ng/dl at 90 minutes, respectively. When last seen on February 1, 1979, the blood pressure was 160/100 mm Hg, while on propranolol, 80 mg QID, but no hydrochlorothiazide. The serum potassium was 5.5 mEq/L. The patient was known to be alive and well as of July 1981. He continued to take propranolol 80 mg QID and hydrochlorothiazide 100 mg QD from another physician because of continued hypertension.

Results

As shown in Table 1, diurnal variation of serum cortisol was exhibited while baseline excretion of urinary 17-hydroxycorticosteroids (17-OHCS), 17-ketosteroids and urinary free cortisol were all within normal limits. Urinary free cortisol and plasma cortisol were suppressed normally on dexamethasone, 0.5 mg every 6 hours, although urinary 17-OHCS failed to show normal suppression. Table 2 shows markedly elevated values of both PA and urinary AER and suppressed PRA. Assuming the upright posture for four hours produced a slight increment in PRA on two occasions. At the same time, PA increased significantly. An attempt to demonstrate autonomy of aldosterone production by three days of saline
Fig. 1. (a) Cords of tumor cells penetrating vascular spaces (arrow heads) in the thick fibrous capsule (FC) which envelops the tumor (T). The residual lumina of the invaded vessels are indicated by arrows. Peri-adrenal adipose tissue (AT) outside the fibrous capsule is shown. (Hematoxylin and Eosin, ×80). (b) The tumor cells are arranged in solid nests and cords separated by prominent fibrous trabeculae and dilated vascular spaces (arrows). (Hematoxylin and Eosin, ×80).
**Table 1. Baseline plasma and urinary glucocorticoid levels and response to low dose dexamethasone suppression.**

<table>
<thead>
<tr>
<th></th>
<th>17-OHCS** (mg/24 hrs)</th>
<th>17-Ketosteroids (mg/24 hrs)</th>
<th>Urinary Free Cortisol (μg/24 hrs)</th>
<th>TV (ml)</th>
<th>Serum Cortisol (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/27/78-8/28/78</td>
<td>10.7 (nl 4-12)</td>
<td>5.5 (nl 10-20)</td>
<td>43 (nl 20-90)</td>
<td>2650</td>
<td>0.800 h=18.0 (nl 8-19)</td>
</tr>
<tr>
<td>8/31/78-9/1/78</td>
<td>8.9</td>
<td>6.7</td>
<td>8</td>
<td>1600</td>
<td>0.800 h= 2.9 (9/1)</td>
</tr>
</tbody>
</table>

* Patient received 0.5 mg dexamethasone q6h from 8 a.m. on 8/30 through 2 a.m. 9/1 and chlordiazepoxide 25 mg hs QD.

** Urinary 17-hydroxycorticosteroids = 17-OHCS.

**Table 2. Recumbent and upright plasma aldosterone and plasma renin activity and 24 hour urinary aldosterone excretion rate.**

<table>
<thead>
<tr>
<th></th>
<th>Plasma Aldosterone (ng/dl)</th>
<th>Plasma Cortisol (μg/dl)</th>
<th>Plasma Renin Activity (ng/ml/hrs)</th>
<th>Serum Na/K (mEq/L)</th>
<th>Aldosterone Excretion rate (μg/24 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/5/78</td>
<td>0.800 h (recumbent)</td>
<td>105 (nl 3-16)</td>
<td>18.0 (μg/dl)</td>
<td>0.1 (μg/dl)</td>
<td>139/ 4.9 (nl 3-12)</td>
</tr>
<tr>
<td>12.00 h (upright)</td>
<td>196.8 (nl 2-3 fold increase)</td>
<td>12.5 (μg/dl)</td>
<td>0.35 (μg/dl)</td>
<td>39 (μg/dl)</td>
<td>105.6</td>
</tr>
<tr>
<td>9/7/78</td>
<td>11.30 h (10 minutes post 40 mg furosemide)</td>
<td>139 (μg/dl)</td>
<td>1.1 (μg/dl)</td>
<td>145/ 4.2 (μg/dl)</td>
<td>90 (μg/dl)</td>
</tr>
<tr>
<td>15.30 h</td>
<td>90</td>
<td>6.9 (μg/dl)</td>
<td>0.08 (μg/dl)</td>
<td>4.2 (μg/dl)</td>
<td>141/ 3.6 (μg/dl)</td>
</tr>
<tr>
<td>9/8/78</td>
<td>0.800 h (recumbent)</td>
<td>124.8 (μg/dl)</td>
<td>18.4 (μg/dl)</td>
<td>0.1 (μg/dl)</td>
<td>167.5 (μg/dl)</td>
</tr>
<tr>
<td>12.00 h (4 hrs upright)</td>
<td>17.5 (μg/dl)</td>
<td>7.2 (μg/dl)</td>
<td>0.18 (μg/dl)</td>
<td>8.0 (μg/dl)</td>
<td>141/ 3.6 (μg/dl)</td>
</tr>
</tbody>
</table>

* From 9/3 through 9/9, the patient was maintained on a 5 Gm NaCl diet with KCL, 40 mEq BID. Two liters of normal saline were administered IV on 9/6. Pulmonary edema developed the night of 9/6.

Loading was aborted by the patient developing left ventricular failure after the first day (Table 2). At least partial refractoriness of PRA to stimulation was confirmed by obtaining a value of 0.07 ng/ml/h on 8/28/78 three and a half hours after administration of 20 mg furosemide IV and assuming the upright posture at 8 a.m. Five weeks following surgery the upright PA level was approximately 6% of the average pre-operative values and demonstrated impaired responsiveness to ACTH.

