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Mechanism of impaired growth hormone secretion in patients with Cushing's disease

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The function of the growth hormone-releasing hormone (GHRH)-growth hormone (GH) axis in Cushing's disease was studied by monitoring (a) the GH responses to GHRH loading and L-dopa loading, (b) the GHRH response to L-dopa loading, and (c) the daytime profiles of plasma GH concentration. GH release following GHRH and L-dopa was blunted in patients as compared to that in age-matched control subjects. However, GHRH release following L-dopa was similar in patients and controls. The plasma GH levels in four patients measured every 20 min by a highly sensitive immunoradiometric assay for GH showed pulsatile GH secretion at low levels during the observation period. These results indicate that GHRH release from the hypothalamus is preserved in patients with Cushing's disease, and support the hypothesis that glucocorticoid inhibits GH secretion by altering the hypothalamic somatostatin tone.

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Patients with Cushing's disease show blunted growth hormone (GH) responses in various provocative tests including insulin-induced hypoglycemia (1, 2), arginine loading (1), and L-dopa loading (3). Smals et al. (4) reported that they also show a reduced GH response to growth hormone-release hormone (GHRH). The reason for impaired GH secretion in patients with Cushing's disease is, however, still unknown, and there are no reports of studies on the functional change of their GHRH–GH axis.

We found previously that the GHRH level in the plasma increased before or simultaneously with increase in GH secretion during slow wave sleep or oral L-dopa loading (5), indicating that oral L-dopa should be useful as stimuli in GHRH provocative tests (6). Therefore, in this work, to elucidate the mechanism of impairment of GH secretion in patients with Cushing's disease, we studied the function of their GHRH–GH axis by analysing (a) the GH responses of the patients to GHRH loading and L-dopa loading, (b) the GHRH response to L-dopa loading, and (c) the daytime profile of their plasma GH concentration. Plasma GH was measured with a highly sensitive immunoradiometric assay (IRMA).

Materials and methods

Subjects

As shown in Table 1, one male and six female patients with Cushing's disease (aged 29–51 years) were studied. Cushing's disease was diagnosed on the basis of the symptoms, laboratory findings and pituitary imaging. Five patients had received no therapy, and two patients had undergone transsphenoidal adenomectomy but with insufficient effect. All the patients had hypercortisolemia. Tests on GHRH loading and L-dopa loading were performed at rest after overnight fasting.

As controls, 10 age-matched subjects (33–51 years) and 12 normal elderly subjects (70–85 years) were also studied.

The study was approved by the Human Subjects Protection Committee, School of Medicine, University of Tokushima, and informed consent was obtained from the patients.

GHRH test

A dose of 100 μg of synthetic GHRH (1–44)NH_2 (GRF Sumitomo 100; Sumitomo Pharmaceutical Co., Osaka, Japan) was administered intravenously and before and 15, 30, 60, 90 and 120 min after its administration blood samples were collected in pre-cooled polypropylene tubes containing 1.2 mg EDTA and 500 KIU aprotinin per ml of blood. The plasma was separated and stored at −40°C until assay of plasma GH concentration.

L-dopa loading test

A dose of 500 mg of L-dopa (Daiichi Pharmaceutical Co., Tokyo, Japan) was given orally and blood samples were collected at 0, 30, 60, 90 and 120 min for determinations of plasma GHRH and GH concentrations.

Table 1. Clinical and hormonal data on the patients with Cushing's disease.

Case no.	Age	Sex	BMI (kg/m^2)	Plasma ACTH (ng/l)	Plasma TSH (mU/l)	Serum T_3 (μg/l)	Serum T_4 (μg/l)	Plasma cortisol (nmol/l)	Urinary 17-OHCS (mg/day)			Pituitary tumor
									Basal	Dex. 2 mg	Dex. 8 mg	
1	51	M	23.6	97·3	0.8	0.9	67	802.8	14.5	4.0	1.4	Basophilic microadenoma
2	31	F	22.5	97.0	1.0	0.6	79	819.3	12.2	5.4	4.3	5 × 5 mm by MRI
3	44	F	27.3	131.4	1.0	0.8	90	857.9	13.1	11.8	6.5	Not detectable by MRI
4	41	F	23.4	82·4	4.3	0.8	77	769.7	12.6	7.0	2.4	Microadenoma by surgery
5	51	F	23.4	70.3	1.0	1.0	80	789.0	15.7	6.4	2.6	Not detectable by CT
6	29	F	28.1	97.5	4.2	1.0	100	1103.4	17.8	7.8	—	Basophilic microadenoma
7	36	F	27.3	146.0	1.1	0.9	90	924.1	21.1	19.8	8.8	Not detectable by CT

BMI: body mass index; Dex: dexamethasone suppression test; MRI: magnetic resonance imaging; CT: computed tomography; —: not examined.

