

Effects of Reserpine Treatment on Pituitary-Adrenocortical Axis in Patients with Cushing's Disease

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Abstract. Effects of reserpine treatment, not associated with pituitary irradiation, on the pituitary-adrenocortical axis in a total of 37 untreated patients with Cushing's disease were evaluated. With short-term treatment (2 mg daily for 2 weeks, $n=36$), basal excretion of urinary 17-OHCS significantly decreased from 11.2 ± 5.2 mg/day/m² (body surface area) (mean \pm SD) to 9.6 ± 4.4 mg/day/m² ($P < 0.01$), and metyrapone-induced incremental responses of urinary 17-OHCS decreased from 58.4 ± 41.4 mg/3 days/m² to 45.9 ± 29.8 mg/3 days/m² ($P < 0.05$). Long-term treatment (1.7 ± 0.3 mg/day for a mean of 15.8 ± 19.9 weeks) induced a marked reduction in plasma cortisol, and 24-h urinary 17-OHCS and/or free cortisol in 4 of 8 patients examined. Long-term reserpine administration caused normal suppression of plasma cortisol (or 11-OHCS) in 3 of 9 patients with 1 mg, and in all of 5 patients with an 8 mg overnight dexamethasone suppression test. Plasma ACTH response to CRH was evidently decreased in one patient evaluated one month after the initiation of reserpine. The circadian rhythm of plasma cortisol was normal in one patient when the basal glucocorticoid level became normal with reserpine treatment. The present findings suggest that reserpine itself contributes in a causal fashion to the effectiveness of our regimen, reserpine and pituitary irradiation, for some Cushing's disease patients in whom it is effective.

Key words: Cushing's disease, Reserpine, CRH, Cortisol, Dexamethasone.

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IN 1975, our group presented a new treatment with long-term reserpine administration in combination with pituitary irradiation for Cushing's disease [1]. Recent long-term follow-up studies in patients treated with reserpine and a single course of pituitary irradiation gave very good results [2, 3]: with our regimen, there is a higher remission rate than with pituitary irradiation alone [4, 5] or pituitary irradiation with an adrenal blocking drug [6–8], and preserves the normal hypothalamo-pituitary-adrenocortical axis as well as other pituitary

hormones [2, 3]. In spite of these results, the role(s) of reserpine in our method has not been studied and had remained unclear. The results of *in vivo* and *in vitro* studies on the effect of reserpine action on hypothalamic CRH are controversial [9–11]. *In vitro* evidence, however, suggests that reserpine directly inhibits ACTH release from the removed pituitary adenoma or peradenomatous tissue of Cushing's disease [12]. In this study, to clarify the part played by reserpine in the results obtained with our therapeutic regimen, we determined the effect of short-term and/or long-term reserpine treatment alone, not associated with pituitary irradiation, on the hypothalamo-pituitary-adrenal axis in untreated patients with Cushing's disease.

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Subjects and Methods

Patients (Table 1)

Thirty-seven patients with Cushing's disease (9 men and 28 women aged 15 to 59 with a mean of 29.6 ± 10.2 [mean \pm SD] years old) were involved in this study. All the patients had the procedures, therapeutic outcomes, and adverse effects of the treatment explained to them, and gave oral informed consent.

The diagnosis was confirmed with characteristic clinical features and endocrine data; high basal 24-h urinary 17-OHCS excretion and plasma cortisol or 11-OHCS, abnormal suppression of plasma cortisol or 11-OHCS with dexamethasone overnight suppression test, loss of diurnal rhythm of plasma cortisol or 11-OHCS (data not shown), and/or an increased or high-normal level of plasma ACTH (data not shown). Routine radiological studies showed no abnormality of the sella turcica in any patient. No pituitary tumor was detected in 6 of the patients examined by computerized tomography of the pituitary, or in one (patient 3, Table 2) by additional magnetic resonance imaging (MRI). A pituitary tumor ($12.4 \times 11.1 \times 6.2$ mm), however, was detected in one patient (patient 4, Table 2) by MRI [13].

Short-term reserpine treatment (Table 1)

Reserpine, 2 mg daily in 3 divided doses, was administered orally for 2 weeks to all but one of 37 patients (patient 12, Table 2). The mean basal urinary 17-OHCS value for 3 days before reserpine and that for 3 days from the 7th to 9th day after the initiation of short-term reserpine treatment were compared. The metyrapone test (described below) was performed twice, before and during short-term reserpine administration. On the second metyrapone test, metyrapone was administered on the 10th and 11th days after the start of reserpine administration, and the responses of urinary 17-OHCS were compared with that before reserpine treatment. CRH test was performed twice in 2 patients before and on the 8th day of short-term reserpine treatment.

