The Pituitary ACTH, GH, LH, FSH, TSH and Prolactin Reserves in Patients with Cushing's Syndrome

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Synopsis

In order to know the pituitary reserves of ACTH, GH, LH, FSH, TSH and prolactin in patients with Cushing's syndrome, the responses of these hormones to hydrocortisone, lysine-8-vasopressin (LVP), insulin-induced hypoglycemia, luteinizing hormon-releasing hormone (LH-RH) and thyrotropin releasing hormone (TRH) were examined before and after treatment. Fourteen patients with Cushing's disease (adrenal hyperplasia), 3 patients with adrenal adenoma and one patient with adrenal carcinoma were investigated. Before treatment, sufficient response of plasma ACTH to LVP was observed in patients with Cushing's disease, while no response was observed in 3 patients with adrenal adenoma. There was no significant difference in the responses of other pituitary hormones between the patients with Cushing's disease and the patients with adrenal adenoma. The response of plasma GH to insulin-induced hypoglycemia was impaired in most these patients. The response of plasma TSH to TRH was impaired in 6 of 8 patients tested. The response of plasma LH and FSH to LH-RH were preserved in 6 and 5 of 8 patients, respectively. The response of plasma prolactin to TRH was normal in most patients tested. After treatment, the improvements of the impaired responses of GH, TSH, LH and FSH were observed. Therefore, the impaired reserve observed in these patients before treatment seemed to be due to the hypercortisolemia. If the difference of the suppressibility of these pituitary hormones by cortisol may be judged simply from our observation, the order of the suppressibility is supposed to be ACTH, GH, TSH, LH and FSH, and then prolactin.

The disorders in pituitary ACTH release and adrenocortical cortisol secretion characterize Cushing's syndrome. Increased or high normal plasma ACTH concentrations, impaired suppression of ACTH and cortisol release following relatively low dose dexamethasone administration, and hyperresponsiveness to metyrapone and lysine-8-vasopressin (LVP) are characteristic findings in patients with pituitary dependent Cushing's syndrome (Cushing's disease or adrenal hyperplasia). While, in patients with adrenal tumors (adrenal adenoma and adrenal

carcinoma), undetectable level of plasma ACTH, no suppression of cortisol release following high dose dexamethasone administration and no response of ACTH and cortisol to metyrapone and LVP are characteristic.

In recent years, the development of the sensitive and reliable radioimmunoassay for several pituitary hormones, in addition to the synthesis of hypothalamic releasing hormones has permitted the evaluation of the pituitary reserves for these hormones. To assess the pituitary reserves of these hormones in patients with Cushing's syndrome, we examined the responses of plasma

ACTH, GH, LH, FSH, TSH and prolactin to hydrocortisone, LVP, insulin-induced hypoglycemia, luteinizing hormone-releasing hormone (LH-RH) and thyrotropin releasing hormone (TRH). A comparison was made between patients with Cushing's disease and adrenal tumors. Another comparison was also made between the responses before and after treatment.

Patients and Methods

Eighteen patients with Cushing's syndrome were investigated. Fourteen patients had Cushing's disease, 3 had adrenocortical adenoma and one had adrenal carcinoma. The pituitary-adrenal functions in these patients are listed on Table 1 which were examined in their untreated stage. The diagnosis of Cushing's syndrome was made based on the clinical pictures, *i. e.*, elevated rates of cortisol secretion, elevated

levels of urinary 17-KGS and plasma cortisol, the absence of normal diurnal rhythm of plasma cortisol and the absence of substantial decrease in plasma cortisol and urinary 17-KGS after the administration of a low dose of dexamethasone (1~2 mg/day). The more detailed diagnosis of "Cushing's disease" was made by demonstrating a suppressive effect of high dose of dexamethasone and also by demonstrating stimulation under the influence of metyrapone and LVP (Table 1, Fig. 1). Patient TM and EF had been diagnosed at another hospital. The diagnosis was also confirmed by adrenal surgery in 10 patients, 6 with Cushing's disease, 3 with adrenal adenoma and one with adrenal carcinoma. Surgical treatment consisted of unilateral total adrenalectomy for adrenocortical tumors and bilateral total adrenalectomy for Cushing's disease. Patient ST was first treated with pituitary irradiation, and later, with bilateral total adrenalectomy because of relapsing. Patient SK had been first suspected to have adrenal hyperplasia and treated with pituitary irradiation. After that, she was correctly diagnosed to have adrenal adenoma and treated with unilateral adrenalectomy.

