Secretory Regulation of 19-Hydroxyandrostenedione in Normal Man

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Abstract. To evaluate the secretory regulation of 19-hydroxyandrostenedione (19-OH-AD), its plasma concentration was measured before and after stimulation and inhibition tests for the ACTH-adrenal axis and the renin-angiotensin system in 50 normal subjects. Basal levels of plasma 19-OH-AD did not correlate with either those of plasma renin activity (PRA) or the plasma aldosterone concentration (PAC), but positively correlated with those of plasma cortisol. Plasma 19-OH-AD was stimulated by 0.25 mg ACTH-(1-24) and was suppressed by 1 mg dexamethasone (DEX) as were plasma cortisol and PAC. On the other hand, with 2-h standing alone or iv 40 mg furosemide plus 2-h standing, plasma 19-OH-AD and cortisol did not increase but PRA and PAC did. With iv furosemide plus 2-h standing with 3 mg DEX pretreatment, plasma 19-OH-AD and cortisol did not respond either, but PRA and PAC increased. With 25 mg oral captopril following 1-h standing with 3 mg DEX pretreatment, plasma 19-OH-AD and cortisol did not change but PAC decreased. These results indicate that the secretion of 19-OH-AD is mainly under the control of the ACTH-adrenal axis rather than the renin-angiotensin system.

Key words: 19-hydroxyandrostenedione, ACTH-adrenal axis, Renin-angiotensin system, Aldosterone, Captopril test.

THE STEROID. 19-hydroxyandrost-4-ene-3,17-dione (19-OH-AD), has been reported by Sekihara to be a more potent sodium-retaining and hypertensinogenic agent than deoxycorticosterone acetate in intact rats [1-3]. Simultaneous administration of 19-OH-AD and subthreshold dose of aldosterone increases the mineralocorticoid action of aldosterone in adrenalectomized rats, but 19-OH-AD alone does not [4]. Adrenal immunohistochemistry in dogs revealed that 19-OH-AD, as well as the other C19 steroids, is mainly produced in the zona reticularis [5].

Clinical studies by Sekihara et al. on the secreting mechanism of the secretion of 19-OH-AD in normal man have been done. They have shown that 19-OH-AD is secreted directly from the adrenal cortex and its secretion is under the control of both the ACTH-adrenal axis and the renin-angiotensin system [3, 6, 7]. Their data in normal subjects, however, did not necessarily show a dose-dependent response of 19-OH-AD to angiotensin II infusion [7]. Furthermore, during upright posture for 3 h in normal subjects, it decreased in some, although PRA and PAC increased in all [7]. In the in vitro study by Higuchi and colleagues [8], the production of 19-OH-AD from cultured human adrenal cells was promoted in the presence of ACTH but not angiotensin II.

In the present study, we measured the plasma...
concentration of 19-OH-AD before and after several stimulation and suppression tests of the ACTH-adrenal axis and the renin-angiotensin system to evaluate the main regulator of 19-OH-AD secretion in normal man.

Subjects and Methods

Subjects

Fifty normal subjects—36 males aged 20 to 52 (28.4±7.1 yr; mean±SD) and 14 females aged 17 to 43 (27.1±7.7 yr)—were studied. There was no significant age difference between both sexes. All were normotensive and free from renal, hepatic and cardiac diseases. They were given a diet containing 10 to 13 g/day of sodium chloride during the study.

Test procedures

The peripheral blood samples for various basal hormone levels were obtained between 0800 and 0900 h after at least 30 min rest in the fasting state. For an ACTH stimulation test, 0.25 mg ACTH-(1-24) (Cortrosyn, Daiichi Pharmaceutical Co., Tokyo, Japan) was administered iv between 0800 and 0900 h to 5 subjects and blood was drawn before and 1 h after the injection. A DEX suppression test was performed in 12. Blood samples were taken between 0800 and 0900 h before and after an oral dose of 1 mg DEX the preceding night at 2300 h.

Postural stimulation tests were done as follows: 2-h standing alone in 9 subjects, iv furosemide plus 2-h standing in 10, and iv furosemide plus 2-h standing with DEX pretreatment in 8. These postural maneuvers were started between 0800 and 0900 h after basal plasma samples were taken. Forty mg furosemide was injected just before 2-h standing. DEX pretreatment was performed to eliminate the effect of endogenous ACTH. The subjects received 2 mg DEX at 2300 h on the preceding day and 1 mg at 0700 h on the test day prior to iv furosemide plus 2-h standing. A second sample was taken after 2-h standing.

