Aldosterone response to adrenocorticotrophin and furosemide in primary aldosteronism after prolonged spironolactone treatment

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We evaluated the effects of prolonged spironolactone treatment on aldosterone secretion in patients with primary aldosteronism. The patients were hospitalized and underwent a furosemide test with or without dexamethasone, as well as an adrenocorticotrophin (ACTH) test. In untreated patients, neither plasma renin activity (PRA) nor plasma aldosterone showed a response in the furosemide test. In patients receiving spironolactone, furosemide increased significantly both the PRA and the plasma aldosterone concentration (from 2.6 ± 0.8 to 7.0 ± 2.0 µg·l⁻¹·h⁻¹, p < 0.05) and from 345.6 ± 55.8 to 492.7 ± 76.8 ng/l (p < 0.05), mean ± SEM, respectively). Dexamethasone administration had no effect on the results of the furosemide test (p > 0.1). However, dexamethasone tended to decrease the basal plasma aldosterone concentration in the untreated patients, but not in the patients receiving spironolactone. In the ACTH test, the plasma aldosterone concentration increased significantly in the untreated patients (from 549.0 ± 69.8 to 1169.3 ± 165.5 ng/l, p < 0.01), but there was no significant aldosterone response in the spironolactone-treated patients (from 885.5 ± 204.9 to 1260.3 ± 289.2 ng/l, p > 0.1). We conclude that aldosterone secretion is mainly dependent on ACTH in the untreated patients with primary aldosteronism and is more strongly regulated by the renin–angiotensin system during spironolactone treatment.

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In patients with aldosterone-producing adenomas, ACTH plays a more important role than the renin–angiotensin system in aldosterone secretion (1–6). Spironolactone (SPL) is an antimineralocorticoid agent that is widely used to treat hyperaldosteronism and essential hypertension (7). However, surprisingly, few studies have assessed the response of aldosterone to ACTH and the activity of the renin–angiotensin system in patients with primary aldosteronism on SPL treatment. Our previous study revealed that aldosterone secretion is affected significantly by sodium ion intake in patients with primary aldosteronism receiving SPL. In patients with a sodium intake of 80 mEq/day, the plasma aldosterone level during SPL treatment was the same as in untreated patients, but plasma aldosterone levels were higher in patients with a low sodium intake of 15 mEq/day (8). In addition, other studies of ours have shown that the plasma renin activity (PRA) increases to normal during SPL therapy (9, 10). These data suggest that the renin–angiotensin system may be restored to normal by long-term treatment with SPL and then might modulate aldosterone secretion. Accordingly, the present study was performed to investigate the response of aldosterone to exogenous ACTH and furosemide before and during SPL treatment in patients with primary aldosteronism. To exclude the effect of endogenous ACTH on the furosemide standing test, it was performed with and without dexamethasone administration. Also, because basal aldosterone secretion and its secretion in response to bolus ACTH are enhanced by a low Na⁺ intake in normal subjects (11), we conducted the ACTH test with both a low Na⁺ intake of 15 mEq/day and a high Na⁺ intake of 342 mEq/day.

Subjects

Twelve patients with primary aldosteronism were enrolled in this study. The diagnosis was based on the presence of hypertension, hypokalaemia, a high plasma aldosterone concentration, low PRA, detection of an adenoma by adrenal scintigraphy and/or computed tomography and determination of the adrenal venous aldosterone concentrations. All patients were operated on and the diagnosis of adrenal adenoma was confirmed by pathological examination. The patient profiles are summarized in Table 1. Clinical data were obtained on the first hospital day.
Table 1. Clinical profiles of the patients with primary aldosteronism.  

