Differences in Pathological Findings and Growth Hormone Responses in Patients with Growth Hormone-Producing Pituitary Adenoma

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Abstract. Plasma growth hormone (GH) responses to various stimuli were examined in 21 patients with GH-producing pituitary adenomas, classified into three types by the immunohistochemistry of cytokeratin and the glycoprotein hormone α-subunit distribution. Seven type 1 adenomas were exclusively composed of cells in which the cytokeratin formed a dot-like pattern; they were chromophobic to hematoxylin and eosin (H&E), occasionally positive for GH, and almost completely negative for the α-subunit. Thirteen type 2 adenomas were composed of cells with cytokeratin that had a perinuclear distribution; they were eosinophilic to H&E, and diffusely positive for both GH and the α-subunit. One patient had a type 3 adenoma which had a mixed pattern of intracellular cytokeratin distribution and was chromophobic and eosinophilic to H&E. Clinically, type 1 is characterized by earlier onset, larger tumor size, and more frequent aggressive extension. Paradoxical GH responses to TRH and OGTT were seen in 1 of 6 patients (16.7%) of type 1 and 8 of 9 patients (88.9%) of type 2, and 0% of type 1 and 62.5% of type 2, respectively. Type 2 cases showed higher plasma GH response to GH-releasing hormone, and a tendency to greater suppression of plasma GH by bromocriptine compared with type 1. Octreotide acetate administration revealed that the nadir/basal ratio of plasma GH levels was 42.9±6.6% in type 1 and 13.5±5.8% in type 2. These results suggest that there is a pathophysiological difference between these two distinct types of GH-producing pituitary adenomas.

Key words: Acromegaly, Cytokeratin, Immunohistochemistry, Growth hormone (GH), Somatostatin.

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SANO *et al.* recently reported that growth hormone (GH)-producing pituitary adenomas could be classified into at least two types according to the intracytoplasmic distribution of immunoreactive cytokeratin [1]. Cytokeratin is considered to have a certain unknown function within the cells besides forming their framework. In GH-producing pituitary adenomas, a distinct distribution of cytokeratin has been reported, and a functional abnormality of these cells has been suggested.

On the other hand, abnormalities of GH secretion in acromegalic patients include a high plasma GH level, impaired inhibition or paradoxical increase in GH after glucose ingestion [2], and stimulation of GH release by thyrotropin-releasing hormone (TRH) [3–5], or various GH response to GH-releasing hormone (GHRH) [6]. Bromocriptine [7, 8] and the somatostatin analogue octreotide acetate suppress GH release in patients with acromegaly [9, 10]. However, heterogeneous responses to these provocative and suppressive

BANDO *et al.*

Table 1. Summary of immunohistochemical findings, plasma hormone levels, and radiological staging of patients with acromegaly

Type	No.	Age	M/F	Cytokeratin[b]	GH	PRL	α-Subunit	GH (ng/ml)	PRL (ng/ml)	Radiological staging[c]
		Patient		Immunohistochemistry[a]				Plasma hormone level		
Type 1	1	36	F	Dot	++	−	−	79.5	ND	III
	2	40	F	Dot	+++	−	−	200.4	28.9	III
	3	37	M	Dot	++	−	−	51.3	5.3	III
	4	40	M	Dot	++	−	−	59.9	10.8	II
	5	48	F	Dot	++	+	−	22.6	56.5	II
	6	40	F	Dot	++	−	−	77.0	3.1	III
	7	36	F	Dot	+	++++	+	19.8	180	III
Type 2	8	67	F	P.D.	++++	−	++++	25.9	7.9	I
	9	33	F	P.D.	++++	+	−	48.4	62.2	II
	10	55	M	P.D.	++++	−	+++	33.3	6.0	II
	11	47	M	P.D.	++++		++	80.6	5.6	IV
	12	32	F	P.D.	++++	−	−	19.8	20.4	II
	13	25	F	P.D.	+++	+++	+	32.1	ND	I
	14	56	F	P.D.≫dot	++	++	++++	10.1	ND	II
	15	61	F	P.D.≫dot	++++	+	+++	45.8	11.2	II
	16	35	F	P.D.≫dot	+++	+	++++	78.9	7.5	III
	17	62	M	P.D.≫dot	+++	−	+++	7.8	4.9	II
	18	65	F	P.D.≫dot	+++	+	++++	9.8	10.6	II
	19	47	M	P.D.≫dot	++++	−	++	364.1	2.3	IV
	20	32	F	P.D.≫dot	++++	++	+++	74.5	68.8	III
Type 3	21	70	F	P.D.<dot	++	+	−	18.8	10.0	II

