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Low Plasma 19-Hydroxyandrostenedione Levels in Patients with Aldosterone-Producing Adenoma

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Abstract. The present study was performed to clarify changes in plasma levels of 19-hydroxyandrostenedione (19-OH-AD), an amplifier of aldosterone and a possible hypertensinogenic steroid, during several tests for the renin-angiotensin system in 20 patients with aldosterone-producing adenoma (APA) and to determine whether 19-OH-AD participates in the etiology of the hypertension in this disorder. Basal plasma 19-OH-AD levels in patients with APA were significantly lower than those in 50 normal subjects, and correlated positively with basal plasma cortisol levels ($r=0.45$, $P<0.05$). Plasma 19-OH-AD levels were not changed significantly by 2-h standing during which plasma renin activity (PRA) remained suppressed. With 40 mg iv furosemide plus 2-h standing during which PRA remained suppressed, plasma 19-OH-AD levels increased significantly with a concomitant significant increase in plasma cortisol. With dexamethasone pretreatment, however, such positive responses of plasma 19-OH-AD and cortisol disappeared. After the removal of the APA with the adjacent adrenal tissue, PRA and plasma aldosterone concentrations became normal or low-normal, but plasma 19-OH-AD and cortisol did not change as compared with the preoperative levels. There were no significant correlations between basal plasma 19-OH-AD levels and mean blood pressure either before or after the adrenal operation. These findings suggested that 1) the secretion of 19-OH-AD in patients with APA is reduced due to the chronically suppressed renin-angiotensin system, 2) but is still concomitantly under the control of the ACTH-adrenal axis and 3) 19-OH-AD may, at least, not play an important causative role in the hypertension commonly observed in patients with APA.

Key words: 19-Hydroxyandrostenedione, Primary aldosteronism, Aldosterone, Hypertension.
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IT HAS BEEN demonstrated that 19-hydroxy-androst-4-ene-3, 17-dione (19-OH-AD) amplifies the mineralocorticoid activity of a subthreshold dose of aldosterone in adrenalectomized rats [1]. Moreover, 19-OH-AD has recently been shown to have more potent sodium-retaining and hypertensinogenic actions than deoxycorticosterone acetate or aldosterone in intact rats [2–4]. In normal subjects, the secretion of 19-OH-AD was initially reported to be under the control of both the ACTH-adrenal axis and the renin-angiotensin system [4, 5]. We have demonstrated, however, that the main regulator of 19-OH-AD secretion in normal subjects is the ACTH-adrenal axis rather than the renin-angiotensin system [6].

In regard to hypertension in man, higher plasma 19-OH-AD levels have been reported in normal- or low-renin essential hypertension [4], high-renin hypertension [7] and hypertensive pregnant women [8] than those in the respective normal controls. Primary aldosteronism due to

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APA is known to be a hypertensive disorder with aldosterone excess. It has been impossible, however, to induce reproducible hypertension in healthy subjects by continuous administration of aldosterone [9, 10]. In rats, hypertension could be induced only in those sensitized by unilateral nephrectomy, by concomitant administration of isotonic saline by mouth, or by using pharmacological doses of aldosterone [11]. This difficulty in inducing hypertension is most likely explained by the ability of the kidney to escape from the mineralocorticoid action, implying that patients with primary aldosteronism due to APA may have another factor which prevents escape from the mineralocorticoid action of chronically increased aldosterone or that they may have another hypertensinogenic factor [12], which operates in conjunction with chronic hyperaldosteronemia in raising the blood pressure.

We speculated that 19-OH-AD may be such an associated hypertensinogenic steroid and may account for the hypertension commonly observed in these patients by amplifying the action of aldosterone which is excessively and chronically produced by APA and/or by preventing escape from chronic hyperaldosteronism. In the present study, we evaluated plasma 19-OH-AD levels in the peripheral blood in patients with APA to clarify 1) how its secretion is regulated and 2) whether it plays an important role in the etiology of hypertension in patients with APA.

