11 β -Hydroxylase in Mitochondrial Fractions of Functioning and Non-Functioning Adrenocortical Tumors

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NAGANUMA, H., OJIMA, M. and SASANO, N. *11 β -Hydroxylase in Mitochondrial Fractions of Functioning and Non-Functioning Adrenocortical Tumors.* Tohoku J. exp. Med., 1988, **155** (1), 81-96 — The activity of 11 β -hydroxylase was investigated in the mitochondrial fractions of 19 surgically resected adrenocortical tumors associated with Cushing's syndrome (4 adenomas and 2 carcinomas), primary aldosteronism (8 adenomas), adrenogenital syndrome (AGS) (2 carcinomas) and no hormonal symptoms (3 adenomas). Five adrenal cortices from patients with mammary carcinoma, renal cell carcinoma and pheochromocytoma were used for the normal control. The activities of 11 β -hydroxylation of deoxycorticosterone and of 11-deoxycortisol in the control adrenal cortices were 0.66-2.16 pmole/mg protein/min (mean: 1.28 pmole/mg protein/min) and 0.25-0.77 pmole/mg protein/min (mean: 0.56 pmole/mg protein/min), respectively. The activities in adenomas and carcinomas associated with Cushing's syndrome were in the range of normal control. The activities in aldosteronomas were significantly higher in 4 cases than those of the normal control and in the range of the normal control in 4 cases, suggesting that the higher activity of 11 β -hydroxylase is one of the important factors causing mineralocorticoid excess. The activities in adrenocortical carcinomas with AGS were significantly lower, corroborating clinical findings of androgen excess with suppressed production of mineralocorticoid or glucocorticoid. The activities in two cases of nonfunctioning adenomas were in the range of the normal control, but the third case showed higher activity than the normal control. These results show that the abnormal activity of mitochondrial 11 β -hydroxylase exists in some aldosteronomas, non-functioning adrenocortical adenomas and carcinomas with AGS. ——— adrenals; 11 β -hydroxylase; enzyme activity; adrenocortical tumors; mitochondrial fraction

Adrenocortical tumors have been generally classified into 4 types according to clinical findings; tumors with 1) glucocorticoid excess (Cushing's syndrome), 2) mineralocorticoid excess (primary aldosteronism), 3) androgen excess (adrenogenital syndrome (AGS)) and 4) no endocrine manifestations. Many

Received January 22, 1988; revision accepted for publication April 21, 1988.

investigators tried to relate this classification to histologic features of tumors (Brode et al. 1962; Neville and Symington 1966, 1967; Kano and Sato 1977; Sasano et al. 1980; Tuchiya et al. 1980), but the endocrine manifestation is not always consistent with morphology of tumors. Very interestingly several ultra-structural studies revealed that the shape of mitochondria varied remarkably from case to case of adrenocortical adenoma and carcinoma (Sasano et al. 1980; Tuchiya et al. 1980). This finding implies some possible abnormality of mitochondrial steroidogenic enzyme activities. The steroidogenic property in adrenocortical carcinoma was explained to be related with the defect of several