Daytime profile of plasma GH concentrations

After overnight fasting, four patients were fitted with an indwelling cannula in the antecubital vein. After resting in bed for 30 min, blood samples were collected every 20 min from 08.00 to 17.40 for determination of plasma GH concentration.

Determination of plasma GH

Plasma GH concentration was measured using a radioimmunoassay (RIA) kit, HGH-II (Dianabot Co., Tokyo, Japan). The sensitivity of the assay was 0.3 μg/l and the intra- and interassay coefficients of variation were 5.7–6.3% and 3.4–5.6%, respectively (6). The daytime profile of plasma GH concentration was determined with a highly sensitive GH IRMA kit (Daiichi Radioisotope Institute D-9111). The lower detection limit was 0.01 μg/l, and the intra- and interassay coefficients of variation were 5.1% and 6.8%, respectively (7, 8).

Determination of plasma GHRH

Plasma GHRH concentration was determined using the double antibody method reported previously (9). For plasma GHRH extraction, 1 ml of plasma was acidified with 80 μl of solution containing 80% 1N HCl, 5% formic acid, 1% trifluoro-acetic acid (TFA), and 1% NaCl. Then it was applied to a Sep-Pak C18 cartridge, and washed in 0.1% TFA; the material was eluted in 90% methanol/0.1% TFA. With the assay, GHRH could be measured in the range 1.5–200.0 pg/tube, and when 0.3 ml of plasma per tube was used the minimum detectable concentration of plasma GHRH was 5 ng/l. The intra- and interassay coefficients of variation were 5.0–8.4% and 5.3–8.5%, respectively. The recovery rate was 78–83% at concentrations of 12.5–50.0 ng/l. The dilution curve of normal plasma extracts was parallel to the standard curve in RIA. The elution profile of the plasma extracts of a normal subject on a Sephadex G-50 fine column showed one peak of GHRH immunoactivity at the same position as synthetic GHRH(1–44)NH_2(9).

Statistical analysis

The significances of differences between data were examined using Student's unpaired *t*-test. Values in the text and figures are shown as means ± SEM. The daytime profile of plasma GH concentration was analysed by cluster analysis (10).

Results

Plasma GH response to GHRH loading

The plasma GH levels in the seven patients with Cushing's disease increased from a basal value of 1.1 ± 0.1 μg/l to 6.3 ± 3.2 (2.8–12.2) μg/l after the administration of GHRH (1–44)NH_2. This response was significantly ($p<0.01$) lower than that of 10 age-matched controls aged 33–51 years (basal value 1.8 ± 0.3 μg/l; peak value 29.1 ± 4.7 μg/l) (Fig. 1). There was no significant difference between the plasma GH responses of the patients and 12 elderly normal subjects aged 70–85 years (basal value 0.9 ± 0.2 μg/l; peak value 10.7 ± 2.3 μg/l).

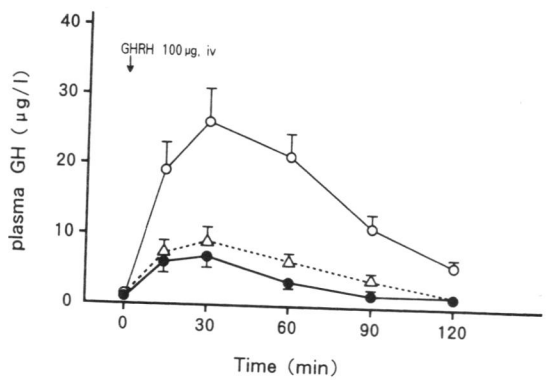

Fig. 1. Plasma GH response after intravenous administration of 100 μg GHRH in seven patients with Cushing's disease (●), 10 age-matched controls (○), and 12 normal elderly subjects (△). Points and bars are means and SEM.

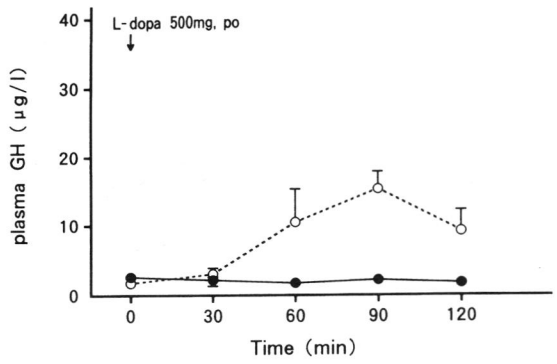

Fig. 2. Plasma GH response to L-dopa in patients with Cushing's disease (●) and age-matched controls (○). A dose of 500 mg L-dopa was given orally. Points and bars are means and SEM.