Subjects and Methods

Subjects

Twenty patients with APA—7 men aged 33 to 63 yr and 13 women aged 25 to 65 yr—were enrolled in the present study. All were hospitalized and given a daily diet containing 10 g sodium chloride. Three patients had been receiving spironolactone and one furosemide. These diuretics were discontinued on admission, and this study was performed at least 2 weeks after the admission. As shown in Table 1, 18 of 20 patients (Cases 1–18) were confirmed to have unilateral adrenal adenomas by surgery. An APA was removed with the adjacent adrenal tissue in all the 18 patients. After the adrenal operation, PRA normalized in all patients and the plasma aldosterone concentration (PAC) declined to the normal or subnormal range within 2±3 weeks (mean±SD). Serum potassium also became normal without potassium supplement after the operation within 1±1 week. The existence of unilateral adrenal tumors in the other 2 patients (Cases 19 and 20) was strongly suspected as a result of computed tomography (CT) (15×15×15 mm on the left side in Case 19 and 8×8×8 mm on the left side in Case 20) and adrenal venous sampling. PAC and plasma cortisol levels during an ACTH infusion at a rate of 50 μg/h in Case 19 were 82,000 pg/m*l* and 518 μg/d*l* in the left adrenal vein and 602 pg/m*l* and 18.1 μg/d*l* in the inferior vena cava (IVC), respectively. The ratio of PAC (pg/m*l*) to plasma cortisol (μg/d*l*) in the left adrenal vein in this patient was 158, which was within the range of the ratio in the left adrenal vein obtained in 4 patients with left APA in our laboratory (between 51 and 181, unpublished data). Because Case 20 exhibited an allergic reaction to ACTH-(1–24) in the intracutaneous test, the adrenal sampling was performed without the ACTH infusion. PAC and plasma cortisol levels in the patient were 2,600 pg/m*l* and 6.3 μg/d*l* in the left adrenal vein and 105 pg/m*l* and 2.1 μg/d*l* in the IVC, respectively. The ratio (410) of PAC (pg/m*l*) to plasma cortisol (μg/d*l*) in the left adrenal vein in this patient was compatible with that in patients with left APA according to the criteria of Iwaoka *et al.* (a ratio above 50 in the left adrenal vein indicates the presence of left APA) [13]. All the patients had high PAC (more than 138 pg/m*l*) and low PRA (less than 0.4 ng/m*l*/h).

Test procedures

A peripheral blood sample for the measurement of basal levels of various hormones, aldosterone, 19-OH-AD, cortisol and PRA, was obtained between 0800 and 0900 h after at least a 30 min rest in the fasting state. These hormones were measured in the same blood sample before and after the tests. We evaluated changes in plasma 19-OH-AD with the following 3 kinds of postural stimulations for the renin-angiotensin system: a) 2-h standing alone in 8 patients, b) iv furosemide plus 2-h standing in 12, and c) iv furosemide plus 2-h standing with dexamethasone (DEX) pretreatment in 8. These postural maneuvers were started between 0800 and 0900 h after the first plasma sample was taken. In tests b) and c), 40 mg furosemide was injected just before 2-h standing.

In test c), 3 mg DEX p.o. in 3 daily doses for one to 2 days was administered to eliminate the effect of endogenous ACTH. A second sample was taken after 2-h standing in all the 3 tests.

In 10 (Cases 1–10) of 18 patients who received the operation, basal plasma steroid levels and PRA in the pre- and postoperative periods were compared. The postoperative sample was obtained from 2 to 13 weeks (5±4 weeks) after the operation when PRA was more than 0.3 ng/ml/h. All samples were drawn with EDTA as an anticoagulant, immediately centrifuged and stored at −20°C until assayed. The mean blood pressure on 3 consecutive days was adopted as the mean blood pressure in all patients. Details of the various tests for the renin-angiotensin system and the ACTH-adrenal axis performed in normal subjects were reported previously [6], and the results were used as the control data in the present study. Informed consent was obtained from each subject before the test procedures.

Chemicals

Aldosterone and 19-OH-AD were obtained from Sigma Chemical Co. (St. Louis, Mo, U.S.A.). [6, 7-^{3}H]-19-OH-AD (SA, 1850.0 GBq/mmol), [1, 2-^{3}H]-aldosterone (SA, 1994.3 GBq/mmol) and [1, 2, 6, 7-^{3}H]-cortisol (SA, 3256.0 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 [5] was generously supplied by Dr. H. Sekihara of the University of Tokyo Faculty of Medicine. Anti-aldosterone and anti-19-OH-AD antisera were diluted to 1:20,000 and 1:200,000, respectively, with borate buffer (0.05 M: pH 7.8).