TABLE 1. *Clinical data of the patients included in this study*

Case	Age	Sex	Disease	PRA (ng/ml /hr)	PAC (pg/ ml)	F (μ g/ 100 ml)	17OHCS (mg/day)	17KS (mg/ day)
1) A.T.	57	F	MC					
2) T.S.	65	F	MC					
3) R.M.	36	F	Pheo.					
4) K.N.	42	F	Pheo.					
5) M.O.	73	F	RCC					
6) N.O.	32	F	Cushing	Trace	330	23.7	13.2	3.6
7) S.I.	30	F	Cushing	—	—	25.6	16.2	3.6
8) E.N.	34	F	Cushing	0.2	54	26.0	12.5	4.9
9) S.E.	37	M	Cushing	—	—	31.4	23.8	27.2
10) A.A.	34	F	PA	—	662	—	4.9	5.2
11) S.M.	27	F	PA	Trace	235	15.3	6.5	7.2
12) T.F.	49	M	PA	Trace	200	12.3	—	—
13) C.I.	28	F	PA	0.3	354	8.7	—	—
14) T.K.	40	M	PA	Trace	186	13.0	6.9	6.8
15) Y.N.	46	F	PA	Trace	400	7.3	3.9	4.4
16) M.I.	39	F	PA	2.2	370	9.3	5.8	3.5
17) Y.H.	27	F	PA	0.2	310	—	3.3	4.6
18) T.S.	27	M	Cushing†	2.1	23	12.6	9.6	15.6
19) R.Y.	41	F	Cushing*	—	17	40.8	52.0	123.5
20) S.I.	41	F	AGS*	3.9	262	24.3	4.0	23.7
21) T.T.	56	F	AGS*	5.7	85	19.9	22.4	145.9
22) K.O.	55	F	HT	Trace	79	16.5	6.6	6.4
23) T.I.	59	M	PC	<0.2	<20	5.5	4.8	7.6
24) I.O.	65	F	DM	—	<20	11.6	5.7	4.2

MC, mammary carcinoma; Pheo., pheochromocytoma; RCC, renal cell carcinoma; AGS, adrenogenital syndrome; Cushing, Cushing's syndrome; PA, primary aldosteronism; HT, hypertension; PC, pancreatic cyst; DM, diabetes mellitus.

*carcinoma; †borderline malignancy; —, not examined.

enzymes (Lipsett and Wilson 1962 ; Lipsett et al. 1963 ; West et al. 1964 ; O'Hare et al. 1979), but it remains obscure whether the same property in adrenocortical adenoma is due also to some enzyme abnormality. In the present study, we analyzed the activities of 11 β -hydroxylase in the mitochondrial fraction of adrenocortical adenomas and carcinomas from patients with various kinds of clinical manifestations.

MATERIALS AND METHODS

Sex and age of the patients along with the relevant clinical data and the histology of the adrenocortical tumors are listed in Tables 1 and 2. The surgical materials from 19

TABLE 2. *Pathological data of adrenocortical tumors and adrenocortices of various disease*

Case	Disease	Tumor size (cm)	Histology
1) A.T.	MC		Normal adrenal gland
2) T.S.	MC		
3) R.M.	Pheo.		
4) K.N.	Pheo.		
5) M.O.	RCC		Atrophic adrenal gland
6) N.O.	Cushing	2.0 $\times$ 2.7 $\times$ 3.0	Adrenocortical adenoma
7) S.I.	Cushing	2.5 $\times$ 3.0 $\times$ 3.2	
8) E.N.	Cushing	1.8 $\times$ 2.0 $\times$ 3.0	
9) S.E.	Cushing	2.5 $\times$ 3.7 $\times$ 3.8	Atypical adrenocortical adenoma
10) A.A.	PA	1.1 $\times$ 1.8 $\times$ 2.1	Adrenocortical adenoma
11) S.M.	PA	0.9 $\times$ 1.6 $\times$ 1.8	
12) T.F.	PA	0.8 $\times$ 0.9 $\times$ 1.0	
13) C.I.	PA	1.0 $\times$ 2.2 $\times$ 2.3	
14) T.K.	PA	1.0 $\times$ 1.2 $\times$ 1.4	
15) Y.N.	PA	1.8 $\times$ 1.8 $\times$ 2.0	
16) M.I.	PA	1.4 $\times$ 1.6 $\times$ 2.1	
17) T.H.	PA	1.0 $\times$ 1.5 $\times$ 1.5	
18) T.S.	Cushing	2.2 $\times$ 2.5 $\times$ 3.0	Borderline malignancy
19) R.Y.	Cushing	6.2 $\times$ 6.8 $\times$ 8.0	Adrenocortical carcinoma
20) S.I.	AGS	5.5 $\times$ 12.0 $\times$ 17.0	Adrenocortical carcinoma
21) T.T.	AGS	7.0 $\times$ 8.5 $\times$ 10.5	Adrenocortical carcinoma
22) K.O.	HT	1.3 $\times$ 1.5 $\times$ 2.2	Adrenocortical adenoma
23) T.I.	PC	1.8 $\times$ 1.8 $\times$ 2.0	
24) I.O.	DM	2.3 $\times$ 2.3 $\times$ 2.6	