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age (years) (mmHg)</th>
<th>Blood pressure (mEq/l)</th>
<th>K (mEq/l)</th>
<th>PAC (ng/l)</th>
<th>PRA (µg·1⁻¹·h⁻¹)</th>
<th>Side of adenoma</th>
<th>Blood pressure (mmHg)</th>
<th>K (mEq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F</td>
<td>46</td>
<td>190/118</td>
<td>2.8</td>
<td>520</td>
<td>0.3</td>
<td>Left</td>
<td>130.80</td>
<td>4.2</td>
</tr>
<tr>
<td>2.</td>
<td>F</td>
<td>53</td>
<td>170/106</td>
<td>1.8</td>
<td>230</td>
<td>0.1</td>
<td>Left</td>
<td>140/90</td>
<td>4.0</td>
</tr>
<tr>
<td>3.</td>
<td>M</td>
<td>49</td>
<td>208/128</td>
<td>3.1</td>
<td>1092</td>
<td>0.2</td>
<td>Right</td>
<td>144/80</td>
<td>4.1</td>
</tr>
<tr>
<td>4.</td>
<td>M</td>
<td>43</td>
<td>160/110</td>
<td>3.5</td>
<td>441</td>
<td>0.5</td>
<td>Right</td>
<td>140/90</td>
<td>4.0</td>
</tr>
<tr>
<td>5.</td>
<td>F</td>
<td>54</td>
<td>150/80</td>
<td>3.2</td>
<td>291</td>
<td>0.3</td>
<td>Left</td>
<td>132/82</td>
<td>4.9</td>
</tr>
<tr>
<td>6.</td>
<td>M</td>
<td>44</td>
<td>178/114</td>
<td>2.4</td>
<td>311</td>
<td>0.1</td>
<td>Right</td>
<td>114/84</td>
<td>4.1</td>
</tr>
<tr>
<td>7.</td>
<td>F</td>
<td>43</td>
<td>140/96</td>
<td>3.3</td>
<td>210</td>
<td>0.1</td>
<td>Right</td>
<td>120/80</td>
<td>4.2</td>
</tr>
<tr>
<td>8.</td>
<td>F</td>
<td>33</td>
<td>160/100</td>
<td>3.4</td>
<td>509</td>
<td>0.1</td>
<td>Left</td>
<td>133/90</td>
<td>4.0</td>
</tr>
<tr>
<td>9.</td>
<td>M</td>
<td>40</td>
<td>178/106</td>
<td>2.6</td>
<td>1043</td>
<td>0.5</td>
<td>Left</td>
<td>133/90</td>
<td>4.0</td>
</tr>
<tr>
<td>10.</td>
<td>M</td>
<td>30</td>
<td>190/110</td>
<td>2.8</td>
<td>1993</td>
<td>0.4</td>
<td>Left</td>
<td>133/90</td>
<td>4.0</td>
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<tr>
<td>11.</td>
<td>M</td>
<td>36</td>
<td>208/120</td>
<td>2.7</td>
<td>204</td>
<td>0.3</td>
<td>Right</td>
<td>120/80</td>
<td>4.2</td>
</tr>
<tr>
<td>12.</td>
<td>F</td>
<td>57</td>
<td>178/100</td>
<td>2.3</td>
<td>847</td>
<td>0.3</td>
<td>Right</td>
<td>133/90</td>
<td>4.0</td>
</tr>
</tbody>
</table>

PA: primary aldosteronism; SPL: spironolactone; PA+SPL: primary aldosteronism with spironolactone treatment; PAC: plasma aldosterone concentration; PRA: plasma renin activity; K: serum potassium.

Examinations were performed at Gunma University Hospital after obtaining the informed consent of the patients, and the study was approved by the Gunma University Hospital Ethics Committee. Five patients were tested both before and during treatment with SPL, another five patients were only tested before treatment and two were only tested during treatment. The daily dose of SPL in the seven patients tested during treatment ranged from 75 to 225 mg. Blood pressure and hypokalaemia were well controlled for more than 3 months by this regimen.