Long-term reserpine treatment (Table 2)

Fourteen patients were involved in this study. They were newly diagnosed patients except one, patient 8 in Table 2, who took bromocriptine for 8 months at a maximum daily dose of 25 mg just before reserpine treatment, but it failed to normalize plasma and urinary glucocorticoid levels [14]. Reserpine was readministered to 4 patients, in

Table 1. Clinical and biochemical characteristics of the 36 patients with Cushing's disease in whom the effect of short-term reserpine was evaluated

	Group I		Group II	
	Before reserpine	During reserpine	Before reserpine	During reserpine
Number of patients	15		21	
Age (years)	29.8 ± 8.5		30.3 ± 11.2	
Sex (male/female)	5/10		3/18†	
Duration of disease (years)	3.2 ± 2.4		4.2 ± 3.0	
Mean basal 24-h urinary 17-OHCS for 3 days (mg/day/m ²)	10.5 ± 5.0	$8.6 \pm 4.3^*$	11.8 ± 5.4	10.4 ± 4.4
Increment in urinary 17-OHCS in metyrapone test (mg/3 days/m ²)	79.4 ± 48.8	$40.1 \pm 31.2^{**}$	$43.4 \pm 28.5^\dagger$	50.1 ± 28.9
Basal plasma cortisol or 11-OHCS (μ g/dl)	34.9 ± 12.8		32.9 ± 17.1	
Decrement in plasma cortisol or 11-OHCS after 8 mg dexamethasone (μ g/dl)	17.5 ± 10.9		20.0 ± 10.0	

Mean \pm SD. *and ** indicate statistically significant difference between before and during reserpine treatment ($P < 0.05$ and $P < 0.01$, respectively). † indicates statistical significance between Group I and Group II. Groups I and II; See the text.

Table 2. Characteristics of the 13 patients in whom changes in basal glucocorticoid levels and the results of various pituitary loading tests were evaluated during long-term reserpine treatment

Patient	#	Group	Sex	Age	Duration of disease	Prior to reserpine treatment				During short-term reserpine treatment			During long-term reserpine treatment				
						Urinary 17-OHCS (mg/m ²)		Plasma cortisol or 11-OHCS (μg/dl)		Urinary 17-OHCS (mg/m ²)		Interval between short- and long-term reserpine treatment	Duration of reserpine treatment	Total dose of reserpine			
						Metrapone test		Dexamethasone test		Metrapone test							
						Basal	Increment	Basal	Dexamethasone After 1 mg	Basal	Total				Increment		
						(day)	(/3 days)	(/3 days)		(day)	(/3 days)	(/3 days)					
1 ^a	I	M		30.0	2	8.6	70.8	*37.0	*34.0	*17.0	51.0	21.0	0	8	84		
2 ^{a,b,c,d}	I	F		30.5	1	8.3	43.0	33.5	18.0	8.5	29.3	10.1	0	80	892		
3 ^{a,b}	II	F		42.0	6	14.4	128.6	85.4	27.0	5.0	8.2	108.4	83.9	1	7	45	
4 ^{*,a,b}	II	F		37.0	2	14.4	92.7	49.7	65.1	50.3	21.3	90.9	61.1	0	3	23	
5 ^{a,b,c}	I	M		31.9	6	21.3	88.9	25.0	24.7	24.2	22.1	18.2	69.9	15.3	12	30	430
6 ^{a,b,c}	II	M		30.5	1	9.2	43.5	15.9	23.5	15.3	4.1	7.7	38.9	15.8	5	11	162
7 ^{a,c,d}	II	M		20.4	1	6.8	33.1	12.7	31.5	8.3	13.2	10.3	44.7	13.8	0	12	150
8 ^a	II	F		41.5	4	12.8	74.8	36.4	15.2	18.6	6.5	10.3	74.8	43.9	0	11	119
9 ^b	I	M		19.9	4	11.2	98.6	65.0	*21.0	*11.0	*1.0	5.3	30.9	15.0	0	24	336
10 ^b	II	F		42.4	7	7.5	58.4	35.9	*38.0	*37.0	*20.0	4.8	50.6	36.2	0	4	56
11 ^b	I	F		17.8	3	5.8	71.4	54.0	*21.0	*14.0	*1.0	4.5	50.6	37.1	0	10	144
12 ^b	—	M		23.5	3	7.0	30.4	9.4	**19.3	**9.2	**14.5	—	—	0	8	85	
13 ^b	II	F		36.8	6	4.2	30.5	17.9	13.7	9.5	10.5	4.5	34.4	20.9	6	9	130
14 ^c	II	F		35.1	1	9.1	130.7	103.4	18.5	10.7	2.1	8.7	112.7	86.6	0	4	56
Normal range						1.5 ~5.1	32.8 ~47.1	25.0 ~39.3	6.0 ~20.0	<5							

a, patients in whom changes in basal glucocorticoid levels were measured during long-term reserpine treatment;

b, patients undergoing overnight dexamethasone suppression test during long-term reserpine treatment;

c, patients undergoing GH provocation tests with arginine and/or insulin-induced hypoglycemia during long-term reserpine treatment;

d, patients in whom circadian rhythm of plasma cortisol was evaluated during long-term reserpine treatment;

e, patient undergoing CRH test during long-term reserpine treatment;

