Somatostatin Receptor Subtypes mRNA in TSH-Secreting Pituitary Adenomas: A Case Showing a Dramatic Reduction in Tumor Size During Short Octreotide Treatment

KAZUHIKO HORIGUCHI, MASANOBU YAMADA, RYOHEI UMEZAWA, TETURO SATOH, KOSHI HASHIMOTO, MASAKI TOSAKA*, SHOZO YAMADA** AND MASATOMO MORI

Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan
*Department of Neurosurgery, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan
**Department of Hypothalamic and Pituitary Surgery, Toranomon Hospital, Minato-ku, Tokyo 105-8470, Japan

Abstract. TSH-secreting adenoma is a rare pituitary adenoma, and the expression levels of the specific subtypes of somatostatin receptors (sstr) mRNAs have remained obscure. To determine the quantitative expression of the sstr1-5 mRNAs in TSH-secreting adenomas that may be related to the efficacy of treatment with a somatostatin analogue, expression of the sstr1-5 mRNAs was examined and compared in TSH-secreting adenomas and other pituitary adenomas. The pituitary adenomas were obtained at transsphenoidal surgery from 4 cases of TSH-secreting adenoma, including 1 patient showing a significant shrinkage of the tumor size after only 10 days of octreotide treatment, 2 patients without tumor size reduction and 1 patient without treatment, and 5 GH-secreting adenomas, 6 prolactinomas, 5 nonfunctioning adenomas, 4 ACTH-secreting adenomas and normal pituitaries at autopsy from 4 normal subjects. In comparison to the normal pituitary, sstr2A>sstr1>sstr5>sstr3 mRNAs were expressed in the TSH-secreting adenomas examined. No expression of sstr2B or sstr4 mRNA was observed. The expression level of sstr2 mRNA was significantly higher than those in normal pituitary, prolactinomas, ACTH-secreting and nonfunctioning pituitary adenomas. The patient with marked shrinkage of the tumor showed the highest expression of both sstr2 and sstr5 mRNAs among all the cases of pituitary adenoma. A TSH-secreting tumor without shrinkage showed a similar expression level of sstr2 mRNA. These findings demonstrated that TSH-secreting adenomas express sstr1, 2A, 3 and 5 mRNAs, predominantly sstr2A, and in addition to the expression of sstr2 mRNA, the expression level of sstr5 mRNA may be a factor affecting the tumor shrinkage by somatostatin analogues against TSH-secreting adenomas.

Key words: TSH-secreting adenoma, Sstr, Octreotide

THYROTROPIN (TSH)-secreting pituitary adenomas are a rare form, representing less than 1% of all pituitary adenomas [1, 2]. The molecular mechanisms underlying their tumorigenesis and the molecular nature of these tumors are not well understood [3]. The presence of somatostatin receptors on TSH-secreting adenomas demonstrated by in vitro studies and octreotide scintigraphy has led to treatment with somatostatin analogs such as octreotide and lanreotide [4, 5]. Indeed, treatment with these somatostatin analogues suppresses TSH secretion in more than 90% of patients, normalizes the serum thyroid hormone level in about 70% of the patients, and decreases the adenoma size in about 50% of cases [1, 5–7]. Somatostatin (SRIF) is widely distributed throughout the central nervous system and peripheral tissues [8]. Since SRIF inhibits the secretion of hormones from the anterior pituitary and other endocrine organs, SRIF and its analogue have been used to treat the
patients with pituitary adenomas, particularly GH-secreting adenomas and gastroenteropancreatic tumors, including insulinomas and gastrinomas [4]. SRIF acts through a family of G-protein-coupled membrane receptors containing seven transmembrane domains, and five distinct SRIF receptor (sstr) subtypes have been identified. It has been reported that octreotide and lanreotide bind with high affinity to sstr2 and less efficiently to sstr5 [9]. Several studies have evaluated the expression of sstr subtypes in human pituitary adenomas, but discrepancies remain regarding their distribution [10–16]. It is, however, apparent that the majority of pituitary tumors express sstr2 mRNA, whereas sstr4 mRNA expression has only been reported in a few cases.