Methods

Furosemide standing test

All patients were maintained on a constant metabolic diet throughout the study with daily sodium and potassium intakes of 85 and 80 mEq, respectively. No experimental procedures were performed in the first 6 days after admission. On the 7th day, the patients ate lunch and then rested in the supine position for 1 h. Blood was collected to determine the basal values of each hormone. Blood was collected again after the injection of 40 mg of furosemide with the patient ambulatory for 2 h. Each patient then underwent a second furosemide standing test under dexamethasone treatment. To suppress endogenous ACTH, 0.5 mg of dexamethasone was administered orally at 4 h intervals on days 13 and 14. Then the furosemide test was carried out on day 14.

Adrenocorticotrophin test

Three untreated patients and one patient receiving SPL did not consent to the ACTH test or were otherwise unavailable. The study was first performed with a low Na⁺ intake (15 mEq/day) and was repeated with a high Na⁺ intake (342 mEq/day). The daily K⁺ intake was 80 mEq/day regardless of the sodium ion intake. The low Na⁺ regimen was begun on day 15 and the ACTH test was performed on day 23. Breakfast was withheld and all patients remained supine throughout the test, which started in the early morning. Blood samples were

Table 2. Basal plasma renin activity, plasma aldosterone and cortisol concentrations of spironolactone-treated and untreated patients with primary aldosteronism with and without dexamethasone administration in the furosemide test.

<table>
<thead>
<tr>
<th></th>
<th>Without DEX</th>
<th>With DEX</th>
<th>Without DEX</th>
<th>With DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>469.1 ± 102.7</td>
<td>319.5 ± 43.4</td>
<td>345.6 ± 55.8</td>
<td>332.0 ± 46.4</td>
</tr>
<tr>
<td>PAC (ng/l)</td>
<td>0.2 ± 0.05*</td>
<td>0.2 ± 0.1*</td>
<td>2.6 ± 0.8</td>
<td>5.8 ± 2.0</td>
</tr>
<tr>
<td>PRA (µg·1⁻¹·h⁻¹)</td>
<td>90 ± 15</td>
<td>33 ± 12*</td>
<td>142 ± 33</td>
<td>70 ± 23*</td>
</tr>
</tbody>
</table>

PA: primary aldosteronism; DEX: dexamethasone; PA+SPL: primary aldosteronism with spironolactone treatment; PAC: plasma aldosterone concentration; PRA: plasma renin activity. All values are the mean + SEM: *p < 0.05, vs without dexamethasone; **p < 0.01, PA vs PA+SPL.
generated angiotensin I per millilitre of plasma during 1 h of incubation. The plasma aldosterone concentration was determined by radioimmunoassay after chromatographic separation, as described previously (12), and the plasma cortisol concentration was measured using a commercial radioimmunoassay kit (Eiken Immunochem Lab., Tokyo, Japan).

**Statistics**

Data were expressed as the mean ± SEM. The Wilcoxon test was used to compare changes from baseline in the response of each hormone to each test. The differences in hormonal values were assessed also by the Wilcoxon test between patients with and without dexamethasone treatment in the furosemide test or with a low and high Na⁺ intake in the ACTH test. Differences in basal hormonal values before and during SPL treatment were assessed by the Mann-Whitney test. A level of p < 0.05 was considered to indicate statistical significance.

**Results**

**Furosemide standing test**

The basal values of each hormone in the furosemide standing test are shown in Table 2. Although the mean basal plasma aldosterone concentration was higher in the SPL-untreated patients not given dexamethasone than those given dexamethasone, the difference was not significant. The basal PRA was increased significantly by SPL treatment independently of dexamethasone administration, but SPL did not affect the basal cortisol level irrespective of dexamethasone.

Dexamethasone administration suppressed endogenous ACTH because the plasma cortisol concentration was decreased significantly in both the SPL-treated and SPL-untreated patients. However, the basal plasma aldosterone concentration showed no significant difference between these two, irrespective of dexamethasone administration.

