Endocrinol. Japon. 1989, 36 (5), 705–720

Evaluation of Hypothalamic-Pituitary Function in a Combination of Tests with Four Hypothalamic Releasing Hormones and L-Dopa in Normal Subjects and in Patients with Hypothalamic and/or Pituitary Disorders

HIROSHI BANDO, RYUICHI YAMASAKI and SHIRO SAITO

First Department of Internal Medicine, School of Medicine, The University of Tokushima, 3-18 Kuramoto-cho, Tokushima 770, Japan

Abstract

Hypothalamic-pituitary function was evaluated in a combination of tests with four hypothalamic releasing hormones (4RHs) and L-dopa in normal subjects and in patients with hypothalamic and/or pituitary disorders. Plasma concentrations of anterior pituitary hormones (GH, ACTH, TSH, PRL, LH and FSH) were measured before and after simultaneous iv administration of GHRH, CRH, TRH and LHRH. In addition, changes in the plasma levels of GHRH and GH were investigated before and after oral administration of L-dopa. Normal subjects showed appreciable responses to both tests. In five patients with hypothalamic disorders, the response of plasma anterior pituitary hormones varied, but plasma GHRH and GH did not respond to L-dopa. Patients with idiopathic and postpartum hypopituitarism showed low response to 4RHs or none at all, but L-dopa evoked a normal GHRH response in 2 of the 4 cases having no GH response. In the patients with hypopituitarism due to resection of a pituitary tumor, the response of anterior pituitary hormones to 4RHs was low, and L-dopa administration induced a normal GHRH and low GH response in 5 out of the 7 cases. After 4RHs administration, the patients with ACTH deficiency syndrome showed different patterns of impaired ACTH secretion, and isolated, combined or limited ACTH reserve. Seven patients with anorexia nervosa showed exaggerated GH, delayed TSH and FSH, low ACTH and LH, that is, normal PRL response to 4RHs, but no response of plasma GHRH or GH to L-dopa, suggesting the presence of hypothalamic dysfunction. These results indicate that the combination of the 4RHs test and L-dopa test is a simple and useful means for evaluating hypothalamic-pituitary function by measuring the response of plasma GHRH and six anterior pituitary hormones in the patients with endocrine disorders.

Following the discovery of TRH (1969), LHRH (1970) and CRH (1981), GHRH was isolated in 1982 by Guillemin *et al.* and Rivier *et al.* Since then, there has been considerable advance in studies on the hypothalamic control of anterior pitui-

Received May 12, 1989

tary hormone secretion. One of the clinical applications of these hypothalamic releasing hormones (RHs) was simultaneous intravenous administration of 4RHs (GHRH, CRH, TRH, LHRH) to evaluate pituitary function (Sheldon *et al.*, 1985; Cohen *et al.*, 1986). On the other hand, we have investigated the mechanism of GHRH release and its physiopathological role in normal and diseased states using a sensitive and specific radioimmunoassay (RIA) for GHRH (Saito *et al.*, 1984; Sano *et al.*, 1986; Yamasaki *et al.*, 1988a). First we found that oral administration of L-dopa induces GH secretion by stimulating hypothalamic GHRH release (Saito *et al.*, 1984). This was confirmed by Donnadieu *et al.* (1985) and Chihara *et al.* (1986). In this study, we describe the usefulness of the combination of tests with 4RHs and L-dopa to evaluate hypothalamic-pituitary function in patients with hypothalamic and/or pituitary disorders.

Materials and Methods

Subjects and Protocols

Six normal male adults aged 20–25 years without obesity or endocrine disorders were examined as controls. The patients studied consisted of 5 hypothalamic disorders, 11 hypopituitalism of various causes, 3 ACTH deficiency syndrome and 7 anorexia nervosa (Table 1). The diagnosis was confirmed by clinical manifestations and data on 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.

The tests were performed during bed rest after an overnight fast. At least 30 min prior to the start of each test, a 21-gauge indwelling needle was inserted into the antecubital vein and blood samples were taken through the cannula serially before and after the administration of 4RHs or L-dopa.

Combined administration of four hypothalamic releasing hormones

Synthetic preparation of GHRH (1–44) NH_2 (100 μg, Sumitomo Pharmaceutical Co., Osaka, Japan), CRH (100 μg, Peptide Institute Inc., Japan), TRH (500 μg, Takeda Pharmaceutical Co., Ltd, Tokyo, Japan) and LHRH (100 μg, Tanabe Pharmaceutical Co., Ltd, Tokyo, Japan) were simultaneously administered intravenously. CRH was dissolved in sterile distilled water, and filtered on a Millipore filter before use. Blood samples were obtained serially at 0, 15, 30, 45, 60, 90 and 120 min after the administration, and plasma concentrations of anterior pituitary hormones (GH, ACTH, TSH, PRL, LH, FSH) were measured.

Administration of L-dopa

A dose of 500 mg L-dopa was administered orally to the subjects, and blood samples were obtained serially at 0, 30, 45, 60, 75, 90 and 120 min to measure the plasma concentrations of GHRH and GH.

Radioimmunoassay for plasma GH, ACTH, TSH, PRL, LH and FSH

The following RIA kits were used to measure the plasma hormone concentration: GH, HGH-II Dainabot, Tokyo, Japan; ACTH, ACTH RIA-II, Compagnie Oris Industry S. A., France; TSH, MH-TSH, Corning, Ciba-Corning-Diagnostics, U. S. A.; PRL, Prolactin RIA Kit, Sorin Biomedica, France; LH and FSH LH and FSH RIA Kit, Daiichi Radioisotope, Tokyo, Iapan.

Radioimmunoassay for plasma GHRH

The extraction of GHRH from the plasma was done as described previously (Saito *et al.*, 1984; Yamasaki *et al.*, 1988b). In brief, samples of 3.5 ml of plasma were mixed with 7 ml of cold acetic acid-acetone solution (3 : 100, vol/vol) and the supernatant was mixed twice with 20 ml of petroleum ether. After the ether layer was carefully removed and the remaining acetone was eliminated by evaporation, the aqueous portion was lyophilized. The residue was dissolved in assay buffer and subjected to RIA for GHRH.

Synthetic GHRH (1–44) NH_2 (Takeda Chemical Industries, Ltd, Osaka, Japan) was labelled with ^{125}I by the chloramine T method and the iodinated product was purified on a 1×10 cm caboxymethyl cellulose column (CM 23, What-

Table 1. List of the patients

	case No.	Diagnosis	Age (yr)	Sex M/F	Height (cm)	Body Weight (kg)	plasma cortisol (μg/dl)	serum T_4 (μg/dl)
Hypothalamic disorder	1	Suprasellar germinoma	15	M	165	53	5.0	4.6
	2	Hand-Schüller-Christian disease	21	F	151	49	4.7	7.7
	3	Craniopharyngioma	26	M	166	56	1.4	4.8
	4	Craniopharyngioma	52	F	147	52	3.9	4.3
	5	Prader-Willi syndrome	21	F	139	64	8.2	7.3
Hypopituitarism	6	Idiopathic hypopituitarism	58	F	153	56	24.1*	7.8*
	7	Sheehan's syndrome	71	F	156	55	1.2	4.4
	8	Sheehan's syndrome	61	F	155	42	6.7*	8.4*
	9	Sheehan's syndrome	52	F	155	48	2.7	2.6
	10	Pituitary apoplexy due to unknown etiology	71	M	148	52	2.6	5.3
	11	Pituitary apoplexy due to unknown etiology	72	F	150	55	8.2*	9.4*
	12	GH-secreting adenoma	35	M	173	76	1.2	6.9
	13	GH-secreting adenoma	34	F	161	71	10.3*	6.9
	14	Non-functioning adenoma	46	M	161	67	9.9*	4.9
	15	Non-functioning adenoma	35	M	169	79	4.4	4.8
	16	Non-functioning adenoma	52	M	165	77	5.0	2.9
ACTH deficiency syndrome	17	Isolated ACTH deficiency	49	F	149	49	<1.0	5.7
	18	ACTH deficiency associated with GH deficiency	54	M	170	50	<1.0	6.0
	19	Limited ACTH reserve	48	M	168	56	2.9	6.2
Anorexia nervosa	20	Anorexia nervosa	16	F	165	30	13.5	7.2
	21	Anorexia nervosa	16	F	146	26	15.2	7.6
	22	Anorexia nervosa	17	F	159	39	21.4	7.0
	23	Anorexia nervosa	15	F	156	41	20.9	8.4
	24	Anorexia nervosa	28	F	152	25	12.9	9.3
	25	Anorexia nervosa	20	F	157	34	19.6	9.1
	26	Anorexia nervosa	19	F	157	33	24.5	3.5