a The degree of staining is expressed as follows: −, no positive cells; +, occasional; ++, frequent; +++, many; ++++, most cells are positive.
GH, growth hormone; PRL, prolactin; ND, not done.

b Dot, cytokeratin was stained in a dot-like pattern; P.D., cytokeratin was stained with perinuclear distribution; P.D.≫dot, cells with dots were seen in only a few adenoma cells; P.D.<dot, cells with dots were seen more often than those with perinuclear distribution.

c Radiological staging was according to Hardy [18].

agents have been shown and no universal trend has been established [11–17].

In the present study, we investigated differences in pathological findings, clinical features and plasma GH responses to various loading tests in patients with GH-producing pituitary adenomas.

Materials and Methods

Patients

Twenty-one patients with acromegaly (Table 1) were examined before and after transsphenoidal adenomectomy. The diagnosis of acromegaly was made on the basis of clinical and endocrinological findings, and the adenoma tissues were obtained at surgery for light microscopic and immunohistochemical studies. The study was approved by the Human Subjects Protection Committee, School of

Medicine, University of Tokushima, and informed consent was obtained from all patients.

Plasma levels of GH and prolactin

Plasma levels of GH and prolactin (PRL) before and after provocative and suppression tests were determined by radioimmunoassay (RIA).

Blood samples were taken from the antecubital vein between 0800 and 0900 h after an overnight fast for measurement of basal plasma levels. Evaluations were made on at least 3 separate days and the average value was calculated. Plasma GH response to 500 μg TRH (Takeda Pharmacoceutical Co., Ltd., Osaka, Japan) and 100 μg GHRH (Sumitomo Pharmacoceutical Co., Ltd., Tokyo, Japan) given iv for 15 s was studied at 0, 15, 30, 60, 90 and 120 min after the injection. GH response to oral administration of 75 g glucose was studied at 0, 30, 60, 90, 120 and 180 min after.

Response to bromocriptine (2.5 mg po; Parlodel®, Sankyo Pharmacoceutical Co., Ltd., Tokyo, Japan) or octreotide acetate (50 μg sc; Sandostatin, Sumitomo Pharmacoceutical Co., Ltd.) was studied 0, 1, 2, 4, 6 and 8 h after treatment. These tests were performed from 0800–0900 h on separate days.

Hormone assay

The plasma GH level was measured with a GH-IRMA kit (Daiichi Radioisotope, Tokyo, Japan). The sensitivity was 0.05 ng/ml and the within-assay coefficient of variation was less than 7%. The plasma PRL level was measured with a double-antibody RIA kit supplied by Dainabbot Co., Ltd. (Osaka, Japan).

Radiological evaluation

Preoperative radiological evaluation included skull x-ray examination and computed tomography, with magnetic resonance imaging (MRI) in selected patients. Hardy's criteria [18] were used for radiological classification. In brief, the stage I tumor was less than 10 mm in diameter and the stage II tumors greater than 10 mm in diameter; both were within the sella. Stage III tumors show signs of localized invasion of the sella, and stage IV tumors signs of diffuse destruction of the sella.

