A STUDY ON DEHYDROEPiANDROSTERONE SULFATE IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Alterations in the concentration of plasma dehydroepiandrosterone sulfate (DHEA-S) and cortisol in patients with essential hypertension (EH) were investigated. The subjects were 25 patients with EH: 7 of low plasma renin activity (PRA) group, 10 of normal PRA group and 8 of high PRA group; 7 normal subjects were used as the controls. Plasma DHEA-S and cortisol were measured before and after the following tests: (1) circadian rhythm (6:00, 16:00 and 24:00), (2) furosemide (0.7 mg/kg) test, (3) ACTH (12.5 IU/4 hr) infusion test, (4) dexamethasone (1.0 mg) test, (5) furosemide (0.7 mg/kg) test under dexamethasone (1.0 mg) treatment, (6) metopirone (1.5 g) test, (7) angiotensin II (8 ng/kg/min, 30 min) infusion test and (8) saline (1000 ml/hr) test.

The alterations of the endogenous ACTH-adrenal hormone system as well as the renin-angiotensin-aldosterone system induced by these tests did not cause significant changes in plasma levels of DHEA-S in the 3 groups with EH. However, significant enhancement of plasma DHEA-S was observed after both the administration of exogenous ACTH and angiotensin II. It is considered that the responsiveness of DHEA-S to ACTH may increase in the low and normal PRA groups and that the responsiveness of DHEA-S to angiotensin II may increase in the high PRA group.

Based on these results, it is suggested that plasma DHEA-S hardly or only partially participates as a causal factor of EH.

DEHYDROEPiANDROSTERONE (DHEA) and dehydroepiandrosterone sulfate (DHEA-S) are principal steroids produced in the adrenal cortex. However, their physiological functions and secretory status have not been precisely determined. In 1963, Sharma et al. reported that DHEA and DHEA-S inhibited 11- hydroxylase in vitro using bovine adrenal gland. Furthermore, in 1968, Nowaczynski et al. described a decreased urinary excretion rate of DHEA-S in patients with essential hypertension (EH). In 1969, Fracchini et al. indicated that DHEA inhibited 11β-hydroxylation in vivo also. In 1970, Shao et al. found that the secretion rates of DHEA-glucuronide and DHEA-S were significantly higher in patients with EH than in normal subjects. The discrepancy between their results and those of Nowaczynski et al. suggested increased protein binding of DHEA-glucuronide and DHEA-S and decreased renal clearance in patients with EH. On the other hand, in 1975, Sekihara et al. who developed a radioimmunoassay for DHEA-S, found that the mean level of plasma

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DHEA-S was significantly higher in patients with EH. However, systematic studies on DHEA-S in EH were not reported. We therefore investigated responses of the plasma concentration of DHEA-S and plasma cortisol to the alteration of endogenous or exogenous ACTH-adrenal hormone system and renin-angiotensin-aldosterone system induced by administration of furosemide, ACTH, dexamethasone, metopirone, angiotensin II and saline.

**MATERIALS AND METHODS**

**Subjects**

Sixteen normal male subjects aged from 28 to 45 (average: 37.5 ± 10.8, mean ± SD) without evidence of metabolic, endocrine or cardiovascular disorders, and 25 male patients with EH aged from 20 to 45 (average: 34.5 ± 8.3) were studied. The hypertensive patients were divided into low, normal and high PRA groups according to the response of PRA to intravenous administration of 0.7 mg/kg of furosemide as described previously. According to our criteria of 7 of the hypertensive patients were of low PRA, 10 of normal PRA and 8 of high PRA. Antihypertensive therapy was withheld for at least 3 weeks prior to the present study.

Complete clinical examinations had excluded known causes of secondary hypertension. Investigation was conducted on both the normal subjects and patients with EH with a diet containing 7–10 g of NaCl daily.

**Test Procedures**

1) Circadian rhythm: Blood samples were taken at 6:00, 16:00 and 24:00 on recumbency. All samples were drawn after one hour recum-bency.