* during cortisol and/or thyroxine supplement

man, Maidstone, England). The anti-GHRH serum (RAS-8061, Peninsula Lab, San Carlos, CA ; lot No. 004118) used in this study failed to crossreact with various neuropeptides and recognized the N-terminal and part of the middle portion of the amino acid sequence of hGHRH (1–44) NH_2. When synthetic GHRH (1–44) NH_2 was used as the standard, the sensitivity of this assay was 4 pg/tube. Since the extract from 1 ml of plasma was dissolved in 100 μl of assay buffer and used for the assay as well as 100 μl of standard, the least detectable value was 4 pg/ml. Antibody bound and free tracers were separated by double antibody method. The recovery of 30 pg of synthetic GHRH (1–44) NH_2 added to 1 ml of plasma was 59.5±2.1%. The intra- and inter-assay coefficient of variation were less than 10%.

Statistical analysis

Data are expressed as means±SE. Undetectable plasma hormone concentrations were assigned as a value of the detection limit of the assay to calculate mean values and increases. The shaded areas indicating the normal range in figures are expressed as the maximum and minimum responses to the tests in normal subjects. The significance of differences in values in different groups was analyzed by Student's *t*-test.

Results

Normal subjects

The responses of anterior pituitary hormones to simultaneous intravenous administration of 4RHs in six normal subjects are shown in Fig. 1. Plasma levels of GH, ACTH, TSH, PRL, LH and FSH increased and peaked at: GH, 25.4± 5.5 ng/ml at 45 min; ACTH, 62.0±10.7 pg/ml at 30 min; TSH, 15.4±1.8 ng/ml at 30 min; PRL, 60.8±11.4 ng/ml at 15 min; LH, 67.8±13.7 mIU/ml at 30 min; and FSH, 20.6±3.7 mIU/ml at 30 min. Changes in plasma GHRH and GH after oral administration of L-dopa in six normal subjects are shown in Fig. 2. Basal and peak plasma GHRH were 11.2±2.4 pg/ml, and 31.4±5.2 pg/ml, respectively. The plasma GHRH concentration peaked usually between 30–90 min after L-dopa administration, which was 2–4 times the basal level. Basal and peak plasma GH were less than 1 ng/ml and 23.0±6.1 ng/ml, respectively. The peak plasma GH concentration was synchronized or 15–30 min behind the peak plasma GHRH concentration.

Patients with hypothalamic disorders

The results of the 4RHs test in 5 patients with hypothalamic disorders (Cases 1–5) including 1 suprasellar germinoma, 1 Hand-Schüller-Christian disease, 2 craniopharyngioma and 1 Prader-Willi syndrome are shown in Fig. 3. The plasma GH response was normal or low, and the plasma ACTH response was almost normal. Plasma TSH showed a various responses: exaggerated (cases 1 and 5), delayed (cases 3 and 4) and normal (Case 2). Basal plasma PRL was increased in 3 out of 5 patients, who also showed a exaggerated response to 4RHs. Plasma LH and FSH did not respond in cases 1, 2 and 3, and were subnormal in the other 2 cases.

In contrast, the L-dopa test revealed no plasma GHRH or GH response in any of these patients (Fig. 4). In addition, insulin hypoglycemia and arginine load failed to evoke GH release in cases 1, 2 and 5 who received these tests (data not shown).

Patients with hypopituitarism

The results of the 4RHs test in 4 patients with idiopathic or postpartum hypopituitarism (case 6–9) are shown in Fig. 5. Plasma GH showed no response in any, and plasma ACTH showed a low response or none at all. Plasma TSH and PRL showed a low response or none at all. Plasma LH and FSH showed almost no response. On the other hand, L-dopa administration induced normal plasma GHRH response in 2 cases but no response in 2 cases. Plasma GH did not increase in any

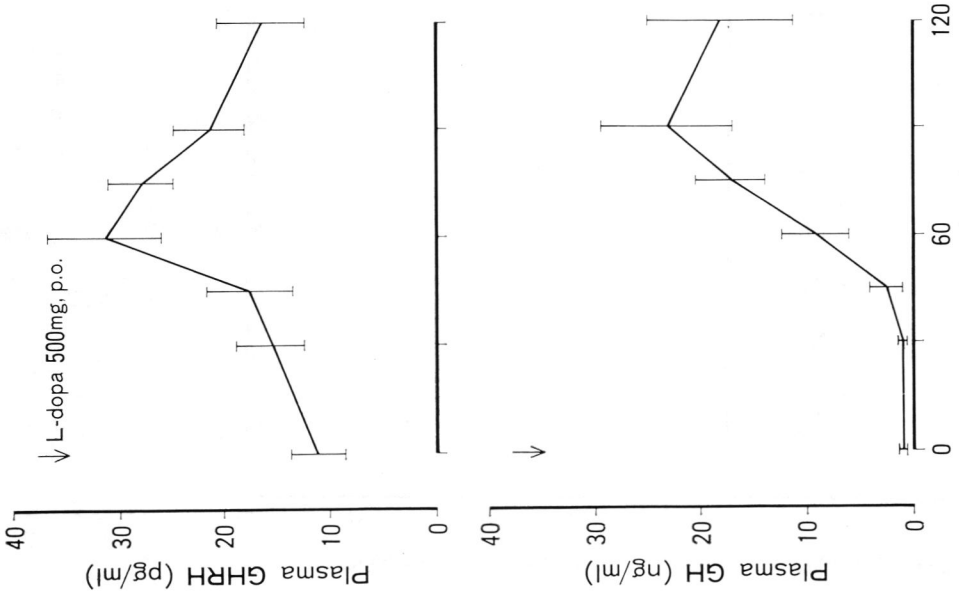

Fig. 2. Effect of L-dopa on plasma GHRH and GH in six normal subjects. Points and bars represent the mean±SE.

