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CORRELATION BETWEEN PLASMA LEVELS OF ACTH AND CORTISOL IN BASAL STATES AND DURING THE CRH TEST IN NORMAL SUBJECTS AND PATIENTS WITH HYPOTHALAMO-PITUITARY DISORDERS

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Using a new ACTH-immunoradiometric assay (IRMA), we measured plasma ACTH levels in the basal states and during CRH test in normal subjects and the patients with hypothalamo-pituitary disorders. The basal levels of plasma ACTH in 76 normal young (25–45 yr) and 140 elderly (60–85 yr) subjects were 23.1±13.6, and 17.5±11.2 pg/m*l*, respectively. The plasma ACTH levels were less than detection limit (5 pg/m*l*) in 3 patients with isolated ACTH deficiency, and less than 10 pg/m*l* in 6 of 7 patients with hypopituitarism. A significant correlation was observed between the basal levels of plasma ACTH and of cortisol in two age groups, with almost the same regression line, showing no age-related decline in the plasma levels of ACTH and cortisol. In 2 normal subjects and 2 patients with Cushing's disease, synchronized secretions of ACTH and cortisol were observed between 0800h and 1800h. In normal subjects and the patients with pituitary disorders, a significant correlation was observed between the Area Under the Curve's for plasma ACTH and cortisol during the CRH test. The correlation constant was higher in normal subjects, but lower in the patients with acromegaly, non-functioning pituitary tumor, and Cushing's disease in this order, suggesting low sensitivity of the pituitary-adrenal axis in these patients. These results suggest that the ACTH-IRMA kit provide reliable data for clinical investigation, and that the secretions of ACTH and cortisol correlate each other in basal states and during the CRH test in the patients with pituitary disorders as well as in normal subjects.

Key words: CRH—ACTH—Cortisol—Aging—Cushing's disease—Immunoradiometric assay (IRMA)

ACTH and cortisol play important roles in the pathophysiology of the pituitary-adrenal axis in normal and diseased states. The plasma concentration of ACTH has been measured by radioimmunoassay (RIA)[2,4,19], but it has not always given satisfactory results by non-specific interference of the plasma and the assay sensitivity. In addition, there has been contraversy concerning age-related changes in the plasma levels of ACTH and cortisol[1,5,9,18,23,24]. Recently, the immunoradiometric assay (IRMA) for ACTH has been developed by Ratter *et al.* [20], and followed by providing the ACTH-IRMA kit [10,27]. The ACTH-IRMA kit "Mitsubishiyuka" developed recently, showed high sensitivity, specificity and precision as reported previously[11]. Using this kit, we monitored the changes in the plasma ACTH levels in basal states and during the CRH test in normal and diseased states, and evaluated the correlation between the plasma levels of ACTH and cortisol.

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Materials and Methods

Subjects

Seventy-six young subjects aged 20–45 years and 140 elderly subjects aged 60–85 years who were nothing particular in physical, biochemical and hormonal examination, were examined as normal controls. The patients with hypothalamo-pituitary disorders (Table 1) were diagnosed by clinical manifestations and hormonal and morphological examinations. The study was approved by the Human Subjects Protection Commitee, School of Medicine, the University of Tokushima, and the informed consent for the study was obtained from all volunteers and patients.