2) Furosemide test: After recumbency from 7:00, a baseline sample was drawn at 9:00. Furosemide (0.7 mg/kg) was then administered intravenously and after 2-hour ambulation, a blood sample was again collected. The 2 samples were used for PRA assay as well as determination of the levels of DHEA-S. The patients with EH were divided into low, normal and high PRA groups according to the PRA response, as described previously.

3) ACTH infusion test: The baseline sample was drawn at 9:00 on recumbency since 7:00.

![Fig.1. Plasma levels of DHEA-S at 9:00 after one hour recumbency in normal subjects and in hypertensive patients with low, normal and high PRA. Results are summarized as mean ± SD; n = number of determinations.](image-url)
A 4-hour infusion of 12.5 IU of ACTH (Acthar, Armour Pharmaceutical Co.) in 200 ml of 5% dextrose was then begun, and 2 other samples were collected 2 and 4 hour later.

4) Dexamethasone test and furosemide plus dexamethasone test: one mg of dexamethasone was administered at 21:00 orally and the baseline samples were drawn at 9:00 on the next day. Immediately afterwards, furosemide (0.7 mg/kg) was administered intravenously, and following 2-hour ambulation, another blood sample was collected.

5) Metopirone test: One and a half grams of metopirone were administered at 21:00 orally and on the next morning, a blood sample was drawn at 9:00 on recumbency.

6) Angiotensin II infusion test: Immediately after drawing the baseline blood sample at 13:00 on recumbency, angiotensin II (8 ng/kg/min, Hypertensin, Ciba Co.) was infused intravenously.

### TABLE II

<table>
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<tr>
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<th>Changes in plasma levels of DHEAs and cortisol by various procedures</th>
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<td>Plasma DHEA-S level (µg/dl)</td>
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<td>Low PRA</td>
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<td>Circadian rhythm</td>
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<td>24:00</td>
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<td>ACTH infusion test</td>
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<td>Dexamethasone test</td>
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<td>Furosemide test with dexamethasone</td>
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<td>Angiotensin II infusion test</td>
<td>0 (min)</td>
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<td>15</td>
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<td>Saline infusion test</td>
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Results are given as mean ± SD.

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for 30 min and plasma samples were collected 15, 30 and 45 min later.

7) Saline infusion test: The baseline sample was drawn at 9:00 on recumbency since 7:00, and 1000 ml of saline was then infused intravenously for one hour. A second blood sample was collected 60 min later.

The plasma levels of DHEA-S were measured by the radioimmunoassay technique reported previously by Kokubu et al.7

Plasma cortisol and PRA were measured with a CEA-IRE-SORIN kit.

Statistical comparisons were made by Student's t test.

RESULTS

Age, Blood Pressure and Serum Electrolytes

The clinical and laboratory findings are shown in Table I. There was no significant difference in the mean ages of the 4 groups. Both the systolic and diastolic blood pressure levels were significantly higher in the patients with EH than those of the normal subjects, but there was no significant difference among the 3 groups of EH. The patients with low PRA revealed significantly higher serum sodium levels than the normal subjects and 2 other hypertensive groups. However, the serum potassium levels were not significantly different among the 4 groups (Table I).

Plasma Levels of DHEA-S at 9:00 after One-hour Recumbency (Fig. 1)

The mean levels of DHEA-S were 168 ± 49 µg/dl (mean ± SD) in normal subjects and 176 ±

<table>
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<th>TABLE III</th>
<th>PERCENTILE INCREASES OF PLASMA DHEA-S AND CORTISOL AFTER 2 AND 4 HOURS OF ACTH (12.5 IU/4 hr) INFUSION IN HYPERTENSIVE PATIENTS WITH LOW, NORMAL AND HIGH PRA</th>
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<tr>
<td></td>
<td>Low PRA (n = 7)</td>
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<td>DHEA-S (%)</td>
<td>2 (hr)</td>
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<td></td>
<td>4</td>
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<tr>
<td>Cortisol (%)</td>
<td>2 (hr)</td>
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</table>

Results are given as mean ± SD; n = number of determinations.

* = p < 0.05, significant as compared with percentile increases of plasma cortisol after 2 hours of ACTH infusion in normal and high PRA groups.

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the 7 other tests are summarized in Table II.