Hormone assays

PRA was measured with a radioimmunoassay (RIA) kit from Dainabot RI Laboratory (Tokyo, Japan). Plasma cortisol levels were measured directly with a RIA kit from Baxter Ltd. (Tokyo, Japan). PAC was measured with specific RIA after separation by HPLC as well as plasma 19-OH-AD measurement in the same sample, as described in detail previously [6]. In short, 1 m*l* plasma was extracted twice with 10 m*l* dichloromethane and the extract was subjected to high performance liquid chromatography (HPLC; 2150 HPLC pump with Spherisorb 5 μm ODS 2 column, Pharmacia LKB Biotechnology, Uppsala, Sweden; solvent, 32% methanol, 8% tetrahydrofuran, 60% water; flow rate 0.6 m*l*/min). The fractions corresponding to aldosterone and 19-OH-AD were collected and measured with specific RIAs. The recovery rate of aldosterone or 19-OH-AD during the extraction and the separation procedure in each sample plasma was calculated with the following formula:

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

R-C-S-P: recovery rate of [^{3}H]cortisol in each sample plasma

Statistical analysis

Group data were presented as the mean±SD throughout the study. Wilcoxon's signed-rank test was used for comparison of the changes before and after the 3 kinds of postural maneuvers and the adrenal operation. Wilcoxon's rank-sum test was used for the comparison of the data among the maneuvers because some of the patients examined were different. P values below 0.05 were considered statistically significant.

Results

Basal plasma 19-OH-AD levels (Table 1)

The mean basal plasma 19-OH-AD level in the patients with APA was 33±8 pg/m*l*. There was no significant difference according to sex (35±7 pg/m*l* in 7 men and 32±9 pg/m*l* in 13 women) as in normal subjects [6]. As shown in Table 1, the mean basal level in the patients was significantly lower ($P<0.01$) than that (41±12 pg/m*l*) in the

Table 1. Clinical characteristics and endocrine data in the 20 patients with aldosterone-producing adenoma (APA)

Case		Age (yr old)	Sex	Duration of HT (yr)	Mean BP (mmHg)	Serum Na (mEq/*l*)	Serum K (mEq/*l*)	Plasma 19-OH-AD (pg/m*l*)	Plasma cortisol (μg/d*l*)	PRA (ng/m*l*/h)	PAC (pg/m*l*)	Side of APA	Size of APA (mm)
1	œ	50	M	2	121	144	3.4	31	7.8	<0.1	313	left	15×12×8
2	¶œ	42	F	6	122	141	3.7	27	7.7	<0.1	180	right	7×7×7
3	¶œ	41	M	1	124	142	2.1	33	8.2	<0.1	390	left	20×15×12
4	¶#œ	25	F	5	102	144	2.6	39	11.5	<0.1	442	left	19×16×11
5	§¶#œ	46	F	7	113	142	3.4	33	10.9	0.3	495	right	11×11×11
6	§¶#œ	57	F	5	129	142	2.5	42	10.0	<0.1	420	left	18×10×10,10×8×7,6×5×5
7	¶#œ	58	M	8	105	143	2.0	36	17.1	<0.1	713	left	14×11×11
8	§¶#œ	38	F	5	109	144	2.8	17	6.7	<0.1	348	left	15×12×9,9×7×5
9	¶œ	51	M	8	107	143	3.3	35	12.5	0.2	142	right	10×9×6
10	§#œ	52	M	10	132	141	3.8	34	14.2	<0.1	297	right	8×8×5
11	§¶#	26	F	1	120	140	3.3	40	15.2	<0.1	300	left	10×8×7
12	#	50	F	12	105	142	3.0	20	11.6	<0.1	138	left	12×9×9
13	§	40	F	8	122	142	2.6	38	7.9	0.3	313	right	18×14×13
14		28	F	1	114	139	3.6	25	6.3	<0.1	179	right	12×9×7
15		39	F	16	111	138	2.5	23	5.2	<0.1	162	right	15×12×10
16		33	M	1	117	140	3.3	48	11.9	0.4	165	right	17×12×11
17		44	F	8	120	145	2.4	45	17.7	<0.1	139	right	18×17×9
18	¶	63	M	3	114	148	1.8	30	19.7	<0.1	138	right	22×18×16
19	§¶	30	F	10	109	141	3.6	35	8.4	<0.1	211	left†	15×15×15 by CT
20	§¶	65	F	16	102	142	3.8	38	12.3	0.2	240	left†	8×8×8 by CT
Mean±SD		44±12*		7±5	115±9*	142±2	3.0±0.6*	33±8*	11.2±4.0		296±151*		
Mean±SD in 50 normal subjects		28±7			80±7	140±3	4.0±0.3	41±12	13.7±3.7	1.1±1.0	79±35		