ACTH reserve was tested by the single intravenous injection of 4 pressor unit LVP. Blood samples were

Table 1. Indices of pituitary-adrenocortical function in patients with untreated Cushing's syndrome

Name	Sex	Age	Diagnosis	Plasma cortisol (μ g/dl)		Plasma ACTH (pg/ml)	Response to dexamethasone		Response to 3 g metyrapone
				9 a.m.	11 p.m.		2 mg/day	8 mg/day	
1. ST	F	21	CD	38.9	13.3	/	(-)	(+)	(++)
2. KS	M	27	"	22.3	/	/	(-)	(+)	(+)
3. KM	M	47	"	21.6	15.0	195.0	(-)	(-)	(++)
4. RY	F	22	"	27.2	21.6	/	(-)	(+)	(++)
5. TF	F	21	"	23.4	17.8	/	(+)	(+)	(+)
6. KI	M	16	"	47.7	19.5	/	(-)	(-)	(+)
7. NM	M	30	"	25.9	21.7	/	(-)	(-)	(++)
8. MN	F	13	"	12.3	14.4	/	(-)	(+)	(++)
9. MT	F	21	"	24.9	24.6	/	(-)	(+)	(++)
10. KT	F	26	"	26.8	12.4	178.6	(-)	(-)	/
11. TS	F	36	"	13.2	15.1	100.0	(-)	(+)	(++)
12. SF	F	22	"	19.0	11.7	193.8	(-)	(+)	(++)
13. SK	F	21	AA	20.8	18.5	ND	(-)	(-)	(-)
14. YO	F	26	"	24.7	24.0	"	(-)	(-)	(-)
15. SI	M	36	"	19.6	12.5	"	(-)	(-)	(-)
16. MM	M	3	AC	24.2	28.8	"	*(+)	**(-)	(-)
Normal				3.7 ~14.5	0 ~7.0	ND ~100	(+)	(+)	(+)

Abbreviations; CD: Cushing's disease, AA: adrenal adenoma, AC: adrenal carcinoma, ND: not detectable level (≤ 20 pg/ml)

The responses to dexamethasone and metyrapone were examined by measuring urinary 17-KGS after the oral administration of dexamethasone and metyrapone.

* response to 1 mg/day dexamethasone, ** response to 4 mg/day dexamethasone.

Case KM and NM showed sufficient suppression in urinary 17-KGS to 16 mg/day dexamethasone.

Case TF showed no suppression to 1 mg/day dexamethasone.

Fig. 1. Plasma ACTH and cortisol in response to 4U of lysine-8-vasopressin. ● : Cushing's disease, ○ : adrenal adenoma

taken for ACTH and cortisol estimations before and 15, 30, 60 min after the injection. Plasma ACTH was also determined following the intravenous infusion of 150 mg hydrocortisone over 2 hours in patients with adrenal hyperplasia.

Growth hormone reserve was tested by insulin-induced hypoglycemia after intravenous injection of regular insulin (0.1~0.15 U/kg of bwt). Blood samples were obtained before and 30, 60 and 90 min after the injection.

LH and FSH reserves were examined by the responses to LH-RH. TSH and prolactin reserves were tested by the responses to TRH. Synthetic LH-RH (100 μ g) and synthetic TRH (500 μ g) were administered as a single intravenous injection and blood samples were obtained for LH, FSH, TSH and prolactin estimations before and 15, 30, 45, 60, 90 and 120 min after the injection. Thirty-three μ g of LH-RH and 100 μ g of TRH were administered to one patient with adrenal carcinoma aged 3 years. The normal range of LH and FSH responses were determined from the responses in normal males and those in females with normal menstrual cycle at their follicular phase.