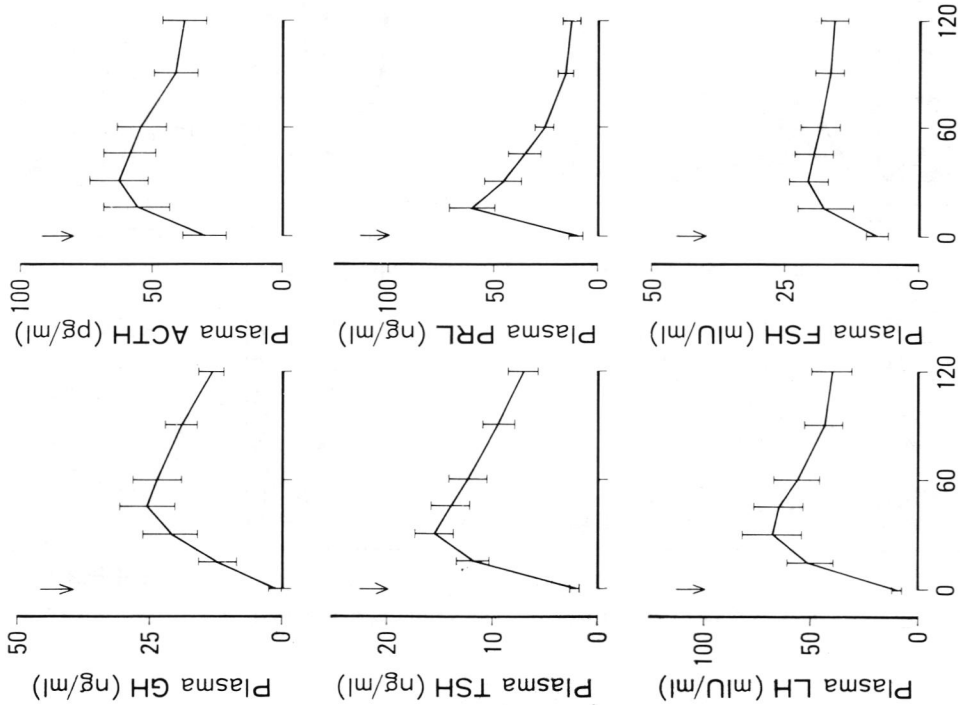

Fig. 1. Effect of simultaneous administration of four hypothalamic releasing hormones (TRH, LHRH, CRH and GHRH) on the plasma concentration of anterior pituitary hormones in six normal subjects. Points and bars represent the mean±SE.

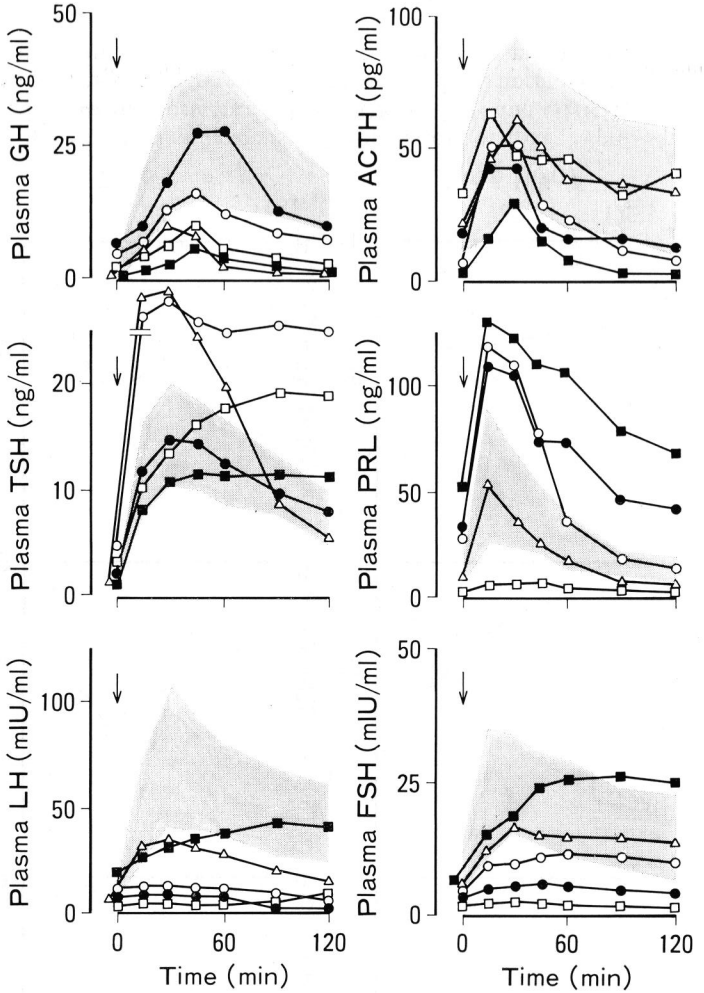

Fig. 3. Effect of simultaneous administration of four hypothalamic releasing hormones (TRH, LHRH, CRH and GHRH) on the plasma concentration of anterior pituitary hormones in five patients with hypothalamic disorders. The shaded area represents the maximum and minimum response in normal subjects. ○—○ Suprasellar germinoma (case 1) ●—● Hand-Schüller-Christian disease (Case 2) □—□ Craniopharyngioma (case 3) ■—■ Craniopharyngioma (case 4) △—△ Prader-Willi syndrome (case 5)

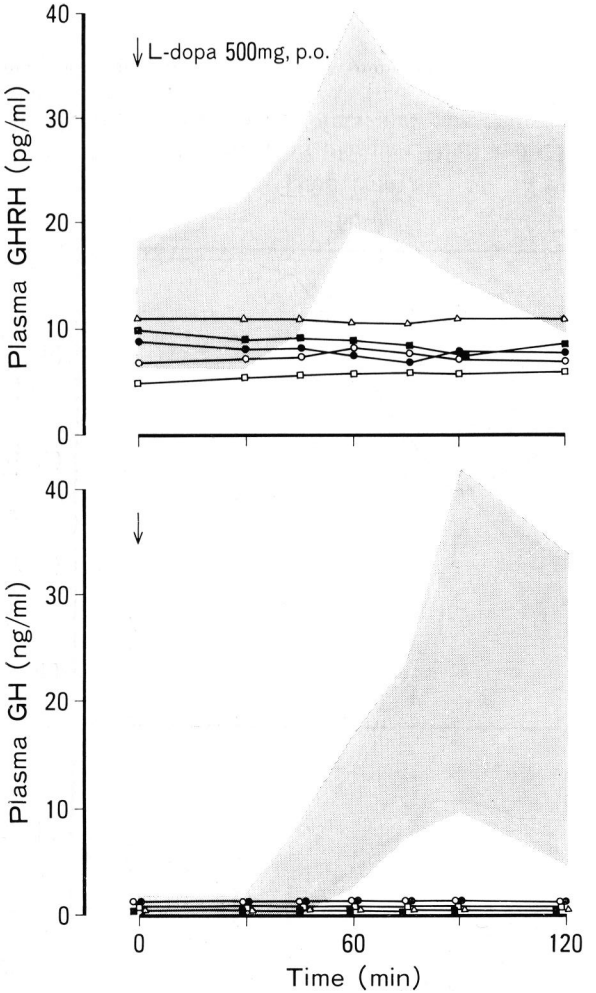

Fig. 4. Effecct of L-dopa on plasma GHRH and GH in five patients with hypothalamic disorders. The shaded area represents the maximum and minimum response in normal subjects.
○—○ Suprasellar germinoma (case 1) ●—● Hand-Schüller-Christian disease (case 2) □—□ Craniopharyngioma (case 3) ■—■ Craniopharyngioma (case 4) △—△ Prader-Willi syndrome (case 5)