Captopril test following 1-h standing with DEX pretreatment was performed in 8 subjects. Captopril, an angiotensin converting enzyme inhibitor, was used to suppress angiotensin II production. The procedure was as follows: 3 mg DEX was administered to eliminate the effect of endogenous ACTH as mentioned above. To enhance the response of angiotensin II, 1-h standing from 0800 to 0900 h was done before captopril administration. Basal blood samples were drawn at 0900 h. The subjects then received 25 mg captopril orally to suppress the stimulated angiotensin II and were recumbent for another 1 h. Second samples were drawn at 1000 h.

All samples were drawn with EDTA as an anticoagulant, immediately centrifuged and stored at -20°C until assayed. Informed consent was obtained from all the subjects prior to all tests.

Chemicals

Aldosterone and 19-OH-AD were obtained from Sigma Chemical Co. (St. Louis, Mo. U.S.A.). [6,7-3H]19-OH-AD (SA, 1,850.0 GBq/mmol), [1,2-3H]aldosterone (SA, 1,994.3 GBq/mmol) and [1,2,6,7-3H]cortisol (SA, 3,256.0 GBq/mmol) were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.). All solvents were of HPLC grade and purchased.

Antisera

Anti-aldosterone antiserum was obtained from Teikoku Hormone Mfg. Co., Ltd. (Tokyo, Japan). Antiserum to 19-OH-AD-3-oxime-BSA [7] was generously supplied by Dr. H. Sekihara, University of Tokyo Faculty of Medicine. For use in RIA, anti-aldosterone antiserum and anti-19-OH-AD antiserum were diluted 1:20,000 and 1:200,000, respectively, with borate buffer (0.05 M; pH 7.8).

Measurement of PRA and plasma cortisol level

PRA was measured with a RIA kit from Dainabot RI Laboratory (Tokyo, Japan). The plasma cortisol level was measured directly with a RIA kit from Baxter Ltd. (Tokyo, Japan).

Measurement of PAC and plasma 19-OH-AD level

A) Retention time of aldosterone, 19-OH-AD and cortisol on HPLC

To determine the retention time, 1 ml control plasma from a normal subject containing 2,000 dpm each of [1,2-3H]aldosterone, [6,7-3H]19-OH-
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AD and [1,2,6,7-3H]cortisol was extracted twice with 10 ml dichloromethane. The extract was washed once with 1 ml distilled water, evaporated to dryness and subjected to high performance liquid chromatography (HPLC; 2150 HPLC pump with a Spherisorb ODS2 5 μm column, Pharmacia LKB Biotechnology, Uppsala, Sweden; solvent, 32% methanol, 8% tetrahydrofuran, 60% water; flow rate 0.6 ml/min). All fractions were collected every 10 sec and counted with a scintillation spectrophotometer (Packard Minaxiβ Tri-Carb 4000, Packard Instrument Co., Downers Grove, IL). The retention time of these steroids in the HPLC is shown with the data for the various other steroids in Table 1. The HPLC completely separated aldosterone, 19-OH-AD and cortisol.

B) Determination of recovery rates of [3H]aldosterone, [3H]19-OH-AD and [3H]cortisol during extraction and separation

Since complete separation of these 3 steroids was achieved with the present HPLC procedure, [1,2-3H]aldosterone, [6,7-3H]19-OH-AD and [1,2,6,7-3H]cortisol were added together, not separately, to 1 ml control plasma. The plasma was extracted and separated in the same way as mentioned above prior to the separation of sample plasma. The mean recovery rates were 76±4 (±SD) % for [3H]aldosterone, 80±3% for [3H]19-OH-AD and 70±5% for [3H]cortisol (n=8). The reason why the recovery rate of [3H]cortisol was determined will be described in C).