Plasma ACTH, GH, LH, FSH, TSH and prolactin were measured by the radioimmunoassay techniques. The radioimmunoassay kit for human ACTH was obtained from the Radio Chemical Center (England). The kit for human GH from the Dainabot Radioisotope Lab. and the kits for human LH, FSH, and TSH from the Daiichi Radioisotope Labs. The

reagents for prolactin assay was supplied by National Institute of Health (U.S.A.). The standard for ACTH assay was human ACTH (R.C.C.), that for GH assay NIH-GH-HS 1523D (Wilhelmi), that for LH and FSH assays human pituitary extract (Calbiochem), that for TSH assay human TSH (Calbiochem), that for TSH assay human TSH (Calbiochem) and that for prolactin assay HPr V-L-S #1 (N.I.H.).

Results

Pituitary ACTH reserve

As shown in Table 1, patients with Cushing's disease had high resting levels of plasma ACTH, while all patients with adrenal tumors had undetectable levels. Normal or increased urinary 17-KGS responses to oral administration of metyrapone and sufficient plasma ACTH response to LVP were observed in Cushing's disease, while no response to metyrapone and LVP was observed in adrenal adenoma (Table 1, Fig. 1). After treatment, more elevation of resting ACTH levels was found in totally adrenalectomized Cushing's disease (360.1 ± 253.8 pg/ml, mean $\pm$ SD). The intravenous infusion of 150 mg of hydrocortisone over 2 hours produced no fall in plasma ACTH until 180 min in a patient with Cushing's disease treated with pituitary irradiation, while the decrease of plasma ACTH was found by 60 min in 3 totally adrenalectomized Cushing's disease. Significant decrease in plasma ACTH was not found by 30 min in these patients, while it was already found by 30 min in 3 patients with Addison's disease whose basal ACTH levels were also high (Fig. 2).

Pituitary GH reserve

The mean plasma basal GH in 17 untreated patients with Cushing's syndrome was 1.3 ± 1.2 ng/ml (mean $\pm$ SD), while that in 9 treated patients was 2.5 ± 1.6 ng/ml. Before treatment, insulin test was performed in 9 Cushing's diseases, 3 adrenal adenomas and one adrenal carcinoma. Hypoglycemia in which blood glucose levels fell by more

than 30% of the resting level was observed in response to insulin in 11 of these 13 patients. The mean decrease of blood glucose was $37.3 \pm 12.7\%$. Before treatment, the significant response of plasma GH to insulin-induced hypoglycemia was not found in most patients regardless of the etiology but a girl patient aged 13 who showed a normal response. After bilateral total adrenalectomy, one patient (TM) showed a excessive response, while another patients (TS) showed a impaired response. Two patients treated with pituitary irradiation showed no responses. One patient (SK) treated with unilateral adrenalectomy showed a normal response (Fig. 3).

Pituitary LH reserve

The response of plasma LH to LH-RH was tested before therapy in 4 Cushing's

diseases, 3 adrenal adenomas and one adrenal carcinoma (Table 2, Fig. 4). One patient with Cushing's disease (EF) and one child patient with adrenal carcinoma (MM) showed subnormal responses. One patient with Cushing's disease (SF) and one patient with adrenal adenoma (SK) showed excessive LH response. Patient SK also showed a excessive response after treatment. The other 4 patients showed normal responses. After the effective treatment, most of the patients had normal responses, regardless of the etiology and the treatment. In 2 male patients totally adrenalectomized showed excessive LH responses.

