NOTE

Evaluation of growth hormone-releasing peptide-2 for diagnosis of thyrotropin-producing pituitary adenomas

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Abstract. Thyrotropin (TSH)-producing adenomas are a rare cause of hyperthyroidism and are a type of functional pituitary adenoma. The diagnosis of TSH-producing adenoma is a challenging problem in clinical endocrinology. Since growth hormone-releasing peptide-2 (GHRP-2) fails to induce TSH secretion in normal subjects, the effect of GHRP-2 on TSH levels was therefore examined in patients with TSH-producing adenomas. A total of 5 patients (4 women and 1 man) referred to our departments for further evaluation of pituitary hormones were followed-up using the GHRP-2, TSH-releasing hormone (TRH), octreotide, and bromocriptine tests to examine and evaluate TSH secretory dynamics in TSH-producing adenomas. Of 5 patients, 2 (40%) showed such a significant response, defined as a >50% increase in serum TSH level above baseline in the GHRP-2 test. Additionally, 1 patient showed a 48% increase in serum TSH level. In 1 patient whose adenoma was completely removed, basal serum concentrations of TSH were sufficiently suppressed after the operation, and serum TSH levels failed to increase in response to GHRP-2 administration. In 4 patients (80%), a poor response of serum TSH levels was observed in the TRH test. In 2 out of 5 patients (40%), serum TSH levels were significantly decreased following octreotide administration. No patient demonstrated a significant response to the bromocriptine test. In addition to TRH test, the GHRP-2 test as a potential diagnostic tool for TSH-producing pituitary adenomas.

Key words: Growth hormone-releasing peptide, Thyrotropin, Hypothalamus, Pituitary adenoma

THYROTROPIN (TSH)-PRODUCING ADENOMAS

can cause a chronic elevation in thyroid hormones that may be accompanied by clinical thyrotoxic signs and symptoms [1]. Pituitary adenomas are a rare cause of hyperthyroidism and result from the functional TSH-producing form of pituitary adenomas [2]. TSH secretion is normally tightly regulated by negative feedback from thyroid hormones, but TSH-producing adenomas may disrupt these feedback mechanisms. The following are

considered the diagnostic criteria for TSH-producing pituitary adenoma [3]: 1) normal to high levels of TSH in the blood despite high levels of thyroid hormones; 2) presence of a pituitary adenoma on magnetic resonance imaging; and 3) positive immunological staining results for TSH. However, the diagnosis of TSH-producing adenoma can sometimes be a challenging problem in clinical endocrinology [4].

Growth hormone (GH)-releasing peptides (GHRPs) are synthetic peptides that induce a strong response in the release of GH in both animals and humans [5], and act *via* a receptor that is normally specific for ghrelin [6]. GHRPs and ghrelin also stimulate the release of adrenocorticotrophic hormone (ACTH) *via* corticotropin-releasing factor and/or arginine vasopressin in the hypothalamus [7, 8]. Among the GHRPs, GHRP-6 has been studied extensively in Europe [5], while GHRP-2 is currently available for clinical use in Japan [9] and may

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be useful for the diagnosis of secondary adrenal insufficiencies such as hypothalamic disorders and pituitary damage [10]. The blood levels of GH, ACTH, and prolactin, but not TSH, are increased after GHRP-2 injection in healthy men [11]. In the present study, we examined whether serum TSH levels were paradoxically increased in response to GHRP-2 administration in TSH-producing adenomas, and evaluated the GHRP-2 test as a potential diagnostic tool for TSH-producing pituitary adenoma.

Patients and Methods

Patients

A total of 5 patients (4 women and 1 man) referred to our departments in Hirosaki University School of Medicine & Hospital and Yamagata University School of Medicine & Hospital for further evaluation of pituitary hormones were followed-up using the GHRP-2, TSH-releasing hormone (TRH), octreotide, and bromocriptine tests to examine and evaluate TSH secretory dynamics in TSH-producing adenomas. This study was approved by the Ethics Committee of the Hirosaki University School of Medicine (No. 2017-1080), and written informed consent was obtained from all participants.

TSH response tests

GHRP-2 test

A single dose (100 µg) of GHRP-2 (Kaken Seiyaku Co., Tokyo, Japan) was injected intravenously under fasting conditions. Blood samples were taken before and at 15, 30, 45, and 60 min after injection, and serum TSH, GH, cortisol, and ACTH levels were determined.

TRH test

A single dose (500 µg) of TRH (Tanabe Pharma, Osaka, Japan) was injected intravenously under fasting conditions. Blood samples were taken before and at 15, 30, 60, 90, and 120 min after injection, and serum TSH levels were determined. An increase of more than two-fold in serum TSH concentration, compared with the basal level, was considered to be a significant response.

