RESPONSES OF ALDOSTERONE-PRODUCING ADENOMAS TO ACTH AND ANGIOTENSINS

By
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ABSTRACT

To elucidate the control mechanism of aldosterone production in primary aldosteronism, in vivo and in vitro studies were done in 7 patients with aldosterone-producing adenomas. In the in vivo study, plasma aldosterone was stimulated more significantly by $\frac{1-24}{a}$ synthetic ACTH than by angiotensin II or furosemide. Diurnal variations of plasma aldosterone, which were studied in 4 patients, were similar to those seen in normal controls. In agreement with the results in the in vivo study, the in vitro study also revealed ACTH stimulated aldosterone and deoxycorticosterone (DOC) from the adenoma more markedly than angiotensin II or III. There was no adenoma which was more sensitive to angiotensin II or III than to ACTH. From these results it is considered that changes in plasma aldosterone induced by the exogenous administration of angiotensin II or ACTH in patients with aldosterone-producing adenoma are mainly based on changes in aldosterone production in the adenoma. Furthermore, in patients with an aldosterone-producing adenoma in whom diurnal variations of plasma aldosterone similar to those in normal subjects are observed, responses of aldosterone to angiotensin II are supposed to be less than those to ACTH.
It is known that the control mechanism of aldosterone in patients with an aldosterone-producing adenoma is different from that in patients with hyperplasia. In patients with primary aldosteronism due to hyperplasia, it is reported that angiotensin II plays an important role in control of aldosterone (Wisgerhof et al. 1978). On the other hand, several studies suggested that ACTH may play an important role in control of aldosterone in patients with an aldosterone-producing adenoma, since diurnal variations of plasma aldosterone in the patients are similar to those seen in normal subjects (Kem et al. 1973, 1978) and furthermore since aldosterone production in patients with an aldosterone-producing adenoma is reduced by administering dexamethasone or glucocorticoids (Newton & Laragh 1968; Slaton et al. 1969). However, contrary results are reported. Spark et al. (1969) reported that angiotensin II is much more important in control of aldosterone in patients with an aldosterone-producing adenoma. Furthermore, a recent study by Wenting et al. (1978) has suggested that even in patients with an aldosterone-producing adenoma, there is a dissociation of the responses of aldosterone to ACTH and to angiotensin II in some cases, and the presence or absence of ACTH-dependency cannot be used as an absolute criterion for differentiating adenoma from non-adenoma cases. In this study, the control mechanism of aldosterone in patients with an aldosterone-producing adenoma was investigated in both in vivo and in vitro systems.

**MATERIALS AND METHODS**

Seven patients with primary aldosteronism (3 men and 4 women) were studied. Main laboratory findings of these patients are summarized with the amounts of aldosterone and DOC in each adenoma in Table 1 and Table 2. In 5 of the 7 patients effects of ACTH, furosemide and angiotensin II on plasma aldosterone were studied before surgery. Before these tests all medication was interrupted for at least 2 weeks. After this these stimulation tests were carried out on a regular sodium diet (150-200 mEq./day of sodium). On this diet urinary excretion of sodium was between 120 and 180 mEq./day in all patients. In the stimulation test by ACTH, 0.25 mg of a synthetic \( \frac{1-21}{a} \) ACTH (Cortrosyn) was given by im administration. Blood samples were drawn just before and one hour after the administration. In the stimulation by angiotensin II (Hypertensin, Ciba), it was infused at a dose which increased 20 mmHg of diastolic blood pressure above the base line for one hour. In the stimulation by furosemide, 40 mg of furosemide was administered iv. Blood samples were drawn before and two hours after the administration. These tests were taken every other day in the supine position, in the order of the administration of angiotensin II, ACTH and furosemide.