Pathological evaluation

Surgically removed pituitary adenomas were studied by light microscopy and immunohistochemistry by means of methods described previously [1]. Immunohistochemistry was performed by the avidin-biotin-peroxidase complex (ABC) method with an ABC kit (Vector Lab, Burlingame, CA, USA). The following primary antibodies were used: anti-human cytokeratin CAM 5.2 (25 μg/ml, Becton-Dickinson, San Jose, CA, USA) which is specific for cytokeratins 8 and 18, and anti-α-subunit of monoclonal antibody to the α-subunit of glycoprotein (1:1,000, ICN Immunobiologicals, Lisle, IL, USA). The following primary antisera were used: anti-GH (1:800, DAKO, Santa Barbara, CA, USA), and anti-PRL (1:2,000, DAKO, Santa Barbara, CA, USA). Details of the procedure have been described elsewhere by Sano *et al.* [1].

Statistical analysis

Data are expressed as the mean ± SD. Student's *t*-test was used to compare plasma hormone levels in the two groups and analysis of variance was used to compare the differences at different time points.

Results

Pathology of GH-producing pituitary adenoma

GH-producing pituitary adenomas were divided into type 1 (7 cases), type 2 (13 cases), and type 3 (1 case) according to the histological and immunohistochemical findings (Table 1).

Type 1 adenomas showed a paranuclear nodular reaction by cytokeratin staining (Fig. 1a) and chromophobic cells with an intracytoplasmic unstained nodular (dot-like) area and an irregular nucleus (Fig. 1b) following hematoxylin and eosin (H&E) staining. The area positive for cytokeratin was consistently unstained by H&E. Although the number of GH-immunoreactive cells varied from case to case, it was generally less in type 1 than in type 2 (Fig. 1c). The α-subunit was negative in six of seven cases (Fig. 1d).

Type 2 adenomas showed signs of a partial or complete perinuclear distribution surrounding the nucleus following cytokeratin staining (Fig. 2a) and eosinophilic granular cytoplasma and a round nucleus following H&E staining (Fig. 2b), with no unstained area of the kind seen in type 1 adenomas. Immunohistochemical study revealed a larger number of cells positive for GH in all cases (Fig. 2c), and staining was positive for α-subunit in 11 of 13 cases (Fig. 2d).

Type 3 showed a mixed pattern of intracellular cytokeratin distribution and was chromophobic and eosinophilic to H&E stain. Immunohistochemical study revealed the presence of positive cells for GH and PRL, but no cells reactive for the α-subunit.

