Studies of Impaired Aldosterone Response to Spironolactone-Induced Renin and Potassium Elevations in Adenomatous but Not Hyperplastic Primary Aldosteronism

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SUMMARY Spironolactone (SPL) corrects hypertension, hypokalemia, and hyporeninemia in patients with primary hyperaldosteronism (PHA) by blocking mineralocorticoid (MCH) receptors. We evaluated the effect of continuous SPL treatment (100 to 300 mg/day for 7 days to 9 years) on plasma renin (PRC), potassium, aldosterone (PA), 18-hydroxycorticosterone (18-OHB), deoxycorticosterone (DOC), and corticosterone (B) concentrations and 24-hour urinary excretion of aldosterone (UA) in 24 patients with PHA (15 with an aldosterone-producing adenoma [APA] and nine with idiopathic PHA [IHA]). Despite the normalization of PRC and K in both APA and IHA patients by SPL, UA and PA failed to increase in APA (55.8 ± 8.8 to 51.4 ± 7.3 µg/24 hr and 54.0 ± 9.4 to 44.6 ± 6.2 ng/dl, respectively) in contrast to rises in IHA patients (22.3 ± 2.5 to 69.3 ± 10.3 µg/24 hr and 16.0 ± 1.0 to 49.9 ± 9.9 ng/dl). Similar corrections with amiloride (20-40 mg/day for 2 months) in one patient with APA produced a three- to fourfold increase in UA and PA. In addition, while on SPL the characteristic fall or no change in PA and 18-OHB during upright posture persisted in all APA patients despite further increases in PRC (4.48 ± 1.15 to 7.86 ± 1.89) and K (4.0 ± 0.1 to 4.3 ± 0.1). The patterns of the aldosterone precursors, DOC, B, and 18-OHB, and their ratios to acute stimulation with cosyntropin were not altered by SPL. Thus, SPL treatment causes a sustained impairment of the aldosterone secretory response to normalized PRC and K, but not to ACTH stimulation, only in patients with APA. (Hypertension 5 (supp V): V-115-V-121, 1983)

KEY WORDS • mineralocorticoid hormones • aldosterone-producing adenoma • idiopathic hyperaldosteronism • inhibition of aldosterone production • amiloride • spironolactone bodies

SPIRONOLACTONE (SPL) has been used for the medical management of disorders with aldosterone excess since 1960.1 Its antimineralocorticoid effect results in correction of hypertension and hypokalemia in most patients with primary aldosteronism.2 A favorable response to SPL treatment can, in fact, be used as a good predictor of the postoperative effect of removal of an aldosterone-producing adenoma (APA).2,3 The principal mechanism of action of SPL is competitive antagonism for the mineralocorticoid hormone (MCH) receptor at the target cell, especially in the kidney.1,4 In vitro studies show that SPL, or some of its metabolites, may also inhibit aldosterone biosynthesis in the incubated adrenal tissue.5,6 Impaired conversion of labeled corticosterone to aldosterone suggested a predominant involvement of the mitochondrial 11β- and 18-hydroxylase systems.1,6 Other possible sites of enzymatic inhibition have been reported for hydroxylation at positions 17α and 21 and corticosterone-methyloxidase (CMO) II.7 We report the effect of SPL treatment on aldosterone production and on the integrity of the zona glomerulosa biosynthetic pathway in 24 patients with primary aldosteronism due to APA and to idiopathic hyperaldosteronism (IHA).
Subjects and Methods

Patients

Twenty-four patients with hypertension, hypokalemia, renal potassium wasting, and suppressed plasma renin concentration (PRC) were diagnosed as having primary aldosteronism. Fifteen (11 women and four men, 25 to 56 years of age, median age, 32 years) had an APA, established by computed tomography and/or adrenal vein catheterization and subsequently proved by surgery in 14 of the 15 patients. Nine (six women and three men, 33 to 59 years of age, median, 41 years) showed no evidence of adrenocortical masses by 131I iodocholesterol or computed tomographic scanning. A diagnosis of IHA was suggested by the MCH profile and hormonal responses to posture. Clinical data for all patients are summarized in table 1.

All studies were performed in the General Clinical Research Center at San Francisco General Hospital Medical Center after patients were equilibrated on a constant metabolic diet containing a fixed electrolyte content of 120 mEq of sodium and 50-70 mEq of potassium/day. Daily plasma levels and total urinary excretion of sodium and potassium were checked to assess equilibration. Informed written consent was obtained from each patient after the protocol had been approved by the Committee on Human Research of the University of California, San Francisco.