Pituitary FSH reserve

Before treatment, the resting levels of plasma FSH were normal or decreased, while normal basal levels were observed

Fig. 2. The suppression of plasma ACTH following the intravenous infusion of 150 mg hydrocortisone over 2 hours in patients with Cushing's disease and Addison's disease. The numerals indicate the levels of plasma ACTH (pg/ml). The basal levels of plasma ACTH were 195 pg/ml (KM), 249 pg/ml (TS), 198 pg/ml (KI), 800 pg/ml (KS), 875 pg/ml (MO), 1093 pg/ml (SO), 513 pg/ml (TK). PI: pituitary irradiation, BTA: bilateral total adrenalectomy

after treatment. Plasma FSH response to LH-RH was tested in the same patient whose LH responses were examined. One patient with adrenal hyperplasia (KT) showed a hyper-response and one patient with adrenal carcinoma (MM) showed a hypo-response. Four patients showed normal responses. In patients EF and SI, rises of plasma FSH were not significant. After treatment, 5 patients with Cushing's disease and one patient with adrenal adenoma showed normal responses. However, in patient TM, no significant rise of plasma FSH was observed. Patient SK showed a excessive FSH response. (Table 3, Fig. 4)

No significant difference was observed in LH and FSH responses between Cushing's disease and adrenal adenoma.

Fig. 3. Plasma GH in response to insulin-induced hypoglycemia before and after treatment.

—•— : Cushing's disease,
 - - - : adrenal adenoma,
 x - - - x : adrenal carcinoma

Pituitary TSH reserve

The resting levels of plasma TSH were normal or undetectable. Before treatment, the response of plasma TSH to TRH was examined in 4 Cushing's diseases, 3 adrenal adenomas and one adrenal carcinoma. Three patients with Cushing disease and 3 patients with adrenal adenoma showed subnormal responses. Low but normal response was observed in one patient with Cushing's disease and one patient with adrenal carcinoma. After treatment, 3 of 5 totally adrenalectomized Cushing's diseases showed normal responses, while 2 patients had subnormal responses. One patient treated with pituitary irradiation had a normal response. One of 2 patients with adrenal adenoma treated with unilateral adrenalectomy had a normal response while another patient (SK) who was diagnosed to have primary hypothyroidism showed significantly elevated levels of plasma TSH. Except this case (SK), the mean resting levels before and after treatment were 2.1 ± 2.7 (SD) $\mu\text{U/ml}$ and 3.4 ± 4.0 (SD) $\mu\text{U/ml}$, and the mean peak levels before and after treatment were 8.9 ± 4.5 $\mu\text{U/ml}$ and 16.6 ± 6.1 $\mu\text{U/ml}$, respectively. No significant difference was found in TSH responses between Cushing's syndrome and adrenal adenoma. (Table 4, Fig. 5.)

Plasma prolactin reserve

The resting levels of plasma prolactin were normal or high. Three of 4 patients with Cushing's disease had high resting levels. Before treatment, the response of plasma prolactin to TRH was examined in 4 Cushing's diseases, 2 adrenal adenomas and one adrenal carcinoma. The responses of plasma prolactin in these 7 patients were almost within normal range. However, patients with Cushing's disease whose basal levels were high (KI, TS) showed subnormal rises of plasma prolactin. After treatment, 3 patients treated with bilateral total adrenalectomy and a patient treated with pituitary

Fig. 4. Plasma LH (left panels) and FSH (right panels) in response to the intravenous administration of 100 μ g synthetic LH-RH before and after treatment. Case MM was administered 33 μ g of LH-RH. — : Cushing's disease, ---o--- : adrenal adenoma, x---x : adrenal carcinoma