To measure the basal levels of plasma ACTH and cortisol, the blood was with-

Table 1.
Responses of plasma ACTH and cortisol during CRH test

	Case No.	age (yr)	sex M/F	plasma ACTH basal (pg/ml)	plasma ACTH peak (pg/ml)	plasma ACTH AUC (pg·hr/ml)	plasma cortisol basal (pg/ml)	plasma cortisol peak (pg/ml)	plasma cortisol AUC (pg·hr/ml)
normal	1	24	M	46.3	84.7	36.0	11.3	17.8	9.2
	2	20	M	17.2	59.4	45.4	20.5	36.4	20.7
	3	21	M	35.3	75.0	30.4	15.7	19.4	3.2
	4	20	M	10.3	47.8	58.4	5.2	23.0	24.8
	5	22	M	5.1	23.3	17.5	17.2	21.1	4.2
	6	23	M	26.9	60.0	46.1	10.0	22.3	14.9
Cushing's disease	7	26	F	89.9	142.2	41.1	30.0	51.4	29.9
	8	42	F	70.2	93.5	26.7	21.0	35.5	12.2
	9	43	F	97.7	189.1	39.0	18.8	40.3	21.0
	10	36	F	41.8	139.6	67.6	15.1	32.4	21.9
	11	34	F	146.9	264.9	26.7	37.1	56.5	21.4
	12	36	F	115.0	232.0	68.0	35.6	57.6	15.5
	13	48	F	60.0	129.0	58.9	23.5	43.8	25.7
	14	52	M	29.0	182.0	133.5	23.8	35.6	11.6
acromegaly	15	58	F	7.5	32.3	24.2	11.7	32.9	24.9
	16	33	F	20.0	28.8	4.4	8.0	18.6	13.2
	17	71	F	23.0	47.6	27.3	19.5	32.9	13.4
	18	45	M	9.5	18.2	4.8	17.0	25.0	4.0
	19	35	F	24.5	64.8	48.0	13.2	17.4	3.7
	20	61	F	29.1	53.5	30.6	14.8	19.6	9.1
	21	31	F	31.5	59.0	13.2	11.0	19.1	9.0
non-functioning	22	49	M	23.2	38.5	27.6	8.2	18.8	14.2
pituitary tumor	23	52	M	26.9	81.9	46.3	11.0	22.5	6.6
	24	47	M	20.4	55.8	34.5	10.8	22.5	11.6
	25	47	M	17.1	65.9	41.3	9.5	15.0	7.2
	26	62	F	29.0	29.7	0.2	19.2	21.3	1.9
	27	29	M	16.3	29.9	6.7	15.9	18.9	2.1
	28	59	F	31.4	80.3	46.1	9.3	28.9	20.9
hypopituitarism	29	53	F	8.2	8.8	0.5	1.0	1.8	0.7
	30	42	M	8.7	9.5	0.2	3.1	3.5	0.2
	31	33	F	4.0	14.3	8.8	1.0	1.3	0.3
	32	16	M	7.0	16.2	11.2	1.8	3.3	0.8
	33	21	M	3.4	3.5	0.1	1.1	1.1	0.1
	34	37	F	5.5	6.8	0.3	1.0	1.3	0.2

drawn from the antecubital vein into polystyrene tube containing 1 mg EDTA-2Na and 400 untis Trasylol/m*l* of plasma keeping rest at 0800–0900 h after overnight fast. The tube was centrifuged immediately, and the separated plasma was frozen at −70°C until hormone assay.

Daytime profile of plasma levels of ACTH and cortisol

Two normal men and 2 patients with Cushing's disease were studied. After overnight fast and at rest, a 21-gauge indwelling needle was inserted into the antecubital vein and the blood sample was drawn every 20 min between 0800–1800h.

CRH test

CRH (100 μg, Peptide Institute Inc., Japan) was dissolved in sterile distilled water, and filtered on a Millipore filter before use. The CRH test was performed at bed rest after an overnight fast. At least 30 min prior to start the test, a 21-gauge indwelling needle was inserted into the antecubital vein. CRH was given to the subjects at 0800–0900h, and the blood samples were drawn at 0, 15, 30, 60, 90, 120 min to measure the plasma levels of ACTH and cortisol.

Immunoradiometric assay (IRMA) of human ACTH

The plasma ACTH levels were measured by ACTH IRMA kit "Mitsubishiyuka" as reported previously[11]. This kit used a monoclonal and polyclonal antisera raised against synthetic human ACTH(1–39). A monoclonal antibody, specific for the 18–39 position of aminoacid sequence of human ACTH, was immobilized on polystyrene beads as solid phase, and a polyclonal antiserum specific for the 1–24 sequence was radiolabelled with ^{125}I. The radioactivity on the solid phase is proportional to the amount of ACTH present in the specimen. The minimum detection limit of this assay was approximately 5 pg/m*l*, and the ranges of the intra- and interassay coefficients of variation were 3.1–6.7% and 12.6–14.1%, respectively.