Baseline Determinations

One or more baseline determinations of plasma electrolytes, steroids (cortisol, corticosterone [B], 18-hydroxycorticosterone [18-OHB], deoxycortic-
sterone [DOC], 18-hydroxydeoxycorticosterone [18-OHDOC], and aldosterone), and PRC were performed in each patient before and during continuous treatment with SPL in doses ranging from 100–300 mg/day for periods of 7 days to 9 years (median, 3 months) in APA patients and 1½ months to 8 years (median, 9 months) in IHA patients. In one patient with APA, SPL treatment was discontinued after 8 days due to development of a morbilliform skin rash. The patient was switched to amiloride, 20–40 mg/day. Control measurements were obtained at 10 days and 2 months on this regimen (fig. 1). The adenomas and contiguous adrenal glands were examined by electron microscopy for SPL bodies.

**Studies (Maneuvers)**

**Postural Stimulation**

Postural stimulation of the renin system was assessed by measuring PRC, electrolytes, and the above steroids in the overnight recumbent and fasting patient at 0800 hours and again 2 to 4 hours after quiet ambulation. This maneuver was carried out in all APA and IHA patients before initiation of SPL treatment and again in the APA patients during SPL treatment.

**Cosyntropin Stimulation**

Cosyntropin stimulation of the adrenal cortex was evaluated in eight APA patients before and in six APA

![Graph showing sodium and potassium balances and hormonal changes in a patient during treatment with spironolactone and amiloride](http://hyper.ahajournals.org/)

**Figure 1.** Comparison of sodium and potassium balances and hormonal changes in a patient with an aldosterone-producing adenoma during treatment with spironolactone (Aldactone) and amiloride. Note the increase in urinary (U) and plasma aldosterone levels occurred only during amiloride treatment. Aldo = aldosterone; PRC = plasma renin concentration; V = volume.
and five IHA patients during SPL treatment to assess the magnitude of the steroid responses and to amplify any possible interference of SPL action with enzyme activities in steroid biosynthesis. Recumbent steroid samples were obtained before and 1 hour after intravenous administration of 250 μg of cosynotropin.

**Methods**

Plasma and urinary sodium and potassium were measured by internal standard flame photometry. Urinary aldosterone excretion was assessed by the radioimmunoassay of the aldosterone 18-glucuronide after 24 hours of hydrolysis at pH 1 and chromatographic isolation. Plasma renin concentration was determined by the radioimmunoassay of angiotensin I generated in an excess of sheep substrate. Plasma cortisol and B were measured by competitive protein binding analysis. Plasma DOC, 18-OHDOC, 18-OHB, and aldosterone were determined in a single sample by specific radioimmunoassays of the separated and derived steroids. Paired and unpaired Student’s t-tests were used, respectively, to compare the responses of each parameter to stimulation, intragroup comparisons to parameters before and during SPL treatment, and intergroup differences in each parameter.

**Results**

**Baseline Determinations**

Clinical data for the 24 patients with primary aldosteronism due either to APA or IHA are summarized in table 1. Plasma renin concentrations were similar whereas urinary and plasma aldosterone (p < 0.01) and plasma 18-OHb (p < 0.02) levels were significantly higher in patients with APA than in those with IHA (table 2). Other aldosterone precursors, DOC and B, were also higher in patients with APA, but not significantly different from patients with IHA. Cortisol and 18-OHDOC levels were within normal limits and similar in both groups. Treatment with SPL significantly decreased blood pressure* (p < 0.001) and plasma sodium levels (p < 0.001, only in APA patients), while it significantly increased PRC and plasma potassium concentrations (p < 0.001) (table 2). However, urinary and plasma aldosterone levels remained unchanged in patients with APA but increased threefold in patients with IHA (p < 0.001 and < 0.01, respectively). Although not reaching statistical significance, 18-OHB also increased fourfold in patients with IHA but not in patients with APA. Cortisol, B, DOC, and 18-OHDOC were not significantly affected during SPL treatment in both groups of patients. The one APA patient who received amiloride after discontinuing SPL had a significant increase in urinary and plasma aldosterone levels without a greater increase in PRC (1.4 to 1.9) and potassium (3.6 to 3.7, fig. 1) than seen with SPL over a similar period. After 2 months on 40 mg/day of amiloride, the levels reached values that were more than threefold higher with an additional increase in PRC (from 1.9 to 3.2) and potassium (from 3.7 to 4.0).

**Response to Posture**

Upright position for 2 to 4 hours did not significantly increase the suppressed renin levels in APA patients before SPL treatment (fig. 2). Concomitantly, plasma aldosterone and 18-OHB levels did not increase and actually decreased together with cortisol, B, DOC, and 18-OHDOC during the normal circadian rhythm of ACTH. Cortisol and B fell significantly (p < 0.05 and < 0.02, respectively). Repeat postural stimulation during SPL treatment induced a further increase in PRC (p < 0.005) and plasma levels of potassium (p < 0.001). Plasma aldosterone and 18-OHB in particular did not change.