Table 2. Plasma LH response to LH-RH in patients with Cushing's syndrome

Name	Sex	Age	Diagnosis	Treatment	LH(mIU/ml) response to LH-RH						Maximum increment
					0	15	30	45	60	90	
(min after 100 μ g of LH-RH injection)											
a) before treatment											
1. KT	F	26	AH		11.7	46.0	75.0	64.5	56.0	59.0	63.3
2. TS	F	36	"		13.0	49.0	63.0	50.0	42.0	26.0	50.0
3. EF	F	36	"		11.9	24.5	22.5	/	19.5	19.0	12.6
4. SF	F	22	"		41.2	240.0	215.0	/	142.0	118.0	198.8
5. SK	F	21	AA		40.0	271.0	368.0	250.0	197.0	182.0	328.0
6. YO	F	26	"		8.7	65.0	55.0	33.0	32.0	47.0	56.3
7. SI	M	36	"		21.0	54.0	50.0	43.0	42.0	43.0	33.0
8. *MM	M	3	AC		4.2	9.0	17.2	/	12.8	9.3	13.0
b) after treatment											
1. KS	M	35	CD	BTA	18.1	321.0	340.0	/	215.0	104.0	321.9
2. KI	M	18	"	"	10.5	68.0	93.0	66.0	51.0	53.0	82.5
3. TM	M	19	"	"	32.0	170.0	210.0	130.0	110.0	122.0	178.0
4. TS	F	36	"	"	24.5	76.0	83.0	/	67.0	92.0	67.5
5. ST	F	30	"	BTA+PI	17.5	52.0	62.0	56.0	49.0	43.0	44.5
6. KM	M	50	"	PI	21.0	27.0	48.0	42.0	44.0	45.0	27.0
7. YO	F	26	AA	UA	35.0	82.0	138.0	/	102.0	72.0	103.0
8. SK	F	21	"	UA+PI	51.0	105.0	242.0	/	330.0	310.0	279.0
Normal					5.2	36.9	44.0	28.8	27.6	21.0	38.8
					~24.8	~180	~150	~129	~100	~84.0	~162.9

Abbreviations; BTA: bilateral total adrenalectomy, PI: pituitary irradiation,

UA: unilateral total adrenalectomy.

* Case MM was administered 33 μ g of LH-RH.

Table 3. Plasma FSH response to LH-RH in patients with Cushing's syndrome

Name	Sex	Age	Diagnosis	Treatment	FSH(mIU/ml) response to LH-RH						Maximum increment
					0	15	30	45	60	90	
(min after 100 µg of LH-RH injection)											
a) before treatment											
1. KT	F	26	CD		13.5	28.0	39.5	47.0	49.5	56.0	42.5
2. TS	F	36	"		5.9	12.3	16.8	17.0	22.5	20.0	16.6
3. EF	F	36	"		13.3	16.4	15.1	/	14.7	14.7	3.1
4. SF	F	22	"		7.7	12.8	15.7	/	12.0	14.2	8.0
5. SK	F	21	AA		10.9	17.1	22.7	24.5	26.5	24.0	15.0
6. YO	F	26	"		1.0	11.1	18.6	22.3	23.5	27.8	26.8
7. SI	M	36	"		10.4	9.8	14.0	14.6	14.4	15.6	5.2
8. *MM	M	3	AC		3.6	4.4	6.6	/	3.8	8.4	4.8
b) after treatment											
1. KS	M	35	CD	BTH	12.3	18.9	29.3	/	15.3	22.5	17.0
2. KI	M	18	"	"	13.2	22.0	31.0	40.0	29.0	17.0	26.8
3. TM	M	19	"	"	16.0	16.2	16.9	16.9	16.6	16.0	0.9
4. TS	F	36	"	"	11.7	22.5	25.0	22.2	23.0	22.5	13.3
5. ST	F	30	"	BTA+PI	12.2	17.0	24.0	21.0	22.5	18.4	11.8
6. KM	M	50	"	PI	15.5	13.8	16.8	24.6	22.0	20.0	9.1
7. YO	F	26	AA	UA	16.8	21.0	28.0	/	18.3	25.4	11.2
8. SK	F	21	"	UA+PI	25.8	30.4	35.2	/	43.0	36.0	17.2
Normal					5.5	7.0	10.0	8.8	11.7	9.5	3.2
					~21.0	~30.5	~38.0	~35.0	~30.0	~39.0	~18.0

* Case MM was administered 33 µg of LH-RH.