#, see the text for Group I and II. M, male patient; F, female patient; mg/m², mg per body surface area (m²).

*, patient whose pituitary tumor was confirmed to be larger than 1 cm in diameter by MRI.

*, plasma 11-OHCS; **, plasma cortisol measured by a competitive protein binding assay.

addition to short-term reserpine treatment, at intervals of 1 to 12 weeks. In the other 10 patients reserpine administration was continued after the completion of short-term reserpine trial without intervals, and the duration of reserpine treatment was counted from the beginning of short-term treatment in these cases. An attempt was made to administer a maximum daily dose of up to 2.0 mg. However, the dose had to be altered during the course of the treatment in some patients because of adverse effects, which were described in our previous reports [1–3], and was adjusted to 0.5–2.0 mg/day, as shown in Fig. 1. That is, the dose was reduced following the appearance of side effects within a short period, and reincreased with the disappearance. A constant daily dose of 2 mg was maintained in 7 patients (patients 5, 6, 9, 10, 11, 13, 14), and 1.5 mg in 2 patients (patients 1 and 12). The mean daily dose was 1.7 ± 0.3 mg (1.0–2.0 mg/day). Except for one patient (patient 3), 1.0–2.0 mg of reserpine was administered throughout the study. The mean duration of reserpine administration was 15.8 ± 19.9 (3 to 80 weeks, median 9) weeks. The clinical and biochemical characteristics of these 14 patients are listed in Table 2. In 8 of them, changes in the basal levels of plasma cortisol or 11-OHCS, 24-h urinary 17-OHCS excretion and/or 24-h urinary free cortisol excretion were evaluated. Various examinations were done, i.e. overnight dexamethasone suppression test in 10 patients, CRH test in one, and tests of plasma cortisol circadian rhythm in 2, plasma GH responses to l-arginine in 3, and plasma GH responses to insulin-induced hypoglycemia in 3.

Hormone assays and endocrine assessment

Basal plasma hormone levels were measured at 0800 h - 0900 h. Plasma 11-OHCS, which was used as an index of plasma cortisol until 1973, was measured [15]. Plasma cortisol was measured by a competitive protein binding assay (CPBA) [16] until 1975, and thereafter by means of commercially available radioimmunoassay (RIA) kit (Daiichi Radioisotope Laboratory, Tokyo). Normal ranges of plasma cortisol (CPBA and RIA) and 11-OHCS levels measured by these respective methods fell into the same range of 6 to 20 $\mu\text{g/dl}$. Urinary 17-OHCS was measured by the method of Silber and Porter [17]. Urinary free cortisol,

plasma GH and ACTH were measured with commercially available RIA kits, as previously reported [3, 14].

The overnight dexamethasone suppression test was conducted by the method already mentioned elsewhere [1, 3, 18–20]. The metyrapone test was performed by the previously reported method [1]. Briefly, urine specimens for the measurement of 17-OHCS were collected for 3 consecutive days before metyrapone administration as a baseline, and for another 3 consecutive days, 2 days during and one day following metyrapone (3 g/day) administration. The difference between the response and the baseline was taken as the increment of urinary 17-OHCS (mg/3 days/m^2 body surface area) in the metyrapone test. CRH test, and arginine infusion test and insulin tolerance test for studies of plasma GH responses were performed as in previous studies [1, 14].

Statistical analysis

Student's paired and unpaired *t*-test were used for statistical analysis of the hormonal data. Comparison of two parts of independent samples was analyzed by Chi square test. Data were expressed as the mean \pm SD. Statistical significance was defined as $P < 0.05$.

Results

1) Effects of Short-Term Reserpine Treatment

Change in mean basal 24-h urinary 17-OHCS excretion

The mean pre-reserpine value for 24-h urinary 17-OHCS for 3 days in the 36 patients was 11.2 ± 5.2 mg/day/m^2 , and that during reserpine treatment was significantly decreased to 9.6 ± 4.4 mg/day/m^2 ($P < 0.01$) (median of the change: -1.9 mg/day/m^2). The mean % change was -9.3% (median: -17.9% , range: -52.7% to $+39.6\%$).