Radioimmunoassay of plasma cortisol

Plasma cortisol concentration was measured using Cortisol RIA kit, Daiichi Radioisotope, Tokyo, Japan.

Statistical analysis

Data are expressed as means±SD. Undetectable plasma hormone concentration was assigned as a value of the detection limit of the assay to calculate means±SD. The significance of difference of values in different groups was analyzed by Student's t-test.

Each individual ACTH and cortisol profile was analyzed to determine the frequency of episodic hormone secretion. An objective peak detection algorithm (Cluster analysis) was used[26]. In the Cluster program, a power function fit of local variance, a one by one point cluster size, and a statistic of either 2.32 or 1 for significant increases/decreases was used for in 20-min sampling, a 1×1 cluster size and *t* statistic of 1 have been found to minimize both type I and type II errors in pulse detection[25,26].

Results

Plasma ACTH levels in normal subjects and patients with hypothalamo-pituitary disorders

The plasma ACTH levels at 0800–0900 AM in normal subjects and patients with endocrine disorders were shown in Fig. 1. The plasma ACTH levels in the young and elderly men were 23.1±13.6 pg/m*l* and 17.5±11.2 pg/m*l*, respectively, and there was no difference between the two age groups. The plasma ACTH levels were elevated in the patients with Cushing's disease, ectopic ACTH syndrome, Addison's disease or anorexia nervosa. In contrast, the plasma ACTH levels were less than detection limit (5 pg/m*l*) in 3 patients with isolated ACTH deficiency, and less than 10 pg/m*l* in 6 of 7 patients with hypopituitarism.

Correlation between plasma levels of ACTH

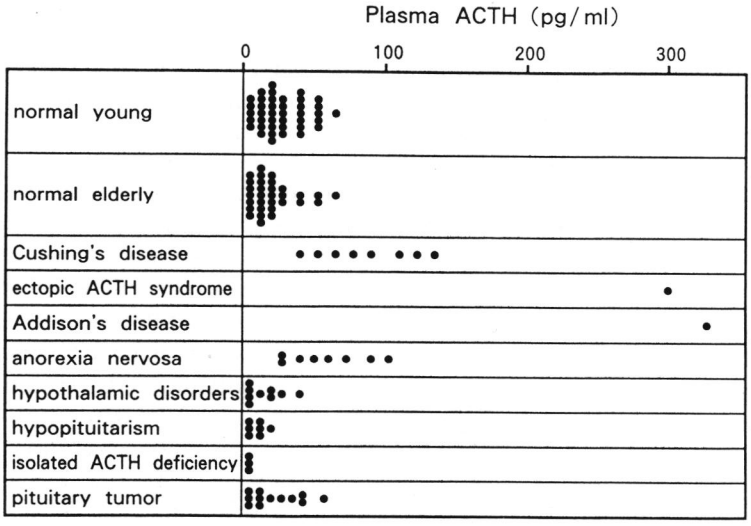

Fig. 1. Plasma ACTH levels in normal subjects and patients with hypothalamo-pituitary disorders. Hypothalamic disorder includes: suprasellar germinoma 2, craniopharyngioma 2, Prader-Willi syndrome 2, Hand-Schüller-Christian disease 1, Kallmann syndrome 2.