Clinical features of the patients

Type 1 included two men and five women, with an average age of 39.5±4.2 yr. Type 2 included four men and nine women, with an average age of 47.4±14.6 yr.
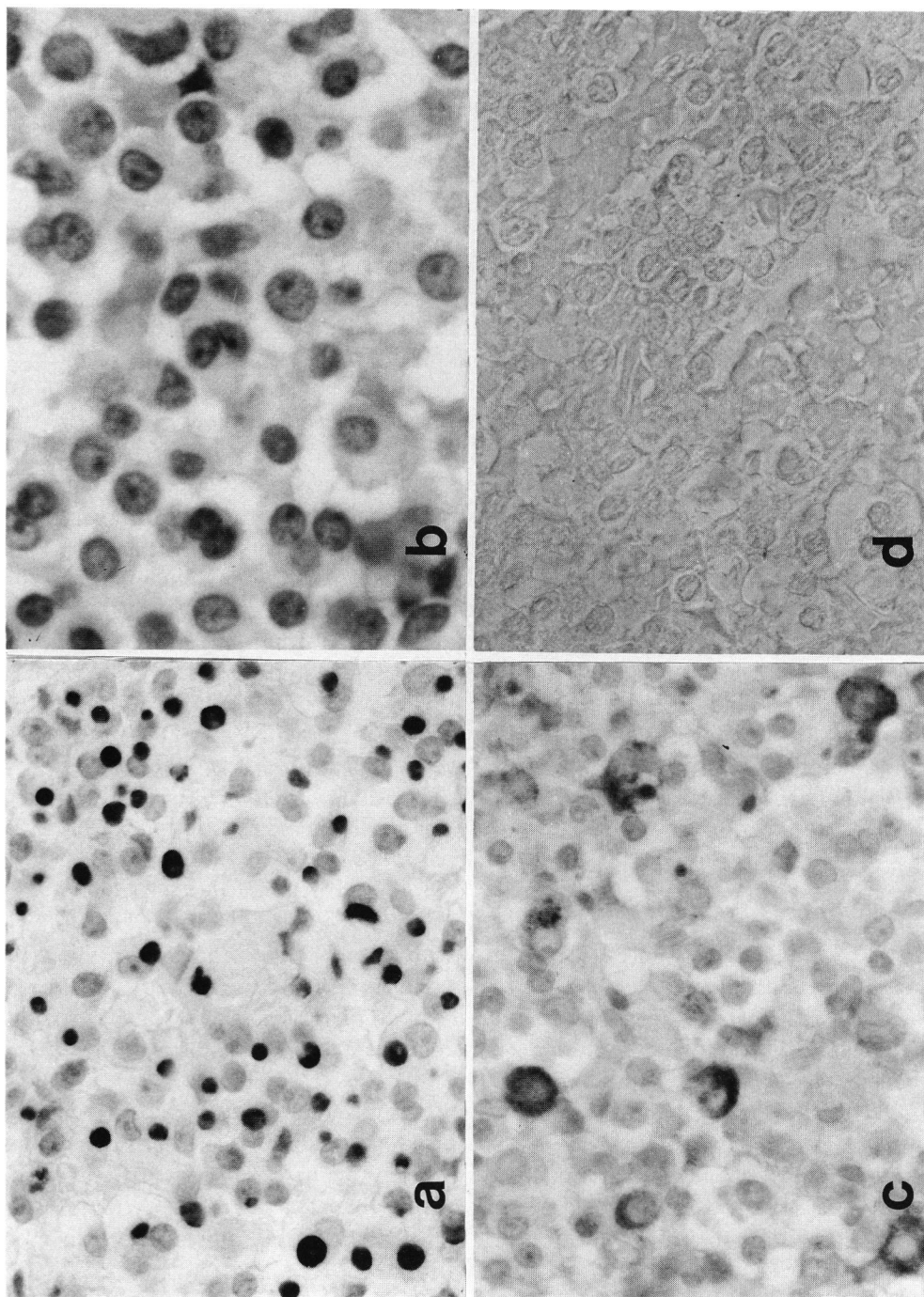
Fig. 1. Histological findings of growth hormone (GH)-producing pituitary adenomas of type 1. a: Immunohistochemistry of cytokeratin revealed evident paranuclear reaction of cytokeratin immunostaining. b: Chromophobic adenoma cells have an intracytoplasmic, unstained nodular area and an