Octreotide test

A single dose (100 µg) of octreotide acetate (Novartis Pharma, Basel, Switzerland) was injected subcutaneously under fasting conditions. Blood samples were taken before and at 2, 4, 6, and 8 h after injection, and serum TSH levels were determined. A decrease of more than 50% in serum TSH concentration, compared with the basal level, was considered to be a significant response.

Bromocriptine test

A single dose (2.5 mg) of the dopamine receptor agonist bromocriptine mesylate (Novartis Pharma, Basel, Switzerland) was administered *per os* under fasting conditions. Blood samples were taken before and at 1, 2, 4, 6, and 8 h after injection, and serum TSH levels were determined. A decrease of more than 50% in serum TSH concentration, compared with the basal level, was considered to be a significant response.

Results

Patient characteristics

The characteristics of the 5 patients are shown in Table 1. The 5 patients included 1 male and 4 females, with ages ranging from 32 to 61 years. All patients had pituitary macroadenomas (>1 cm), and their tumor sections were confirmed the expression of the TSH by TSH immunostaining.

TSH response to GHRP-2

Since GHRP-2 fails to induce TSH secretion in normal subjects, the effect of GHRP-2 on TSH levels was examined in the patients. No patient showed a greater than 2-fold increase in TSH following the GHRP-2 test. When a greater than 50% increase in serum TSH level following the GHRP-2 test compared with the basal level was considered a significant response, 2 of 5 patients (40%; Cases 1 and 2) showed such a response. Additionally, 1 patient showed a 48% increase in serum TSH level (Case 4).

In Case 1, serum TSH levels were increased in response to intravenous TRH administration (Table 1), and were paradoxically increased in response to the administration of 100 µg GHRP-2 (Fig. 1A). Octreotide administration tended to decrease serum TSH levels (0 h, 4.87 µIU/mL; 8 h [nadir], 2.83 µIU/mL). Bromocriptine administration produced no observable effect on TSH levels (0 h, 7.69 µIU/mL; 8 h [nadir], 7.03 µIU/mL), although it decreased serum prolactin concentrations (0 h, 13.9 ng/mL; 6 h [nadir], 2.0 ng/mL). The diagnosis of TSH-producing pituitary adenoma was made and the adenoma was incompletely resected through transsphenoidal neurosurgery. At 3 weeks after the operation, the basal serum concentrations of free thyroxine (fT4, 2.36 ng/dL), free triiodothyroxine (fT3, 6.47 pg/mL), and TSH (7.53 µIU/mL) remained elevated. Serum TSH levels were slightly elevated in response to GHRP-2 (Fig. 1A). The residual tumor was treated by injections of octreo-

Table 1 Clinical and laboratory features. Changes in serum TSH levels in response to each test are indicated at the “basal level/peak or nadir level.”

Case	Age (years)	Sex (M/F)	GHRP-2 test										Tumor size (mm)	fT3/fT4 ratio (pg/mL)/ (ng/dL)
			Pre-operation		Post-operation		TRH test		Octreotide test		Bromocriptine test			
			Increase (%)	TSH (μIU/mL) base/peak	Increase (%)	TSH (μIU/mL) base/peak	Response	TSH (μIU/mL) base/peak	Response	TSH (μIU/mL) base/nadir	Response	TSH (μIU/mL) base/nadir		
1	52	F	198	5.81/11.50	188	3.46/6.51	+	7.35/16.53	–	4.87/2.83	–	7.69/7.03	Macro (18 × 20 × 13)	3.14
2	32	F	172	4.08/7.00	133	0.06/0.08	–	3.72/4.96	+	4.78/2.29	–	2.47/2.17	Macro (16 × 12 × 16)	3.43
3	61	F	106	4.45/4.71		NP	–	3.99/4.71	–	3.78/2.14	–	4.74/3.65	Macro (20 × 15 × 16)	2.11
4	40	F	148	7.96/11.81		NP	–	9.06/17.7	–	7.22/3.87		NP	Macro (20 × 18 × 17)	2.60
5	44	M	132	4.06/5.37	114	0.76/0.87	–	6.73/7.08	+	5.20/2.44	–	6.05/3.97*	Macro (7 × 11 × 11)	3.04

A significant response is indicated as +. No significant response is indicated as –. Test not performed is indicated as NP.

GHRP-2, growth hormone-releasing peptide-2.

* Cabergoline 0.25 mg

Fig. 1 GHRP-2 test in the pre- and post-operative periods. Changes in serum TSH, GH, cortisol, and ACTH levels in response to GHRP-2 are shown as open circles, closed circles, open squares, and closed squares, respectively. (A) Case 1. (B) Case 2.

tide long-acting release (LAR) (10 mg/month).