In contrast, in patients with IHA before SPL treatment, upright posture stimulated basal levels of aldosterone (16.0 ± 1.0 to 30.4 ± 4.7, p < 0.02) and 18-OHB (24.1 ± 4.7 to 42.9 ± 11.2, p > 0.05) as well as PRC (0.82 ± 15 to 1.68 ± 0.41, p < 0.02) and...
TABLE 2. Comparisons of Plasma (P) and Urinary (U) at Parameters (means ± se) in Patients with Primary Aldosteronism Due to an Aldosterone-Producing Adenoma (APA) or Idiopathic Hyperaldosteronism (IHA)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with APA (n = 15)</th>
<th>Patients with IHA (n = 9)</th>
<th>p value for comparisons between APA and IHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Off SPL</td>
<td>On SPL</td>
<td>Off SPL</td>
</tr>
<tr>
<td>PRC (ng/ml/hr)</td>
<td>0.58 ± 0.11</td>
<td>4.64 ± 1.15</td>
<td>0.82 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>&lt;0.005</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PK (mEq/liter)</td>
<td>3.0 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PNa (mEq/liter)</td>
<td>142.0 ± 0.4</td>
<td>139.0 ± 0.6</td>
<td>140.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U aldosterone (µg/24 hr)</td>
<td>55.8 ± 8.8</td>
<td>51.4 ± 7.3</td>
<td>22.3 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P aldosterone (ng/dl)</td>
<td>54.0 ± 9.4</td>
<td>44.6 ± 6.2</td>
<td>16.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>P 18-OHB (ng/dl)</td>
<td>141.4 ± 21.4</td>
<td>124.3 ± 20.9</td>
<td>24.1 ± 4.7†</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P cortisol (µg/dl)</td>
<td>11.7 ± 1.0</td>
<td>12.9 ± 1.0</td>
<td>10.2 ± 1.1†</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P DOC (ng/dl)</td>
<td>20.5 ± 3.7</td>
<td>17.1 ± 3.5</td>
<td>8.0 ± 2.6†</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 18-OHDOC (ng/dl)</td>
<td>9.3 ± 1.6</td>
<td>11.3 ± 2.2</td>
<td>3.9 ± 0.9†</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P B (ng/dl)</td>
<td>374.0 ± 54</td>
<td>518.0 ± 108.0</td>
<td>157.0 ± 43.0§</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>116.4 ± 2.9</td>
<td>94.1 ± 2.2</td>
<td>116.2 ± 2.2</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
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</tbody>
</table>

*p = 7; n = 4; †n = 5; §n = 6; ‡n = 3. Most patients with IHA also required a second nondiuretic antihypertensive drug to produce adequate control of blood pressure.

plasma potassium (3.4 ± 0.1 to 3.7 ± 0.1, p < 0.02). Cortisol, B, DOC, and 18-OHDOC decreased during this period.

Response to Cosyntropin

Administration of cosyntropin acutely and significantly stimulated all steroids in patients with APA (n = 8) before SPL treatment without affecting PRC, sodium, or potassium levels (fig. 3). The magnitudes of the cortisol, B, DOC, and 18-OHDOC responses were similar to those observed in normal subjects. The percentage increases in aldosterone (120%) and 18-OHB (204%) levels were also within normal limits. During SPL treatment, six patients with APA had similar or even greater but not statistically significant increases in aldosterone (153%) and 18-OHB (247%) levels. The responses of cortisol, B, DOC, and 18-OHDOC were similar to preSPL treatment. Plasma renin concentration, sodium, and potassium did not change. Analysis of steroid ratios showed no evidence of SPL-induced enzymatic block in 11β-hydroxylation (DOC:B), 18-hydroxylation (CMO I, DOC: 18-OHDOC), and 18-dehydrogenation (CMO II, 18-OH:aldosterone).

Cosyntropin stimulation in five patients with IHA during SPL treatment also increased all steroids significantly (except aldosterone). The magnitudes of the responses were similar to those in normal subjects and slightly smaller than those in the APA patients. The percentage increases in aldosterone (102%) and 18-OHB (173%) were also diminished compared with those in patients with APA.