The plasma levels of cortisol decreased significantly between 6:00 and 24:00 in the 3 groups of EH, but no significant difference was observed among the 3 groups of EH.

On the other hand, the plasma levels of DHEA-S at 16:00 were increased slightly, but not significantly, in the 3 groups of EH.

**Changes of Plasma DHEA-S and Cortisol after the Furosemide Test (Fig. 3)**

The plasma levels of cortisol tended to increase slightly in the 3 groups of EH, but the change was not statistically significant.

The plasma levels of DHEA-S tended to increase in the low and normal PRA groups and decrease in the high PRA group, but none of these changes were statistically significant.

**Changes of Plasma DHEA-S and Cortisol after the ACTH Infusion Test (Fig. 4)**

The plasma levels of DHEA-S and cortisol increased significantly in the 3 groups of EH. Table III shows the percentile increase from the baseline levels of plasma DHEA-S and cortisol in the 3 groups of EH. The mean percentile increase in cortisol after 2 hours in the low PRA group was significantly higher than those in the other 2 groups, but the percentile increases at 4 hours were not significantly different among the 3 groups of EH. Also, the plasma levels of DHEA-S and cortisol were not significantly correlated with each other.

**Changes of Plasma DHEA-S and Cortisol after the Dexamethasone Test (Fig. 5)**

The plasma levels of cortisol decreased to 10, 16 and 32% of each baseline level in the low, normal and high PRA groups, respectively, but no significant difference was observed among the 3 groups of EH.

On the other hand, the plasma levels of DHEA-S in the 3 groups of EH after dexamethasone treatment did not decrease significantly.

**Changes of Plasma DHEA-S and Cortisol after the Furosemide Test following Dexamethasone Treatment (Fig. 6)**

There was no significant difference in the plasma levels of DHEA-S and cortisol before and after the furosemide test following dexamethasone treatment and, no significant difference existed in the plasma levels of DHEA-S and cortisol before and after the test among the 3 groups of

44 μg/dl in the patients with EH. Among the patients with EH, the levels were 179 ± 34, 169 ± 57 and 179 ± 42 μg/dl in the low, normal and high PRA groups, respectively. No statistically significant difference was observed between the normal subjects and each of the 3 groups of EH.

**Circadian Rhythm of Plasma DHEA-S and Cortisol (Fig. 2)**

The plasma levels of DHEA-S and cortisol in relation to circadian rhythm and before and after

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**Fig. 7.** Plasma levels of DHEA-S and cortisol before and after administration of metopirone (1.5 g/day) in hypertensive patients with low, normal, and high PRA. Results are given as mean ± SD; n = number of determinations.

**Fig. 9.** Plasma levels of DHEA-S and cortisol before and after infusion of saline (1000 ml, 1 hr) in hypertensive patients with low, normal, and high PRA. Results are given as mean ± SD; n = number of determinations. * = p < 0.05, significant as compared with levels of cortisol before infusion of saline.

**Changes of Plasma DHEA-S and Cortisol after the Angiotensin II Infusion Test (Fig. 8)**

The plasma levels of DHEA-S 15 min after the infusion increased significantly to 130% of the baseline level in the high PRA group.

On the other hand, the levels of DHEA-S in the normal PRA group tended to increase, although no significant difference was found. In the low PRA group, the levels of DHEA-S did not change appreciably throughout the test.

The plasma levels of cortisol 15 min after the infusion increased significantly to 180% of the baseline levels in the high PRA group, but no significant changes were observed in the 2 other groups.

There was no significant correlation between DHEA-S and cortisol in all 3 groups of EH.

**Changes of Plasma DHEA-S and Cortisol after the Saline Infusion Test (Fig. 9)**

The plasma levels of cortisol in the low and normal PRA groups after the saline infusion decreased significantly to 59 and 67% of their baseline levels, respectively; however, those in the high PRA group did not change significantly.

No significant change in the plasma levels of DHEA-S was observed after the infusion in the 3 groups of EH.