Changes in urinary 17-OHCS response to metyrapone (Table 1)

In the pre-reserpine period, both the total and the increment in urinary 17-OHCS excretion in 3 days during the metyrapone test in the 36 patients were 91.7 ± 43.7 mg/3 days/m^2 and 58.4 ± 41.4

mg/3 days/m², respectively. With reserpine administration, there were significant decreases in both the total to 74.7 ± 36.4 mg/3 days/m² ($P < 0.01$) and increment to 45.9 ± 29.8 mg/3 days/m² ($P < 0.05$), respectively. The mean % change in these two parameters were -12.4% (median: -16.5%, range: -75.3% to +46.5%), and -11.4% (median: -10.5%, range: -91.6% to +91.7%), respectively.

A preliminary study on the reproducibility of the metyrapone test in Cushing's disease patients confirmed that the variation in the response of urinary 17-OHCS was within $\pm 20\%$ in most cases and did not exceed $\pm 30\%$ [1]. Based on these findings, 36 patients were divided into 2 groups. Group I included the patients in whom the increment in urinary 17-OHCS response to metyrapone test during short-term reserpine administration decreased more than 30% compared with that before reserpine. Group II consisted of the other 21 patients in whom the incremental response of urinary 17-OHCS during reserpine administration was decreased but less than 30% or increased compared with that in the pre-reserpine period. As shown in Table 1, age, the duration of the disease, basal hormonal values (basal urinary 17-OHCS, basal plasma cortisol or 11-OHCS) before metyrapone in these 15 patients in Group I, but not the male to female ratio, were similar to those in 21 patients in Group II. Short-term reserpine treatment significantly suppressed both the mean basal value ($P < 0.01$) and incremental response to metyrapone of urinary 17-OHCS in Group I ($P < 0.05$). The responses of urinary 17-OHCS to metyrapone before reserpine administration were significantly higher in Group I than in Group II ($P < 0.05$). With respect to the suppression of plasma cortisol or 11-OHCS in an 8 mg dexamethasone suppression test before reserpine, however, there was no difference between the 2 groups.

CRH test

ACTH response to CRH in 2 patients during reserpine treatment became higher in one (patient 3, Table 2), [before reserpine: basal 106 pg/ml (normal range: 10–50), peak 477 pg/ml (normal range: 52–100), during reserpine: basal 119 pg/ml, peak 742 pg/ml], but smaller in another (patient 4, Table 2) (before reserpine: 139 pg/ml, peak 366 pg/ml, during reserpine: basal 92 pg/ml,

peak 273 pg/ml).

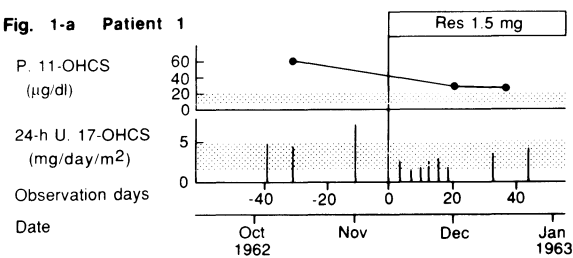
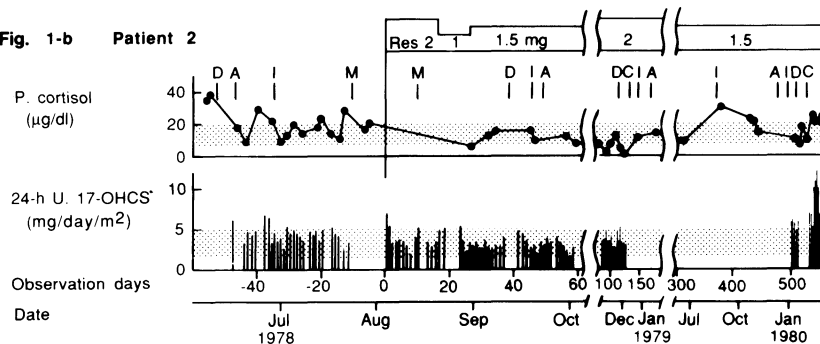
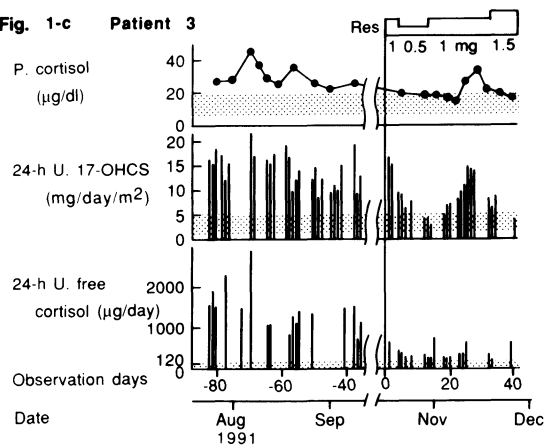
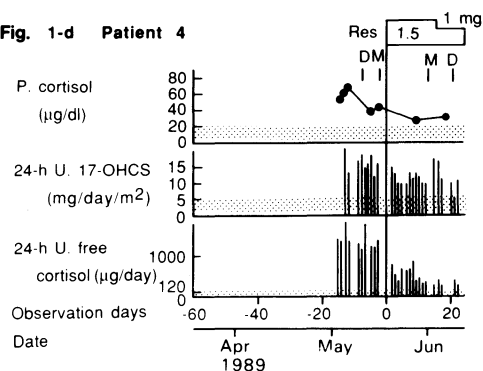
2) Effects of Long-Term Reserpine Treatment

