A Functional Thyrotropin- and Growth Hormone-Secreting Pituitary Adenoma with a Ultrastructurally Monomorph Feature: A Case Study

YUKAKO OZAWA, TORU KAMEYA*, AKIRA KASUGA, HEIJI NARITAKA**, NAOKO KANDA, HIROSHI MARUYAMA, AND TAKAO SARUTA

Abstract. A 38-yr-old female with a TSH- and GH-secreting pituitary adenoma is described, who had both overt symptoms, hyperthyroidism and acromegaly. Her serum TSH was not suppressed despite high concentrations of free T3 and free T4, and her α-subunit/TSH molar ratio was high. Her serum GH was consistently high, and was not suppressed by an oral glucose tolerance test. Preoperative testing revealed that, although the TSH response was impaired, TSH, α-subunit and GH were increased by TRH injection, and that these hormones were reduced by bromocriptine or somatostatin analog. Although she did not have hyperprolactinemia, the in vitro culture and immunohistochemical studies revealed that the adenoma cells produced and released PRL, in addition to TSH, α-subunit and GH. Immunohistochemical studies showed the presence of GH in the cytoplasm of many adenoma cells. TSHβ-positive adenoma cells were less frequently seen than GH-positive adenoma cells. No cells showed the coexistence of GH and TSHβ, and a few cells were positive for PRL. By electron microscopy, the adenoma was found to be composed of a single cell type resembling thyrotrophs, and did not have any characteristics of somatotrophs. This case was considered to be of interest, because the adenoma was ultrastructurally monomorphous, but immunohistochemically polymorphous.

Key words: Pituitary adenoma, GH, TSH, Acromegaly, Hyperthyroidism

Received: July 30, 1997
Accepted: January 12, 1998
Correspondence to: Dr. Yukako OZAWA, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan
had overt symptoms of hyperthyroidism and acromegaly. We examined the effects of hypothalamic releasing hormone, bromocriptine, or somatostatin analog on the adenoma in *in vivo* functional tests. To examine the function of the adenoma cells *in vitro*, we cultured cells and measured the levels of secreted pituitary hormones in the medium. We also performed morphological studies including immunohistochemistry and electron microscopy to examine whether this adenoma consisted of monomorphous or polymorphous cells.

**Case Report**

*Patient*

In 1994, a 38-yr-old woman consulted her physician for gradual acral enlargement accompanied by palpitation and increased perspiration. Her serum GH was high, and computerized tomography revealed a pituitary tumor. Her serum thyroid hormones were slightly high, although TSH levels were not suppressed. She was diagnosed with acromegaly owing to a pituitary adenoma, along with Graves’ disease. An antithyroid agent improved the symptoms, but her thyroid gland became enlarged, and she was referred to our hospital for further examination. Physical examination showed acromegalic features, such as increase in the size of the fingers, pronounced nose and thick lips. The thyroid gland was soft and extremely enlarged. Her hands were warm and moist, and had a slight tremor, but there was no exophthalmos or pretibial dermopathy. Her blood pressure and heart rate were high (140/90 mmHg and 120 beats/min, respectively). Her basal metabolic rate was +17%. Her visual field was normal. Laboratory examinations showed slightly increased alkaline phosphatase (464 IU/l) and low total cholesterol (94 mg/dl).

Her GH remained consistently high (Table 1). An oral 75g glucose tolerance test revealed impaired glucose tolerance (plasma glucose; 180 mg/dl at 120 min), but GH was not suppressed by high serum glucose. The level of insulin like growth factor I was 944 ng/ml (79–383 ng/ml). Her TSH which was high on admission was reduced to the normal range and her thyroid gland decreased in size after discontinuation of the antithyroid agent (Table 1). TSH was not suppressed despite the high concentrations of thyroid hormones (TSH 3.7 mU/l, free T3 9.3 pg/ml, free T4 3.4 ng/dl). The α-subunit (2.94 ng/ml) and α-subunit/TSH molar ratio (7.6) were high. The levels of LH (7.0 IU/l), FSH (4.2 IU/l), PRL (14.7 ng/ml), and ACTH (21 pg/ml) were normal.

An MRI scan revealed a large homogenous sellar mass with bony destruction. The maximal diameter was 5.4 cm. Thyroid scintiscan showed an enlarged thyroid gland with homogeneous uptake; 123I uptake was +82% at 24-h. Antithyrogloblin, antimicrosomal autoantibody, and TSH receptor antibody were negative. Anti-T3, anti-T4 and human anti-mouse antibodies were also negative. Sex hormone-binding globulin was 130 nmol/l (18.6–117). The serum osteocalcin level was 23 ng/ml (2.3–9.9).