M, male; F, female; HT, hypertension; BP, blood pressure; 19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration; CT, computed tomography. §, tested with 2-h standing (ref. Table 2); ¶, tested with iv furosemide plus 2-h standing (ref. Table 2); #, tested with iv furosemide plus 2-h standing with dexamethasone pretreatment (ref. Table 3); œ, additionally evaluated after the operation (ref. Table 4). †, tumor localization was confirmed by additional adrenal venous sampling. *, $P<0.01$ *vs.* 50 normal subjects.

normal subjects previously reported by us [6]. We did not find any age-related change in plasma 19-OH-AD levels in normal subjects aged between 18 and 62 yr [6]. However, since the mean age of the present 20 patients was significantly higher than that of the 50 normal subjects, we selected 10 normal subjects aged 30 to 52 yr (39±8 yr, which was not statistically different from the mean age of the patients, 44±12 yr). The mean basal level in the age-matched normal subjects (47±10 pg/m*l*) was also significantly higher than that in the patients.

On the other hand, there was also a positive correlation between basal plasma 19-OH-AD and cortisol levels in the patients ($r=0.45$, $P<0.05$) as well as in normal subjects [6], but no correlation was found between basal plasma 19-OH-AD levels and PAC, as can be calculated by the data in Table 1.

Two-h standing (Table 2)

PRA remained suppressed in all patients during the test. There was no change in plasma 19-OH-AD levels before and after the test. Plasma cortisol levels did not change, either. However, PAC increased in 7 of 8 patients examined, although the increase in the mean PAC was not significant.

Furosemide injection plus 2-h standing (Table 2)

PRA remained suppressed in all patients tested. Plasma 19-OH-AD increased significantly ($P<0.01$) as did plasma cortisol ($P<0.01$). In addition, PAC increased in 10 of 12 patients, with a significant increase in the mean value ($P<0.01$).

Correlation between the concentrations of plasma 19-OH-AD and aldosterone obtained 2 h after the start of stimulation tests with 2-h standing or furosemide plus 2-h standing (Fig. 1)

Table 2. Endocrine data before and after 2-h standing and iv furosemide plus 2-h standing

	Plasma 19-OH-AD (pg/ml)		Plasma cortisol (μg/dl)		PAC (pg/ml)	
	basal	after	basal	after	basal	after
1) 2-h standing (n=8)						
Mean±SD	39± 9	47±10	12.1±4.9	10.8±3.5	265±158	430±196
2) iv furosemide plus 2-h standing (n=12)						
Mean±SD	34± 8	54±19*	10.3±3.1	14.7±4.3*	319±175	481±247*

19-OH-AD, 19-hydroxyandrostenedione; PAC, plasma aldosterone concentration.
*, $P<0.01$ *vs.* own basal value.