Changes in basal glucocorticoid values in urine and plasma (Table 2 and Fig. 1)

In 8 patients, daily variations in both plasma and urinary glucocorticoid levels before and during long-term reserpine treatment were assessed. In patient 1 (Fig. 1-a), both plasma 11-OHCS and 24-h urinary 17-OHCS were nearly normal during reserpine treatment for 2 months. In patient 2 (Fig. 1-b), reserpine also suppressed both parameters to the normal range and her clinical remission persisted for 1.5 years. Thereafter, both plasma cortisol and 24-h urinary 17-OHCS excretion gradually increased to above the normal range even though reserpine administration was continued. In patient 3 (Fig. 1-c), 24-h urinary free cortisol and 17-OHCS, and plasma cortisol markedly decreased: the latter two parameters in particular often entered the normal range. In patient 4 (Fig. 1-d), plasma cortisol, 24-h urinary free cortisol and 17-OHCS decreased obviously, but did not reach the normal range during reserpine administration for 3 weeks. In patient 5 (Fig. 1-e), the normalization of plasma cortisol was observed from 100th day until 150th day after the initiation of reserpine. In patient 6 (Fig. 1-f), reserpine was administered for about 3 months, but did not cause an evident decrease in either plasma cortisol or 24-h urinary 17-OHCS. In patient 7 (Fig. 1-g), only transient but repeated reductions to the normal range of both plasma cortisol and 24-h urinary 17-OHCS were observed during reserpine administration for about 3 months. In patient 8 (Fig. 1-h), reserpine treatment for 2.5 months did not result in any continuous effect. The basal levels of plasma and/or urinary glucocorticoid never increased in any patient with long-term reserpine treatment, compared with those before treatment.

Overnight dexamethasone suppression test (Tables 2 and 3, Fig. 1).

The test was performed at 12.9 ± 16.1 (from 3 to 61) weeks after the reserpine treatment. As shown in Table 3, in 3 patients (patients 2, 9 and 10) out of 9 patients (patients 2–6 and 9–12) normal

Fig. 1-a Patient 1**Fig. 1-b Patient 2****Fig. 1-c Patient 3****Fig. 1-d Patient 4**