<table>
<thead>
<tr>
<th>Table 1. Serum hormone levels and parameters of thyroid function while taking and after discontinuation of methimazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameter</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>GH</td>
</tr>
<tr>
<td>f-T3</td>
</tr>
<tr>
<td>f-T4</td>
</tr>
<tr>
<td>TSH</td>
</tr>
<tr>
<td>α-subunit</td>
</tr>
<tr>
<td>α/α-TSH molar</td>
</tr>
<tr>
<td>BMR</td>
</tr>
<tr>
<td>123I uptake (24 h)</td>
</tr>
<tr>
<td>Total cholesterol</td>
</tr>
</tbody>
</table>

MMI, methimazole; ND, not determined.
TSH- AND GH-SECRETING PITUITARY ADENOMA

Functional test

A TRH infusion test was performed by infusing 500 μg TRH i.v. over a one minute period and blood samples were obtained to measure TSH, PRL and GH at 0, 15, 30, 60, 90 and 120 min, and α-subunit at 0 and 15 min. To investigate the effect of exogenous T3 on the responses of these hormones to TRH, the TRH infusion test was also done after a daily 50 μg oral administration of T3 for 8 days. A GnRH infusion test (200 μg i.v.) was performed in which blood samples were obtained for TSH, GH, α-subunit, LH and FSH at the same intervals as in the TRH test. A GHRH infusion test (100 μg i.v.) was performed in which blood samples were obtained for TSH and GH at 0, 15, 30, 60, 90, and 120 min, and for α-subunit at 0 and 120 min. Bromocriptine administration (2.5 mg. p.o.) was followed by venous blood sampling at 0, 60, 120, 180, 240 and 360 min for TSH, GH and PRL, and at 0 and 240 min for α-subunit. Octreotide acetate (50 μg) was administrated subcutaneously, followed by venous blood sampling at 0, 1, 2, 4, 6, 8 and 24 h for TSH and GH, and at 0 and 2 h for α-subunit.

Morphologic studies

For light microscopy, pieces of tumor tissue were fixed in 10% buffered formalin or U-Fix (Sakura-Seiki, Co., Tokyo) and embedded in paraffin. For immunohistochemistry of anterior pituitary hormones including GH, PRL, LHβ, FSHβ, TSHβ, α-subunit and ACTH, respective antisera (NADDKD, Bethesda, U.S.A. and DAKO, Kyoto, Japan) were used in the avidin-biotin-peroxidase complex method as described elsewhere [18]. 3',5'-diaminobenzidine (DAB) was used for visualization. For electron microscopy, small pieces of tumor tissue were fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide, dehydrated and embedded in epoxy-resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEM 1200 EX electron microscope.

In vitro studies

On April 20, 1994, transsphenoidal surgery was performed. Tissue culture was performed as previously described [19]. In brief, immediately after removal by surgery, the pituitary adenoma tissue was placed in ice-cold Dulbecco's minimal essential medium (Life Technologies, Inc, Grand Island, NY, USA) containing 10% fetal calf serum, 1 × 10⁶ U/l penicillin, and 100 mg/l streptomycin. The tissue was cut into small pieces and incubated with 0.1% collagenase (type IV, Worthington Biochemical Corporation, Freehold, NJ, USA) at 37 °C for 1 h, followed by gentle mechanical dispersion with a Pasteur pipette. After washes, 5 × 10⁶ cells suspended in 2 ml of medium were precultured in a 24-well plate for 6 days, with medium change every 2 days, at 37 °C in an atmosphere of 5% CO₂ in air. After the preculture, the tissue was incubated for 2 days, and the medium was collected for hormone assays of GH, TSH, α-subunit, PRL, LH, and FSH. A sample of pituitary adenoma tissue of a patient with acromegaly was used as a control. The adenoma cells were found to produce only GH by immunohistochemistry. All the tests were performed in quadruplicate, and the results were described as the mean ± standard deviation.

Assays

Serum GH, TSH, FSH, and LH were determined by immunoradiometric assay with commercial kits (Daiichi RI Laboratory, Tokyo, Japan). The crossreactivity of TSH with LH, FSH or hCG in the assays was negligible. Serum α-subunit levels were also determined by an immunoradiometric assay (Medical System Service Kanagawa, Kanagawa, Japan). PRL was determined by a time resolved fluoro-immunoassay with a commercial kit (Kabi Pharmacia Diagnostics, Tokyo, Japan). Free T3 and T4 were measured by RIAs (Ortho-Clinical Diagnostics KK, Tokyo, Japan). The α-subunit/TSH molar ratio was calculated on the basis of the following molecular weight values: TSH, 28,000, and α-subunit, 14,700 (1 ng TSH corresponding to 4.93 μU).