With respect to TSH-secreting adenomas, only two case reports showing the expression of the specific subtypes of sstr mRNA in TSH-secreting adenoma have been available to date [17, 18]. Even in these available reports, only a single case of TSH-secreting adenoma was evaluated. Furthermore, in these studies, the expression of sstr mRNA was studied using simple RT-PCR. Therefore, a precise comparison of the expression levels of specific subtype of sstrs in the TSH-secreting adenomas with those in other pituitary adenomas could not be carried out. The recently developed TaqMan probe technique provides a way to precisely measure the amount of mRNA even from a small mRNA sample. Furthermore, in the present study we had obtained a specimen of a TSH-secreting adenoma from a patient showing a dramatic reduction in tumor size after only 10 days of treatment with octreotide, and, in addition, specimens from 2 patients with no change of the tumor size after treatment and from 1 patient with no treatment. Therefore, we measured sstr1-5 mRNA levels in these TSH-secreting adenomas and then compared them with each other and with those in other pituitary adenomas.

**Subjects and Methods**

**Subjects**

As shown in Tables 1 and 2, the samples included 4 cases of TSH-secreting tumors, 5 GH-secreting adenomas, 6 prolactinomas, 5 nonfunctioning tumors, 4 ACTH-secreting tumors and 4 normal pituitaries. To exclude the possibility of contamination by the normal pituitary, we used macroadenomas throughout this study. Pituitary adenoma tissues were obtained from patients at the time of transsphenoidal surgery. Informed consent was obtained from each family, and this study was approved by the ethical committee on human research of Gunma University.

**RNA extraction and real-time PCR**

Some specimens of the pituitary adenomas were immersed in RNAlater (Qiagen, Tokyo, Japan) at the time of the operation; otherwise, specimens were frozen in liquid nitrogen immediately after removal at the operation. Total RNA was prepared from each adenoma and normal pituitary using ISOGREN (Nippongene, Toyama, Japan) according to the manufacturer’s instructions.

After cDNAs were reverse-transcribed from 300 ng of total RNA (TaqMan Reverse Transcription Reagents, Applied Biosystems, Tokyo, Japan), 0.5 µl of the product was subjected to real-time PCR. All reactions were performed in triplicate using TaqMan probes and an Applied Biosystems 7500 sequence detection system. TaqMan probes for somatostatin receptor sstr1 (Hs 00265617), sstr2 (Hs 00265647), sstr3 (Hs 00265633), sstr4 (Hs 00265639), sstr5 (Hs 00265624) and GAPDH (Hs 99999905) were purchased from Applied Biosystems. The expression level of each mRNA relative to that of GAPDH was calculated using a standard curve, and the relative quantification method described in ABI User Bulletin #2. All experiments were repeated at least twice.

To distinguish sstr2A mRNA from sstr2B mRNA, a simple RT-PCR was performed with primers 5' GAT GATCACCATGGCTGTG 3' and 5' CAGGCATG TCCCTCTTC 3', and the expected size of the amplified fragments of sstr2A and 2B mRNAs were 892 bp and 549 bp, respectively.

**Statistical analysis**

Results are expressed as mean ± SEM. Statistical analysis was performed by ANOVA followed by Wilcoxon’s signed rank test or by Student’s t test. A p-value less than 0.05 was considered statistically significant.
A 56-year-old man who had hand tremors, depression and sleeplessness consulted a psychologist. He was found to have an elevated serum thyroid hormone level, and was then referred to our department. The patient had a history of paroxysmal atrial fibrillation and had consulted a cardiologist since he was 53 years old. Physical examination revealed that his pulse rate was 90/min and irregular, a diffuse goiter and mild edema in the pretibial region. He did not have exophthalmus. His electrocardiogram showed atrial fibrillation. Laboratory data showed mild hypoproteinemia with 3.7 g/dl serum albumin level and low cholesterol level. Serum free T3 and T4 levels were 8.3 pg/ml (normal: 2.4–4.5) and 4.0 ng/dl (normal: 0.8–2.1). However, his serum TSH level, 4.74 mU/ml (normal: 0.5–5.5), was within normal limits, showing SITSH. His growth hormone level was normal and serum prolactin was slightly elevated (12.3 ng/ml).