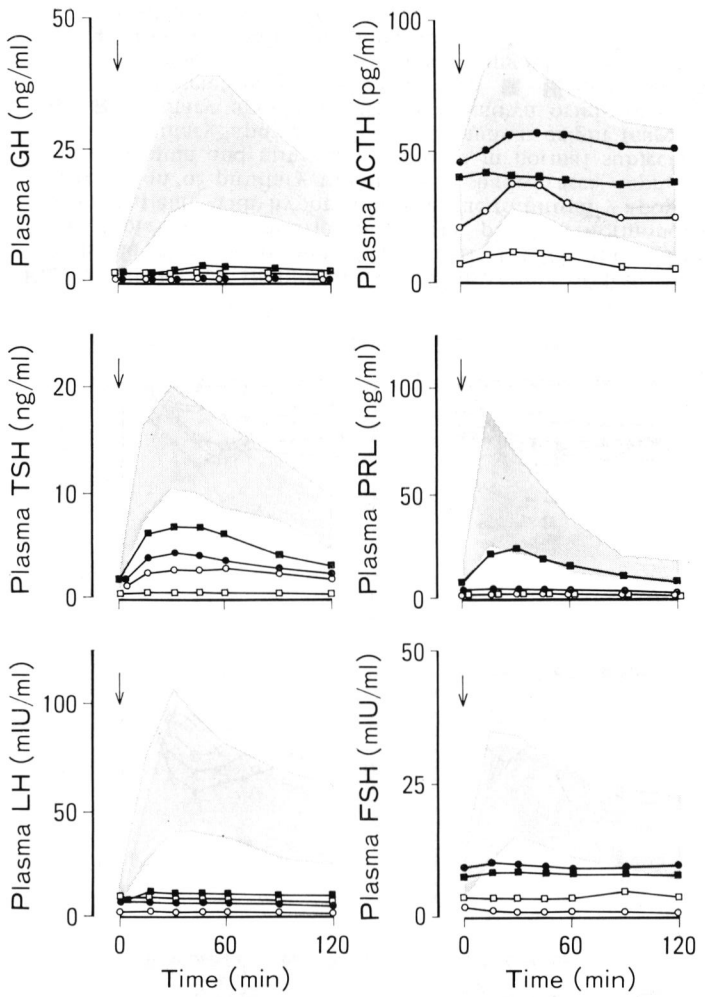

Fig. 5. Effect of simultaneous administration of four hypothalamic releasing hormones (TRH, LHRH, CRH and GHRH) on the plasma concentration of anterior pituitary hormones in four patients with idiopathic or postpartum hypopituitarism. The shaded area represents the maximum and minimum response in normal subjects.
○—○ Idiopathic hypopituitarism (case 6) ●—● Sheehan's syndrome (case 7) □—□ Sheehan's syndrome (case 8) ■—■ Sheehan's syndrome (case 9)

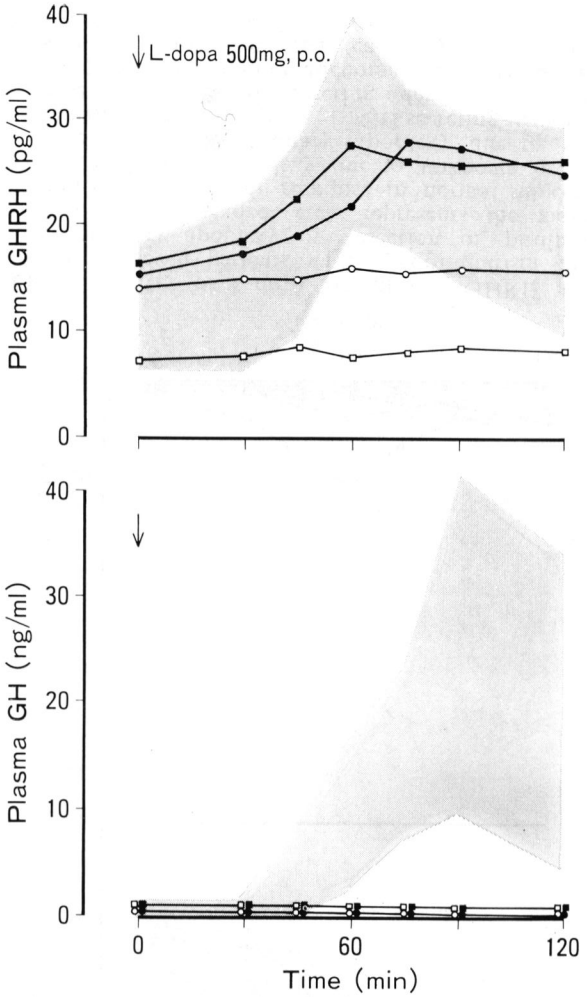

Fig. 6. Effect of L-dopa on plasma GHRH and GH in four patients with idiopathic or postpartum hypopituitarism. The shaded area represents the maximum and minimum response in normal subjects.
○—○ Idiopathic hypopituitarism (case 6) ●—● Sheehan's syndrome (case 7) □—□ Sheehan's syndrome (case 8) ■—■ Sheehan's syndrome (case 9)

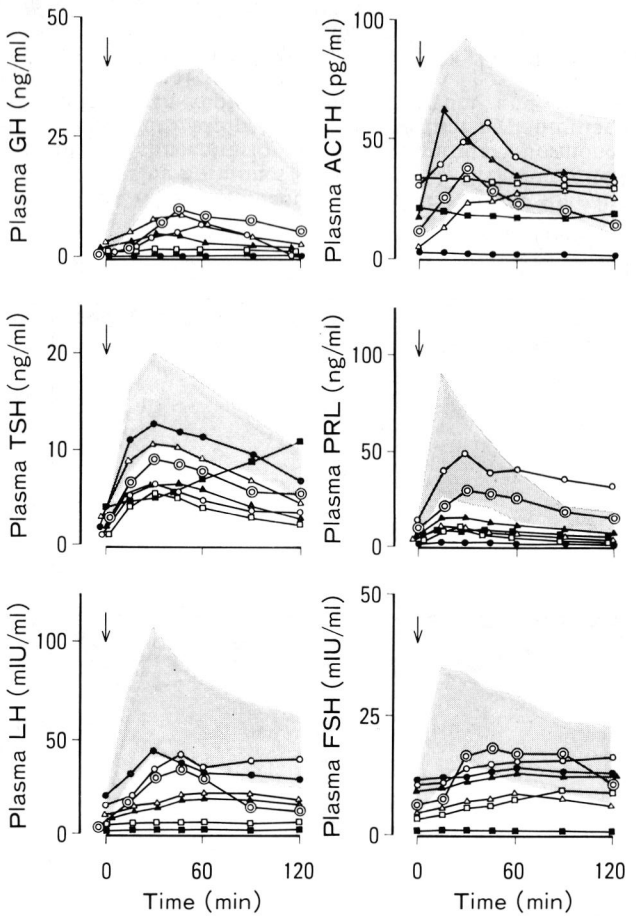

Fig. 7. Effect of simultaneous administration of four hypothalamic releasing hormones (TRH, LHRH, CRH and GHRH) on the plasma concentration of anterior pituitary hormones in seven patients with hypopituitarism due to pituitary apoplexy or resection of pituitary tumor. The shaded area represents the maximum and minimum response in normal subjects. ○—○ Pituitary apoplexy due to unknown origin (case 10) ●—● Pituitary apoplexy due to unknown origin (case 11) □—□ GH-secreting adenoma (case 12) ■—■ GH secreting adenoma (case 13) △—△ Non-functioning adenoma (case 14) ▲—▲ Non-functioning adenoma (case 15) ◎—◎ Non-functioning adenoma (case 16)