The results of the furosemide test are summarized in Fig. 1. Administration of furosemide did not affect the PRA in the SPL-untreated patients but increased it significantly in the SPL-treated patients both with and without dexamethasone (from 2.6 ± 0.8 to 7.0 ± 2.0(p < 0.05) and from 5.8 ± 2.0 to 10.8 ± 2.0 µg·l⁻¹·h⁻¹ (p < 0.05), respectively). The plasma aldosterone concentration was unaffected by furosemide in the SPL-untreated patients but was increased significantly in the SPL-treated patients both with and without dexamethasone (from 345.6 ± 55.8 to 492.7 ± 76.8, (p < 0.05) and from 332.0 ± 46.4 to 423.6 ± 34.1 ng/l (p < 0.05), respectively). The serum cortisol concentration was unaffected by furosemide in all groups.
Table 3. Basal plasma renin activity, plasma aldosterone and cortisol concentrations of spironolactone-treated and untreated patients with primary aldosteronism in the ACTH test on low and high Na⁺.

<table>
<thead>
<tr>
<th></th>
<th>PA</th>
<th></th>
<th>PA+SPL</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Na⁺</td>
<td>High Na⁺</td>
<td>Low Na⁺</td>
<td>High Na⁺</td>
</tr>
<tr>
<td>PAC (ng/l)</td>
<td>549.0 ± 69.8</td>
<td>469.1 ± 90.5</td>
<td>885.5 ± 204.9</td>
<td>433.3 ± 59.7</td>
</tr>
<tr>
<td>PRA (µg·l⁻¹·h⁻¹)</td>
<td>0.3 ± 0.2**</td>
<td>0.2 ± 0.1</td>
<td>7.0 ± 2.0</td>
<td>3.03 ± 2.06*</td>
</tr>
<tr>
<td>Cortisol (µg/l)</td>
<td>71 ± 18</td>
<td>97 ± 16*</td>
<td>119 ± 13</td>
<td>122 ± 18</td>
</tr>
</tbody>
</table>

*PA: primary aldosteronism; PA+SPL: primary aldosteronism with spironolactone treatment; PAC: plasma aldosterone concentration; PRA: plasma renin activity. All values are the mean ± SEM. *p < 0.05, low Na⁺ intake vs high Na⁺ intake. **p < 0.01. PA vs PA+SPL.

Adrenocorticotrophin test

The basal values of each hormone in the ACTH test are shown in Table 3. The increase of the basal plasma aldosterone concentration with SPL treatment (from 549.0 ± 69.8 to 885.5 ± 204.9 ng/l) was not statistically significant. The basal PRA was only increased significantly by SPL treatment with a low Na⁺ intake in accordance with the results of the furosemide standing test (from 0.3 ± 0.2 to 7.0 ± 2.0 µg·l⁻¹·h⁻¹, p < 0.05). In contrast, a high Na⁺ intake decreased the basal PRA and plasma aldosterone concentration in the SPL-treated patients.

The response of each hormone to ACTH injection is summarized in Fig. 2. Bolus intravenous injection of ACTH elevated the PRA in the SPL-untreated patients with a low Na⁺ intake, but did not alter PRA in the other groups. Injection of ACTH increased the serum cortisol concentration in all groups. The plasma aldosterone concentration of the SPL-untreated patients was increased significantly by ACTH stimulation independently of Na⁺ intake. However, although the mean plasma aldosterone concentration of the treated patients also increased after ACTH stimulation, the increase was not statistically significant.

The percentage increase of plasma aldosterone in response to ACTH stimulation is shown in Fig. 3. The percentage increase tended to be smaller during SPL treatment (p = 0.10).