**DISCUSSION**

The biosynthesis and secretion of adrenal androgen as well as cortisol are under the regula-
tory control of ACTH, but no mechanism of negative feedback to ACTH secretion has been detected.\(^9\) DHEA-S is biosynthesized at the zona fasciculata and reticularis of the adrenal cortex, and the main pathway in the synthesis is via pregnenolone, and 17α-OH-pregnenolone. The sulfate pathway has been reported also. In this pathway, cholesterol sulfate remains as sulfate ester and is converted to DHEA-S directly.\(^6\) DHEA-S is metabolized to the free form by sulfatase in the liver and skin, etc., and then converted to Δ^4^-androstenedione by the conjugated reaction with irreversible Δ^3^-3β-hydroxysteroid dehydrogenase and Δ^5^-Δ^4^-isomerase. Next, Δ^4^-androstenedione is reduced to androsterone and eticholanolone, etc., 17 to 40% of which is excreted into the urine. However, approximately 5 to 10% of the DHEA-S itself is excreted.\(^7\)

The human peripheral concentrations of DHEA-S in both sexes increase rapidly at the age of puberty, reach a peak at about 20 years old and then decrease approximately linearly and gradually with aging. The physiological function of DHEA-S is not yet well understood. Sekihara et al\(^5\) have reported that the serum DHEA-S level in patients with EH was significantly higher than that in normal subjects. On the other hand, Holland et al\(^11\) indicated that no difference in plasma concentration was noted between patients with EH and normal subjects. Furthermore, Lipsett et al\(^12\) found that when DHEA was given in normal subjects, there were no measurable alterations in steroidogenesis and mineral balance.

On the other hand, 16β-OH-DHEA derived from DHEA was discovered by Shackleton et al\(^13\) in 1967, and Sennett et al\(^14\) reported in 1975 that this steroid reduced the urinary ratio of Na/K in adrenalectomized rats. It had a mineralocorticoid bioactivity of 1/40 of that of aldosterone, and its level in the urine was found to be elevated in patients with EH. They suggested therefore that this steroid might play a minor role in the pathogenesis of hypertension in patients with low PRA. However, Ogihara et al\(^15\) indicated that there was no change in systolic blood pressure, PRA, serum concentrations of Na and K, or urinary ratio of Na/K after administration of 16β-OH-DHEA to rats. Based on the above findings, it appears unlikely that 16β-OH-DHEA is a direct causative factor in the pathogenesis of EH with low PRA, even if this steroid possesses a mineralocorticoid bioactivity.

In our study, the plasma levels of DHEA-S at rest in the early morning showed no significant difference either between normal subjects and the EH groups, or among all 4 groups involving the normal subjects and low, normal and high PRA goups of EH. These results differ from the findings of Sekihara et al\(^5\) Presumably, this discrepancy could arise from the difference of ages which Sekihara et al\(^5\) compared by classifying subjects into their forties and fifties. On the other hand, we compared them as a group ranging in age from 28 to 45, since our subjects were relatively few in number. Holland et al\(^11\) examined the plasma DHEA-S concentration in normal subjects and patients with EH in relation to sex and age, and they found that there was no significant difference between EH patients and normal subjects or between low and normal PRA groups with EH. It might be considered therefore that the plasma DHEA-S concentration is normal in the great majority of patients with EH, and it seems unlikely that DHEA-S is one of the direct causative factors in the pathogenesis of EH.

The circadian rhythms in plasma levels of DHEA-S in the 3 groups with EH revealed no significant difference at any time. Furthermore, the circadian rhythm in plasma level of DHEA-S did not change significantly among the 3 groups with EH. Yamaji et al\(^16\) measured the circadian rhythm of plasma DHEA-S in 284 control subjects of both sexes and indicated that the values at 9:00 were 11% higher than those at 17:00. On the other hand, Lamb et al\(^17\) found no diurnal variation of plasma DHEA-S in normal females. Also, Rosenfeld et al\(^18\) reported diurnal variation of plasma DHEA synchronous with plasma cortisol, but no diurnal variation in plasma DHEA-S was shown. They concluded that this might be expected since DHEA-S is secreted into a large plasma pool, the half-life of which is of the order of 10–20 hours, and additional contribution to this pool is derived from the conjugation of free DHEA, a process which takes place rapidly.