In Case 2, serum TSH levels were increased by GHRP-2 administration. The pituitary adenoma was completely removed in this case. Basal serum concentrations of TSH were sufficiently suppressed after the operation, and serum TSH levels failed to increase in response to GHRP-2 administration (Fig. 1B).

TSH response to TRH, octreotide, and bromocriptine

In 4 patients (80%), a poor response of serum TSH levels was observed in the TRH test. One of the patients with a significant increase of TSH (Case 1) following the TRH test also showed a significant response to GHRP-2. In 2 out of 5 patients (40%), serum TSH levels were significantly decreased following octreotide administration. One patient with a significant response to octreotide

(Case 2) also had a significant response to GHRP-2, but the other patient with a significant response to octreotide (Case 5) showed no response to GHRP-2. No patient demonstrated a significant response to the bromocriptine test.

Discussion

In contrast to patients with resistance to thyroid hormone, a previous study reported that 81% of TSH-producing adenomas show a poor response of serum TSH levels following the TRH test [12]. Consistent with the study [12], in our study, serum TSH levels showed a poor response following TRH test in 4 out of 5 patients (80%). While the TRH test would be indicative of TSH-producing adenoma [13], pituitary apoplexy might

be caused by TRH test in pituitary macroadenomas. Additionally, the presence of a mutation of thyroid receptor β may be useful to diagnose resistance to thyroid hormone. However, this test would be expensive, and be requested primarily in uncertain cases without a clear-cut macroadenoma, or a first-degree relative with similar characteristics or patients with suspected resistance to thyroid hormone [13].

GHRP-2 is currently available for clinical use in Japan [9] and can be useful in the diagnosis of severe GH deficiency because it is known to stimulate GH release. GHRP-2 is also known to induce ACTH, but not TSH, release in healthy subjects [14, 15]. In fact, Kamoi *et al.* showed that intravenous administration of GHRP-2 failed to modulate plasma TSH levels (from 1.28 ± 0.18 μ IU/mL to 1.32 ± 0.19 μ IU/mL) in Japanese healthy subjects [11]. In the present study, some cases with TSH-producing pituitary adenomas showed a significant increase of serum TSH concentration in response to GHRP-2. However, the mechanisms underlying this response of TSH are unclear. Machado *et al.* reported a positive association between the expression levels of GHRP receptor type 1a and *in vivo* responses to GHRP-6 in Cushing's disease [16]. Therefore, it is plausible that GHRP-2 also stimulates the release of TSH *via* GHRP receptor type 1a in TSH-producing tumors, which would make the GHRP-2 test a useful diagnostic tool for TSH-producing tumors.

Although a greater than 1.5-fold increase in TSH levels was considered to be a significant response in this study, the diagnostic criteria of the GHRP-2 test for TSH-producing pituitary adenomas have yet to be determined. As mentioned before, GHRP-2 did not modulate plasma TSH levels at all in healthy subjects [11]. Therefore, a greater than 1.5-fold increase in TSH levels might be tight criteria. Evaluation in a total of 5 patients is limited, and future research should examine the effect of GHRP-2 in large-scale studies. The GHRP-2 test may be a sensitive screening test for TSH-producing pituitary adenoma and it may be able to discriminate it effectively from thyroid hormone resistance syndrome.

The pituitary adenoma was not removed completely in Case 1 and the basal serum concentrations of TSH

remained elevated even after the operation. Serum TSH levels were also found to be increased in response to GHRP-2 in this case. The inhibitory effect of octreotide and the lack of an effect of a dopamine agonist on TSH levels are typical characteristics of a TSH-producing tumor. Octreotide LAR has been shown to be a useful and safe therapeutic treatment for TSH-producing adenomas after incomplete surgery [17, 18] and was therefore determined to be the most appropriate treatment for the residual tumor in this case. Conversely, in Case 2 with a TSH-producing adenoma, serum TSH levels were increased by the administration of GHRP-2. The pituitary adenoma was completely removed in this case. Basal serum concentrations of TSH were sufficiently suppressed after the operation, and serum TSH levels failed to increase in response to GHRP-2 administration. Although the diagnostic criteria of the GHRP-2 test after operation should be discussed, both suppressed TSH levels and GHRP-2 test may be also useful for evaluate the residual tumor after the operation.

In summary, in addition to TRH test, the GHRP-2 test, currently available for clinical use in Japan, may be a useful diagnostic tool for TSH-producing pituitary adenomas. Although functional TSH-producing adenomas are rare, further evidence supporting its use needs to be generated in future studies.

Compliance with Ethical Standards

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Disclosure potential conflicts of interest

None of the authors have any potential conflicts of interest associated with this research. This study was approved by the Ethics Committee of the Hirosaki University School of Medicine (No. 2017-1080).

Authors' contributions

All authors were concerned with the treatment, drafted the manuscript, and approved the final manuscript.

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