MC, mammary carcinoma ; Pheo., pheochromocytoma ; RCC, renal cell carcinoma ; AGS, adrenogenital syndrome ; Cushing, Cushing's syndrome ; PA, primary aldosteronism ; HT, hypertension ; PC, pancreatic cyst ; DM, diabetes mellitus.

adrenocortical tumors and 5 control adrenal glands were used. There were 2 carcinomas resulting in Cushing's syndrome and 2 resulting in AGS. Four of the adrenocortical adenomas produced Cushing's syndrome, 8 produced primary aldosteronism and 3 were non-functioning. The control adrenal glands were taken from 2 patients with mammary carcinoma, 2 with pheochromocytoma and one with renal cell carcinoma.

Histology of adrenocortical tumors

Adenomas of Cushing's syndrome had predominantly compact cells with focal clear cells. One case (Case 9) of Cushing's syndrome had an atypical adenoma with irregular cell size and hyperchromatic nuclei (Fig. 1). An adrenocortical tumor of borderline malignancy with Cushing's syndrome (Case 18) was small, measuring $2.2 \times 2.5 \times 3.0$ cm in size, and contained nodular foci of solid proliferation of small compact cells and a few mitotic figures (Fig. 2a, b). Aldosteronomas were mainly composed of clear cells with sporadic foci of compact cells. Clear cells occasionally had large nuclei. An adrenocortical carcinoma causing Cushing's syndrome (Case 19) was large, measuring $8.0 \times 6.8 \times 6.0$ cm in size and 127 g in weight. The tumor had pleomorphic cells arranged in alveolar and solid patterns (Fig. 3). Vacuolization of tumor cells was evident. Adrenocortical carcinomas with AGS, weighing 640 g in Case 20 and 300 g in Case 21, had multiple nodules on the cut surface. A tumor in Case 20 was composed of large cells characteristic of tumors in AGS. Cells with hyperchromatic bizarre nuclei were prominent. Case 21 contained small cells with hyperchromatic and bizarre nuclei arranged in trabecular and alveolar patterns (Fig. 4). Non-functioning adenomas were composed mainly of clear cells with small nuclei and partly of compact cells.

Fig. 1. Atypical adenoma of Cushing's syndrome ; Case 9 is mainly composed of compact cells with irregular cell size and hyperchromatic nuclei. Hematoxylin and eosin, $\times 150$.

Fig. 2. a: Adrenocortical tumor of borderline malignancy with Cushing's syndrome; Case 18 contains nodular foci of expansive proliferation of small compact cells. Hematoxylin and eosin, $\times 75$.
b: High magnification of Case 18. An arrow indicates a mitotic figure. Hematoxylin and eosin, $\times 300$.

Fig. 3. Adrenocortical carcinoma with Cushing's syndrome; Case 19 had pleomorphic cells arranging in aleolar and solid paterns. Hematoxylin and eosin, $\times 300$.

Fig. 4. Adrenocortical carcinoma with AGS; Case 21 is composed of irregularly shaped small cells arranging in trabecular and alveolar patterns. Hematoxylin and eosin, $\times 300$.

Isolation of mitochondria

Tissues from the adrenocortical tumors and adrenal cortex removed surgically were rapidly (within 15–20 min) placed in an ice bath. Tissues of the tumor, adjacent adrenal cortex, medulla and capsule were each isolated. The mitochondrial fraction of the tumor and adrenocortical tissues was prepared according to the method of Cammer et al. (1968), with some modifications. Part of the mitochondrial fraction was fixed in 3% glutal aldehyde with cacodylate buffer and examined by an electron microscopy (JEM 100 B, JEOL Ltd., Tokyo). Protein concentration was determined by the method of Lowery et al. (1951) using bovine serum albumin as the standard.