irregular nucleus to H&E stain. c: These cells are predominantly positive for GH immunohistochemically. d: These cells are negative for α-subunit (αSU). (a, c, d: ×400, b: ×800).
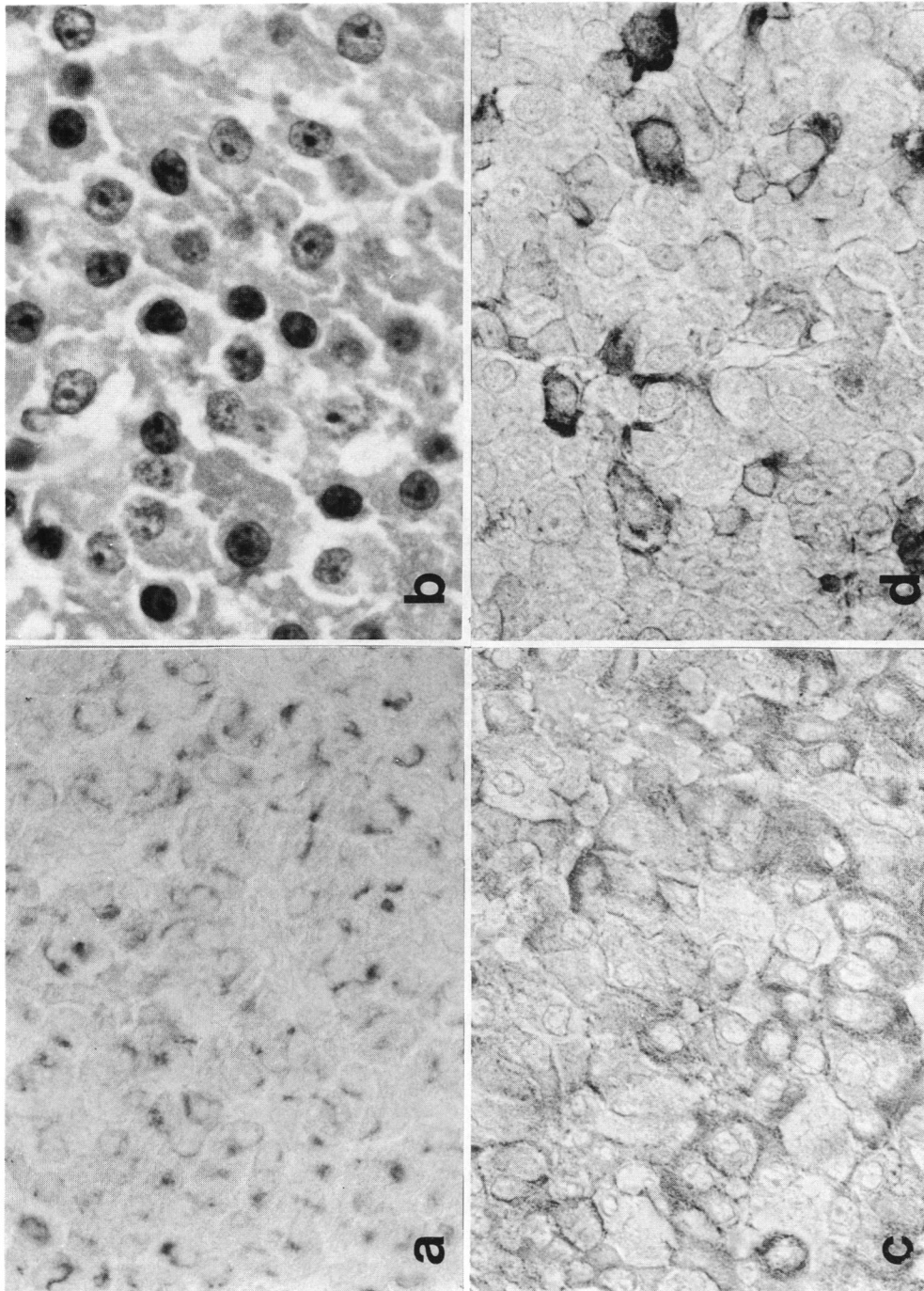
Fig. 2. Histological findings of growth hormone (GH)-producing pituitary adenomas of type 2. a: Immunohistochemistry of cytokeratin revealed immunoreaction diffusely around the nucleus. b: Adenoma cells have eosinophilic granular cytoplasm and a round nucleus. c: Many of these cells are positive for