The tissue study based on previous studies (Kaplan & Bartter 1962; Saruta et al. 1972), was done in the following manner. Approximately 0.5 mm thick slices of adenoma were combined and minced to obtain uniformity. At least 4 vessels containing 100 mg of tissue were prepared. In all the experiments the tissues were pre-incubated...
<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Blood pressure (mmHg)</th>
<th>Serum sodium (mEq/l)</th>
<th>Serum potassium (mEq/l)</th>
<th>Plasma renin activity (ng/ml/h)</th>
<th>Plasma aldosterone (ng/100 ml)</th>
<th>Plasma DOC (ng/100 ml)</th>
<th>Weight of adenoma (g)</th>
<th>Aldosterone in adenoma (µg/g)</th>
<th>DOC in adenoma (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 F</td>
<td>42</td>
<td>210/110</td>
<td>148</td>
<td>3.2</td>
<td>0.4</td>
<td>70.0</td>
<td>6.2</td>
<td>2.6</td>
<td>16.2</td>
<td>0.85</td>
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<td>164/112</td>
<td>150</td>
<td>2.9</td>
<td>0.3</td>
<td>20.0</td>
<td>2.5</td>
<td>3.8</td>
<td>10.2</td>
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<tr>
<td>Case 3 M</td>
<td>46</td>
<td>232/138</td>
<td>145</td>
<td>2.5</td>
<td>0.3</td>
<td>25.0</td>
<td>6.2</td>
<td>2.2</td>
<td>12.6</td>
<td>0.46</td>
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<tr>
<td>Case 4 M</td>
<td>22</td>
<td>174/108</td>
<td>149</td>
<td>2.9</td>
<td>0.8</td>
<td>20.0</td>
<td>14.2</td>
<td>2.4</td>
<td>18.0</td>
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<tr>
<td>Case 5 F</td>
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<td>160/100</td>
<td>148</td>
<td>2.4</td>
<td>0.5</td>
<td>60.0</td>
<td>4.6</td>
<td>4.5</td>
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<td>12.2</td>
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<tr>
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<td>0</td>
<td>41.2</td>
<td>8.6</td>
<td>4.5</td>
<td>6.2</td>
<td>0.52</td>
</tr>
</tbody>
</table>

F: woman; M: man.
Table 2.
Diurnal variation of plasma aldosterone in patients with primary aldosteronism with adenoma.

<table>
<thead>
<tr>
<th>Case number</th>
<th>plasma aldosterone (ng/100 ml)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Case 1</td>
<td>30.5</td>
</tr>
<tr>
<td>Case 2</td>
<td>20.0</td>
</tr>
<tr>
<td>Case 4</td>
<td>18.0</td>
</tr>
<tr>
<td>Case 7</td>
<td>16.4</td>
</tr>
<tr>
<td>Normal controls (n = 3)</td>
<td>4.8 ± 2.5</td>
</tr>
</tbody>
</table>

Case numbers are in accordance with the case numbers in Table 1.
Results in normal controls are expressed as mean ± sd.

for 30 min in 2 ml of Krebs-Ringer bicarbonate medium containing a glucose concentration of 200 mg/100 ml. The pre-incubation medium was then replaced with 2 ml of fresh medium and an appropriate stimulatory agent was added, excepting to the control vessel. Each vessel was gassed with 95% O₂-5% CO₂ and capped tightly. In each adenoma at least 8 vessels were prepared, and each 2 vessels was used for the control, ACTH, angiotensin II or angiotensin III. Incubation was usually for 2 hours.

In all the studies, 25 U of \( \frac{1-24}{a} \) ACTH/g tissue, 20μg of angiotensin II (Hypertensin, Ciba), and angiotensin III (Protein Research Foundation; Osaka) were added as stimulants.

After incubation the medium was decanted and 0.5 ml was analyzed for aldosterone and DOC by radioimmunoassay (Bayard et al. 1970; Fukuchi et al. 1974). One paper chromatographic separation was used before radioimmunoassay (Saruta et al. 1972). The intra-assay variations and inter-assay variations of the measurements by this method were 8.2 ± 3.5 (m ± sd) % and 12.5 ± 4.2 %, respectively. As the basal amounts of aldosterone and DOC were various in each adenoma, the per cent increases from the values in the control vessels were calculated for the effects of ACTH, angiotensin II and angiotensin III on aldosterone and DOC.

In order to compare these results with results in normal adrenal cortex, effects of ACTH, angiotensin II and angiotensin III on aldosterone and DOC were studied, using 3 adrenal glands of 3 patients with breast cancer. Outer slices of the adrenal cortex (approximately 0.3 mm in thickness) were minced and 100 mg of the tissue was placed in each vessel. Successive steps were the same in the study on adenomas.

Statistical analysis was carried out using Student's t-test. Values are presented as mean ± standard deviation.
RESULTS

In vivo experiment

In the control subjects plasma aldosterone was stimulated from 10.2 ± 3.8 to 28.5 ± 5.0 ng/100 ml by ACTH (P < 0.05), 12.2 ± 4.4 to 24.2 ± 8.2 ng/100 ml by angiotensin II (P < 0.05), and 9.8 ± 4.0 to 26.0 ± 5.5 ng/100 ml by furosemide (P < 0.05; shaded parts in Fig. 1). In all of the patients with primary aldosteronism, plasma aldosterone was stimulated significantly by ACTH, but responses of plasma aldosterone to angiotensin II (from 25.6 ± 6.2 to 31.6 ± 6.6 ng/100 ml; P > 0.05) were significantly less than those to ACTH (from 32.4 ± 6.5 to 53.0 ± 12.0 ng/100 ml; P < 0.05), as shown in Fig. 1. Responses of plasma aldosterone to furosemide (from 29.5 ± 7.5 to 39.5 ± 8.5 ng/100 ml; P > 0.05) were similar to those to angiotensin II.