Procedure of incubation

General conditions of the incubation were as follows: The incubation medium contained 20 mM Tris-HCl, 11.5 mM NaCl, 15.7 mM KCl, 70 mM sucrose and 10 mM sodium succinate and was titrated to pH 7.4. The mitochondrial preparation, 0.1 mg/ml was incubated aerobically in a water bath shaker at 37°C for 30 min, in the presence of 18 pmole of [1, 2-³H]-deoxycorticosterone (specific activity, 40.5 Ci/mmmole), or the same dose of [1, 2-³H]-11-deoxycortisol (specific activity, 58.6 Ci/mmmole) in a final volume of 0.5 ml. The reaction was stopped by the addition of 0.5 ml methanol and samples were then frozen at –20°C until extraction.

Isolation of the products

The incubation medium was extracted with five times volume of dichloromethane. The extracts were evaporated to dryness and the residue was redissolved in 200 μ l of the mixture of methanol and dichloromethane. The products were separated by thin layer chromatography (TLC). The extracts were then spotted on silica gel plates with fluorescent indication (20 $\times$ 20 cm, Eastman-Kodak-Company, Rochester, NY, USA) and developed in toluene-methanol 90 : 10. Identification of radioactive steroids was performed by authentic steroids running simultaneously with the labeled fractions. The radiochromatogram was scanned with a TLC scanner (TLC-1, Aloka, Tokyo). Radioactivity was counted in 10 ml of toluene-based liquid scintillator with a liquid scintillation counter (LSC-901, Aloka, Tokyo).

Reagents

[1, 2-³H]-Deoxycorticosterone and [1, 2-³H]-11-deoxycortisol were obtained from New England Nuclear Corporation (Boston, MA, USA). Deoxycorticosterone, corticosterone, 11-deoxycortisol, cortisol and 18-hydroxycorticosterone were obtained from Sigma Chemical Company (St. Louis, MO, USA). Other reagents were commercial products of analytical grade.

RESULTS

Ultrastructural findings of mitochondrial fraction

There was little microsomal contamination. The structure of mitochondrial membrane and cristae was well preserved, but almost all mitochondria showed spherical form (Fig. 5). Mitochondria in aldosteronomas (Fig. 6), Cushing's adenomas, nonfunctioning adenomas were slightly larger than those in the control. In the fractions of Cushing's adenomas, electrondense particles were more prominently observed (Fig. 7). In adrenocortical carcinoma, mitochondria were bizarre in shape with irregular cristae (Fig. 8).

Fig. 5. Electron micrograph (EM) of mitochondrial fraction of human adrenocortex; all mitochondria of various size are round in shape, but mitochondrial membrane and cristae are well preserved. $\times 3,300$.

Fig. 6. EM of mitochondrial fraction of aldosteronoma; almost all mitochondria are larger than those of the control in Fig. 5. $\times 3,300$.

Fig. 7. EM of mitochondrial fraction of Cushing's adenoma ; largest mitochondria and many electron-dense particles. $\times 3,300$.

Fig. 8. EM of mitochondrial fraction of adrenocortical carcinoma with Cushing's syndrome ; mitochondria are bizarre in shape with irregular cristae. $\times 3,300$.

Fig. 9. a: TLC graph of conversion of DOC (deoxycorticosterone) to B (corticosterone) in mitochondrial fraction of human adrenal cortex.
 b: TLC graph of conversion of 11-deoxycortisol (S) to cortisol (F) in mitochondrial fraction of human adrenal cortex.

