STUDIES ON THE IN VITRO PRODUCTION OF ALDOSTERONE AND OTHER CORTICOIDS IN THE ADRENAL ADENOMA OF A PRIMARY ALDOSTERONISM

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Since the first description of primary aldosteronism by Conn in 1955, about 30 cases have been reported in Japan.

This paper deals with the in vitro production of steroids from either an adenomatous or non-adenomatous portion of the adrenal of primary aldosteronism with typical clinical symptoms.

From our experimental results as well as histological findings, the pathogenesis of aldosteronism and its related factors are discussed.

CASE REPORT

A 38-year-old Japanese male had been treated for several years against hypertension. Weakness of the upper extremities was occasionally complained, but no paralysis noted. The therapy given being noneffective, the patient came to our clinic with complaints of headache, dizziness, insomnia, anxiety, thirst, muscular asthenia, etc. Examinations at the time of admission (Table 1) showed hypertension (181/116), hypokalemic alkalosis, hyposthenuria which was found unresponsible to pitressin, a diminution in concentration power, polyuria, muscular asthenia, an increase of secretion and excretion of aldosterone, all of which suggested primary aldosteronism. Pyelography and retroperitoneal pneumography were performed for the possible presence of adrenal tumor. The result revealed the left nephroptosis and hypertrophy of the left adrenal which supported the diagnosis of primary aldosteronism. Left unilateral adrenalectomy was carried out. The removed tumor was round and yellowish in color, weighing 2.2 g and measuring approximately 15 × 12 mm in diameter. Removal of the left adrenal led to a regression of the patient’s symptomatic manifestations (Table 1 and Fig. 1).

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Table 1. Clinical examination

<table>
<thead>
<tr>
<th>Blood chemistry</th>
<th>concentration test</th>
</tr>
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<tbody>
<tr>
<td>total protein</td>
<td>I 1015</td>
</tr>
<tr>
<td>rest N</td>
<td>II 1018</td>
</tr>
<tr>
<td>A/G ratio</td>
<td>III 1016</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urine examination</th>
<th>Endocrine examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>daily amount</td>
<td>urinary 17 KS 10.7 µg/day (3.6)</td>
</tr>
<tr>
<td>specific gravity</td>
<td>urinary 17 OHCS 8.0 µg/day (6.9)</td>
</tr>
<tr>
<td>Na/K-ratio</td>
<td>urinary aldosterone 5.5, 11.7 µg/day (3.3, 2.9)</td>
</tr>
<tr>
<td>albumin trace</td>
<td>aldosterone secretion rate 920 µg/day</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal function test</th>
<th>Regitin test</th>
<th>Pitressin test</th>
<th>ACTH-Z test</th>
<th>SU-4885 test</th>
<th>Suppression test due to dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenolsulphathalein</td>
<td>negative response</td>
<td>negative response</td>
<td>normal response</td>
<td>normal response</td>
<td></td>
</tr>
<tr>
<td>renal plasma flow</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>glomerular filtration rate</td>
<td>14.75 ml/min. (15.7)</td>
<td></td>
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</table>

( ): postoperative value.

MATERIALS AND METHODS

Material: The removed adrenal was separated into adenomatous and non-adenomatous parts immediately after operation. The separated two parts of the adrenal were used for in vitro incubation experiments and histological studies.

Incubation method: The sliced material was incubated at 37°C in a glucose-Krebs-bicarbonate buffer solution, 5 ml/500 mg of adrenal tissue. The solution was replaced by another in 1 hr. The solution processed during the subsequent hour was combined with the former solution to be treated as follows.

Extraction, separation and determination of corticosteroids: A crude extract was obtained by extraction of the incubation medium at pH 1 with 5 vol. of chloroform and was preliminarily purified on florisil column. Aldosterone, cortisol, cortisone and corticosterone fractions were separated with two paper chromatographic systems, i.e. butyl acetate-formamide-water system (Mattox, 1958) and Bush B3 system (Bush, 1952). These steroids were measured by the reaction with blue tetrazolium (Nowaczynski et al., 1957).

Bioisntthetic activity of aldosterone in vitro: In order to examine the biosynthetic activity of aldosterone originating in corticosterone in the removed adrenal tissue, 0.5 µC of corticosterone-4-C14 (specific activity: 44.6 µC/mg) was added to the incubation medium and the radioactivity of the aldosterone fraction was determined.
Fig. 1. Postoperative course
RESULTS

1) The adenomatous tissue of the removed adrenal produced a large amount of corticosterone as well as aldosterone while the production of cortisol and cortisone was scarcely observed (Fig. 2).

![Tissue Steroids Production Table](image)

<table>
<thead>
<tr>
<th>tissue</th>
<th>steroids</th>
<th>0</th>
<th>5</th>
<th>10</th>
</tr>
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<tr>
<td>adenoma</td>
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</tr>
<tr>
<td>aldosterone</td>
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<tr>
<td>cortisol</td>
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</tr>
<tr>
<td>cortisone</td>
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</tr>
<tr>
<td>corticosterone</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>total</td>
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</tr>
<tr>
<td>non-adenomatous</td>
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<td></td>
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</tr>
<tr>
<td>adrenal tissue</td>
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</tr>
<tr>
<td>aldosterone</td>
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<td>cortisol</td>
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<td>cortisone</td>
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<tr>
<td>corticosterone</td>
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<tr>
<td>total</td>
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(μg/g of adr. weight 2 hrs.)