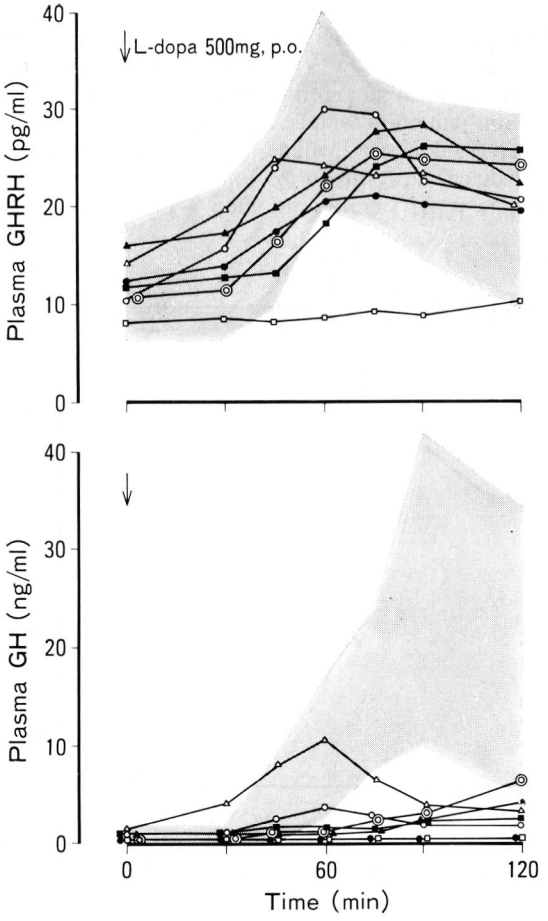

Fig. 8. Effect of L-dopa on plasma GHRH and GH in seven patients with hypopituitarism due to pituitary apoplexy or resection of pituitary tumor. The shaded erea represents the maximum and minimum response in normal subjects. ○—○ Pituitary apoplexy due to unknown origin (case 10) ●—● Pituitary apoplexy due to unknown origin (case 11) □—□ GH secreting adenoma (case 12) ■—■ GH-secreting adenoma (case 13) △—△ Non-functioning adenoma (case 14) ▲—▲ Non-functioning adenoma (case 15) ◎—◎ Non-functioning adenoma (case 16)

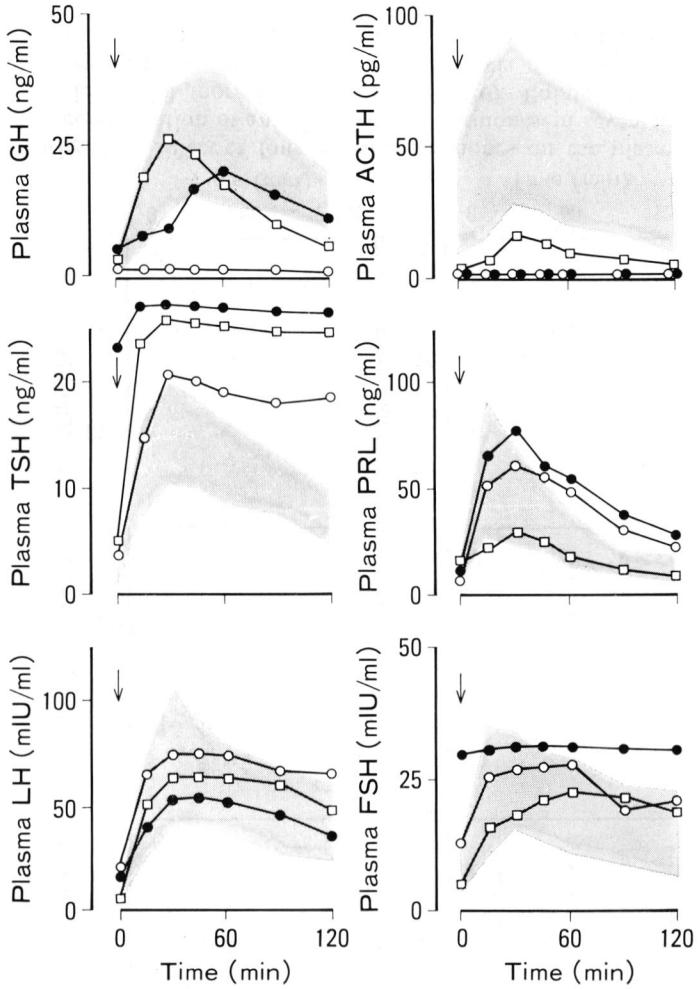

Fig. 9. Effect of simultaneous administration of four hypothalamic releasing hormones (TRH, LHRH, CRH and GHRH) on the plasma concentration of anterior pituitary hormones in three patients with ACTH deficiency syndrome. The shaded area represents the maximum and minimum response in normal subjects. ○—○ Isolated ACTH deficiency (case 17) ●—● ACTH deficiency associated with GH deficiency (case 18) □—□ Limited ACTH reserve (case 19)

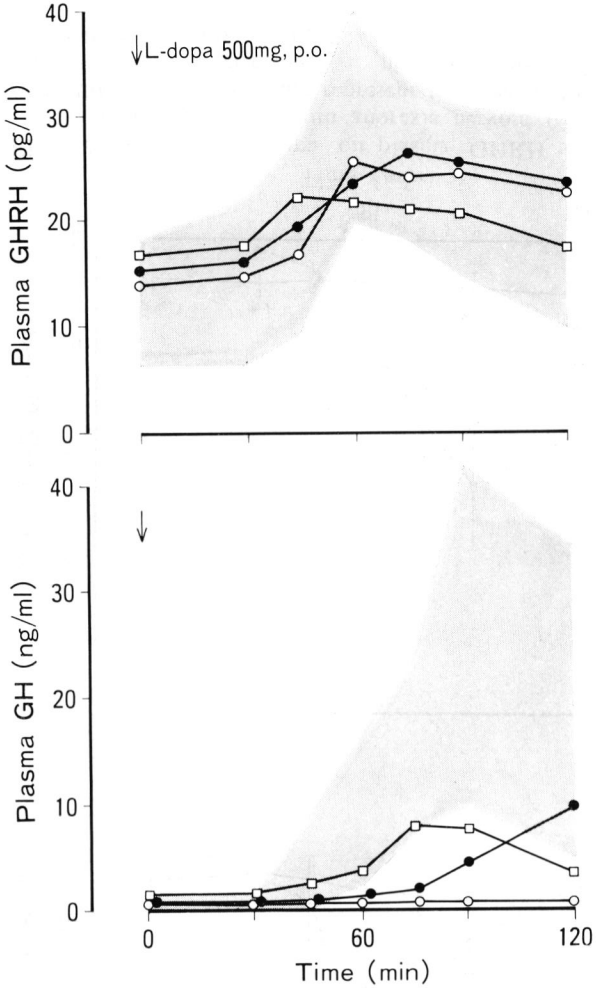

Fig. 10. Effect of L-dopa on plasma GHRH and GH in three patients with ACTH deficiency syndrome. The shaded area represents the maximum and minimum response in normal subjects. ○—○ Isolated ACTH deficiency (case 17) ●—● ACTH deficiency associated with GH deficiency (case 18) □—□ Limited ACTH reserve (case 19)

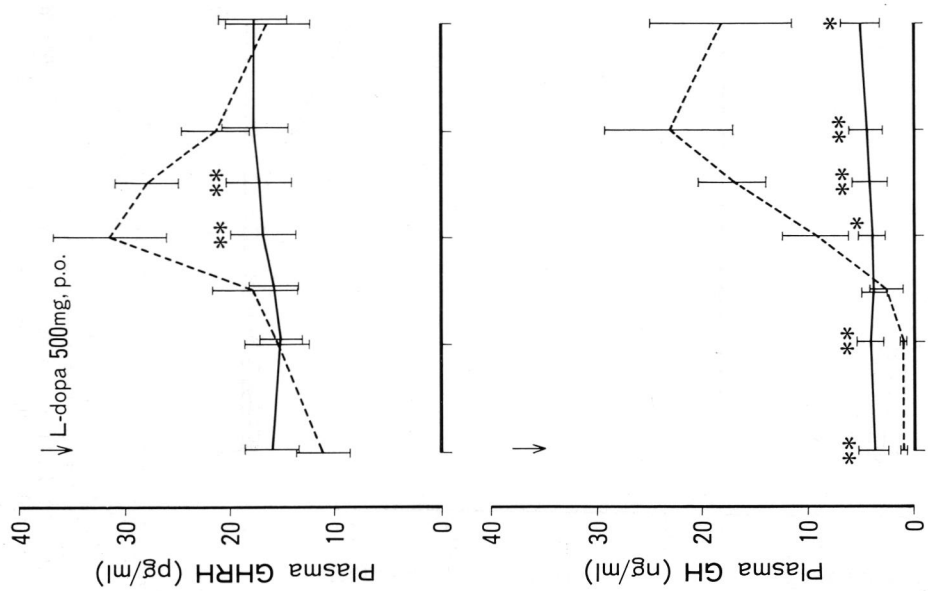

Fig. 12. Effect of L-dopa on plasma GHRH and GH in seven patients with anorexia nervosa (case 20–26). Points and bars represent the means±SE. (——: anorexia nervosa, ····· normal, *: $p<0.05$, **: 0.01 vs normal subjects)