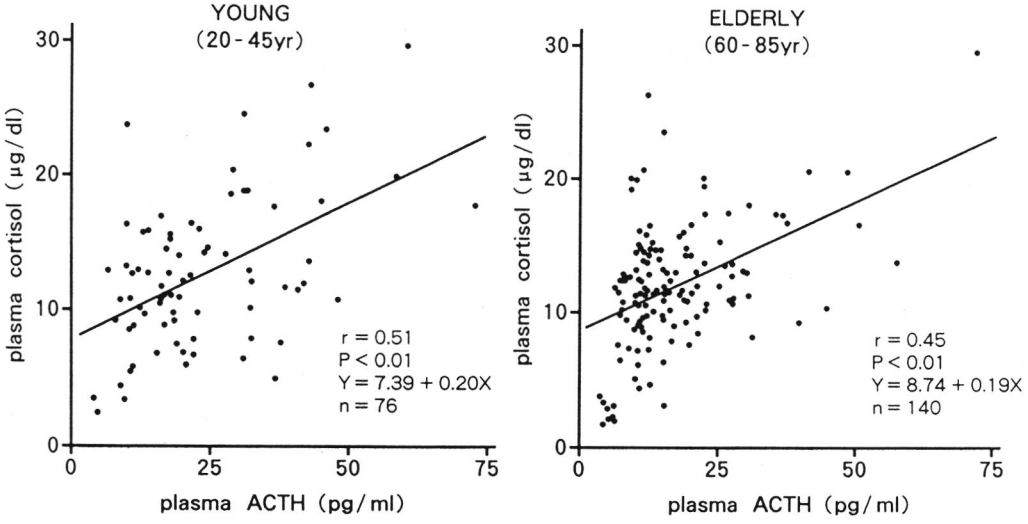

Fig. 2. Correlation between plasma levels of ACTH and cortisol in normal young (40 males and 36 females, 20–45yr) and elderly (76 males and 64 females, 60–85yr) subjects.

and cortisol in normal young and elderly men

The correlation between the levels of ACTH and cortisol at 0800h–0900h in normal young and elderly men were shown in Fig. 2. Positive correlation were observed between them ($r=0.51$, $p<0.01$ and $r=0.45$, $p<0.01$, respectively) in each group, with almost the same regression lines.

Daytime profile of plasma ACTH and cortisol levels and correlation of them between in normal subjects and in patients with