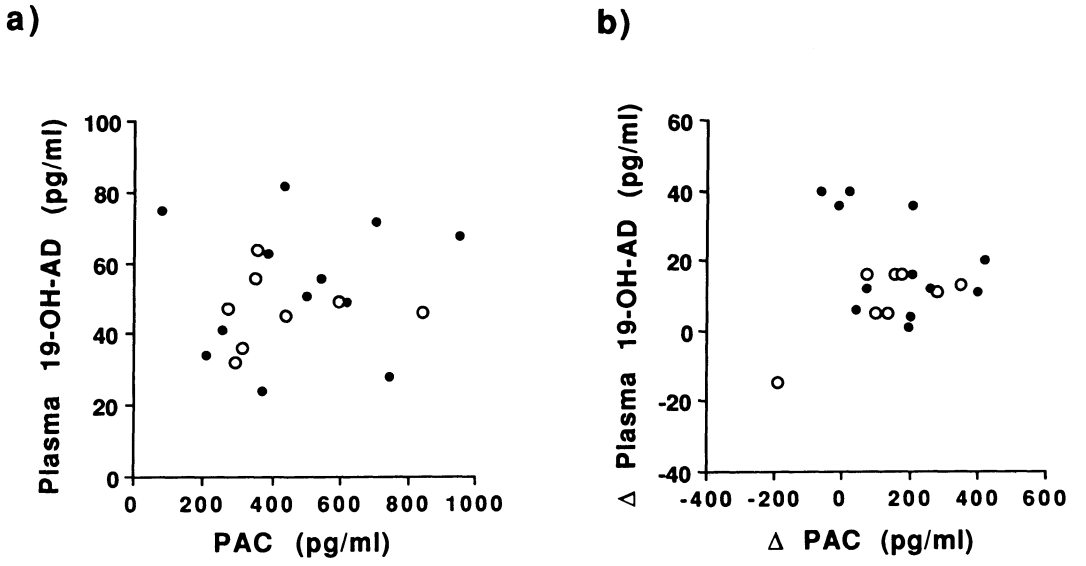

Fig. 1. Correlations between plasma 19-hydroxyandrostenedione (19-OH-AD) and plasma aldosterone concentrations (PAC) in the tests with 2-h standing (○) and furosemide plus 2-h standing (●). a, values after 2-h; b, the incremental values (△) between basal and 2 h after.

There were no significant negative correlations between the concentrations of plasma 19-OH-AD and aldosterone measured 2 h after the start of these two tests, 2-h standing and furosemide plus 2-h standing (Fig. 1a). And no significant negative correlation was observed between the values when there was a change (△) in plasma 19-OH-AD and aldosterone between basal and 2 h after the start of these 2 tests (Fig. 1b).

Furosemide injection plus 2-h standing with DEX pretreatment (Table 3)

Pretreatment with DEX suppressed basal plasma cortisol (2.8±1.5 *vs.* 10.3±3.1 μg/dl) and also decreased basal plasma 19-OH-AD (21±8 *vs.* 34±8 pg/ml). Even with DEX pretreatment, basal PRA remained suppressed in all the patients. Under DEX pretreatment, plasma 19-OH-AD as well as cortisol levels did not change during iv furosemide plus 2-h standing. To compare the effect of DEX pretreatment in the same patients, we evaluated plasma 19-OH-AD and cortisol in 6 patients (Cases 4 to 8 and 11) who were examined on both conditions. Significant increases in both plasma 19-OH-AD and cortisol were also observed with iv furosemide plus 2-h standing in these 6 patients (36±14 to 55±24 pg/ml, $P<0.05$ and 10.0±3.8 to 15.0±3.8 μg/dl, $P<0.05$, respectively) as seen in all 12 patients. When 6 patients were pretreated with DEX, however, the increases in both plasma 19-OH-AD and cortisol disappeared (21±9 to 26±12 pg/ml for plasma 19-OH-AD and 2.2±1.4 to 2.6±1.7 μg/dl for plasma cortisol).

Table 3. Endocrine data before and after iv furosemide plus 2-h standing with dexamethasone pretreatment

Case	Plasma 19-OH-AD (pg/ml)		Plasma cortisol (μg/dl)		PRA (ng/ml/h)		PAC (pg/ml)	
	basal	after	basal	after	basal	after	basal	after
4	34	46	1.2	1.4	<0.1	0.2	361	723
5	22	15	1.7	1.8	<0.1	<0.1	384	494
6	28	26	4.4	5.6	<0.1	<0.1	379	669
7	14	27	1.6	2.0	<0.1	0.2	244	236
8	12	13	3.2	3.8	<0.1	<0.1	462	592
10	23	35	0.5	0.5	<0.1	<0.1	154	250
11	16	29	0.9	1.2	<0.1	<0.1	142	230
12	15	13	2.6	2.9	<0.1	0.5	258	379
Mean±SD	21±8**	26±12	2.8±1.5**	3.2±1.6			298±116	447±201*

19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration. *, $P<0.05$ *vs.* own basal value. **, $P<0.01$ *vs.* the basal value in iv furosemide plus 2-h standing test (ref. Table 2).