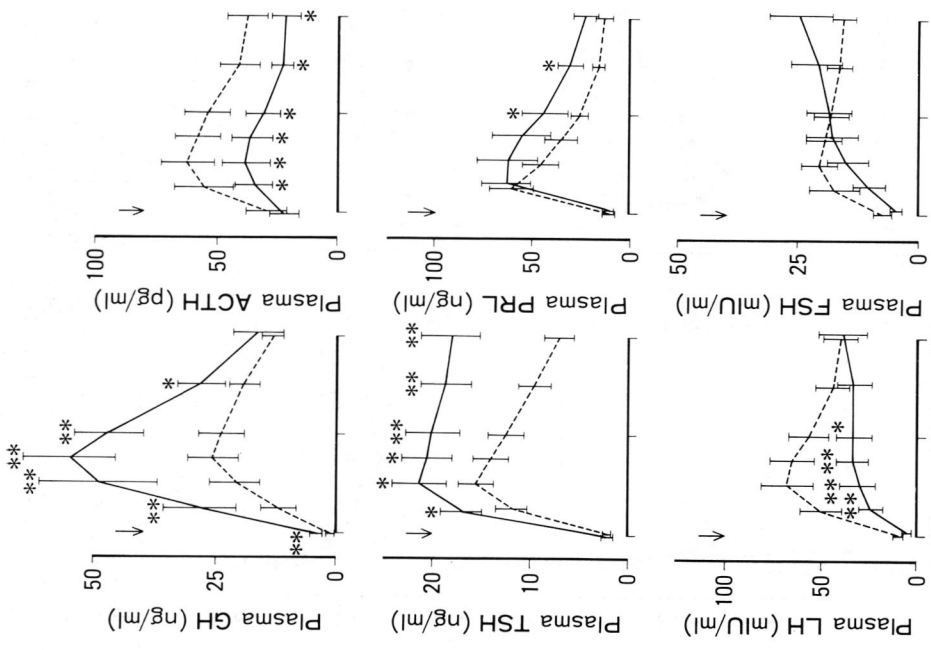

Fig. 11. Effect of four releasing hormones on the plasma concentration of anterior pituitary hormones in seven patients with anorexia nervosa (case 20–26). Points and bars represent the mean±SE. (——: anorexia nervosa, ····· : normal, *: $p<0.05$, **: $p<0.01$, vs normal subjects)

of the patients (Fig. 6).

The results of the 4RHs test in 7 patients with hypopituitarism due to pituitary apoplexy (cases 10 and 11) or due to resection of a pituitary tumor (cases 12–16) are shown in Fig. 7. Plasma GH showed a low response or none at all. Four cases revealed a subnormal ACTH response with an increase in plasma cortisol (data not shown), and in three cases there was no ACTH or cortisol response. Plasma TSH showed a normal or subnormal response in 6 cases, and case 12 showed a delayed response. Plasma PRL showed a low response in 5 out of 7 patients. Plasma LH showed no response in cases 12 and 13 and a subnormal response in the other 4 cases. Plasma FSH showed no response in case 13 and other 4 cases a subnormal response. In contrast, L-dopa test revealed a normal response of plasma GHRH in 6 out of 7 patients, but a normal response of plasma GH was found only in case 14 and in the others there was almost no plasma GH response (Fig. 8).

Patients with ACTH deficiency syndrome

The results of the 4RHs test in 3 patients with ACTH deficiency syndrome (cases 17, 18 and 19) are shown in Fig. 9. In case 17, all anterior pituitary hormones except ACTH showed normal responses. Case 18 had no plasma ACTH or GH response. The basal plasma ACTH level is not detectable in case 19, but his plasma ACTH and cortisol were slightly increased after the administration of 4RHs (data not shown). In addition, plasma TSH showed a rather strong response in these 3 cases. On the other hand, plasma GHRH showed a subnormal response to L-dopa in all 3 patients (Fig. 10). Plasma GH did not increase in case 8, and there was only a subnormal increase in the other 2 cases.

Patients with anorexia nervosa

The results of the 4RHs test in 7 patients with anorexia nervosa (case 20–26) are shown in Fig. 11. Basal and peak plasma GH concentrations were 4.2 ng/ml and 55.4 ng/ml, respectively, and significantly higher than in normal controls ($p<0.01$). Peak plasma ACTH was lower than that of the controls ($p<0.05$). Plasma TSH showed a hyper- and delayed response. Peak plasma PRL was similar to that of the controls, but reached later. Plasma LH showed a low ($p<0.01$) and delayed response, and plasma FSH also showed a delayed response.

As shown in Fig. 12, basal plasma GHRH was slightly higher than that of the controls, but the difference was not significant. Plasma GHRH and GH did not increase after L-dopa administration. Peak GHRH and GH were significantly lower than those of the controls ($p<0.01$, both).

Discussion

Until recently, the function of the anterior pituitary has been evaluated by provocative tests with various secretagogues including hypothalamic releasing hormones. However, this requires several loading tests to examine a patient. Then TRH and LHRH were combined with each other (Saito *et al.*, 1972) and with insulin (Lufkin *et al.*, 1983) as a single test of the release of multiple anterior pituitary hormones. After the discovery of CRH and GHRH, Sheldon *et al.* (1985), Cohen *et al.* (1986) and Kogure (1987) reported that the combined administration of 4RHs caused no apparent inhibition or synergism with respect to the individual hormone responses compared to the separate administration of TRH, LHRH, CRH and GHRH. Therefore, they concluded that the 4RHs test is a rapid, safe and useful test of combined anterior pituitary function. The dose used

in our study on the combined test was 100 μg for LHRH, CRH and GHRH and 500 μg for TRH, and proved sufficient to stimulate the release of LH, FSH, ACTH, GH and TSH in normal subjects.

On the other hand, we have developed a RIA for GHRH and investigated the correlation between plasma GHRH and plasma GH in normal and diseased states, including idiopathic pituitary dwarfism, acromegaly and ectopic growth hormone-releasing hormone (GHRH) syndrome (Saito *et al.*, 1984; Sano *et al.*, 1984, 1986, 1987; Yamasaki *et al.*, 1988a, b). It was reported that clonidine, insulin and ornithine increased plasma GH, but not plasma GHRH (Donnadieu *et al.*, 1985; Tapanainen *et al.*, 1988). However, we found that L-dopa stimulates the release of GHRH resulting in an increase in plasma GH (Saito *et al.*, 1984), and this was confirmed by Donnadieu *et al.* (1985), Chihara *et al.* (1986) and Tapanainen *et al.* (1988). Plasma GHRH reached a peak 30 to 90 min after L-dopa administration. This is at least in part due to the difference in the time taken to absorb L-dopa from the intestine in each subject. The increase in plasma GHRH is synchronized with or followed by that of plasma GH in normal subjects, suggesting that GH response to L-dopa is mediated by the release of GHRH from the hypothalamus. Therefore, the L-dopa test is thought to be useful in evaluating hypothalamic-pituitary function on the basis of the response of plasma GHRH and GH. Thus, we applied the combination of the 4RHs test and L-dopa test to evaluate hypothalamic-pituitary function in the patients with hypothalamic and/or pituitary disorders.