C) Correction of the variation in the recovery rate of aldosterone or 19-OH-AD among sample plasma

In our pilot study, [3H]aldosterone and [3H]19-OH-AD were added to each sample plasma to correct the inter-sample variation in the recovery rates. However, the radioactivity left in the sample, though a small amount, interfered in each RIA. Thus, we used [1,2,6,7-3H]cortisol as an indicator to correct the variation in sample plasma. Namely, 1 ml sample plasma containing 2,000 dpm [1,2,6,7-3H]cortisol alone was extracted and separated, and the fractions corresponding to not only aldosterone and 19-OH-AD but also cortisol were collected. The fraction of cortisol was counted and the recovery rate of [3H]cortisol in sample plasma (R-C-S-P) was determined. The fraction of aldosterone or 19-OH-AD in the same sample was replaced in ethanol and stored at -40°C until assayed by RIA. The final recovery rate for aldosterone or 19-OH-AD during the extraction and the separation in sample plasma was calculated with a following formula:

\[ \text{Recovery rate of aldosterone or 19-OH-AD in sample plasma} = \frac{\text{R-C-S-P}}{\text{Recovery rate of [3H]cortisol in control plasma}} \]

The mean final recovery rates for aldosterone and 19-OH-AD in 185 sample plasma calculated with the formula were 72±8% and 77±8% (±SD), respectively, which were almost the same as the mean recovery rates for [3H]aldosterone (76±4%) and [3H]19-OH-AD (80±3%) obtained with control plasma as mentioned in B). The value for aldosterone and 19-OH-AD measured by RIA in sample plasma was corrected with the recovery rates for aldosterone and 19-OH-AD, respectively.

D) RIA

The fraction of aldosterone or 19-OH-AD was evaporated to dryness and dissolved in 1 ml ethanol. Duplicate 0.4-ml aliquots of each steroid as well as its duplicate standard were evaporated to dryness, and 0.3 ml diluted antiserum containing 10,000 dpm [6,7-3H]19-OH-AD or [1,2-3H] aldosterone in 0.05% BSA and 0.05% human γ-globulin was added, and the mixture was incubated at 4°C overnight. 0.3 ml saturated ammonium sulfate was then added, mixed on a vortex mixer, and centrifuged for 10 min at 3,000 rpm. Finally, 0.3 ml supernatant was counted.
The intraassay and interassay coefficients of variation during whole procedures were, respectively, 8.5% and 10.9% for aldosterone, and 9.7% and 10.0% for 19-OH-AD. The minimum detectable amount for aldosterone and 19-OH-AD in plasma was 12 pg/ml each.

Statistical analysis

Group data were presented as the mean±SD throughout the study. Wilcoxon’s signed-rank test was used for the comparison of the changes before and after all the maneuvers performed except the ACTH stimulation test. Wilcoxon’s rank-sum test was used to compare the data for the tests because the subjects were different in each test. Paired Student’s t test was used to determine the difference before and after the ACTH stimulation test because the number of subjects examined was small and for further analysis of the data of Sekihara et al. [7]. P values below 0.05 were considered statistically significant.

Results

Basal hormone levels (Table 2)

There was no significant difference according to sex in any of the plasma hormones measured. Basal levels of plasma 19-OH-AD did not correlate with those of PRA or with those of PAC but positively correlated with plasma cortisol (r=0.34, P<0.05).

ACTH stimulation test (Table 3 and Fig. 1-A)

Plasma 19-OH-AD and cortisol rose significantly (P<0.01 and P<0.01, respectively). PAC also increased significantly (P<0.05) but PRA did not change.

DEX suppression test (Table 3 and Fig. 1-B)

Plasma 19-OH-AD, plasma cortisol and PAC declined significantly (P<0.01, P<0.01 and

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number of subjects</th>
<th>Plasma 19-OH-AD (pg/ml)</th>
<th>Plasma cortisol (pg/dl)</th>
<th>PRA (ng/ml/h)</th>
<th>PAC (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>36</td>
<td>41±13</td>
<td>13.6±3.9</td>
<td>1.1±1.1</td>
<td>77±32</td>
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<tr>
<td>Female</td>
<td>14</td>
<td>41±10</td>
<td>13.9±3.1</td>
<td>1.1±0.9</td>
<td>86±42</td>
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<tr>
<td>Total</td>
<td>50</td>
<td>41±12</td>
<td>13.7±3.7</td>
<td>1.1±1.0</td>
<td>79±35</td>
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</table>