Table 4. Clinical characteristics and endocrine data after the removal of aldosterone-producing adenoma (APA) with the adjacent adrenal tissue in the 10 patients with APA

Case	Age (yr old)	Sex	Period after operation (weeks)	Mean BP (mmHg)	Serum Na (mEq/l)	Serum K (mEq/l)	Plasma 19-OH-AD (pg/ml)	Plasma cortisol (μg/dl)	PRA (ng/ml/h)	PAC (pg/ml)
1	50	M	3	110	137	4.6	52	17.9	2.8	62
2	42	F	10	93	139	4.8	27	14.0	1.1	75
3	41	M	2	115	142	4.7	19	9.5	0.7	49
4	25	F	4	81	135	4.1	35	9.3	0.4	50
5	46	F	4	87	135	5.7	32	14.6	0.8	38
6	57	F	4	94	139	4.6	40	13.8	0.8	20
7	58	M	3	90	136	4.6	16	13.9	2.0	38
8	38	F	7	84	141	4.2	15	5.3	0.6	23
9	51	M	2	101	141	4.3	61	21.4	0.6	73
10	52	M	13	108	141	3.8	30	8.5	0.3	53
Mean±SD	46±10		5±4	96±12**	139±3*	4.5±0.5**	33±15	13.0±5.0	1.0±0.8	48±19**
Value (mean±SD) in the preoperative period in the 10 patients				116±11	143±1	3.0±0.6	33±7	10.7±3.3		374±163

M, male; F, female; BP, blood pressure; 19-OH-AD, 19-hydroxyandrostenedione; PRA, plasma renin activity; PAC, plasma aldosterone concentration. *, $P<0.05$; **, $P<0.01$ *vs.* preoperative period.

Interestingly, even in this condition, PAC increased in 7 of 8 patients, with the change in PAC significant ($P<0.05$).

Changes in basal PRA and plasma steroids before and after operation (Table 4)

PRA was normalized and PAC declined to the normal or subnormal range in all patients when measured at a mean time of 5±4 weeks (2 to 13 weeks) after the adrenal operation. In contrast, neither plasma 19-OH-AD nor cortisol changed after the operation as compared to the preoperative period.

A positive correlation was also found between basal plasma 19-OH-AD and cortisol even in the postoperative period ($P<0.01$) as in the preoperative one. However, no correlation was found between basal plasma 19-OH-AD and PAC, or between basal plasma 19-OH-AD and PRA, these

results being the same as those in the normal controls [6].

Relationship between mean blood pressure and plasma 19-OH-AD

There was no correlation between mean blood pressure and basal plasma 19-OH-AD, or between mean blood pressure and the ratio of plasma 19-OH-AD to PAC in patients with APA either before (Table 1) or after (Table 4) the operation.

Discussion

In the present study, basal plasma 19-OH-AD concentrations in patients with APA were found to be significantly lower than those in the normal subjects previously reported by us [6]. This finding may be attributable to the following factors. First, the age difference between normal subjects and patients with APA observed in the present study may be responsible for the difference in basal plasma 19-OH-AD, since a significant age-related decrease in plasma 19-OH-AD levels in normal subjects has been reported similar to that in other plasma androgens [14]. However, our previous study demonstrated no age-related changes in plasma 19-OH-AD levels in normal subjects aged 17 to 52 yr [6]. In addition, plasma 19-OH-AD levels in the age-matched selected patients were also significantly lower than those in the normal subjects. Second, abnormally increased PAC may reduce plasma 19-OH-AD levels in the 3-h standing test in normal subjects as supposed by Sekihara *et al.* [5]. Namely, chronic hyperaldosteronemia, which causes chronically suppressed PRA, may suppress plasma 19-OH-AD levels in patients with APA. In addition, the present study showed that PAC rose in most patients with 2-h standing and this increase became significant with iv furosemide plus 2-h standing. Then, to evaluate whether acutely further increased PAC after these stimulation tests suppresses a change in plasma 19-OH-AD levels, we determined the relationship between the values of plasma 19-OH-AD and aldosterone obtained 2 h after the start of the stimulation tests. There was found, however, no significant negative correlation between them. When the changes in PAC and plasma 19-OH-AD levels during these 2 tests were evaluated, no significant negative correlation was also observed between them. These results suggest that either chronic hyperaldosteronemia or an acute further rise in PAC does not necessarily cause plasma 19-OH-AD to decrease. The reason why the increase in PAC in most of the patients during 2-h standing and iv furosemide plus 2-h standing occurred will be described and discussed in a separate paper. Third, the removal of APA with the adjacent adrenal tissue did not cause any change in plasma 19-OH-AD in the postoperative period when PRA and PAC became normal and low normal, respectively. These results suggest that 19-OH-AD secretion may be reduced by the chronically suppressed renin-angiotensin system.