In the patients with hypothalamic disorders, plasma GH response to the 4RHs test was normal or low. This suggests that these patients have normal or impaired GH secretion secondary to a hypothalamic disorder. However, the L-dopa load failed to increase plasma GHRH and GH in these patients. This finding suggests that hypothalamic GHRH was not released in response to L-dopa because of a hypothalamic disorder, resulting in failure to release GH from the pituitary. Three cases examined by insulin hypoglycemia and arginine load showed no GH response, confirming the existence of a hypothalamic disorder also. Consequently, the patients with hypothalamic disorders seem to have a normal or subnormal response of plasma GH to the 4RHs test and no response to the L-dopa test. Therefore, the combination of both tests is useful and feasible in examining the hypothalamic-pituitary function of the patients with hypothalamic disorders.

Plasma ACTH and cortisol showed almost normal responses in our patients, suggesting that the function of the ACTH-cortisol axis may be preserved for a rather long time, even if the secretions of other anterior pituitary hormones are secondarily impaired by hypothalamic disorders. The increase in basal plasma PRL and the strong response of plasma PRL to 4RHs were observed in cases 1, 2 and 4, probably due to impaired secretion of prolactin inhibiting factor (PIF) from the hypothalamus because of hypothalamic lesions. On the other hand, case 3 had a weak response of plasma PRL to the 4RHs test, suggesting a complication of hypopituitarism caused by invasion of the pituitary by a craniopharyngioma. In a patient with Hand-Schüller-Christian disease, her plasma LH and FSH firstly showed a weak response to the 4RHs test, but responded gradually to normal after repeated pulsatile injections (10 μg every 2 h) of LHRH. This indicates that her hypogonadism is induced by a hypothalamic lesion due to Hand-Schüller-Christian disease contracted at the age of 3. The patient with Prader-Willi syndrome (case 5) had typical clinical signs, and seemed to have mainly hypothalamic dysfunction, because of similar responses of

anterior pituitary hormones to those in the patients with hypothalamic disorders.

As for hypopituitarism, the patients were divided into two groups: one is idiopathic and postpartum hypopituitarism, and the other is hypopituitarism due to pituitary apoplexy and resection of a pituitary tumor. In the 4RHs test, the former showed no response of plasma gonadotropin. In contrast, plasma GHRH was increased in response to L-dopa in 2 of 4 cases, although plasma GH did not increase in all patients. These data indicate that in some cases with the Sheehan's syndrome, secretion of GHRH was impaired, followed by a decrease in GH release and that the release of GH and gonadotropin was more easily blunted than that of ACTH and TSH. Two cases had a normal basal ACTH level, but little response to CRH. This also suggests a reduction in pituitary ACTH reserve in these patients as well as the 2 patients in Fig. 7. In the latter group, the response of plasma anterior pituitary hormones to the 4RHs test was mostly below the normal range. However, plasma GHRH was increased in 6 of 7 cases, although plasma GH was increased in only one case (case 14) in the L-dopa test. These data suggest that the patients with hypopituitarism due to pituitary adenoma have GHRH-secreting capacity unless the tumor invades the hypothalamus.

Stacpoole *et al.* (1982) reported that ACTH deficiency syndrome can be classified into three groups: 1) Isolated ACTH deficiency due to pituitary defect or suprapituitary defect, 2) limited ACTH reserve, and 3) combined pituitary insufficiency. To diagnose this syndrome, provocative tests for all anterior pituitary hormones are required. The combined test make it possible at once, and the L-dopa test is also helpful in investigating the possibility of a complication by a hypothalamic lesion. Case 17 showed no response of ACTH alone and a normal response of other pituitary hormones, indicating typical isolated ACTH deficiency. In case 18, no responses of ACTH or GH to the 4RHs test was observed, and this suggested a combined type of ACTH deficiency syndrome associated with GH deficiency. However, plasma GH responded to GHRH after supplementation with cortisol in this patient. Giustina *et al.* (1989) reported that growth hormone deficiency observed in the patients with idiopathic ACTH deficiency was recovered during glucocorticoid replacement, and suggested that a physiological level of serum cortisol is necessary for GH release in response to secretagogues in man. Furthermore, Hochberg *et al.* (1985) reported patients with both isolated ACTH deficiency and transitory GH deficiency. The pituitary reserve of ACTH and GH in case 18 is similar to the results reported by these authors. Case 19 had clinical manifestations of adrenal insufficiency, decreased urinary excretion of 17-OHCS and an undetectable basal level of plasma ACTH. However, the plasma concentrations of ACTH and cortisol were slightly increased during the combined test, suggesting the condition of limited ACTH reserve described by Stacpoole *et al.* (1982).

These 3 cases had normal thyroid function but showed a strong response of plasma TSH to the 4RHs test. A strong response of both TRH and PRL to TRH was previously reported by Mashito *et al.* (1981) and Gonzalez and Werk (1985) in untreated isolated ACTH deficiency. This may be related to the absence of glucocorticoid because of the normalized response of plasma TSH and PRL after glucocorticoid replacement, but the mechanism is still not known. On the other hand, L-dopa evoked a normal response of plasma GHRH in these three cases, suggesting no apparent lesion in the region of the arcuate nucleus where the GHRH neurons are located. Therefore, the patients seemed to have a pituitary defect. In regard to GH response to L-dopa, only

case 18 (ACTH deficiency with GH deficiency) revealed no response. This finding is compatible with the hypothesis of Giustina *et al.* (1989) and indicates that glucocorticoid replacement is necessary before examining GH-secreting activity in the patients with ACTH deficiency syndrome. This also offers a key to the mechanism of interaction between the GHRH-GH axis and the ACTH-cortisol axis.

In the patients with anorexia nervosa, the 4RHs test revealed a significant change in anterior pituitary function. The increased basal GH concentration and strong response of plasma GH to the 4RHs test are compatible with previous reports (Takano, 1986; Masuda *et al.*, 1988). Plasma ACTH showed a low response, and plasma cortisol did not increase significantly (data not shown). These findings suggest that hypersecretion of CRH may occur in patients with anorexia nervosa (Hotta *et al.*, 1986). Plasma TSH showed a delayed response, that may be related not only to an eating disorder but also to weight loss (Kiyohara *et al.*, 1987). Plasma PRL response was within the normal range as previously reported (Karibe *et al.*, 1988), although Waldhauser *et al.* (1984) described a weak response of PRL to TRH. Decreased response of plasma LH is consistent with previous reports and plasma FSH showed a delayed response similar to plasma LH (Sherman *et al.*, 1975). No responses of plasma GHRH or GH to L-dopa indicate the impairment of hypothalamic function in patients with anorexia nervosa.

In conclusion, we were the first to report the clinical significance of the combination of the combined 4RHs test and L-dopa test, especially by measuring the responses of plasma GHRH and six anterior pituitary hormones. The combination of both tests is a simple and useful means to use in evaluating hypothalamic-pituitary function in normal subjects and in the patients with hypothalamic and/or pituitary disorders.

Acknowledgements

This work was supported by Grants-in-Aid for Research on Intractable Diseases from the Ministry of Health and Welfare of Japan, for Scientific Research (No. 63770852) from the Ministry of Education, Science and Culture of Japan (1988), and for Research on Growth Disorders from the Foundation for Growth Science of Japan (1987).