GH immunohistochemically. d: Many of these cells are positive for α-subunit (αSU). (a, c, d: ×400, b: ×800).
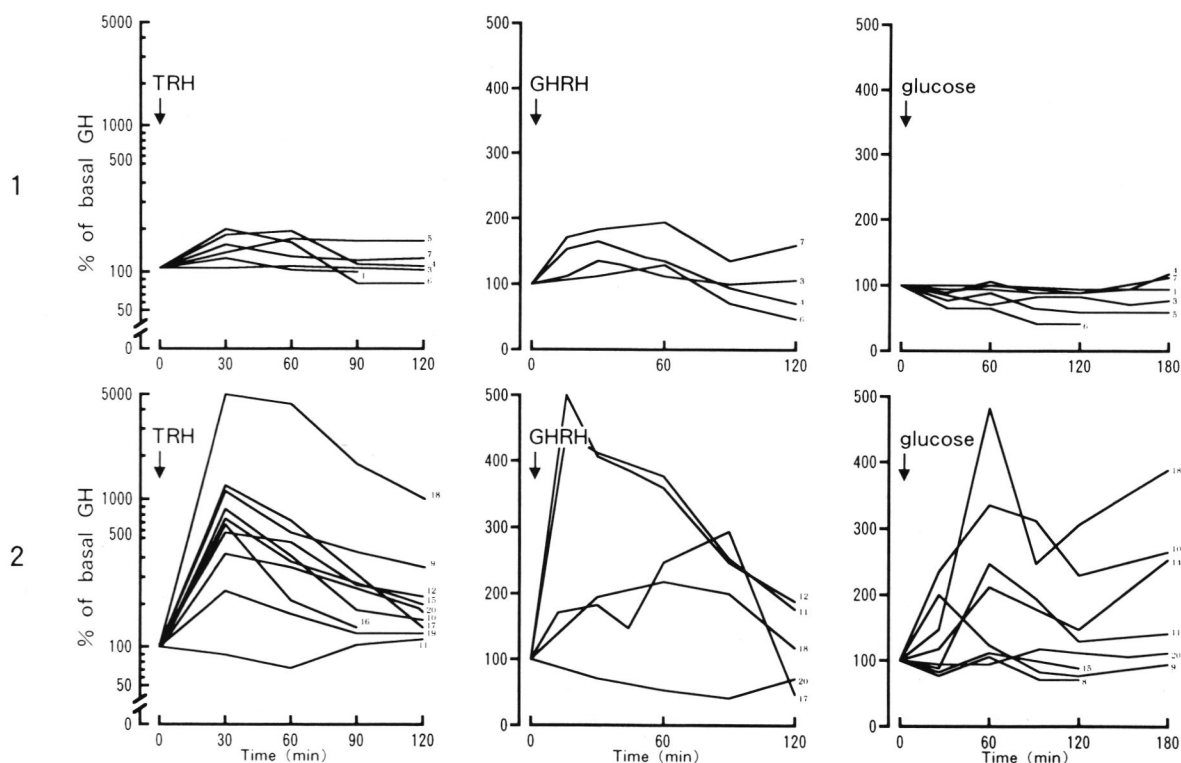
Fig. 3. Plasma growth hormone (GH) responses to thyrotropin-releasing hormone (TRH) (500 μg, iv), GH-releasing hormone (GHRH) (100 μg, iv), and glucose (75 g, po) in patients with GH-producing pituitary adenoma classified as types 1 and 2. The upper and lower panels represent the responses in types 1 and 2, respectively. The case number is indicated in the figure.