Plasma GH response to L-dopa loading

After oral administration of L-dopa, the plasma GH concentrations in the age-matched controls increased from a basal value of 1.3 ± 0.1 μg/l to a peak value of 18.6 ± 7.0 μg/l in 30–90 min, whereas those of patients did not increase significantly (basal value 1.6 ± 0.7 μg/l; peak value 2.2 ± 0.6 μg/l) (Fig. 2).

Plasma GHRH response to L-dopa loading

As shown in Fig. 3, the plasma GHRH concentration in patients with Cushing's disease increased significantly from a basal value of 18.9 ± 1.5 ng/l to 38.9 ± 9.4 ng/l ($p < 0.05$) 60 min after L-dopa loading.

The basal and peak values of plasma GHRH in the L-dopa test in the patients (peak value 40.9 ± 9.1 ng/l) was similar to that in the age-matched controls (basal value 18.2 ± 4.2 ng/l; peak value 36.0 ± 7.8 ng/l) (Fig. 4).

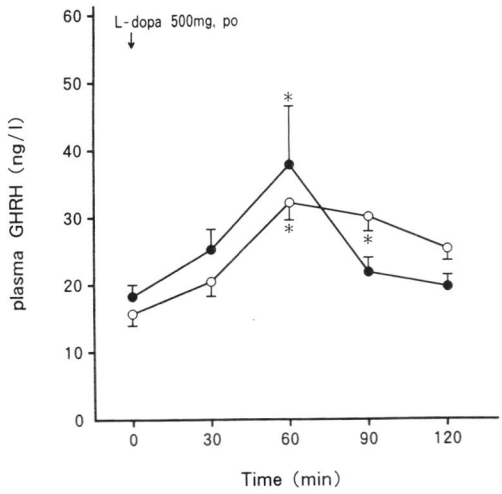

Fig 3. Plasma GHRH response to L-dopa in patients with Cushing's disease (●) and age-matched controls (○). Points and bars are means and SEM. *$p < 0.05$ vs basal level.

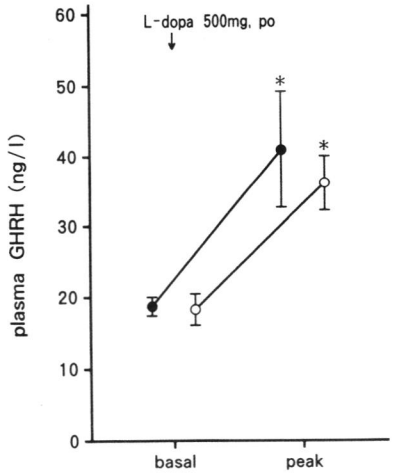

Fig. 4. Basal and peak plasma GHRH levels in the L-dopa test. Values are for patients with Cushing's disease (●) and age-matched controls (○). *$p < 0.05$ vs basal level.

Daytime profile of plasma GH concentration

Fig. 5 shows the daytime profiles of the plasma GH concentration in four patients with Cushing's disease from 08.00 to 17.40. Plasma GH was scarcely detectable with a conventional GH kit, but could be measured during the observation period in all patients with a GH-IRMA kit. Pulsatile secretion of GH was detected by cluster analysis several times a day in these patients.

Discussion

In this study, we demonstrated that GHRH release in response to L-dopa loading in patients with Cushing's disease is similar to that in age-matched subjects, and that the patients show low levels of pulsatile GH secretion during daytime.

Studies on the plasma GHRH level measured by RIA have shown that after L-dopa loading an increase in plasma GHRH occurs after a rise in the plasma GH level (5, 11–14) in normal subjects, but did not in patients with hypothalamic disorders (12). In rats, immunohistochemical study has demonstrated that the L-dopa neurons are mainly present in the arcuate nucleus, together with abundant GHRH neurons (15). These data strongly suggest that L-dopa loading stimulates GH secretion from the hypothalamus, and is thus useful as a GHRH provocative test. Interestingly, we found that after L-dopa loading the increase in plasma GHRH in the patients with Cushing's disease with hypercortisolemia was similar to that in age-matched controls, but that the response of their plasma GH was blunted. This indicates