Values are expressed as the mean±SD. 19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Number of subjects</th>
<th>Plasma 19-OH-AD (pg/ml)</th>
<th>Plasma cortisol (pg/dl)</th>
<th>PRA (ng/ml/h)</th>
<th>PAC (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basal</td>
<td>after</td>
<td>basal</td>
<td>after</td>
<td>basal</td>
</tr>
<tr>
<td>ACTH stimulation test</td>
<td>5</td>
<td>36±13</td>
<td>69±9</td>
<td>11.5±3.7</td>
<td>26.6±5.6***</td>
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<tr>
<td>DEX suppression test</td>
<td>12</td>
<td>42±12</td>
<td>27±8</td>
<td>11.0±3.2</td>
<td>2.0±1.1**</td>
</tr>
<tr>
<td>2-h standing alone</td>
<td>9</td>
<td>43±11</td>
<td>39±17</td>
<td>11.5±4.5</td>
<td>9.7±3.6</td>
</tr>
<tr>
<td>iv furosemide + 2-h standing</td>
<td>10</td>
<td>49±11</td>
<td>45±8</td>
<td>14.7±4.4</td>
<td>12.1±3.4</td>
</tr>
<tr>
<td>iv furosemide + 2-h standing with DEX pretreatment</td>
<td>8</td>
<td>32±6**</td>
<td>34±10</td>
<td>0.8±0.9**</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Captopril test following 1-h standing with DEX pretreatment</td>
<td>8</td>
<td>27±6**</td>
<td>26±8</td>
<td>1.0±0.4**</td>
<td>1.0±0.5</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SD. 19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration. *, P<0.05; **, P<0.01; statistically significant difference as compared with: a, own basal value; b, the basal value in iv furosemide + 2-h standing; c, the basal value in iv furosemide + 2-h standing with dexamethasone (DEX) pretreatment.
Two-h standing alone (Table 3 and Fig. 2)

PRA and PAC rose significantly \( (P<0.01 \text{ and } P<0.01, \text{ respectively}) \) but plasma 19-OH-AD and plasma cortisol did not change significantly.

Plasma 19-OH-AD in 6 (represented as open circles in Fig. 2) out of 9 subjects decreased more than 20% (twice the decrease in the interassay) of the basal level \( (46\pm11 \text{ to } 34\pm11 \text{ pg/ml}, P<0.05, \text{ Group I}) \) as observed by Sekihara et al. \[7\]. Plasma 19-OH-AD in the other 3 subjects (represented as closed circles in Fig. 2) increased \( (37\pm8 \text{ to } 50\pm24 \text{ pg/ml}, \text{ Group II}) \) but the change could not be statistically analyzed because of the small number. Group I had a significant increase in PAC from \( 56\pm16 \text{ to } 134\pm51 \text{ pg/ml} \ (P<0.05) \). In Group II, PAC also rose from \( 91\pm37 \text{ to } 235\pm39 \text{ pg/ml} \) but the change could not be statistically analyzed. Basal PAC in Group I was not significantly different from that in Group II but PAC after 2-h standing in Group II was significantly higher than that in Group I \( (P<0.05) \).

Intravenous furosemide plus 2-h standing (Table 3)

PRA and PAC increased significantly \( (P<0.01 \text{ and } P<0.01, \text{ respectively}) \) and the changes were greater than during 2-h standing alone but plasma 19-OH-AD and plasma cortisol did not change significantly.

Intravenous furosemide plus 2-h standing with DEX pretreatment (Table 3)

Pretreatment with DEX suppressed basal plasma levels of 19-OH-AD and cortisol \( (P<0.01 \text{ and } P<0.01, \text{ respectively, vs. no DEX pretreatment}) \). However, the suppression of plasma cortisol was

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**Fig. 1.** Plasma 19-OH-AD and cortisol levels before and after A) ACTH stimulation test \( (n=5) \) and B) dexamethasone (DEX) suppression test \( (n=12) \). Vertical bars represent the mean±SD. 19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration.

\* \( P<0.01 \text{ vs. before ACTH stimulation or DEX suppression.} \)
more evident than plasma 19-OH-AD as described in the result of DEX suppression test. Following furosemide plus 2-h standing, PRA and PAC increased significantly \((P<0.01\) and \(P<0.01\), respectively) but there was no significant change in plasma levels of 19-OH-AD and cortisol.

**Captopril test following 1-h standing with DEX pretreatment (Table 3 and Fig. 3)**

Basal PRA and PAC increased significantly \((P<0.01\) and \(P<0.01\), respectively, vs. DEX pretreatment alone) due to the preceding 1-h standing even in subjects pretreated with DEX. On the other hand, plasma levels of 19-OH-AD and cortisol after 1-h standing were suppressed by DEX pretreatment \((P<0.01\) and \(P<0.01\), respectively, vs. no DEX pretreatment). The subsequent captopril administration raised PRA and lowered PAC significantly \((P<0.01\) and \(P<0.01\), respectively). In contrast, it did not cause any change in the plasma level of 19-OH-AD or cortisol.