Results

Endocrine studies

Initial studies suggested that this patient had hyperthyroidism due to TSH-secreting pituitary adenoma, since her serum TSH levels were measurable, despite the high concentration of
thyroid hormones, and the α-subunit/TSH molar ratio was more than 1. The hyperthyroid state was confirmed by high sex hormone-binding globulin [20] and osteocalcin concentrations [21]. The adenoma concomitantly secreted GH, since the patient had acromegalic features, and high GH and IGF-I.

Intravenous TRH induced a significant increase in serum GH (+426%, 13.4 to 70.5 ng/ml), α-subunit (+127%, 2.94 to 6.69 ng/ml) and PRL (+135%, 13.9 to 32.7 ng/ml), but induced only a slight increase in serum TSH (+70%, 3.7 to 6.8 mU/l) (Fig. 1). After T3 administration, there were no significant changes in thyroid hormone levels (free T4, 9.3 to 9.6 ng/dl), basal metabolic rate (17% to 16%), 123I uptake at 24 h (82% to 81%), and TRH infusion test (Fig. 1). The GH, TSH, and α-subunit levels were not affected by GHRH injection (Fig. 2). GnRH infusion did not affect the levels of GH and TSH, but slightly increased the level of α-subunit (+60%, 2.41 to 3.97 ng/ml). Bromocriptine administration induced a paradoxical decrease in serum GH (−82%, 13.2 to 2.5 ng/ml). It also reduced TSH (−65%, 3.7 to 1.3 mU/l), α-subunit (−35%, 3.05 to 2.01 ng/ml), and PRL (−76%, 15.2 to 3.7 ng/ml) (Fig. 2). Octreotide acetate injection reduced GH (−71%, 6.3 to 1.8 ng/ml), TSH (−50%, 1.9 to 0.8 mU/l) and α-subunit (−31%, 2.15 to 1.49 ng/ml) (Fig. 2).

Morphologic studies

On light microscopy, the tumor consisted mainly of chromophobic cells and with some eosinophilic cells. It was not pleomorphic and contained few mitotic figures. Immunoperoxidase staining revealed that an appreciable number of adenoma cells contained immunoreactive GH, TSHβ, or α-subunit in the cytoplasm. GH-positive cells were much more numerous than TSHβ-positive cells, although some TSHβ-cell rich areas were occasionally found. A small number of cells that were immunostained positive for PRL were also observed. Other pituitary hormones such as LH, FSH and ACTH were not detected. Consecutive sections revealed that GH-positive cells were not positive for TSHβ, and that TSHβ-positive cells were not positive for GH. There were no cells which coexpressed GH and TSHβ, despite an extensive review of over 5 section pairs.

Fig. 1. Responses of TSH, GH, PRL after TRH injection (500 µg). The dotted line and solid line show the responses before and after oral T3 administration, respectively.
immunostained for GH and TSHβ (Fig. 3). By electron microscopy, we found that the tumor consisted of one cell type characterized by the presence of dense-core secretory granules (100–200 nm), and a lack of GH-type large secretory granules and of fibrous bodies characteristic of sparsely granulated GH-cell adenomas (Fig. 4). No abnormal exocytoses were seen.

**In vitro studies**

When cultured in vitro, the adenoma fragments had a high secretory activity (Table 2). Only a few fibroblasts were seen. The levels of GH, TSH, PRL and α-subunit in the medium were 336 ± 8.7 ng/ml, 10.7 ± 1.9 mU/l, 13.8 ± 2.6 ng/ml and 10.5 ± 2.1 ng/ml, respectively. The α-subunit/TSH molar ratio was high (9.6). In contrast, the control adenoma cells from an acromegalic patient released GH but no other pituitary hormones.

**Discussion**

We reported a patient with a TSH- and GH-secreting pituitary adenoma who manifested the symptoms of both hyperthyroidism and acromegaly. It has been reported that approximately 40% of TSH-secreting pituitary adenomas concomitantly produced other pituitary hormones [3, 4], but there have only been about 30 cases which showed symptoms of both hyperthyroidism and acromegaly [7–12]. Furthermore, only a few researchers have performed in vitro culture or intensive morphologic studies of such multihormone-producing adenoma cells.