Discussion

In this study, to evaluate the effect of SPL on aldosterone secretion we used the bolus intravenous injection of ACTH and the injection of furosemide with standing to stimulate aldosterone. Although a plasma aldosterone response to the upright position has been reported in primary aldosteronism (13, 14), the response is generally smaller in untreated patients with primary aldosteronism than in patients with other forms of hyperaldosteronism. In this study, we found that the plasma aldosterone concentration of the SPL-untreated patients was not increased by the furosemide test, in agreement with previous reports (1, 4), while it rose significantly during SPL treatment. During SPL treatment, the basal PRA and K levels also rose to normal. The furosemide test showed that PRA was greatly stimulated in the SPL-treated patients, while it remained suppressed in the SPL-untreated patients. These data suggest that the plasma aldosterone response is induced by transient and significant activation of the renin–angiotensin system in patients with primary aldosteronism on treatment with SPL.

In contrast, Kater et al. (15) reported that the plasma aldosterone concentration did not change in response to standing after SPL treatment. This discrepancy may be due to differences in the duration of SPL treatment, the sodium intake, and the strength of the stimulus used.

As the plasma aldosterone response to the furosemide test was unrelated to dexamethasone treatment, endogenous ACTH may not be involved in the response to this test. However, the decrease of the basal plasma aldosterone concentration after dexamethasone administration tended to be more prominent in the SPL-untreated than the SPL-treated patients. Dexamethasone decreased the basal plasma aldosterone in seven out of 10 patients not on SPL therapy and in only two out of seven patients receiving SPL. A similar result was reported by Ganguly et al. (4), suggesting that treatment with SPL may modify the plasma aldosterone response to ACTH stimulation. The ACTH test revealed the effect of SPL more clearly. In the patients not on SPL, plasma aldosterone increased significantly after the bolus of ACTH while the response was not as pronounced during SPL treatment. Thus, they hyperresponsiveness of aldosterone to ACTH in patients with primary aldosteronism was attenuated by SPL treatment. The difference in basal PRA and plasma aldosterone levels between SPL-untreated patients with a low Na⁺ or a high Na⁺ intake was significant, but the PRA or aldosterone response was the same irrespective of Na⁺ intake. Hence, the renin–angiotensin system appeared to have no effects on the attenuation of aldosterone hyperresponsiveness by SPL therapy.

The mechanism by which SPL acts to reduce the response of aldosterone to ACTH may be explained by the inhibition of steroidogenesis. The degradation of adrenal microsomal and mitochondrial P-450 by SPL...
has been proposed as the reason why this drug inhibits steroidogenesis. In fact, in vitro studies have shown that the activity of P-450C21 (16,17), P-45011β (18) and 17-α hydroxylase (19) are all reduced by SPL. On the other hand, adrenal cytochrome P-450 activity in connection with steroidogenesis is increased in aldosteronoma (20, 21). These findings suggest that the decrease in the hyperactivity of adrenal microsomal and mitochondrial P-450 noted in aldosteronoma patients on SPL therapy may be the mechanism that blunts the

Fig. 2. Response to bolus injection of ACTH in patients with untreated primary aldosteronism (PA) and treated patients receiving spironolactone (SPL). The test was carried out with a low (15 mEq/day) or high (243 mEq/day) Na⁺ intake. Columns and bars represent the mean ± sem of each hormone concentration. *p < 0.05, before vs ACTH injection.

Fig. 3. The percentage increase in plasma aldosterone in response to a bolus injection of ACTH in patients with untreated primary aldosteronism (PA) and patients receiving spironolactone (SPL). Columns and bars represent the mean ± sem of the percentage increase in the plasma aldosterone concentration after ACTH injection. *p = 0.1, untreated PA vs SPL treatment.

hyperresponsiveness of aldosterone to ACTH in patients with untreated primary aldosteronism.

In conclusion, we documented an enhanced effect of the response of the renin–angiotensin system on aldosterone and reduced hyperresponsiveness of aldosterone to ACTH in patients with primary aldosteronism receiving SPL therapy. Thus, SPL not only controls the blood pressure but also normalizes the response of aldosterone to the renin–angiotensin system and ACTH in primary aldosteronism. Normalization of the effect of the renin–angiotensin system may be involved in the clinical activity of this agent.

References


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