In vitro experiment

In the tissue studies using normal adrenal cortex, the production rate of aldosterone per gram of adrenal cortex during 2 hours of incubation ranged from 0.06 to 0.12 μg/g tissue, and the production rate of DOC per gram of adrenal cortex ranged from 0.05 to 0.11 μg/g tissue. The effect of 25 U of ACTH/g tissue on the production of aldosterone was similar to that of 20 μg/g tissue of angiotensin II or angiotensin III, although DOC was markedly stimulated by ACTH only (Fig. 2).

![Graphs showing effects of ACTH, angiotensin II, and furosemide on plasma aldosterone levels.](image)

*Fig. 1.* Effects of ACTH (Cortrosyn 0.25, im), angiotensin II (a one-hour infusion to increase 200 mmHg of diastolic pressure), and furosemide (40 mg, iv) on plasma aldosterone in patients with an aldosterone-producing adenoma.
Direct effects of ACTH, angiotensin II and angiotensin III on aldosterone-producing adenomas and normal adrenal cortex. In each adenoma or each adrenal gland, effects of ACTH, angiotensin II and angiotensin III on aldosterone or DOC were compared with the control without these agents. Results are expressed as the mean ± standard deviation.

In the study on adenomas, basal amounts of aldosterone and DOC were various in each adenoma. The production rate of aldosterone per gram of adenoma during two hours of incubation ranged from 0.81 µg/g to 2.10 µg/g tissue. The production rate of DOC ranged from 0.06 µg/g to 0.11 µg/g tissue. Differing from the results of the findings on normal adrenal glands, aldosterone in the adenoma responded more significantly to AGTH (P < 0.05). Responses of aldosterone to angiotensin II or angiotensin III were slight, and there was no significant difference between the effects of angiotensin II and angiotensin III (P > 0.05). DOC in the adenoma also responded more significantly to ACTH than to angiotensin II or angiotensin III (P < 0.05; Fig. 2).

**DISCUSSION**

In this in vivo study plasma aldosterone was stimulated less by angiotensin II than by ACTH. The amount of angiotensin II infused in all patients was approximately 5 to 6 ng/kg/min, which was less than that employed by Spark et al. (1969). However, as this amount of angiotensin II was almost equally
potent to 0.25 mg of ACTH in stimulation of plasma aldosterone in normal subjects (Saruta et al. 1975), it was considered that, in this study, the response of plasma aldosterone to angiotensin II was less sensitive than the response to ACTH in patients with an aldosterone-producing adenoma. It is not certain, however, whether ACTH stimulated aldosterone production in the adenoma or in the adrenal glands. The direct effects of ACTH and angiotensin II on aldosterone-producing adenomas were then studied. In this in vitro experiment the effects of angiotensin III, which is known to be equipotent to angiotensin II (Blair-West et al. 1971; Spielman et al. 1974; Saruta et al. 1977) in the stimulation of aldosterone, were also studied.

The amounts of ACTH, angiotensin II and angiotensin III which were employed in this study were almost equally potent in stimulation of aldosterone on the outer cortex of normal adrenal glands. On the other hand, aldosterone production in the adenomas was stimulated more significantly by ACTH than by angiotensin II or angiotensin III. There was no adenoma in which aldosterone was much more significantly stimulated by angiotensin II than ACTH. As the diurnal variations of plasma aldosterone which were studied in 4 of 7 patients before surgery, showed the pattern similar to those seen in normal subjects, it might be considered that adenomas in the patients with the diurnal variations of plasma aldosterone similar to those in normal subjects are less sensitive to angiotensin II than ACTH. Although Wenting et al. (1978) reported a few patients in whom diurnal variations of plasma aldosterone were different from those in normal subjects and a significant response to angiotensin II was observed, and Carey et al. (1979) reported 2 patients with an aldosterone-producing adenoma in whom plasma aldosterone responded well to angiotensin III, there was no such a patient in this study.

In this in vitro study, effects of ACTH and angiotensin II or angiotensin III on DOC production were also studied in order to see the early steps of aldosterone production. Just as seen in the outer cortex of normal adrenal glands, DOC in the adenoma was stimulated more significantly by ACTH than by angiotensin II. As aldosterone in all of the adenomas in this study was less sensitive to angiotensin II than ACTH, DOC in the adenomas might be more significantly stimulated by ACTH. It is interesting to study the early steps of aldosterone production in patients with primary aldosteronism in whom aldosterone is more sensitive to angiotensin II than ACTH.

From these results it is concluded that changes in plasma aldosterone induced by the exogenous administration of ACTH or angiotensin II in patients with an aldosterone-producing adenoma are mainly based on changes in aldosterone production in the adenomas. In all of the 7 patients with an aldosterone-producing adenoma, responsiveness of aldosterone and DOC to angiotensin II was less than that to ACTH.
REFERENCES


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