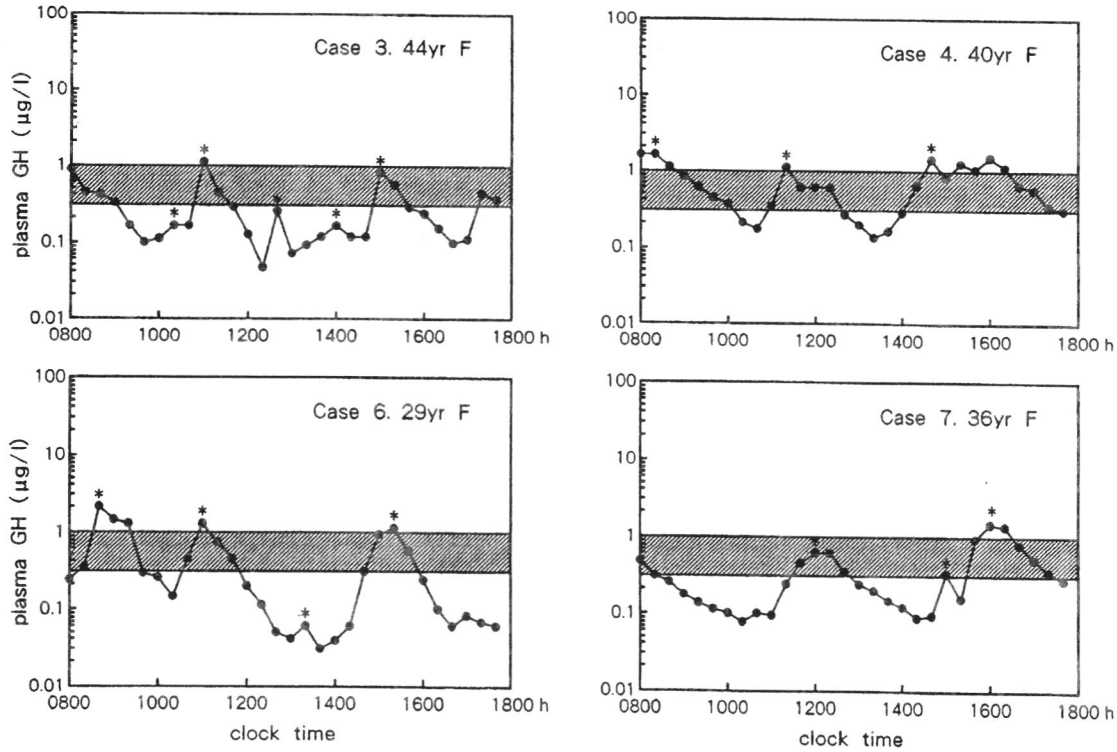

Fig. 5. Daytime profile of plasma GH concentration in four patients with Cushing's disease. Note the logarithmic ordinate. The shaded areas represent the boundary between the least detectable and undetectable range in the conventional GH assay. The asterisks indicate GH pulses demonstrated by cluster analysis.

that the capacity to release GHRH from the hypothalamus in response to L-dopa loading is well preserved in the patients.

Two-thirds of the GH released during a day is secreted during sleep (16). But the profile of plasma GH during the daytime has not been precisely defined because the plasma GH levels are too low to measure by conventional GH RIAs. Drobny et al. (17) collected 20 ml blood samples every 2 h, and determined their plasma GH concentrations by RIA. They found that basal plasma GH level was highly variable below 1 μg/l. Pulsatile secretion of GH was observed in a study in which GH was measured every 15 min for 3 h (18). In addition, using a GH-IRMA, Winer et al. (19) found that the plasma GH concentration shows pulsatile change at levels of between 0.04 and 20.00 μg/l. Wehrenberg et al. (20) reported that administration of anti-somatostatin antibody to unanesthetized rats resulted in a persistent increase of GH secretion, but that administration of monoclonal antibody against GHRH completely inhibited pulsatile GH secretion. As we reported previously, passive immunization of rats with polyclonal antibody against GHRH inhibited pulsatile secretion of GH in a dose-dependent manner (21). These results suggest that pulsatile GH secretion is regulated by GHRH released from the hypothalamus. We demonstrated by cluster analysis that pulsatile secretion occurs at a low level in patients with Cushing's disease. This suggests that GHRH stimulates pulsatile GH release even in patients with Cushing's disease. With regard to somatostatin-GH interaction, Wehrenberg et al. (22) reported that the GH response to GHRH was blunted in rats treated with dexamethasone, but enhanced by passive immunization with anti-somatostatin antibody, indicating a potentiating effect of dexamethasone on hypothalamic somatostatin tone. In humans, Leal-Cerro (23) reported that administration of pyridostigmine, which inhibits somatostatin secretion from the hypothalamus, enhanced the GH response in normal subjects, but had no effect on the plasma GH level in patients with Cushing's disease, suggesting altered somatostatin tone in the hypothalamus of the patients. In in vitro studies, glucocorticoid was found to enhance GH release from cultured rat and human pituitary cells in the presence of GHRH (24, 25), and to increase the transcription rate of the GH gene and the level of GH mRNA in pituitary tumor cells (26). Thus, glucocorticoids probably enhance GH release at the pituitary level.

As glucocorticoids do not inhibit GH secretion at the pituitary level, and as GHRH-GH interaction is well preserved in patients with Cushing's disease, enhanced basal secretion of somatostatin from the hypothalamus may be at least one cause of the impaired GH secretion in these patients.

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