11 β -hydroxylase

Thin layer chromatogram. The results of the partition chromatography of the incubation materials for conversion from deoxycorticosterone or 11-deoxycortisol in the mitochondrial fraction of the normal control adrenal cortex are shown in Figs. 9a and b. Two peaks were detected on each thin layer chromatogram. Two radioactive steroids were isolated with the authentic steroids. In the incubation with addition of ^3H -deoxycorticosterone, corticosterone was converted from deoxycorticosterone, and in that of ^3H -11-deoxycortisol, cortisol was converted from 11-deoxycortisol. Those two peaks on each chromatogram were detected in every tumor. In five cases of aldosteronomas, a peak other than the peaks of deoxycorticosterone and corticosterone was detected. Isolation with authentic steroids, the peak was determined to be 18-hydroxycorticosterone (Fig. 10). This peak was specific for aldosteronomas and not detected in the other adrenocortical tumors or control adrenocortices under the present incubation conditions. The activity of 11 β -hydroxylation of deoxycorticosterone and of 11-deoxycortisol by mitochondrial fractions of human adrenocortical tumors was compared.

As shown in Table 3, the activities of 11 β -hydroxylation of deoxycorticosterone were 0.66–2.16 pmole/mg protein/min (mean 1.28 pmole/mg protein/min)

Fig. 10. TLC graph of conversion of DOC to B and 18-hydroxycorticosterone in mitochondrial fraction of aldosteronoma.

TABLE 3. *The activity of 11 β -hydroxylase and 18-hydroxylase in mitochondrial fractions*

Case	11 β -Hydroxylase		18-Hydroxylase (pmole/mg prot. /min)
	Substrate DOC (pmole/mg prot. /min)	Substrate S (pmole/mg prot. /min)	
Control	mean $\pm$ s.d.	mean $\pm$ s.d.	mean $\pm$ s.d.
1) A.T.	1.17 $\pm$ 0.14	0.50 $\pm$ 0.05	n.d.
2) T.S.	0.66 $\pm$ 0.18	0.74 $\pm$ 0.23	n.d.
3) R.M.	1.37 $\pm$ 0.10	0.55 $\pm$ 0.14	n.d.
4) K.N.	2.16 $\pm$ 0.31	0.77 $\pm$ 0.24	n.d.
5) M.O.	1.02 $\pm$ 0.22	0.25 $\pm$ 0.08	n.d.
Cushing's adenoma			
6) N.O.	1.10 $\pm$ 0.05	0.71 $\pm$ 0.10	n.d.
7) S.I.	0.72 $\pm$ 0.10	0.53 $\pm$ 0.28	n.d.
8) E.N.	1.37 $\pm$ 0.26	0.43 $\pm$ 0.09	n.d.
9) S.E.	1.65 $\pm$ 0.62	0.91 $\pm$ 0.36	n.d.
Aldosteronoma			
10) A.A.	4.22 $\pm$ 0.72	1.35 $\pm$ 0.26	0.80 $\pm$ 0.10
11) S.M.	2.50 $\pm$ 0.79	1.85 $\pm$ 0.14	0.74 $\pm$ 0.28
12) T.F.	3.67 $\pm$ 0.92	3.88 $\pm$ 1.36	1.21 $\pm$ 0.05
13) C.I.	3.69 $\pm$ 0.83	1.23 $\pm$ 0.11	0.43 $\pm$ 0.14
14) T.K.	1.87 $\pm$ 0.05	0.62 $\pm$ 0.06	0.55 $\pm$ 0.03
15) Y.N.	5.29 $\pm$ 1.20	0.68 $\pm$ 0.04	n.d.
16) M.I.	2.31 $\pm$ 0.70	0.77 $\pm$ 0.32	n.d.
17) Y.H.	0.92 $\pm$ 0.11	2.43 $\pm$ 0.25	0.48 $\pm$ 0.05
Adrenocortical carcinoma with Cushing's syndrome			
18) T.S.	2.15 $\pm$ 0.81	0.57 $\pm$ 0.13	n.d.
19) R.Y.	0.97 $\pm$ 0.31	0.57 $\pm$ 0.07	n.d.
Adrenocortical carcinoma with AGS			
20) S.I.	0.33 $\pm$ 0.21	0.27 $\pm$ 0.07	n.d.
21) T.T.	0.37 $\pm$ 0.05	0.32 $\pm$ 0.14	n.d.
Non-functioning adenoma			
22) K.O.	0.97 $\pm$ 0.37	0.43 $\pm$ 0.19	n.d.
23) T.I.	1.37 $\pm$ 0.10	0.55 $\pm$ 0.14	n.d.
24) I.O.	4.57 $\pm$ 0.93	2.47 $\pm$ 0.08	n.d.