As shown in Fig. 1, the MRI study showed a macroadenoma with sagittal, axial, and coronal diameters of 12 × 20 × 20 mm (tumor volume calculated using the DeChiro and Nelson formula: 10,112 cm$^3$) which

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**Case Reports**

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As shown in Fig. 1, the MRI study showed a macroadenoma with sagittal, axial, and coronal diameters of 12 × 20 × 20 mm (tumor volume calculated using the DeChiro and Nelson formula: 10,112 cm$^3$) which
pushed the optic chiasm slightly upward, and showed that the pituitary stalk was shifted to the left. On day 10 after octreotide treatment (300 µg/day subcutaneous injection), the size of the tumor was decreased to 12 × 10 × 18 mm (4,127 cm³), and the size did not change after another 14 days of treatment. The thyroid hormone level and TSH level also became completely normal after 10 days of treatment. After 43 days of treatment, the patient underwent transsphenoidal surgery. The immunohistochemical study of the excised tumor revealed that most of the cells were TSH immunopositive, and a few ACTH- and GH-positive cells were also observed.

The serum TSH level was reduced to less than 0.05 at 3 days after surgery and subsequently became normal in 3 weeks.

Data regarding 3 other TSH-secreting adenomas are listed in Table 1. Patients 2 and 3 did not show any significant reduction in tumor size after octreotide treatment (300 µg/day for 19 and 28 days, respectively). Patient 4 was subjected to the surgery without octreotide treatment due to heart complications. The tumor sizes of these patients were 20 mm, 40 mm and 55 mm maximum diameter, respectively.

### Results

**Expression of somatostatin receptor subtypes mRNAs in the TSH secreting adenomas**

We first evaluated the expression levels of the mRNAs for each subtype of somatostatin receptor in the TSH-secreting adenomas. As shown in Table 1, significant expression of all sstr subtype mRNAs, except for sstr4, was observed in all 4 TSH-secreting adenomas. Comparison of these levels with those in normal pituitaries revealed that, although the expression level differed in each adenoma, the level of sstr2 mRNA in all cases was significantly higher than that in normal pituitaries (p<0.05) (Fig. 2B). Since we could not distinguish sstr2A from sstr2B with the TaqMan probe used for real-time PCR, we performed an RT-PCR experiment with primers that could distinguish between sstr2A and sstr2B mRNAs. As shown in Fig. 3, significant expression of sstr2A mRNA was observed in all TSH-secreting adenomas, but no amplification of sstr2B mRNA.

Although the levels were lower than those in normal pituitaries, sstr5 mRNA was expressed in the TSH-secreting adenomas. Interestingly, the levels of both sstr2 and 5 mRNAs in the patient showing a significant reduction in tumor size were the highest as shown by the large black circles in Fig. 2B and 2D. Sstr 1 mRNA was also expressed in the tumors, and its expression level was similar to that in the normal pituitary. The level in the above patient (No. 1) was lowest among those in the TSH-secreting adenomas, suggesting that the expression of sstr1 mRNA was not responsible for the tumor shrinkage by octreotide treatment. Furthermore, low expression of sstr3 and lack of detectable expression of sstr4 were seen in the TSH-secreting adenomas examined.

**Comparison of the levels of sstr1-5 mRNAs in the TSH-secreting adenomas with those in other pituitary adenomas**

We examined the expression of all sstr1-5 mRNAs
sstr mRNAs In TSHOMAS

20 other pituitary adenomas and 4 normal pituitaries. The patient profiles, including age, gender, pathological staining and basal hormone levels, are shown in Table 2.