Table 4. Plasma TSH response to TRH in patients with Cushing's syndrome

Name	Sex	Age	Diagnosis	Treatment	TSH(μ U/ml) response to TRH						Maximum increment
					0	15	30	45	60	90	
(min after 500 μ g of TRH injection)											
a) before treatment											
1. KI	M	18	CD		5.0	5.7	7.2	/	5.8	5.6	2.2
2. KT	F	26	"		6.0	7.4	15.0	16.0	14.5	13.0	10.0
3. TS	F	36	"		u.d	3.0	3.1	3.6	5.0	/	5.0
4. SF	F	22	"		u.d	8.0	8.6	/	3.9	3.7	8.0
5. SK	F	21	AA		8.6	11.0	12.0	9.6	9.8	7.8	3.4
6. YO	F	26	"		4.0	u.d	3.8	2.0	u.d	2.5	-0.2
7. SI	M	36	"		u.d	u.d	4.8	4.1	7.4	2.4	7.4
8. *MM	M	3	AC		u.d	14.0	13.5	/	9.8	4.6	9.8
b) after treatment											
1. KS	M	35	CD	BTA	2.5	11.0	9.8	/	7.2	5.2	7.3
2. TM	M	19	"	"	12.5	20.0	18.5	/	11.0	6.8	7.5
3. KI	M	18	"	"	2.2	13.0	19.0	18.0	16.5	16.5	16.8
4. TS	F	36	"	"	u.d	3.6	6.6	/	4.9	4.8	6.6
5. ST	F	30	"	BTA+PI	2.0	17.0	18.0	12.0	7.8	11.0	18.0
6. KM	M	50	"	PI	4.9	11.9	25.0	/	6.2	5.0	20.1
7. YO	F	26	AA	UA	u.d	13.0	16.5	/	3.4	7.0	16.5
8. SK	F	21	"	UA+PI	105	210	240	/	250	220	145
Normal					0	16.0	16.0	14.0	9.4	6.2	8.2
					$\sim$ 10.0	$\sim$ 22.6	$\sim$ 27.0	$\sim$ 25.0	$\sim$ 24.0	$\sim$ 18.5	$\sim$ 23.4

* Case MM was administered 100 µg of TRH.

irradiation showed normal resting levels and responses. One patient (SK) with adrenal adenoma treated with unilateral adrenalectomy who had primary hypothyroidism showed excessive response (Table 5, Fig. 5).

Discussion

The basal levels of plasma ACTH were increased or high normal in our patients with Cushing's disease and undetectable in patients with adrenal tumors. Sufficient pituitary response of ACTH to LVP in Cushing's disease and no response of ACTH to LVP in adrenal tumors were demonstrated. These findings were already reported in the previous literature (Ney *et al.*, 1963; Graber *et al.*, 1965; Demura *et al.*, 1966; Demura *et al.*, 1972; Crougns *et al.*, 1973)

In patients with totally adrenalectomized Cushing's disease, the infusion of 150 mg hydrocortisone did not suppress plasma ACTH by 30 min, while the suppression was already observed by 30 min in 3 patients with Addison's disease whose basal ACTH levels were also high. This finding suggests the abnormality in the feedback mechanism of ACTH secretion continuing after the clinically effective treatment. One Cushing's disease treated with pituitary irradiation showed no suppression until 180 min. This impaired suppression was due to the ineffective treatment of pituitary irradiation.

The lack of normal growth hormone responsiveness to insulin induced hypoglycemia in our patients is in agreement with that reported in the literature (Frantz *et al.*, 1964; Hartog *et al.*, 1964; James *et*

Fig. 5. Plasma TSH (left panels) and prolactin (right panels) in response to the intravenous administration of 500 μg synthetic TRH before and after treatment. Case MM was administered 100 μg of TRH. —•—•—: Cushing's disease, ○—○—○: adrenal adenoma, x—x—x: adrenal carcinoma