Several diagnostic tests, such as sensitive TSH radioimmunoassay techniques or α-subunit/TSH molar ratio, have proved useful in the evaluation of hyperthyroidism, but most patients with TSH-secreting pituitary adenoma are still misdiagnosed, because the TSH level usually seems to be normal. The patient in this report was also misdiagnosed and treated initially with an antihyperthyroid agent. Although her thyroid hormone level was normalized by the antihyperthyroid agent, TSH increased to 13 mU/l and her thyroid gland became extremely large. This suggested that the TSH secretion was partially modified by peripheral thyroid hormone, and this was supported by the observation that administration of T3 slightly reduced the TSH level in this case. In the literature, seven of 15 patients with TSH-secreting tumor had similar responses [11].
In this patient, TRH injection increased the levels of TSH, α-subunit and GH, although the TSH response was impaired. Kuzuya et al. [19] reported that these three hormones were increased by GnRH or GHRH injection in a patient with a TSH- and GH-secreting tumor, but our patient did not show such responses. In the case reported by Beck-Pecco et al. [9], GH and α-subunit levels were decreased by bromocriptine or octreotide, but TSH was unaffected. On the other hand, TSH, α-subunit and GH were reduced by bromocriptine or octreotide in our patient. These findings suggested
that the clinical characteristics of combined TSH- and GH-secreting adenomas are not uniform.

Immunohistochemical examination confirmed that the adenoma of this patient was a combined TSH- and GH-secreting tumor. In vitro studies also revealed that the adenoma cells secreted TSH and GH. Several reports have described the morphologic features of TSH- and GH-secreting adenomas. Kuzuya et al. [19] reported an adenoma consisting of two distinct cell types; thyrotroph-like cells containing TSHβ and somatotroph-like cells containing GH. Carlson et al. [11] and Malarkey et al. [8] reported similar findings to Kuzuya et al., but in the case of Malarkey et al., some cells contained both GH and TSHβ in the same secretory granules. Kovacs et al. [22] reported a monomorphous adenoma composed of thyrotroph-like cells which secreted both GH and TSHβ, and the patient showed signs of acromegaly but not hyperthyroidism. Clone et al. [23] reported a monomorphous adenoma which contained characteristics of thyrotroph, but some adenoma cells had fibrous bodies resembling those seen in sparsely granulated GH cell adenomas, and the patient had hyperthyroidism but not acromegaly. Considering these findings, TSH- and GH-secreting pituitary adenomas may have heterogeneous features with a pathogenesis that differs from case to case. In our case, the adenoma was composed of single thyrotroph-like cells which did not have any features of a densely or sparsely granulated somatotroph. A considerable number of adenoma cells were positive for GH, but the cells were much less frequently positive for TSHβ. No cells coexpressed both GH and TSHβ. Some cells were positive for both GH and α-subunit. The sensitivity of TSHβ staining, including the weak immunogenicity of TSHβ, may have affected the results, but GH-positive cells were distinct from TSHβ-positive cells in this study. This is therefore of particular interest because it was ultrastructurally monomorphous but immunohistochemically polymorphous.

Sanno et al. [24] recently reported that GH and PRL mRNA were expressed in five TSH-secreting pituitary adenomas which were clinically shown to produce TSH alone. It is of interest that all five adenomas expressed Pit-1 protein in their adenoma cells, which is a transcriptional regulator of GH, PRL and TSH [25, 26]. Whether Pit-1 is essentially related to the development of multihormone-producing pituitary adenomas remains unknown, but this report suggest that TSH-secreting adenomas may produce more than one hormone. In our patient, immunohistochemistry and in vitro culture studies confirmed the production of PRL, although the patient did not show any sign of galactorrhea. Whether the adenoma causes acromegaly or galactorrhea in addition to hyperthyroidism may depend on translational conditions, bioactivity or the secretory conditions of those hormones.

Surgery is considered to be the best treatment for TSH-secreting pituitary adenomas [3, 4], but less than 40% of the patients are cured by surgery [6]. In our patient, the adenoma was not completely resected due to its large size and an association with bony destruction. In the literature, there are reports of several medications having been tried for treatment of residual or inoperable tumors. The effect of bromocriptine on TSH-secreting tumors is controversial [1, 11, 14, 27]. Octreotide acetate has been reported to be effective for TSH- and GH-secreting pituitary adenomas [8, 27], and it has also reduced the size of some TSH-secreting [28, 29] or GH-secreting adenomas [30]. Our patient is being treated with bromocriptine, which normalized the levels of GH, but moderate hyperthyroidism remains, and further treatment will be needed.

In summary, biochemical and morphologic findings confirmed that this pituitary adenoma produced TSH and GH, and to a lesser degree PRL. The mechanism by which the potential multihormone producing ability is accelerated and leads to the development of clinical symptoms remains to be determined. Increased awareness of these tumors will contribute to further examination and to prompt diagnosis which will offer the best chance for a surgical cure.

Acknowledgment

We thank Mrs. T. Tsuruta and the staff of the EM Center, Kitasato University School of Medicine, for technical assistance.
References


21. Lukert BP, Higgins JC, Stoskopf MM (1986) Serum osteocalcin is increased in patients with hyperthyroidism and decreased in patients...