The level of sstr1 mRNA in GH-secreting adenomas, prolactinomas and ACTH-secreting adenomas varied even among the same form of adenomas, but many of them were higher than those in the TSH-secreting adenomas (Fig. 2A). On the other hand, very low expression of sstr1 mRNA was observed in the non-functioning adenomas. The level in non-functioning adenomas was significantly different from those in other adenomas and the normal pituitary (p<0.05).

In contrast to the findings for sstr1, the levels of expression of sstr2 mRNA in the TSH-secreting adenomas were significantly higher than those in prolactinomas, ACTH-secreting adenomas and nonfunctioning tumors (p<0.05) (Fig. 2B). Although some GH-secreting adenomas showed low expression of sstr2 mRNA, all 4 TSH-secreting adenomas examined
showed higher expression than the normal pituitary. Markedly low expression of sstr2 mRNA was observed in both prolactinomas and ACTH-secreting adenomas, and the level in the non-functioning adenomas was similar to that in the normal pituitary.

As shown in Fig. 2C, in contrast to sstr1 and 2 expression, the expression level of sstr3 mRNA in most of the adenomas was low, except in non-functioning adenomas. The expression in the TSH-, and ACTH-secreting adenomas and prolactinomas was significantly lower than that in the normal pituitary. Interestingly, however, the expression level in the nonfunctioning adenomas was similar to that in the normal pituitary, and was significantly higher than those in TSH-, ACTH-secreting adenomas and prolactinomas.

With respect to sstr5 mRNA, similar to sstr3 mRNA, the overall expression in all the adenomas was relatively low (Fig 2D). The expression levels observed in ACTH-secreting and nonfunctioning adenomas were significantly low as compared to those in the normal pituitary. Although the level varied even among the same form of adenoma and the normal pituitaries, mild expression was observed in the TSH-secreting and GH-secreting adenomas, and in prolactinomas.

Expression of sstr4 mRNA was not detected in any of the pituitary adenomas examined.

Discussion

We first described a case of TSH-secreting adenoma with a dramatic shrinkage (approximately 60% reduction in volume) of the tumor size after only 10 days of the treatment with octreotide. Although it was reported that octreotide induced partial tumor shrinkage in half of the patients, all the reported cases received long-term therapy [7, 19–23]. The case reported by Erem et al. had the shortest treatment period (6 weeks with 20 mg octreotide-LAR), and the MRI study suggested that this shrinkage might have been due to necrosis induced by the treatment [24]. To our knowledge, the period required for the tumor shrinkage of our case was the shortest reported thus far, suggesting that this tumor had specific characteristics that resulted in tumor shrinkage by octreotide treatment. We also obtained pituitary tumor specimens from 3 other TSH-secreting adenomas; 2 cases were treated with octreotide but did not show tumor volume changes, and 1 case did not receive treatment with octreotide. As expected from the previous reports using octreotide scintigraphy and in vitro studies, sstr2 mRNA was expressed in all these TSH-secreting adenomas examined. Whereas the previous 2 case-reports demonstrated sstr2 mRNA expressions by means of simple RT-PCR, the present study for the first time demonstrated the quantitative expression level of sstr2 mRNA in the TSH-secreting adenomas [17, 18]. Surprisingly, the level was significantly higher than those in the normal pituitary, and was similar and even higher than those of GH-secreting adenomas, providing supporting evidence regarding the high efficacy of octreotide in both TSH- and GH-secreting adenomas.

Furthermore, it is important to note that the patient with marked tumor shrinkage showed the highest level of sstr2 mRNA expression. However, patient No. 3 in Table 1, who did not show the tumor shrinkage also showed a similar expression level of sstr2 mRNA, suggesting that a high level of sstr2 mRNA may not be sufficient for the tumor shrinkage by octreotide treatment.