Administration of furosemide slightly enhanced the plasma DHEA-S in the low and normal PRA groups, and there was an increasing tendency in plasma cortisol in all 3 groups. This effect is thought possibly to result in part from an increase in endogenous ACTH by stress, a decreased circulating plasma volume or an increase in endogenous angiotensin II.

When the endogenous ACTH was suppressed by the administration of dexamethasone, the increasing tendency in plasma cortisol after
furosemide infusion was suppressed. This might be expected since dexamethasone suppressed the stimulated secretion of ACTH by stress.

Administration of exogenous ACTH significantly enhanced the plasma levels of DHEA-S and cortisol in all 3 groups, and the percentile increase of plasma cortisol after 2 hours was significantly higher in the low PRA group than in the 2 other groups. Murakami et al. reported that an excess response of plasma cortisol was observed in patients with EH after ACTH infusion, and they concluded that this excess response might be caused by a modified metabolism of cortisol in addition to an enhanced glucocorticoidogenesis system. Furthermore, Honda et al. reported that when ACTH was infused into EH patients, the rate of increase in plasma DOC level was significantly higher in the low PRA group than in normal subjects, but there was no significant difference between the normal PRA group and normal subjects. They concluded that this reflected a difference in response of the adrenal cortex to ACTH between the low and normal PRA groups and another mechanism of enhanced mineralocorticoid metabolic clearance by ACTH infusion. The response of steroidogenesis to ACTH in the low PRA group might thus be increased not only in terms of mineralocorticoid but also of glucocorticoid. However, the lack of any significant difference in plasma level of cortisol in the circadian rhythm between all 3 groups, might be due to the response to a small alteration in physiological ACTH level.

Administration of dexamethasone to suppress the endogenous ACTH, significantly reduced the plasma level of cortisol in the 3 groups, but did not alter the plasma level of DHEA-S in the 3 groups. Yamaji et al. report that the plasma level of DHEA-S was remarkably suppressed in all cases after the administration of 2 mg of dexamethasone daily for 3 days to normal subjects of both sexes. The reason why the plasma level of DHEA-S did not change with our procedure could be related to the difference in dose and period of administration.

Metopirone treatment to increase endogenous ACTH secretion, did not significantly alter the plasma level of DHEA-S or cortisol. One reason why the plasma level of DHEA-S did not change with this procedure, might be that it did not reflect any alteration of endogenous ACTH. The lack of significant alteration of plasma levels of DHEA-S and cortisol after metopirone treatment might reflect a loss of action of metopirone 12 hours after the administration due to the short action of metopirone.

In the angiotensin II infusion study, the plasma levels of DHEA-S and cortisol were significantly increased 15 min after the angiotensin II infusion in the high PRA group but not in the 2 other groups. Angiotensin II in the high PRA group was thought to exert an effect on the early step as well as the late biosynthetic pathway since as reported elsewhere, angiotensin II infusion to patients with EH significantly enhanced the plasma levels of cortisol, deoxycorticosterone and DHEA-S in the high PRA group but did not significantly alter those in the low or normal PRA group, and the simultaneously measured plasma ACTH did not change in all 3 groups. Mendelsohn et al. reported that sodium deprivation enhanced aldosterone secretion to angiotensin II. Therefore, the serum sodium level represents an important factor in the response to angiotensin II. As shown in Table I, the serum sodium level was significantly lower in the high PRA group than in the 2 other groups, so that enhanced steroidogenesis in the high PRA group could be due in part to the low level of serum sodium, or increased sensitivity of the adrenals.

Saline infusion induced a slight decrease in the levels of plasma DHEA-S in the 3 groups with EH, as well as a significant decrease in plasma cortisol in the low and normal PRA groups but no significant alteration in the high PRA group. These effects on plasma DHEA-S and cortisol are thought to result possibly from an increased circulating plasma volume and a suppression of the renin-angiotensin-aldosterone system. Furthermore, it is considered that the decrease in plasma cortisol might be influenced by the circadian rhythm, also.

In summary, based on the present study and the additional fact that no alteration of steroid induction or mineral balance was observed in normal subjects administered with DHEA, it is suggested that plasma DHEA-S hardly or only partially participates as a causal factor of EH.

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