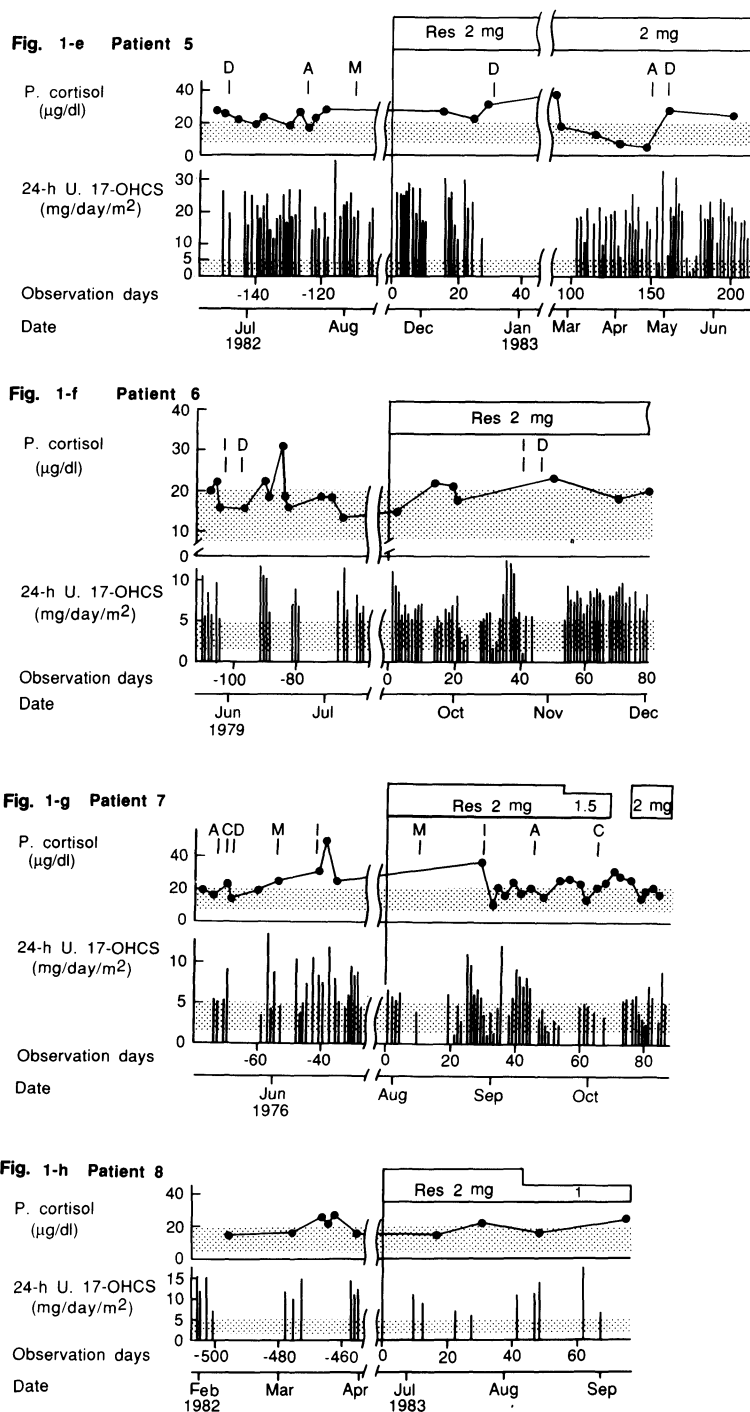


Fig. 1. Effect of long-term reserpine treatment on basal levels of plasma cortisol and 11-OHCS, 24-h urinary 17-OHCS excretion and/or 24-h urinary free cortisol excretion in patients with Cushing's disease. A, arginine load test; C, circadian rhythm of plasma cortisol; D, dexamethasone overnight suppression test; I, insulin-induced hypoglycemia test, M, metyrapone test; P. cortisol, basal level of plasma cortisol; Res, reserpine; 24-h U. 17-OHCS, 24-h urinary 17-hydroxycorticosteroids excretion; 24-h U. free cortisol, 24-h urinary free cortisol excretion. Shaded area shows normal range.

Table 3. Effect of long-term reserpine treatment on suppressibility of plasma cortisol or 11-OHCS with 1 mg or 8 mg dexamethasone overnight suppression test

Patient's number	Group	Before reserpine treatment			During reserpine treatment		
		Basal	Dexamethasone 1 mg	Dexamethasone 8 mg	Basal	Dexamethasone 1 mg	Dexamethasone 8 mg
2	I	33.5	18	8.5	15.8 (6)[59] 6.1 (16)[188] 18 (61)[810]	10.6 <1 9.3	9 <1 <1
3	II	27	22.4	5	32.8 (3)[22]	17.8	3.6
4#	II	65.1	50.3	21.3	31.9 (3)[21]	11.2	4.5
5	I	24.7	24.2	22.1	31 (4)[58] 27.8 (22)[290]	31.9 25.9	
6	II	23.5	15.3	4.1	21.5 (7)[100]	17.1	
9*	I	21	11	7	21 (24)[338]	2	1
10*	II	38	37	20	16 (4)[56]	3.4	
11**	I	21	14	1	12.5 (7)[80]	16	4.8
12**		19.3	9.2	14.5	23 (8)[87]	18	3.3
13	II	11.4	9.5	9.5	8.7 (3)[22]		1.3
Normal range ($\mu\text{g/dl}$)		6–20	<5	<5			

I, belongs to Group I in Table 1; II, belongs to Group II in Table 1; (), weeks after the start of reserpine treatment until the test day; [], total dose (mg) of reserpine administered until the test day; #, patient whose pituitary tumor was confirmed to be larger than 1 cm in diameter by MRI; *, plasma 11-OHCS; **, plasma cortisol by a competitive protein binding assay.

suppression of plasma cortisol below 5 $\mu\text{g/ml}$ with low dose (1 mg) dexamethasone was observed. With high dose (8 mg) dexamethasone, increased suppression of plasma cortisol below 5 $\mu\text{g/dl}$ was observed in all of 5 patients (patients 2, 4, 9, 12 and 13). Normalization of 1 or 8 mg dexamethasone suppression test was observed within 2 months after the initiation of reserpine administration in 4 out of 9 patients, one patient (patient 10) with 1 mg and 3 (patients 4, 12 and 13) with 8 mg dexamethasone test. There was no relationship between the increase in the suppression of plasma cortisol in 1 and/or 8 mg dexamethasone tests and the duration of reserpine administration until the test day, and moreover no parallel changes between the former parameter and the degree of lowering of basal plasma cortisol with reserpine administration were observed in any patient except patient 2 (Fig. 1 and Table 3).