Spironolactone bodies were found with electron microscopy in all 14 adrenal glands examined regardless of the dose or duration of SPL treatment. They were present in adenomatous cells, contiguous adrenocortical cells, and some zona fasciculata cells. There was no apparent correlation between the number of SPL bodies per field and the plasma or urinary aldosterone level in these patients.
Discussion

Treatment with SPL in patients with primary aldosteronism, especially those with an APA, corrects all clinical and biochemical effects of aldosterone excess.1-3 It has been assumed that the mechanism is its competitive antagonism for the MCH receptor, especially in the kidney.1,4 However, the changes in aldosterone during relatively short periods of SPL treatment have been inconsistent in patients with primary aldosteronism, particularly in patients with APA: the renin increases were often not accompanied by aldosterone increases12-15 in contrast to the usual increases in both renin activity and aldosterone production in normal subjects receiving SPL.16 A possible SPL inhibition of aldosterone synthesis by a tumor was hypothesized. This seemed reasonable because in vitro studies with SPL and its metabolites in large concentrations showed evidence of mitochondrial (11β-, 18-) hydroxylase inhibition.1,6 However, this was not observed in vivo. Studies in normal subjects indicated a possible 18-dehydrogenase (CMO II) defect during the initial few days of therapy, but the discrepancy between 18-OHB and aldosterone production quickly disappeared by day 10 of treatment.7 In patients with essential hypertension treated with SPL, no diminution of aldosterone production occurred.17,18 Our studies demonstrate that SPL prevents and sometimes decreases aldosterone levels in patients with APA but not in those with IHA. Long-term treatment does not support an enzymatic inhibition because the levels of MCHs produced by both the zona glomerulosa and the zona fasciculata do not change from pretreatment levels. An effect, if present, is short lived. Spironolactone bodies were present in all adenomas regardless of the duration of therapy. The number of SPL bodies was reported to diminish during treatment of patients with APA suggesting that early in therapy they were actively involved in the inhibition of aldosterone production by a proposed block between B and aldosterone.16 As renin activity increased, aldosterone levels increased to greater than normal levels as the number of SPL bodies decreased.17 The function of the SPL bodies is still not clear.1,15,19,20 In our studies their continued presence offers little substantial evidence that they inhibit aldosterone production. They are also present in the adrenal glands of patients with IHA20 and in the adrenal gland contiguous with an adenoma.6,15,20 The SPL bodies react positively with antialdosterone antibodies19 and, in vitro, demonstrate increased 3β-hydroxysteroid dehydrogenase activity in both adenoma and hyperplastic cells.20 A discrepancy between in vitro and in vivo effects of SPL continues.

After prolonged SPL therapy, both PRC and plasma potassium concentration normalize and blood pressure decreases in both patients with IHA and APA, bearing in mind that a second antihypertensive drug was required in the IHA group. However, only in patients with APA do urinary excretion of aldosterone and plas-
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...levels of aldosterone and 18-OHB fail to increase. Even the response to upright posture with further increases in PRC and potassium fails to effect additional increments in plasma aldosterone and 18-OHB levels. This failure to increase aldosterone by increases in PRC and potassium in patients with APA is supported by the similar failure of exogenous AII to stimulate these steroids in patients with APA21 but not IHA.21, 22 In addition, AII receptors are reduced in number and affinity in isolated adenoma cells.23 However, the ACTH stimulatory mechanism remains intact in both groups. Plasma aldosterone is increased in all patients with IHA and APA. Statistical significance is not achieved because of the limited number of studies and wide range of stimulated levels of PA and 18-OHB.

Potassium loading and increases in serum potassium concentrations have long been known to be effective stimulators of aldosterone production in patients with APA.24 With evidence that the renin system may not be an effective stimulator of the adenoma cells during SPL treatment, inhibition by SPL in the early synthesis of aldosterone in the gonadotropin cells seems a viable alternative. Further support for this proposal is in the observation in one of our patients with APA where both SPL and amiloride controlled the biochemical abnormalities and blood pressure. However, amiloride acts in the luminal side of the distal nephron to interfere with sodium-potassium movement25 and has no known adrenal effect. The prompt and sustained increases in plasma renin activity and plasma and urinary aldosterone levels are in marked contrast to the responses during SPL treatment. This study suggests that the potassium stimulatory mechanism may also be impaired by SPL.

The failure of aldosterone levels in both urine and plasma to increase despite normalization of PRC and potassium levels by prolonged SPL treatment occurs only in patients with APA. Although the mechanism is not completely apparent, a reasonable interpretation of our data is that the potassium-aldosterone stimulatory mechanism in patients with an adenoma is blocked by SPL. This unique dual effect of SPL in patients with APA, as a competitor for the MCH receptor and as an inhibitor of PRC and/or potassium stimulation of aldosterone synthesis, most likely accounts for its effectiveness in the prolonged treatment of patients with APA and can be a useful diagnostic characteristic in ambiguous situations.

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