Table 5. Plasma prolactin response to TRH in patients with Cushing syndrome

Name	Sex	Age	Diagnosis	Treatment	Prolactin (ng/ml) response to TRH					Maximum increment	
					0	15	30	45	60		90
(min after 500 µg of TRH injection)											
a) before treatment											
1. KI	M	18	CD		32.0	33.0	40.0	42.0	42.0	40.0	10.0
2. TS	F	36	"		37.5	43.0	44.0	40.0	/	27.5	5.5
3. KT	F	26	"		11.5	47.5	52.0	45.0	25.0	20.0	40.5
4. EF	F	36	"		23.5	54.0	50.0	/	42.0	39.5	30.5
5. SK	F	21	AA		15.2	63.5	48.0	53.0	44.0	29.5	48.3
6. YO	F	26	"		22.0	86.0	75.0	/	33.0	48.0	64.0
7. *MM	M	3	AC		9.4	42.0	30.5	/	20.0	11.5	32.6
b) after treatment											
1. KS	M	35	CD	BTA	15.0	56.0	37.0	/	20.0	9.4	41.0
2. TM	M	19	"	"	19.5	50.0	40.0	34.0	22.5	19.0	30.5
3. TS	F	36	"	"	14.0	69.0	57.5	/	42.0	42.0	55.0
4. KM	M	50	"	PI	21.0	23.0	36.0	/	30.0	24.0	15.0
5. SK	F	21	AA	UA+PI	30.0	148.0	132.0	/	132.0	80.0	118.0
Normal					6.4	21.0	28.0	19.0	14.0	11.5	19.2
					~21.0	~78.0	~62.0	~58.0	~50.0	~42.0	~65.0

* Case MM was administered 100 µg of TRH.

al., 1968; Strauch *et al.*, 1969; Morrow *et al.*, 1969; Werder *et al.* 1971; Krieger and Glick, 1972; Demura *et al.*, 1972; Werder *et al.*, 1971) reported that the patients with hyper-adrenocorticism showed virtually no growth hormone elevation in response to the stress induced by hypoglycemia, behaving similar to dexamethasone suppressed subjects. Stiel *et al.* (1970) reported that normal subjects treated for 2 weeks with prednisolone in doses of 15 mg per day showed no significant diminution in their spontaneous plasma GH peaks and postulated that the chronicity, the constancy and the severity of glucocorticoid excess might all be the factors in abolishing the plasma GH peaks in Cushing's syndrome. Morrow *et al.* (1969) and Demura *et al.* (1972) observed the abnormal responses to stimuli tended to become normal with improvement after treatment. These findings would imply the abnormal responses of GH, being due to hypercortisolemia. While, James *et al.* (1968) and Krieger and Glick (1972) reported the lack of normal GH response to insulin-induced hypoglycemia in the subjects with Cushing's disease either active or in remission, indicating that the abnormal

responses observed were not secondary to hypercortisolemia alone, and postulated these abnormalities presumes to act via central nervous system releasing mechanism of GH as well as ACTH. In our patients, impaired responsiveness of plasma GH was also found after treatment, but sufficient response was also observed in a totally adrenalectomized patient with Cushing's disease. According to our results and the results in the literature, the hypercortisolemia may play a primary role in abolishing normal response, even though patients with Cushing's disease may also have altered central mechanism regulating GH secretion.

Patient SK and SF showed excessive LH responses. Their menstruations were irregular since their menarche and they were suspected to have primary ovarian dysfunction. Except for these cases and one child case (MM), the mean resting levels of plasma LH before and after treatment were 13.3 ± 4.6 (SD) mIU/ml and 22.7 ± 8.6 (SD) mIU/ml, and those of plasma FSH were 8.8 ± 5.3 mIU/ml and 14.0 ± 2.1 mIU/ml, respectively. The mean peak levels of plasma LH before and after treatment were 56.3 ± 19.3 (SD) mIU/ml and 140.4 ± 103.3 (SD) mIU/ml, and

those of plasma FSH were 27.7 ± 16.6 (SD) mIU/ml and 26.8 ± 7.0 (SD) mIU/ml, respectively. Lower resting levels of plasma LH and FSH before treatment might be due to the suppressive effect of hypercortisolemia, and lower responses (not significant) of plasma LH to LH-RH in untreated Cushing's syndrome might also indicate the suppressive effect of hypercortisolemia. Demura *et al.* (1972) reported that the resting levels and responses of plasma LH to insulin and vasopressin were slightly suppressed in Cushing's syndrome.