Median basal plasma GH levels in types 1 and 2 were 59.9 ng/m*l* and 33.3 ng/m*l*, respectively. Percentages of cases with plasma GH levels > 40 ng/m*l* were 71% in type 1 and 46% in type 2 (no significant difference).

Endocrine testing

Plasma GH response to TRH in types 1 and 2, expressed by the ratio to the basal plasma GH level, is shown in Fig. 3a. In type 1 the peak/basal ratio was never more than 2, whereas in type 2 it was more than 2 in 90% of the patients (significant difference, $P<0.01$).

Plasma GH response ratio for GHRH is shown in Fig. 3b. A peak/basal ratio of more than 2 was 0% in type 1, and 80% in type 2 (significant difference, $P<0.01$).

Plasma GH response to 75 g oral glucose tolerance test (OGTT) is shown in Fig. 3c. No type 1 patients had a paradoxical GH rise (peak/basal ratio of plasma GH level being more than 2). In contrast, five of eight type 2 patients had a paradoxical GH rise.

Plasma GH response to bromocriptine is shown in Fig. 4a. The average suppression rate at 4 h was 62.4±24.1% in type 1 and 44.8±32.4% in type 2, (not significant).

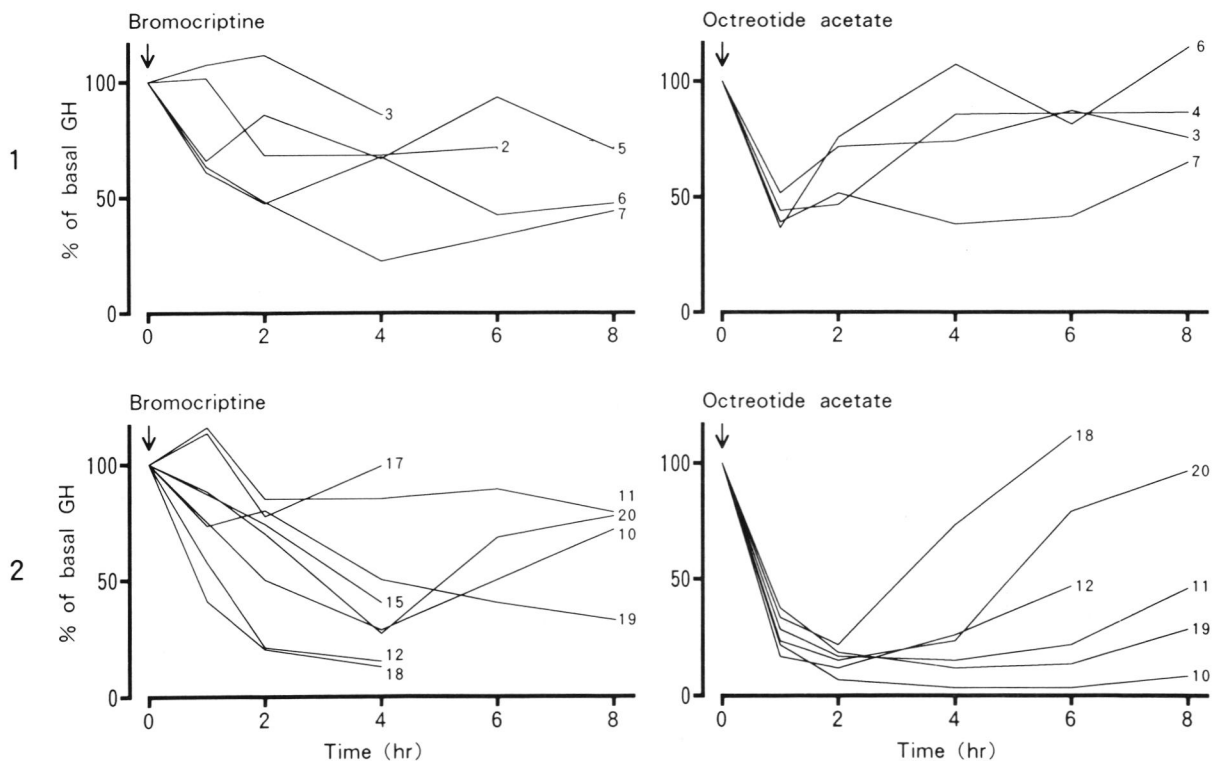The plasma GH response to octreotide acetate is shown in Fig. 4b. The nadir/basal ratio was 42.9±6.6% in type 1 and 13.5±5.8% in type 2 ($P<0.001$). In type 2, the plasma GH level was suppressed to 15.2±5.4% of the basal level 2 h after the administration.