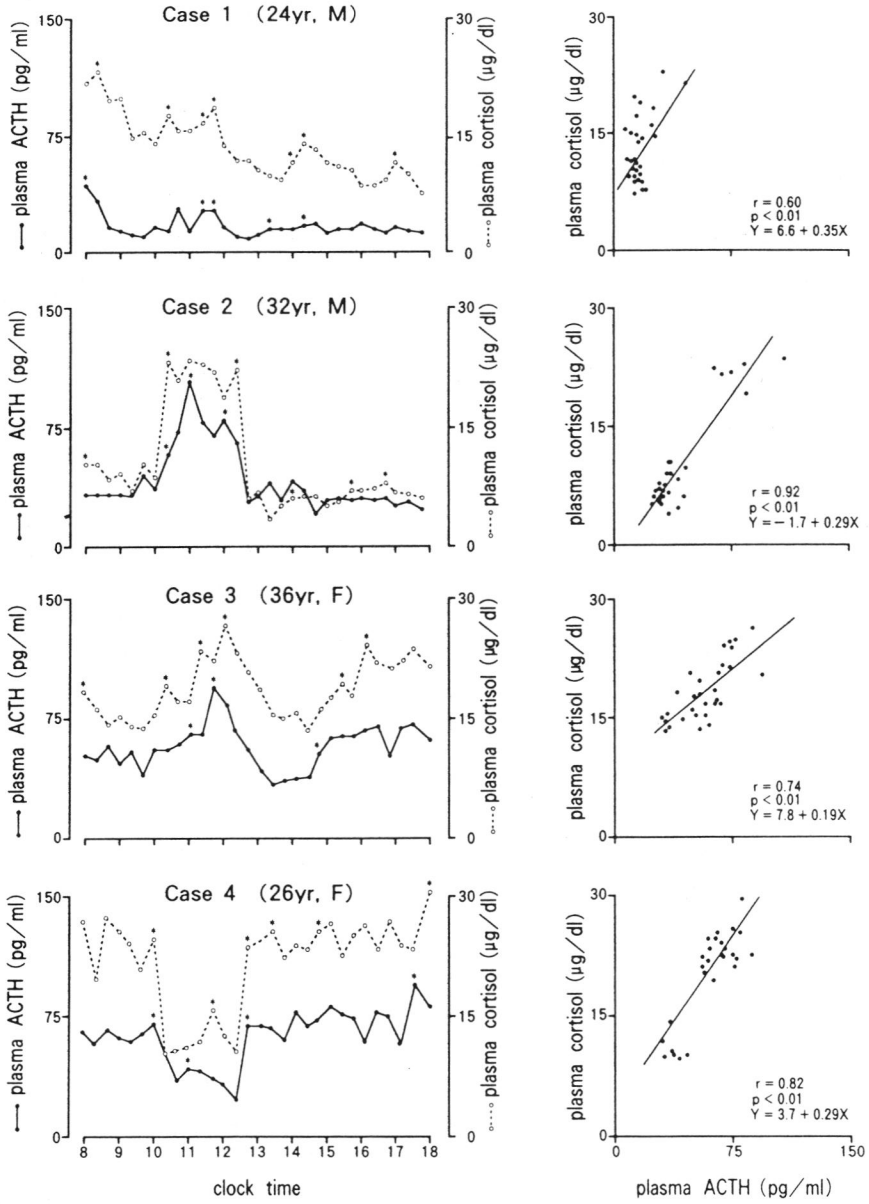

Fig. 3. Daytime profile and correlation of plasma levels of ACTH and cortisol between 0800h and 1800h in normal subjects (case 1 and 2) and patients with Cushing's disease (case 3 and 4). Significant pulses are indicated with asterisks.

Cushing's disease

The responses of plasma ACTH and cortisol during CRH test are shown in Table 1. The changes in plasma ACTH and cortisol concentrations from 800h to 1800h in normal subjects (case 1 and 2) and patients with Cushing's disease (case 3 and 4) were shown in Fig. 3. In addition, the correlations between plasma levels of ACTH and cortisol were shown. The plas-

ma levels of ACTH and cortisol gradually declined from morning to late afternoon in case 1, and rose between 1000h and 1240h in case 2. On the other hand, plasma ACTH and cortisol levels maintained a high level for 10 hours in two patients with Cushing's disease. In these four cases, there was a significant correlation between plasma levels of ACTH and cortisol, and episodic secretions of ACTH and cortisol were almost synchronized.

Correlation between plasma levels of ACTH and cortisol in the patients with pituitary disorders

Correlation between plasma levels of ACTH and cortisol during the CRH test in 6 normal young men and in the patients with pituitary disorders (n=28) was shown in Fig. 4. The plasma levels of ACTH and cortisol were less than normal limit in the patients with hypopituitarism ($p<0.001$), within normal range in the patients with acromegaly or non-function pituitary tumor, and elevated in the patients with Cushing's disease. Plasma ACTH levels were significantly correlated with plasma cortisol levels in these subjects ($r=0.80$, $p<0.01$, $y=0.22X + 6.6$, $n=34$).

Correlation between Area Under the Curves (AUC's) for plasma levels of ACTH and those of cortisol during the CRH test in patients with pituitary disorders

Correlation between AUC's for plasma levels of ACTH and those of cortisol during the CRH test was calculated in normal men (n=6, aged 21–30yr) and in the patients with hypopituitarism (n=6), acromegaly (n=7), Cushing's disease (n=8) and non-functioning pituitary tumor (n=7) (Fig. 5). The AUC's for plasma levels of ACTH and those of cortisol were low in the patients with hypopituitarism ($p<0.01$), in normal

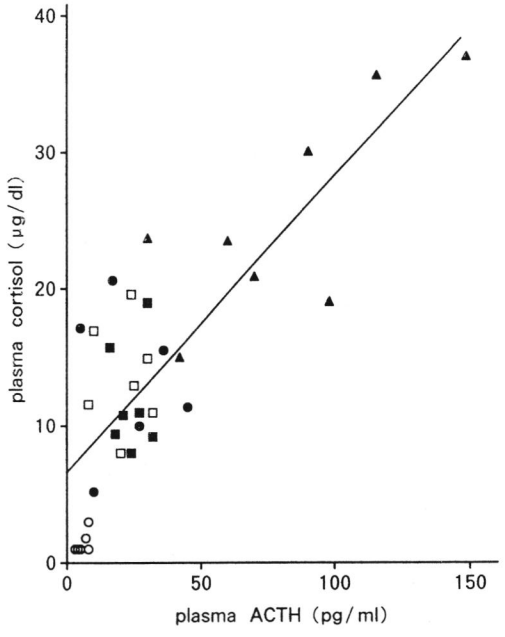

Fig. 4. Correlation between plasma levels of ACTH and cortisol in patients with pituitary disorders.
● normal, ○ hypopituitarism, □ acromegaly, ▲ Cushing's disease ■ non-functioning pituitary tumor