On the other hand, the present results suggest that the secretion of 19-OH-AD in patients with APA is still dependent on the ACTH-adrenal axis based on the following findings: 1) basal levels of plasma 19-OH-AD positively correlated with those of plasma cortisol in both the pre- and postoperative periods and 2) the significant increases in plasma 19-OH-AD as well as plasma cortisol levels, which were observed during iv furosemide plus 2-h standing, disappeared when the patients were pretreated with DEX. Although both the ACTH-adrenal axis and the renin-angiotensin system have been reported as the regulators of 19-OH-AD secretion in man by Sekihara *et al.* [4, 5], our previous study demonstrated that 19-OH-AD secretion in normal subjects is mainly controlled by the ACTH-adrenal axis [6]. This was based on the findings that acute stimulation with an ACTH infusion increased and acute suppression with oral DEX decreased plasma 19-OH-AD as well as plasma cortisol levels and that basal plasma 19-OH-AD correlated positively with basal plasma cortisol in normal subjects. In the present study, a positive correlation between basal plasma 19-OH-AD and cortisol was also seen in patients with APA. Thus, we conclude that chronic suppression of the renin-angiotensin system, not the chronic hyperaldosteronemia, causes plasma 19-OH-AD to decrease, while another crucial secretory mechanism of 19-OH-AD like the ACTH-adrenal axis is still operating in patients with APA.

Cytochrome $P\text{-}450_{11\beta}$ has the capacity to carry out 19-hydroxylase as well as 11β-hydroxylase [15–17]. Since 11β-hydroxylase activity has been shown to be increased in APA [18, 19], it follows that the plasma 19-OH-AD concentrations in

patients with APA should be higher than in normal subjects. However, the opposite finding was observed in the present study and may be attributed to the following factors: 1) $P\text{-}450_{17\alpha}$ is less expressed in APA resulting in less production of androstenedione, the precursor of 19-OH-AD [19], and 2) not $P\text{-}450_{11\beta}$ but $P\text{-}450_{aldo}$ which has been supposed to be a distinct species from $P\text{-}450_{11\beta}$ is expressed much in APA [20]. Therefore, it is suggested that the lower basal level of plasma 19-OH-AD in patients with APA may be not only due to the chronically suppressed renin-angiotensin system but also to lower production.

Sekihara *et al.* reported that daily administration of 19-OH-AD acts directly to raise blood pressure in intact rats [2, 3], in addition to amplifying the action of a subthreshold dose of aldosterone in adrenalectomized rats. According to their study, the hypertensive state induced by the chronic administration of 19-OH-AD in intact rats was similar to that induced by mineralocorticoid excess, and spironolactone inhibited the action of 19-OH-AD [3] . Furthermore, it has been revealed that plasma 19-OH-AD concentrations in patients with normal- and low-renin essential hypertension [4] and in hypertensive pregnant women [8] are higher than those in the respective control subjects. Our results obtained in patients with APA, on the other hand, showed no significant correlation between mean blood pressure and plasma 19-OH-AD or the ratio of plasma 19-OH-AD to PAC in patients with APA either before or after operation.

As is shown by low basal concentrations of plasma 19-OH-AD in patients with APA, the role of 19-OH-AD is equivocal in the blood pressure raising mechanism in the patients. However, 19-OH-AD, as a potentiating steroid on aldosterone or a factor preventing escape from the mineralocorticoid action of aldosterone, may contribute in a causal fashion to the pathogenesis of the hypertension in patients with APA. To further evaluate how 19-OH-AD is involved in hypertension in this disorder, it is necessary to investigate an attitude of 19-OH-AD in normotensive primary aldosteronism [21, 22], idiopathic hyperaldosteronism, and dexamethasone-suppressible hyperaldosteronism (DSH). In DSH, a blood pressure raising steroid has not been found yet [23, 24]. According to our recent study, oral metyrapone causes a decrease in plasma 19-OH-AD in Cushing's disease [25], and hence 19-OH-AD may not be such a steroid in DSH because hypertension in a patient with DSH was produced by daily metyrapone administration [23].

In conclusion, 1) plasma 19-OH-AD concentrations in patients with APA are lower than in normal subjects but 2) are still under the control of the ACTH-adrenal axis, 3) 19-OH-AD may, at least, not play an important causative role in the hypertension in association with chronic hyperaldosteronemia in patients with APA.

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