Since octreotide has been reported to preferentially bind to sstr2 and to bind weakly to sstr5, we next focused on the sstr5 mRNA level. The expression levels of sstr5 mRNA in the TSH-secreting adenomas examined were lower than those in normal pituitaries, but the level in the patient who showed marked tumor shrinkage was again highest even among all of the pituitary adenomas examined, and was higher than those in the other 2 cases without tumor shrinkage. Although octreotide treatment might down-regulate the mRNA levels of sstr2 and 5, the levels in patient No. 4 who was not treated were lower than those in patients No. 1 and 3. In addition, in 5 GH-secreting adenomas in 3 patients receiving octreotide treatment, the levels of sstr2 and 5 mRNA were similar to those in patients not receiving the treatment (data not shown). Although further studies including the protein levels of these sstr subtypes and the mechanisms of the tumor shrinkage, such as cytoplasmic shrinkage or apoptosis, are required, our findings suggest that, in addition to the expression of sstr2, the level of sstr5 might be a critical factor for the tumor shrinkage by octreotide treatment [25].

In addition to sstr2 and 5 mRNAs, sstr1 and 3 mRNAs were also identified in the TSH-secreting adenomas. The level of sstr1 mRNA was relatively lower than those in other pituitary adenomas, but was significantly higher than those in non-functioning adenomas. In contrast, high expression of sstr3 mRNA was ob-
sstr mRNAs In TSHOMAS

served in the non-functioning adenomas, and the level in the TSH-secreting adenomas was significantly lower. Although sstr1-selective activation inhibits hormone secretion and cell viability in GH- and PRL-secreting adenomas, there have been no reports on the pathophysiologic significance of sstr1 and 3 in TSH-secreting adenomas [10]. Furthermore, a somatostatin analogue, SOM230, that has binding affinity to sstr5, 2, 3, and 1, in this order, has been suggested to be a therapeutic candidate for corticotroph tumors, and high expression of sstr5 and 1 mRNAs have been reported [26]. In the present study, expression of sstr1 and 5 mRNAs were also confirmed in ACTH-producing adenomas, but the level of sstr5 mRNA was relatively low. Although the precise mechanism for this discrepancy remains unclear, it may be due to the different levels of serum cortisol or racial differences.

In the case of acromegaly, many studies have been conducted to assess the factors responsible for the responsiveness to octreotide treatment [27–29]. Although heterogeneous expression of somatostatin receptors has been reported, Park et al. found that the octreotide response was positively correlated with the sum of sstr2 and sstr5 mRNA levels, but there was no correlation between sstr2 mRNA levels and basal GH levels [30]. Similarly, Jaquet et al. tested the effect of several compounds, including sstr2- and sstr5-preferential compounds, in cultures of GH-secreting adenomas [31]. They also found high levels of sstr2 and sstr5 expression in octreotide-responsive tumors, while partial responders showed lower expression of sstr2 mRNA. On the basis of these observations, it is apparent that sstr2, at least in part, is important for the responsiveness to octreotide in acromegaly. However, the factors responsible for the tumor shrinkage remain uncertain, and this effect may be independent of the responsiveness of GH or IGF-1 [29]. A recent clinical study of the predictors of tumor shrinkage after primary therapy with somatostatin analogs in acromegaly revealed that 75.5% of the patients had 25% or greater tumor shrinkage after 12 months of somatostatin analogue therapy, and that the best predictor of the tumor shrinkage has been proposed to be the post-treatment IGF-1 level. Accumulation of evidence regarding TSH-secreting tumors will now be required to identify predictors of tumor shrinkage after treatment with somatostatin analogues.

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During the revision of this manuscript, similar results were reported by Yoshihara A et al. showing the importance of sstr5 mRNA expression for the response to octreotide treatment [32]. This work was supported in part by a research grant from Health and Labor Sciences Research Grants, Research on Measures for Intractable Disease, Hypothalamo-Pituitary Dysfunction Research Group (to Masatomo Mori).

**References**