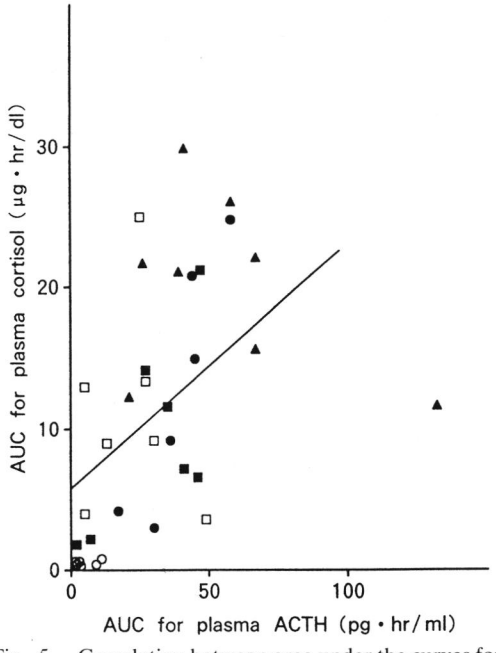

Fig. 5. Correlation between area under the curves for plasma ACTH and cortisol during the CRH test in patients with pituitary disorders.
● normal, ○ hypopituitarism, □ acromegaly, ▲ Cushing's disease ■ non-functioning pituitary tumor

range in the patients with non-functioning pituitary tumor, and high in the patients with Cushing's disease. In the acromegalic patients, the AUC for ACTH was significantly lower than normal ($p<0.05$). Correlation between them was highly positive in normal subjects. The AUC's for plasma ACTH and cortisol in all subjects were significantly correlated ($r=0.52$, $p<0.01$, $Y=0.17X + 5.9$, $n=34$), although rather dispersed distribution was seen in the patients with acromegaly and Cushing's disease.

Discussion

We found that the basal levels of plasma ACTH in normal young and elderly subjects were not significantly different. This finding is consistent with a previous report of no age-related difference in the basal plasma ACTH levels in young (18–30yr) and elderly (70–94yr) subjects[13].

As to age-related change in the basal level of plasma cortisol, contraversial data have been reported; age-related change is present[5,9,18,23] or absent[1,3,24]. Our results showed no significant differences between the plasma levels of cortisol in young and elderly control subjects. Moreover, the regression lines for the correlations between the basal levels of plasma ACTH and cortisol in young and elderly subjects were almost the same ($Y=0.20X + 7.39$, $Y=0.19X + 8.74$). These facts suggest no age-related decline of ACTH-cortisol axis, although the times of the nadir, peak concentration and acrophase of the cortisol level were reported to be significantly earlier in older subjects than in younger ones[22,23].

The basal levels of plasma ACTH were higher than normal range in 6 of 8 patients with Cushing's disease in our series. The patients with ectopic ACTH syndrome and Addison's disease had extremely high plasma ACTH concentrations. In contrast, the basal levels of plasma ACTH were below the detection limit in the patients with isolated ACTH deficiency, and were 10 pg/m*l* in 6 of 7 patients with hypopituitarism. The commercial ACTH kits previously used occasionally gave normal values even in the patients with hypopituitarism. Therefore, the data on plasma ACTH obtained in the study seemed to be reasonable for the diseases.