AGS, adrenogenital syndrome; n.d., not detected.

and those of 11-deoxycortisol were 0.25–0.77 pmole/mg protein/min (mean 0.56 pmole/mg protein/min) in the normal control.

Adrenocortical tumors with Cushing's syndrome. In all adenomas the activities of 11 β -hydroxylation of deoxycorticosterone (0.72–1.65 pmole/mg protein/min) and 11-deoxycortisol (0.43–0.91 pmole/mg protein/min) were in the range of

normal control. Those of deoxycorticosterone and 11-deoxycortisol in carcinoma were 0.97 ± 0.31 pmole/mg protein/min and 0.57 ± 0.07 pmole/mg protein/min, respectively. The tumor of borderline malignancy showed increased activity of 11 β -hydroxylation of deoxycorticosterone (2.15 ± 0.81 pmole/mg protein/min).

Aldosteronomas. In four cases (Cases 10, 12, 13, 15) the activity of 11 β -hydroxylation of deoxycorticosterone was two to three times higher than the normal control (3.67–5.29 pmole/mg protein/min vs. 0.66–2.16 pmole/mg protein/min) and that of 11-deoxycortisol was three to six times higher than the normal (1.23–3.88 pmole/mg protein/min vs. 0.25–0.77 pmole/mg protein/min). In case 17 the higher activity of 11 β -hydroxylation of 11-deoxycortisol than of deoxycorticosterone was obtained. In six of eight cases, 18-hydroxycorticosterone was converted from corticosterone. The activities of 18-hydroxylase were 0.43–1.21 pmole/mg protein/min.

Adrenocortical carcinomas with AGS. Two AGS carcinomas showed lower activities of 11 β -hydroxylase than the normal control.

Non-functioning adenomas. Of the three cases, the activities of two were in the normal control range and of one was higher than those of the normal control.

DISCUSSION

Some adrenocortical tumors produce excessive amounts of steroid and clinically manifest characteristic symptoms. Such excessive production of steroids is considered as a result of the abnormal activity of steroidogenic enzymes in the tumors. Abnormal activities of steroidogenic enzymes in adrenocortical tumors were documented by analyzing plasma steroids (Dufau et al. 1968; Ojima 1978) and the content of steroids in tumor tissues or in the tissue culture media (Bailey et al. 1960; Davignon et al. 1961; Louis and Conn 1961; Ojima 1978; Sasano and Ojima 1980). Many investigators revealed in adrenocortical carcinomas the defects or decrease of 11 β -hydroxylase (Degenhart et al. 1982), 3 β -hydroxysteroid dehydrogenase, 21-hydroxylase and C 17-20 lyase by analysis of urinary steroids (Lipsett and Wilson 1962) and plasma steroids (West et al. 1964; Powell-Jackson et al. 1974; Sasano et al. 1980). It remains unclear, however, whether the abnormality exists in adrenocortical adenomas. In the present paper we analyzed the activities of 11 β -hydroxylase in mitochondrial fractions of adrenocortical tumors in both case of adenoma and carcinoma.

Histological evaluation of the malignant potential is, in general, very difficult in adrenocortical tumors with Cushing's syndrome, especially in tumors of small size. O'Hare et al. (1979) reported that dynamic functional cell culture studies could give some useful parameters in the distinction of benign and malignant tumors, and the abnormalities of steroidogenic enzymes were detectable in malignancy. However, the present study revealed that all the adrenocortical tumors with Cushing's syndrome showed the similar activity of 11 β -hydroxylase to those of the normal control. No decreased activities were observed in even

malignant case and cases (Cases 9 and 18) which had been clinically suspected of malignancy because of the increased urinary 17-KS. Histologic examinations showed atypical adenoma in Case 9 and borderline malignancy in Case 18.