Fig. 2. Corticoids production in adrenal tissue from the patient of primary aldosteronism in vitro

2) Non-adenomatous tissue produced less aldosterone than adenomatous tissue, the ratio being 1:2.5~3, although aldosterone was still produced in a considerable amount. But the non-adenomatous tissue produced a rather small amount of corticosterone while it produced a little more amount of cortisol and cortisone.

3) Incubation of an adrenal slice with corticosterone-4-C\(^{14}\) resulted in the biosynthetic activity of aldosterone in the adenomatous tissue 4~5 times that in the non-adenomatous tissue (Fig. 3).
Fig. 3. Biosynthesis of aldosterone from 4-c14-corticosterone in adenomatous tissue in vitro

Fig. 4. The tumor cells show marked variation in size, but no infiltration into the capsule. (×100)

Fig. 5. The cells in the adenoma Note the cell structure similar to clear cell carcinoma. (×400)
4) Histologically, the tumor contained tumor cells of varying size and was encapsulated by a thin connective tissue. Since there was observed neither mitosis nor intracapsular infiltration of tumor cells, the tumor was suggested to be benign (Fig. 4). The tumor cells were arranged as if in zona glomerulosa; endothelial cells were found between tumor cells. Most of the tumor cells, being varying in size, had a clear cytoplasm so that they were likely to give a confusing impression of a clear cell carcinoma (Fig. 5). The tumor cell contained many sudanophil granules. The contained lipids showed a positive carbonyl staining and double refraction. The above histological findings support a diagnosis of adrenocortical adenoma.

Fig. 6. The hyperplastic cells of the adrenal cortex outside adenoma
Note the growth of tumor cell-like cells. (×100)

Fig. 7. Hypertrophy of the intima of small vessels in the kidney (×400)

The adrenal cortex except for the adenomatous tissue exhibited an atrophy of zona fasiculata. Small groups of apparent tumor cells were scattered among zona glomerulosa and fasiculata. In some parts there were found extending from the capsule to the medulla uniform sized, grouped cells which were arranged in a glomerular shape and possessed of a clear cytoplasm. This finding led to a suggestion of “microscopic adenoma” (Fig. 6).

In the kidney there were found a hypertrophy of the intima of small vessels and hyalinization of glomeruli (Fig. 7).

DISCUSSION

The results of the present experiments give rise to the solution of the following problems: (1) whether the tumor is regulated by the pituitary or not, (2) production of steroids, especially secretion of corticosterone, other than aldosterone, and (3) activity of 18-hydroxylase in the adenomatous and non-adenomatous tissue.

To date, there have been conflicting views on the first problem, some admitting the autonomy of the tumor cell which secretes aldosterone and others' denying this. The patient of the present study excreted 11.7 and 5.5 μg/day of aldosterone under
normal conditions (determined by our modified method of Mattox and Lewbart, 1959), but when body fluid volume decreased by restriction of water intake, an excretion of aldosterone increased to 25.0 µg in 24 hours' urine. Thus a secretion of aldosterone in patients with primary aldosteronism seems to be regulated by changes of hemodynamics due to an alteration of body fluid volume, which, however, does not always seem to deny an autoregulation of aldosterone secretion. Our observations are consistent with the reports of Bartter and Biglieri (1958), Gordon and Eichenholz (1959) and others, who noted an increased secretion of aldosterone following a restriction of sodium intake (Peterson, 1960; Biglieri and Forsham, 1961), an administration of potassium (Eales, 1956; Garrod et al., 1956), bleeding (Biglieri and Forsham, 1961) and an administration of aldosterone antagonists (Peterson, 1960; Biglieri and Forsham, 1961).

The results of the incubation experiments indicated an increased production of aldosterone in the adenomatous tissue. This may be interpreted as evidence of the autonomous hypersecretion of aldosterone in tissues independent of any stimulating factors. Therefore, alterations of aldosterone production in response to changes of hemodynamics seem due to the secretion of aldosterone from the non-adenomatous tissue. Histologically the non-adenomatous portion of the adrenal cortex was found hyperplastic, producing a considerable amount of aldosterone, as was shown on an in vitro incubation. As aldosterone was produced in the hyperplastic tissue as well as adenoma of the adrenal and it was not subjected to the regulation of the pituitary, the hormone was continuously secreted in spite of its high blood level. An apparent contradiction that the non-adenomatous tissue secreted further aldosterone in response to the stimulation of sodium restriction may thus be explained. Though adenoma may well have the autonomy of aldosterone secretion, the normal or the hyperplastic tissue of the adrenal is probably controlled by a normal regulation mechanism of aldosterone.