The daytime profiles of plasma concentrations of ACTH and cortisol were studied in normal subjects and patients with Cushing's disease. The levels of the two changed in parallel and were closely correlated with each other. These findings are not consistent with a previous report of no apparent parallelism between the plasma levels of ACTH and cortisol determined very 30 min for 24 hours[14]. The dissociation between the secretions of cortisol and ACTH secretion was explained by the assay methodology, the timing of collection and processing of the samples[16]. When the secretory patterns of ACTH and cortisol in men were investigated at 5-min intervals, cortisol secretion seemed to begin about 10 min after the initiation of ACTH secretion, but a 20-min sampling program gave a rough secretory pattern[8]. In our protocols, (1) blood sampling was done every 20 min, (2) blood samples were put into the test tube containing EDTA and trasylol and the plasma was immediately frozen until the assay, and (3) ACTH-IRMA kit was used for assay. The measurement of plasma ACTH for 10 hours in daytime provides reliable data for evaluating the profile of ACTH and cortisol secretion.

In 2 normal subjects and 2 patients with Cushing's disease studied, 3–5 episodes of significant episodic ACTH secretion, and 6–7 episodes of significant episodic cortisol secretions were seen within 10 hours. These findings are consistent with the report of Refetoff *et al.*[21] of pulsatile secretions of ACTH and cortisol 10–12 times in 24 hours

in normal subjects and patients with Cushing's disease. Liu *et al.*[17] reported that 62% and 74% of cortisol pulse were preceded by ACTH secretion in normal females and patients with Cushing's disease, respectively. On the other hand, increase in cortisol secretion without any significant change in ACTH secretion was observed after methamphetamine administration, in the early morning and post-prandially[6,7]. They postulated the existence of factors other than ACTH that play a physiological role in cortisol secretion, such as 1) direct sympathetic innervation to the adrenal cortex, 2) indirect sympathetic activation of the adrenal cortex by a paracrine intermediate step involving the adrenal medulla, and 3) humoral factors that modulate adrenal responsiveness to ACTH or directly stimulate the adrenal. Our results showed a significant correlation between the plasma levels of ACTH and cortisol, suggesting the direct activation of cortisol secretion by ACTH.

In a normal male (case 2), the plasma ACTH and cortisol levels showed acute elevation at 10:00–12:40 when the subject was resting but thinking about a serious matter. This suggests that psychological stress can induce acute secretions of ACTH and cortisol. Except in this period, the amplitudes of secretions of ACTH and cortisol were larger in the patients with Cushing's disease than in normal subjects. However, the amplitude of the cortisol pulse did not correlate with that of ACTH, suggesting altered sensitivity of the adrenal cortex to ACTH[8,12,15]. Moreover, an increased ACTH pulse rather than increase in its frequency would be responsible for the elevated cortisol levels in patients with Cushing's disease[17].

A significant correlation was observed between the basal levels of plasma ACTH and cortisol in all the patients studied except Cushing's disease and hypopituitarism. Therefore, in the former, the basal level of plasma ACTH measured with the IRMA kit may reflect, at least in part, the plasma cortisol concentration and the function of the ACTH-cortisol axis.

A correlation between the AUC's of plasma ACTH and cortisol during the CRH test was found in the patients with pituitary disorders. In 6 normal subjects, the correlation coefficient was high, indicating the secretions of ACTH and cortisol were closely parallel. In contrast, the levels of both hormones in the patients with Cushing's disease showed dispersed distributions, indicating variations in the responses of ACTH and cortisol in individual cases. This was probably due to different sensitivities of ACTH-producing pituitary adenomas and the adrenal cortex to CRH. In acromegaly, the AUC for plasma ACTH was significantly lower than normal, although the AUC for plasma cortisol was within normal range. This is probably because excess GH released from the pituitary may affect the ACTH-cortisol axis.

In summary, the plasma ACTH levels measured with the ACTH IRMA kit in normal subjects and patients with hypothalamo-pituitary disorders gave reasonable data for pathophysiology of ACTH secretion. The function of the pituitary-adrenal axis did not show age-related decline, and the daytime profiles of plasma ACTH and cortisol concentrations showed a significant correlation in normal subjects and patients with Cushing's disease. The AUC's for plasma ACTH and cortisol during the CRH test showed various responses corresponding to the diseased states.

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