**Discussion**

The present data suggested that the secretion of 19-OH-AD in normal subjects is closely dependent on the ACTH-adrenal axis: 1) basal levels of plasma 19-OH-AD positively correlated with those of plasma cortisol, and 2) ACTH-(1–24) stimulated and DEX suppressed plasma 19-OH-AD as well as plasma cortisol although the suppressive effect of DEX on plasma 19-OH-AD was the lesser. The latter finding was consistent with that of Sekihara et al. [3, 6, 7].

On the other hand, the regulation of plasma 19-OH-AD by the renin-angiotensin system seems to be less potent. Basal plasma levels of 19-OH-AD did not correlate with either basal PRA or PAC. With regard to stimulation tests such as 2-h standing alone, iv furosemide plus 2-h standing and iv furosemide plus 2-h standing with DEX pretreatment, there was no significant response in plasma 19-OH-AD and cortisol while PRA and PAC increased. Captopril administration following 1-h standing with DEX pretreatment did not

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**Fig. 2.** Various plasma hormone levels before and after 2-h standing alone \((n=9)\). Vertical bars represent the mean±SD. ○, Group I \((n=6)\); ●, Group II \((n=3)\). 19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration. *: \(P<0.01\) vs. before 2-h standing.
affect plasma 19-OH-AD levels whereas PRA rose and PAC declined significantly.

Sekihara et al. reported that angiotensin II infusion raised plasma 19-OH-AD levels in normal subjects in a dose-dependent manner at least up to a rate of 2.0 ng/kg-min but started to decline at a rate of 4.0 ng/kg-min [7]. They also reported that plasma 19-OH-AD increased during 3-h standing in 7 subjects whose basal levels were lower than 45 pg/ml but declined in the other 7 whose basal levels were higher than 45 pg/ml [7]. When we further analyzed their data, both basal PAC and its level after 3-h standing in the latter group were higher than those in the former group (P<0.05 and P<0.01, respectively). Their analysis disclosed that the response of PAC was significant only in the latter group. From these observations, they speculated that 19-OH-AD secretion becomes refractory to high levels of angiotensin II or is inhibited by the increasing levels of aldosterone, or the metabolism of 19-OH-AD becomes accelerated [7].

Similar analysis of our data with 2-h standing showed that in Group I (n=6) whose plasma 19-OH-AD decreased obviously, PAC rose significantly, and in Group II (n=3) whose plasma 19-OH-AD did not change, the change in PAC could not be analyzed but it had a tendency to increase. It seemed that the only difference between our results and those of Sekihara et al. [7] was that the increase in plasma 19-OH-AD in Group II was not significant in our study. This suggests that there is a mechanism of 19-OH-AD secretion in connection with the concomitant increase in angiotensin II and/or aldosterone as previously suggested [7]. However, although the amount in Group II was small, the basal PAC in Group I was not different from that in Group II, and the PAC after 2-h standing in Group II was rather higher than that in Group I (P<0.05). This finding did not necessarily support the speculations by Sekihara et al. [7]. A possible explanation

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**Fig. 3.** Various plasma hormone levels before and after captopril test following 1-h standing with dexamethasone (DEX) pretreatment (n=8). Vertical bars represent the mean±SD. 19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration. *, P<0.01 vs. after 1-h standing with DEX pretreatment.
for the decline in the plasma levels of 19-OH-AD in some of our subjects during the standing maneuver is its diurnal change as in plasma cortisol. The precursor of 19-OH-AD, androstenedione, has been shown to have a diurnal rhythm as in plasma cortisol [9], and plasma 19-OH-AD is closely dependent on ACTH as shown in the present study. In addition, several reports have shown that standing causes an increase in human atrial natriuretic peptide (hANP) [10, 11], which suppresses not only the production of cortisol [12–14] but also that of 19-OH-AD [8], DHEA [12] and aldosterone [12–14]. Further study on the mechanism of 19-OH-AD secretion in connection with the changes in angiotensin II, aldosterone and/or hANP is necessary.

In summary, our results suggested that 19-OH-AD secretion is mainly controlled by the ACTH-adrenal axis rather than the renin-angiotensin system in normal subjects.

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References