Two totally adrenalectomized patients with Cushing's disease (KS, TM) who had been receiving 25~37.5 mg cortisone acetate daily showed hyperresponsiveness of plasma LH to LH-RH. These patients were young and showed no symptoms of hypogonadism. This suggests the possibilities that elevated corticotropin-releasing hormone (CRF) may be responsible for this observation or LH-releasing hormone may also be elevated as well as CRF after bilateral total adrenalectomy.

Otsuki *et al.* (1973) reported all 3 patients with Cushing's syndrome had sub-optimal TSH responses to 100 μ g of TRH. But Hershman *et al.* (1970) and Haigler *et al.* (1971) reported that one patient with Cushing's disease showed a normal response to 1000 μ g of TRH. In our 8 patients who were administered 500 μ g of TRH, 6 showed impaired TSH responses. After treatment, 5 of 8 patients showed normal responses, indicating that hypercortisolemia was directly responsible for the impaired TSH responses in Cushing's syndrome. Nicoloff *et al.* (1970) reported that glucocorticoid administration was accompanied by an acute suppression in both thyroidal iodine release and serum TSH values. Wilber and Utiger (1969) also reported that dexamethasone decreased plasma TSH levels, and concluded from the findings of animal experiments that dexamethasone might act at a supraphypophyseal level, but not at the

pituitary level. However, Otsuki *et al.* suggested from their observation that long term and high dose therapy of glucocorticoid inhibited not only at a supraphypophyseal level, but also at a pituitary level.

Patient SK showed an excessive TSH response after treatment. She was revealed to have primary hypothyroidism. (T_3 -RSU = 22.2%, T_4 = 4.2 μ g/dl) The cause of this primary hypothyroidism is now under investigation. Her subnormal TSH response before treatment was perhaps due to the suppressive effect by cortisol.

Prolactin release inhibiting factor (PIF) is supposed to play a primary role in regulation of prolactin secretion. Therefore, the suppression of PIF may cause a hypersecretion of prolactin. Three patients with active Cushing's disease had elevated resting levels of plasma prolactin. In these patients, their hypothalamic secretion of PIF might be suppressed by cortisol. Peak levels of plasma prolactin in response to 500 μ g of TRH were all within normal range, while the maximum increment was subnormal in 2 patients (KI, TS) who had high resting levels. In these patients whose hypothalamic secretion of PIF was considered to be suppressed by hydrocortisone, pituitary prolactin reserves might also be suppressed by hydrocortisone. If this assumption is the fact in other patients, hydrocortisone may act both at a supraphypophyseal level and at a pituitary level to normalize the pituitary reserves of prolactin in these patients. The excessive response seen in patient SK was perhaps due to her primary hypothyroidism.

In summary, there was no significant difference in the responses of pituitary hormones except for ACTH between the patients with Cushing's disease and the patients with adrenal tumors. Pituitary ACTH reserve was completely suppressed in the patients with adrenal adenoma. Pituitary GH reserve was impaired in most patients. Pituitary TSH reserve was also impaired in most patients except some cases. Pituitary

LH and FSH reserves were preserved in more than half of the patients examined. The pituitary prolactin reserve were normal in most patients. But the possibility that PIF might be suppressed by cortisol was also proposed. Hypercortisolemia is considered to be responsible for the impairments of these pituitary reserves observed in some patients. It may be a danger to discuss the difference of the suppressibility of these pituitary hormones from the above results, since the strength of stimuli used in this study are not identical to release each hormone and the places where these stimuli act on are not the same. However, if this is judged simply from our observation, the order of suppressibility of these pituitary hormones by cortisol might be ACTH, GH, TSH, LH and FSH, and then prolactin.

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