CRH test

In patient 14, CRH test was performed on the 28th day after the initiation of daily 2 mg reserpine. The plasma ACTH response to CRH clearly decreased compared with that in the pretreatment period (before reserpine: basal 28 pg/ml [normal

range: 10–50], peak 611 pg/ml [normal range: 52–100]; during reserpine: basal 22 pg/ml, peak 351 pg/ml).

Circadian rhythm of plasma cortisol (Table 4 and Fig. 1)

In patient 2, the circadian rhythm, tested during reserpine treatment for 5 months (Dec. 1978, 2 mg/day, the total dose of reserpine administered: 224 mg), was normal. Around the test period, the basal level of plasma cortisol had been normal, as seen in Fig. 1-b. On the other hand, when both plasma cortisol and 24-h urinary 17-OHCS rose above normal (Fig. 1-b) during reserpine treatment for 18 months (Jan. 1980, dose of reserpine administered: 855 mg), the normal circadian rhythm was found to be lost. In patient 7, the diurnal pattern of plasma cortisol was abnormal when tested 2 months after the reserpine treatment (Oct. 1976, dose of reserpine administered: 116 mg) as in the pre-treatment period. In that period, basal plasma cortisol had been high (Fig. 1-g).

Plasma GH response to L-arginine or insulin-induced hypoglycemia (Tables 5 and 6, and Fig. 1)

Table 4. Effect of reserpine on the circadian rhythm of plasma cortisol

Patient's number	2		7	
Date	Dec. 1978	Jan. 1980	May. 1976	Oct. 1976
Reserpine	yes	yes	no	yes
Duration (months)	5	18	—	2
Total dose (mg)	224	855	—	116
[Plasma cortisol levels ($\mu\text{g/dl}$)]				
Time	0000	7	25	26
(o'clock)	0200	2	35	41
	0400	1	29	
	0500	3	31	
	0600	8	30	22
	0700	6	33	
	0800	13	30	
	0900	16	30	27
	1000	12	20	25
	1100	10	19	
	1200	13	25	
	1500	8	20	34
	1800	11	37	
	2100	13	23	28

Table 5. Response of plasma GH to arginine load before and during long-term reserpine treatment

Patient's number	Plasma GH (ng/ml)					
	Before treatment		During reserpine treatment			
	Basal	Peak	Basal	Peak	Duration (months)	Total dose (mg)
2	3	4	4	6	1.5	74
			1	12	6	319
			1	13	17	801
5	N.D.	N.D.	1	8	5	306
7	2	9	4	3	2	80
Normal range	N.D.-6	10-50				

N.D., not detected.

The plasma GH response to l-arginine in patient 2 (Fig. 1-b and Table 5) were still absent when tested 1.5 months after the initiation of reserpine (Sept. 1978, dose of reserpine administered: 74 mg), but it became normal 6 (Jan. 1979, dose of reserpine administered: 319 mg) and 17 months (Dec. 1979, dose of reserpine administered: 801 mg) after reserpine treatment. Until the former 2 periods, basal plasma cortisol and 24-h urinary 17-OHCS had been almost normal, but in the latter period they increased again (Fig. 1-b). The

response in patient 5 (Table 5 and Fig. 1-e) became subnormal when tested 5 months after reserpine treatment (Apr. 1983, dose of reserpine administered: 306 mg) in spite of high 24-h urinary 17-OHCS (Fig. 1-e). In patient 7 (Table 2 and Fig. 1-g), the response of plasma GH to l-arginine was still absent 2 months after the treatment (Sept. 1976, dose of reserpine administered: 80 mg). In those days, 24-h urinary 17-OHCS excretion sometimes entered the normal range, although plasma cortisol did not.

Table 6. Response of plasma GH to insulin-induced hypoglycemia before and during long-term reserpine treatment

Patient's number	Plasma GH (ng/ml)					
	Before reserpine treatment		During reserpine treatment			
	Basal	Peak	Basal	Peak	Duration (months)	Total dose (mg)
2	5 (81)	5 (23)	8 (80)	8 (23)	1.5	68
			3 (69)	17 (44)	5	236
			1 (75)	2 (46)	14	640
			2 (80)	4 (28)	17	804
6	1 (76)	3 (24)	1 (76)	3 (20)	1.5	84
7	4 (70)	1 (52)	3 (99)	4 (49)	1	54
Normal range	0–6	10–50				

(), basal or nadir level of plasma glucose (mg/dl) during the test.