An analysis of corticoid patterns of adenoma revealed a great increase of a production of corticosterone and aldosterone in contrast with low values of cortisol and cortisone. The non-adenomatous tissue produced a large amount of aldosterone, though not so prominent as adenoma, but it produced only trace of corticosterone. According to the report of Davignon et al. (1962), aldosterone occupies only about 4% of a total corticoid production and cortisol occupies 58%, cortisone 12% and corticosterone plus compound S 26% in an in vitro production of normal human adrenals. It seems reasonable to consider from the above data that the production of corticosterone and aldosterone was greatly stimulated in the adrenal adenoma studied.

In their analyses of urinary corticoids in primary aldosteronism, Mader and Iseri (1955), Goldsmith et al. (1953) and Nehr (1958) reported an increased production of corticosterone. Simultaneous administration of corticosterone with aldosterone to adrenalectomized rats resulted in a much greater loss of potassium than single administration of aldosterone (Ross, 1960). Consequently, hypersecretion of corticosterone can be regarded as an important factor in the pathogenesis of the disease.

Other authors (Ayres et al., 1958; Nehr, 1958; Gale et al., 1960) reported that they detected no evidence of production of corticosterone in cases of adenoma
and August et al. (1958) and August and Nelson (1959) reported that hyperproduction of aldosterone alone resulted in the development of primary aldosteronism. A high yield of corticosterone, therefore, can be considered not an essential, but only an aggravating factor in primary aldosteronism. Overproduction of corticosterone observed in the present case seems to aggravate signs and symptoms of primary aldosteronism due to a loss of potassium. In addition to the high activity of 18-hydroxylase in adenoma, production of aldosterone seems further stimulated by an increase of an available precursor, corticosterone.

The biosynthetic capacity of aldosterone from corticosterone-4-C\textsuperscript{14} was found greater in the adenomatous tissue than in the non-adenomatous tissue of the adrenal in this patient. This finding was considered as an increased activity of 18-hydroxylase in the tumor tissue. Even in the non-adenomatous tissue of the adrenal in this patient the ratio of aldosterone to a total corticoid production was found higher than normal and histological examinations showed a hyperplasia quite similar to adenoma.

There have been reports of adenoma combined with hyperplasia in the adrenal cortex which led to a question as to whether or not there is an initiating factor for the onset of primary aldosteronism.

Recently, Davis et al. (1961) and Carpenter et al. (1961) reported a humoral stimulating factor of aldosterone secretion in the kidney. It was proved with perfusion experiments that renin or angiotensin was an active substance to stimulate aldosterone secretion (Barter et al., 1961; Biron et al., 1961; Mulrow et al., 1962). It was found in the present authors' experiments that renin failed to increase a production of aldosterone \textit{in vitro} when added to an incubation medium with adrenal slices, but addition of renin together with normal plasma did stimulate the production (Donomae et al., 1962; Kumagai et al., 1962). It was observed that the hypertensive with renal damage secreted aldosterone in larger amounts and that hypertensive dogs of Goldblatt type showed a hypertrophy of zona glomerulosa and hypersecretion of aldosterone (Kumagai et al., 1962; Donomae et al., 1962). Thus, it seems reasonable to conclude that a renal aldosterone stimulating factor is produced from the damaged kidney in such a great amount that it causes hypersecretion of aldosterone (Laragh et al., 1960; Venning et al., 1961).

In primary aldosteronism, one may occasionally observe renal damage induced by hypokalemia. Histological findings in such a case revealed a vacuolar degeneration of the epithelium of proximal renal tubuli, vacuolar or hyaline degeneration of distal tubuli, especially of collecting tubuli, a hyaline degeneration or fibrosis of glomeruli and hypertrophy of the intima of small vessels. It is suggested that the damaged kidney produces a renal stimulating factor of aldosterone in excess, which in turn contributes to the hyperactivity of the adrenal cortex with the exception of adenoma. In addition, aldosterone is devoid of a kind of feedback mechanism which adjusts the blood level of aldosterone such as glucocorticoids; it is still further secreted under continuous stimulation, leading to hyperplasia of the adrenal cortex.
SUMMARY

1) One case of primary aldosteronism was reported. Adenoma of the left adrenal was confirmed on operation.

2) In incubation experiments, overproduction of aldosterone and corticosterone was noted in the adenomatous tissue while cortisol and cortisone were produced only small amounts.

3) The non-adenomatous tissue of the removed adrenal produced aldosterone about 1/3 the adenomatous tissue, although it produced more than normal adrenal tissue. The production of corticosterone, however, was very low.

4) When corticosterone-4-C\textsubscript{14} was added to an incubation medium with slices of adenoma, biosynthetic conversion of the radioactive steroid to aldosterone was greatly enhanced, 5 times as much as in the non-adenomatous tissue. Thus, an autonomous increase of the activity of 18-hydroxylase is reasonably considered.

5) Histologically, hyperplasia was found in zona glomerulosa of the non-adenomatous portion of the removed adrenal cortex, suggesting some stimulating factor which continuously promotes a secretion of aldosterone. The pathogenesis of primary aldosteronism was discussed from a viewpoint of a renal stimulating factor.

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REFERENCES