The activity of mitochondrial 11β -hydroxylation of deoxycorticosterone was significantly higher than the normal control in 4 cases of aldosteronomas. This result suggests that the higher activity of 11β -hydroxylase is one of the important factors causing mineralocorticoids excess in some aldosteronomas. Furthermore, in six (Cases 10, 11, 12, 13, 14, 17) of the eight cases of aldosteronomas, 18 -hydroxycorticosterone was produced under the present condition. Table 3 shows the activities of 18 hydroxylase in various tumors. The higher activity of 18 hydroxylase is also an important factor for excess of aldosterone in some aldosteronomas.

Electron microscopic findings of adrenocortical tumors in our previous study showed that the shape and size of mitochondria is variable from case to case, and that this morphological variation is more prominent in aldosteronomas. These biological and morphological abnormality of mitochondria may be the cause of mineralocorticoid excess. However, in five cases (Cases 10, 11, 12, 13, 17) the results of the activities of 11β -hydroxylase lead to glucocorticoid excess. In the previous study the defect or decrease of 17α -hydroxylase was suspected by analysis of plasma steroids or contents of steroids in the aldosteronoma (Dufau et al. 1968; Ojima 1978). In our above cases the abnormality of 17α -hydroxylase was suspected.

In two cases of adrenocortical carcinomas with AGS, the activities of 11β -hydroxylase were significantly lower than the normal control. In Case 20, plasma aldosterone was high (262 pg/ml), but plasma renin activity was not suppressed (3.8 ng/ml/hr). These data show that the renal artery was compressed by the large tumor and this might have activated the renin-angiotensin system. The decreased 11β -hydroxylase activity in carcinoma with AGS suggests little excess of cortisol in spite of its large size of tumor.

In non-functioning adenomas, two of three had the activity of 11β -hydroxylase of normal control. Kaplan (1967) reported that the aldosterone and corticosterone concentrations in adrenocortical adenomas from patients with essential hypertension were similar to those in the normal adrenal tissue. In this study, however, one case (Case 24) showed a high activity of 11β -hydroxylase. This patient did not show any hormonal symptoms. The plasma cortisol and aldosterone were within the normal levels. Ojima et al. (1978) described three types of non-functioning adenomas; 1) with excess of certain biologically inactive steroids, 2) with excess of the biologically weak steroids and 3) with minimal production of steroids, but this case does not belong to any type above. We suspected the supply of substrates to the tumor was limited or defect of enzymes for early steps of steroidogenic pathway in the tumor.

The results of the present study could be summarized about the activity of

11 β -hydroxylase as follows: 1) An increased activity in four aldosteronomas and a non-functioning adenoma, 2) an activity in the range of normal control in all adrenocortical tumors with Cushing's syndrome and two non-functioning adenomas, and 3) a decreased activity in carcinomas with AGS.

We concluded that the abnormality of enzyme associating with the morphological change of mitochondria exists in some aldosteronomas, some non-functioning adeno and carcinomas with AGS.

Acknowledgment

This study has been supported in part by a grant from the Ministry of Health and Welfare "Disease of steroid hormone" Research Committee, Japan.