The effects of reserpine treatment on plasma GH response to insulin-induced hypoglycemia were evaluated in 3 patients (patients 2, 6 and 7). With the test, plasma glucose decreased to less than 50 mg/dl in all patients. In patient 2 (Fig. 1-b), the GH response was not improved and remained low with the reserpine treatment for 1.5 months (Sept. 1978, dose of reserpine administered: 68 mg) under the condition of normal 24-h urinary 17-OHCS excretion and basal plasma cortisol. However, it became normal after 5 months of the treatment (Dec. 1978, dose of reserpine administered: 236 mg), with normal basal plasma cortisol and 24-h urinary 17-OHCS excretion. An absence of GH response, however, was found 14 months (Sept. 1979, dose of reserpine administered: 640 mg) and 17 months (Dec. 1979, dose of reserpine administered: 804 mg) after the initiation of treatment, although she was in remission. In the other 2 patients (patients 6 and 7, Table 2 and Figs. 1-f, 1-g), GH response remained absent (patient 6: Oct. 1979, dose of reserpine administered: 84 mg, patient 7; Aug. 1976, dose of reserpine administered: 54 mg). Around the test period in these 2 patients, glucocorticoid levels in plasma and urine were still high.

Discussion

We examined the influence of reserpine treatment alone on the pituitary-adrenal axis in Cushing's disease. The present studies on the

effect of short-term and long-term reserpine administration in untreated patients with Cushing's disease indicated that short-term reserpine treatment suppresses both basal 24-h urinary 17-OHCS and the metyrapone-induced urinary 17-OHCS response in about one half of the patients, and long-term reserpine 1) reduces the basal levels of plasma cortisol, 24-h urinary 17-OHCS and/or free cortisol to variable degrees, 2) often normalizes the suppression of plasma cortisol with 1 mg and/or 8 mg dexamethasone, 3) tends to restore the normal circadian rhythm of plasma cortisol, and 4) tends to improve the plasma GH response to l-arginine and/or to insulin-induced hypoglycemia. These findings strongly suggest that reserpine plays a crucial role in improving the efficacy of our treatment regimen [1–3]. On the other hand, the present findings in 14 patients treated with reserpine for a mean of 15.8 ± 19.9 weeks also suggest that the single use of reserpine treatment cannot induce a long-term remission in Cushing's disease, although there may be exceptional cases [21].

The mean daily dose of reserpine in the present study on the long-term effect ranged from 1.0 to 2.0 mg. It seems to be possible that this difference in the mean daily dose might alter the long-term effects in each patient. In fact, when the dose was increased from 1 mg to 1.5 mg in patient 3 during the treatment course, after tolerance of reserpine had been determined, it again suppressed the gradual increase in both plasma and urinary glucocorticoid. The long-term effect of reserpine

alone was independent of its short-term effect on the response of urinary 17-OHCS to metyrapone. Namely, of 4 patients (patients 1–4) in whom long-term reserpine treatment was effective, 2 patients were in Group I and two in Group II. On the other hand, of the other 4 patients in whom the effects were insufficient (patients 5–8), 1 patient was in Group I and 3 in Group II.

Long-term reserpine treatment increased the suppression of the plasma cortisol level with 1 or 8 mg dexamethasone in 4 of 9 patients within 2 months after the reserpine administration. This effect of reserpine is thought to be very advantageous in treating patients. In one patient, long-term treatment with reserpine suppressed plasma ACTH in both the basal level and the response to CRH. Results of *in vivo* and *in vitro* studies of the effects of reserpine on the CRH in the hypothalamus are also controversial [9–11]. However, reserpine directly inhibits the release of ACTH from the pituitary adenoma removed from Cushing's

disease patients [12]. In our *in vitro* experiments using the pituitary gland of normal rats, reserpine also significantly inhibited ACTH release and tended to reduce the plasma ACTH response to CRH [2]. When these results are considered together, reserpine would act directly on the pituitary ACTH-secreting adenoma cells to normalize the feedback regulation of ACTH secretion to decreased or increased plasma glucocorticoid.

Details of the mechanism of plasma GH response to l-arginine or insulin-induced hypoglycemia recovered in some patients are unknown. However, decreased GH secretion in patients with chronic glucocorticoid excess and in Cushing's disease and Cushing's syndrome due to adrenal adenoma could be due to insufficient GHRH release or to a direct toxic action upon somatotrophs [22]. Hence, lowering of the basal glucocorticoid level almost to the normal range around the test period might affect GH secretion.

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