Results of tumor and normal adrenal tissue analysis for aldosterone are as follows. The aldosterone concentration in the present tumor, 0.106 μg/gm, is markedly lower than the previously reported average value of 10.3 μg/gm (Kaplan, 1967), 0.82 μg/gm (Biglieri et al., 1963) and 1.89 μg/gm (Louis and Conn, 1961) in APA. Control adrenal tissue (n=4) showed an average aldosterone concentration of .041 μg/gm, similar to that of .06 μg/gm (Louis and Conn, 1961) but less than 0.27 μg/gm (Biglieri et al., 1963; Kaplan, 1967). However, the weight of the present tumor is 320 grams and the total aldosterone content, therefore, is significantly higher than usually
found in APA. Figure 2 demonstrates specific angiotensin II bound by the present aldosterone-producing carcinoma and, in contrast, a cortisol-producing adenoma, utilizing a single concentration of ligand (20 fmoles). Both tissues were stored frozen prior to use. Binding was highest between ten and fifteen minutes with both tumors, but was 3.6 fold higher with the aldosterone-producing carcinoma. Use of tissues for receptor binding that were previously frozen results in loss of receptor number and lower affinity as compared with use of the same tissues when fresh. Examples of two such experiments, employing normal rat adrenal and human adrenal glomerulosa are presented in Table 3. Each represents a significant loss in the number of receptor sites, whereas only the rat demonstrated a significant increase in $K_d$.

**Discussion**

Several interesting features in this patient deserve comment. Initially, he demonstrates the occurrence of an unusually “pure” form of primary aldosteronism in association with a large adrenal cortical neoplasm. Normal urinary free cortisol, plasma cortisol, diurnal variation of plasma cortisol and response of both to low dose dexamethasone suppression adequately eliminate concurrent excessive cortisol production. The failure of urinary 17-OHCS to normally decrease on low dose dexamethasone may reflect the presence in the urine of chlordiazepoxide metabolites, which are known to interfere with the colorimetric determination (AMA Drug Evaluations 1977). Salassa reported six similar patients with large adrenal cortical tumors and primary aldosteronism culled from a group of 105 patients with primary aldosteronism operated on at the Mayo Clinic over a seventeen year period (Salassa et al., 1974). Unfortunately, neither urinary free cortisol determinations nor dexamethasone suppression testing were performed.

![Fig. 2. Binding of $^{125}$I angiotensin II (20 fmoles) by an aldosterone producing carcinoma and a cortisol producing adenoma. Each value represents the mean plus or minus standard error of triplicate determination.](image)

**Table 3. Comparison of binding constants utilizing fresh vs. frozen human and rat adrenal glomerulosa.**

<table>
<thead>
<tr>
<th>Receptor Number</th>
<th>Human</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>(fmole/mg protein)</td>
<td>FRESH</td>
<td>736</td>
</tr>
<tr>
<td></td>
<td>FROZEN</td>
<td>602</td>
</tr>
<tr>
<td>$K_d$ (nM)</td>
<td>FRESH</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>FROZEN</td>
<td>3.2</td>
</tr>
</tbody>
</table>
in this study although diurnal plasma cortisol variations appeared to exist. Occasional patients with similar presentation, likewise, had normal urinary excretion of 17-ketosteroids and 17-OHCS (Revach et al., 1977; Crane et al., 1965). These patients differed from previously reported cases of adrenal cortical carcinoma associated with primary aldosteronism where elevation in either 17-ketosteroids, 17-OHCS or urinary free cortisol was noted (Alterman et al., 1969; Brooks et al., 1972; Filipiecki et al., 1972).

Secondly, the aldosterone concentration of the present tumor is diminished compared with APA. The striking clinical picture, therefore, is consequent to the large weight of the tumor. In this regard, it is of interest that recalculation of previous data (Louis and Conn, 1961) shows that the mean aldosterone content of 12 APA is 5.3 µg/APA. Allowing for differences in methodology, this value is less than the total content of aldosterone in our patient’s tumor (33.9 µg).

Thirdly, studies of angiotensin II binding to the tumor confirm the presence of angiotensin II receptors on this carcinoma indicating that there is potential for some degree of regulation of hormonal output by angiotensin II. Similar studies of benign aldosterone-producing adenomas have linked the presence of angiotensin II receptors with a sensitive aldosterone response to angiotensin II in vitro (Brown et al., 1980). While the present studies were limited and performed at a single ligand concentration, there was specific binding by the carcinoma which would likely have been higher if the study had been performed using fresh rather than frozen tissue (Table 3). At an equivalent ligand concentration fresh normal human adrenal glomerulosa bound 1060 fm/gm protein and a benign APA bound 685 fm/gm protein at steady state. Hence, the plateau of binding at 470 fm/gm protein by the aldosterone-producing carcinoma was not significantly different from the APA and would have probably been higher had the tissue been studied while fresh. The cortisol-producing adenoma, however, bound significantly less angiotensin II with a plateau of only 130 fm/gm protein.

On the basis of the preceding data, we believe the classical syndrome of primary aldosteronism may be produced by an adrenal cortical carcinoma. The significance of angiotensin II receptors on this as well as benign aldosterone-producing tumors has as yet not been determined (Brown et al., 1980). We suggest the hypothesis that perhaps their presence may explain why some of these neoplasms are associated with a paradoxical rise in plasma aldosterone on assuming the upright posture.

Acknowledgements

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