References

- 1) Bailey, R.E., Slade, C.I., Lieberman, A.H. & Luetscher, J.A. (1960) Steroid production by human adrenal adenomata and non-tumorous adrenal tissue in vitro. *J. clin. Endocr.*, **20**, 457-465.
- 2) Brode, E., Grant, J.K. & Symington, T. (1962) A biochemical and pathological investigation of adrenal tissues from patients with Conn's syndrome. *Acta endocr. (Kbh.)*, **41**, 411-431.
- 3) Cammer, W., Cooper, D.Y. & Estabrook, R.W. (1968) Electrontransport reaction for steroid hydroxylation by adrenal cortex mitochondria. In: *Functions of the Adrenal Cortex*, Vol. II, edited by K.W. McKerns, Appleton-Century-Crofts, New York, pp. 943-992.
- 4) Davignon, J., Tremblay, G., Nowaczynski, W., Koiw, E. & Genest, J. (1961) Parallel biochemical and histological studies of an adrenocortical adenoma from a patient with primary aldosteronism. *Acta endocr. (Kbh.)*, **38**, 207-219.
- 5) Degenhart, H.J., Drop, S.L.S., Hoogerbrugge, J., van Seters, A.P. & de Vries, H.R. (1982) In vitro studies of enzymatic activities in human adrenal tumors. *Horm. Res.*, **16**, 96-99.
- 6) Dufau, M.L., Vilee, D.B. & Kliman, B. (1968) Pregnenolone metabolism in an aldosterone secreting tumor of the adrenal and its adjacent tissue. *J. clin. Endocr.*, **28**, 983-991.
- 7) Kano, K. & Sato, S. (1977) Fine structure of adrenal adenomata causing Cushing's syndrome. *Virchows Arch. [A]*, **374**, 157-167.
- 8) Kaplan, N.M. (1967) The steroid content of adrenal adenomas and measurements of aldosterone production in patients with essential hypertension and primary aldosteronism. *J. clin. Invest.*, **46**, 728-734.
- 9) Lipsett, M.B. & Wilson, H. (1962) Adrenocortical cancer: Steroid biosynthesis and metabolism evaluated by urinary metabolites. *J. clin. Endocr.*, **22**, 906-915.
- 10) Lipsett, M.B., Hertz, R. & Ross, G.T. (1963) Clinical and pathophysiologic aspects of adrenocortical carcinoma. *Amer. J. Med.*, **35**, 374-383.
- 11) Louis, L.H. & Conn, J.W. (1961) Primary aldosteronism: Content of adrenocortical steroids in adrenal tissue. *Recent. Prog. Horm. Res.*, **17**, 415-423.
- 12) Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.
- 13) Neville, A.M. & Symington, T. (1966) Pathology of primary aldosteronism. *Cancer*, **19**, 1854-1868.
- 14) Neville, A.M. & Symington, T. (1967) The pathology of the adrenal gland in Cushing's syndrome. *J. Path. Bact.*, **93**, 19-35.

- 15) O'Hare, M.J., Monaghan, P. & Neville, A.M. (1979) The pathology of adrenocortical neoplasia: A correlated structural and functional approach to the diagnosis of malignant disease. *Human Path.*, **10**, 137-154.
 - 16) Ojima, M. (1978) In vitro and in vivo studies on steroid production by aldosteronoma. *Folia endocr. jap.*, **54**, 246-254. (in Japanese with English abstract)
 - 17) Ojima, M., Saito, M., Itoh, N., Kusano, Y. & Fukuchi, S. (1984) Steroid production in nonfunctioning adrenal tumors. International Congress of Internal Medicine. XVIIth, 218. (Abstract)
 - 18) Powell-Jackson, J.D., Calin, A., Fraser, R., Grahame, R., Mason, P., Missen, G.A.K., Powell-Jackson, P.R. & Wilson, A. (1974) Excess deoxycorticosterone secretion from adrenocortical carcinoma. *Brit. med. J.*, **6**, 32-33.
 - 19) Sasano, N. & Ojima, M. (1980) Steroid hormone-producing tumors. *Igaku-no-Ayumi*, **115**, 635-645. (Japanese)
 - 20) Sasano, N., Ojima, M. & Masuda, T. (1980) Endocrinologic pathology of functioning adrenocortical tumors. *Path. Ann.*, **15**, 105-141.
 - 21) Tuchiya, H., Kawai, K., Harada, T., Shigematsu, K. & Sugihara, H. (1980) Functional pathology of aldosterone producing adenoma. *Acta path. jap.*, **30**, 967-976.
 - 22) West, C.D., Kumagai, L.F., Simons, E.L. & Domingus, O.V. (1964) Adrenocortical carcinoma with feminization and hypertension associated with a defect of 11 β -hydroxylation. *J. clin. Endocr.*, **24**, 567-579.
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