Radiological evaluation

Lesions graded as III and IV were seen in 86% of patients classified as type 1, and in only 31% of type 2 ($P<0.01$) (Table 1).

Discussion

GH-producing pituitary adenoma can be classified grossly into two types based according to the distribution patterns of cytokeratin and GH by means of light microscopy and immunohistoche-

Fig. 4. Plasma growth hormone (GH) responses to bromocriptine (2.5 mg, po) and octreotide acetate (50 μg, sc) in patients with GH-producing pituitary adenoma classified as types 1 and 2. The upper and lower panels represent the responses in types 1 and 2, respectively. The case number is indicated in the figure.

mistry [1]. In this study, we investigated the differences in clinical and endocrinological aspects of the two types in patients with GH-producing pituitary adenoma by performing provocative and suppressive tests.

The 21 patients were divided into three types according to the immunohistochemical patterns of cytokeratin (especially), GH, and the α-subunit. The protein cytokeratin makes up the cytoskeleton of cells and sometimes forms fibrous bodies [19–21] that are rarely seen in normal pituitary cells, but are frequently observed in GH-producing pituitary adenomas; this finding suggests a functional abnormality of GH-producing pituitary adenomas.

Compared with the classification by Kovacs and Horvath [22], type 1 corresponded mainly to sparsely granulated somatotrophic adenoma, including acidophil stem cell adenoma and mixed sparsely GH-PRL cell adenoma. Type 2 seems to correspond mainly to densely granulated somatotrophic adenoma, including mammosomatotrophic cell adenoma and mixed densely granulated GH-PRL cell adenoma. Type 3 may corres-

pond to mixed GH-PRL cell adenoma and to other types [1, 23, 24, 25]. These different cell types seem to reflect pathophysiological as well as histological differences.

When the clinical features of the patients were evaluated, those with type 1 disease tended to be younger and to have a lesion frequently involving the sella turcica, but those with type 2 tended to be older and often had microadenoma.

Plasma GH responses to TRH and GHRH were lower in type 1 than in type 2 cases. Glucose administration induced lower plasma GH levels in type 1 than in type 2. Plasma GH levels in type 1 were not as suppressed as in type 2 after the administration of bromocriptine or octreotide. Although there have been many reports on GH responses to provocative and suppressive agents in acromegalic patients, our results clearly show that the adenoma cells of type 1 and type 2 respond differently to these agents [12–16, 26].

The data suggest that pituitary adenomas of the two types have different abilities to synthesize and secrete GH in these loading tests. The reason for this difference is not yet known, but several

mechanisms can be postulated. First, different cytokeratin distribution may reflect different cellular dysfunction, resulting in changes in sensitivity to stimulatory and inhibitory agents. Second, the characteristics of the receptors to GHRH and somatostatin might be quite different. In particular, the difference in GH response to octreotide suggests that a difference in affinity and the number of somatostatin receptors may exist [27–29]. Third, the amount of production and secretion of GH per cell in type 1 is possibly lower than in type 2, because type 1 was a larger tumor and had roughly the same basal GH level as those of type 2. This may be related to lower GH responses to TRH, GHRH, and glucose in type 1.

Out typing of GH-producing pituitary adenomas seems to be useful for following: 1) a classification based on immunohistochemical findings is technically simpler than that in an electron microscopic study; 2) plasma GH responses to various tests enable us to predict to some degree the immunohistological characteris-

tics of the adenoma; 3) these characteristics would possibly predict tumor size, aggressive extension, postoperative recurrence, and ineffectiveness of bromocriptine and octreotide therapy [30–32]. The differences in cytokeratin distribution might indicate certain dysfunction of the tumor cells, but this remains to be investigated in the future.

In summary, GH-producing pituitary adenoma can be classified into two main types based on the cytokeratin immunohistochemistry; different clinical features and GH responses are observed. These findings provide data on the efficacy of treatment and prognosis in patients with GH-producing pituitary adenoma.

Acknowledgments

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