References

Chihara, K., Y. Kashio, T. Kita, Y. Okimura, H. Kaji, H. Abe and T. Fujita (1986). L-dopa stimulates release of hypothalamic growth hormone-releasing hormone in humans. *J. Clin. Endocrinol. Metab.* **62**, 466–473.

Cohen, R., D. Bouquier, S. Biot-Laporte, E. Vermeulen, B. Claustrat, M. H. Cherpin, P. Cabrera, P. Guidetti, S. Ferry, Ch. A. Bizollon and G. Sassolas (1986). Pituitary stimulation by combined administration of four hypothalamic releasing hormones in normal men and patients. *J. Clin. Endocrinol. Metab.* **62**, 892–898.

Donnadieu, M., D. Evain-Brion, M. C. Tonon, H. Vaudry and J. C. Job (1985). Variations of plasma growth hormone (GH)-releasing factor levels during GH stimulation tests in children. *J. Clin. Endocrinol. Metab.* **60**, 1132–1134.

Giustina, A., G. Romanelli, R. Candrina and G. Giustina (1989). Growth hormone deficiency in patients with idiopathic adrenocorticotropin deficiency resolves during glucocorticoid replacement. *J. Clin. Endocrinol. Metab.* **68**, 120–124.

Gonzalez, J. J. and E. E. Werk (1985). Abnormal thyrotropin and prolactin levels in untreated corticotropin deficiency. *Arch. Intern. Med.* **145**, 356–357.

Guillemin, R., P. Brazeau, P. Boehlen, F. Esch, N. Ling and W. B. Wehrenberg (1982). Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* **218**, 585–587.

Hochberg Am Hardoff, D., D. Atias and A. Spindel (1985). Isolated ACTH deficiency with transitory GH deficiency. *J. Endocrinol.*

Invest. 8, 67–70.

Hotta, M., T. Shibasaki, A. Masuda. T. Imaki, H. Demura, N. Ling and K. Shizume (1986). The responses of plasma adrenocorticotropin and cortisol to corticotropin-releasing hormone (CRH) and cerebrospinal fluid immunoreactive CRH in anorexia nervosa patients. *J. Clin. Endocrinol. Metab.* **62**, 319–324.

Karibe, C., H. Tamai, K. Kiyohara, N. Kobayashi and T. Nakagawa (1988). Prolactin responses to hypoglycemia and thyrotropin-releasing hormone in anorexia nervosa. *Acta Psychiatr. Scand.* 78, 442–447.

Kiyohara, K., H. Tamai, C. Karibe, N. Kobayashi, S. Fujii, O. Fukino, T. Nakagawa, L. F. Kumagai and S. Nagataki (1987). Serum thyrotropin (TSH) responses to thyrotropin-releasing hormone (TRH) in patients with anorexia nervosa and bulimia ; influence of changes in body weight and eating disorders. *Psychoneuroendocrinology* **12**, 21–28.

Kogure, M. (1987). Evaluation of anterior pituitary function by four hypothalamic releasing hormones. *Teikyo Igaku Zasshi* **10**, 433–441. (in Japanese)

Lufkin, E. G., P. C. Kao, W. M. O'Fallon and M. A. Mangan (1983). Combined testing of anterior pituitary gland with insulin, thyrotropin-releasing hormone, and luteinizing hormone-releasing hormone. *Am. J. Med.* **75**, 471–475.

Mashito, T., I. Takada, T. Kenbo and A. Watanabe (1981). Anterior pituitary function before and after treatment in twenty-three patients with isolated adrenocorticotropic hormone (ACTH) deficiency reported in Japan. *Folia Endocrinol. Jap.* **57**, 1659–1671. (in Japanese).

Masuda, A., T. Shibasaki, M. Hotta, H. Suematsu and K. Shizume (1988). Study on the mechanism of abnormal growth hormone (GH) secretion in anorexia nervosa : no evidence of involvement of a low somatomedin-C level in the abnormal GH secretion. *J. Endocrinol. Invest.* **11**, 297–302.

Rivier, J., J. Spiess, M. Thorner and W. Vale (1982). Characterization of a growth hormone-releasing factor from a human pancreatic islet tumor. *Nature* **300**, 276–278.

Saito, S., K. Abe, N. Nagata, K. Tanaka, E. Nakamura and T. Kaneko (1972). Evaluation of pituitary function by use of LH-releasing hormone and thyrotropin-releasing hormone. International Congress Series No. 263, hypothalamic hypophysiotropic hormones, Proceedings of the Conference held at Acapulco, Gro., Mexico, Excerpta Medica, Amsterdam. pp. 276–282.

Saito, H., S. Saito, R. Yamazaki and E. Hosoi (1984). Clinical value of radioimmunoassay of plasma growth-hormone-releasing factor. *Lancet II*, 401–2.

Sano, T., H. Saito, R. Yamazaki, K. Kameyama, M. Ikeda, E. Hosoi, K. Hizawa and S. Saito (1984). Production of growth hormone-releasing factor in pheochromocytoma. *N. Eng. J. Med.* **311**, 1520.

Sano, T., H. Saito, R. Yamasaki, E. Hosoi, K. Kameyama, S. Saito, T. Hirose and K. Hizawa (1986). Production and secretion of immunoreactive growth hormone-releasing factor by pheochromocytoma. *Cancer* **57**, 1788-1793.

Sano, T., R. Yamasaki, H. Saito, T. Hirose, E. Kudo, K. Kameyama, K. Hiraishi, S. Saito and K. Hizawa (1987). Growth hormone-releasing hormone (GHRH)-secreting pancreatic tumor in a patient with multiple endocrine neoplasia type I. *Am. J. Surg. Pathol.* **11**, 810–819.

Sheldon, W. R., C. R. DeBold, W. S. Evans, G. S. DeCherney, R. V. Jackson, D. P. Island, M. O. Thorner and D. N. Orth (1985). Rapid sequential intravenous administration of four hypothalamic releasing hormone as a combined anterior pituitary function test in normal subjects. *J. Clin. Endocrinol. Metab.* **60**, 623–630.

Sherman, B. M., K. A. Halmi and R. Zamundio (1975). LH and FSH response to gonadotropin-releasing hormone in anorexia nervosa : Effect of nutritional rehabilitation. *J. Clin. Endocrinol. Metab.* **41**, 135–140.

Stacpoole, P. W., J. W. Interlandi, W. E. Nicholson and D. Rabin (1982). Isolated ACTH deficiency : A heterogeneous disorder ; Critical review and report of four new cases. *Medicine* **61**, 13–24.

Takano, K. (1986). GRF loading test : Growth hormone releasing factor (GRF). *Nihon Rinsho.* **44**, 532-538. (in Japanese)

Tapanainen, P., M. Knip, P. Lautala and J. Leppaluoto (1988). Variable plasma growth hormone (GH)-releasing hormone and GH responses to clonidine, L-dopa, and insulin in normal men. *J. Clin. Endocrinol. Metab.* **67**, 845–849.

Waldhauser, F., K. Toifl, J. Spona, U. Zeitlhuber, M. Waldhauser and H. Frisch (1984). Diminished prolactin response to thyrotropin and insulin in anorexia nervosa. *J. Clin. Endocrinol. Metab.* **59**, 538–541.

Yamasaki, R., H. Saito, K. Kameyama, E. Hosoi and S. Saito (1988a). Secretion of growth hormone-releasing hormone in patients with idiopathic pituitary dwarfism and acromegaly. *Acta Endocrinol.* **117**, 273–281.

Yamasaki, R., H. Saito, T. Sano, K. Kameyama, K. Yoshimoto, E. Hosoi, M. Matsumura, K. Harada and S. Saito (1988b). Ectopic growth hormone-releasing hormone (GHRH) syndrome in a case with multiple endocrine neoplasia type I. *Endocrinol. Japon.* **35**, 97–109.