RAPID COMMUNICATION

Serum Free Thyrotropin subunit in Congenital Isolated Thyrotropin Deficiency

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Abstract

In two patients with congenital isolated thyrotropin (TSH) deficiency, serum TSH determined by a sensitive immunoradiometric assay (IRMA) was consistently undetectable. The basal levels of serum free TSH- α subunit (TSH- α) determined by a specific radioimmunoassay (RIA) were elevated in the hypothyroid state, and decreased to the undetectable level during displacement therapy with thyroid hormone. The serum free TSH- α significantly increased following intravenous administration of thyrotropin releasing hormone (TRH). Serum free TSH- β subunit (TSH- β) was undetectable. These findings suggest that TSH deficiency in this disease is not due to absence of thyrotroph in the pituitary gland or deficiency of TSH- α , but to abnormalities of the TSH- β gene.

In 1971 we reported the first case of congenital isolated TSH deficiency (Miyai *et al.*, 1971). The probanda were two sisters with congenital nongonitrous hypothyroidism, and their parents' marriage was consanguineous. Serum TSH, assessed by bioassay and RIA, was consistently undetectable in the patients but was normal in their parents. In these cases autosomal recessive inheritance was suggested, but the cause of TSH deficiency remains unclear. Recent developments of immunoassay and gene technology make it possible now to analyze more precisely the pathogenesis of such diseases.

In the present study, we measured serum TSH by a sensitive IRMA and free

TSH- α and- β by a specific RIA in the hypothyroid state and also following administration of TRH or thyroid hormone to the patients. We also analyzed chromosomes by a high-resolution banding technique and genomic DNAs by genomic Southern hybridization technique using human TSH- β gene as a probe.

Materials and Methods

Subjects

Two female siblings with congenital isolated TSH deficiency (case 1, 30 years old; case 2, 28 years old) and their healthy parents were investigated. The parents' marriage was consanguineous with an inbreeding coefficient of 1/64. The case histories, physical examinations

and laboratory findings have been previously described (Miyai *et al.*, 1971, 1976; Hayashizaki *et al.*, 1986). The study was explained to the subjects, all of whom gave informed consent.

Samples

Blood samples were obtained from patients in the euthyroid state during displacement therapy with thyroid hormone, and in the hypothyroid state after discontinuation of the therapy. Blood samples were also withdrawn from patients and their parents before and at intervals after intravenous administration of 500 μ g TRH, or 100 μ g synthetic ovine corticotropin releasing hormone (CRH). Serum samples were then stored at -20°C until the hormone assay.

Hormone assay

The serum TSH concentration was determined by a modified sensitive IRMA (Seth *et al.*, 1984) using a commercial kit [Daichi Radioisotope, Japan].

Free TSH- α was determined by a modified double-antibody RIA [(Kourides *et al.*, 1975). Briefly, a mixture of 50 μ l of serum to be tested or standard human TSH- α [National Institute of Health (NIH), USA], 50 μ l of diluted anti-human TSH- α rabbit serum [UCB Bioproducts (UCB), Belgium], 50 μ l of diluted normal rabbit serum and 100 μ l of buffer was incubated at room temperature for 3 h. Then 50 μ l of ^{125}I -labeled human TSH- α [NIH] was added and the mixture incubated overnight. The bound and free forms were separated by adding the second antibody (goat anti-rabbit IgG), and the radioactivity was counted in the precipitate. The "true" concentration of free TSH- α was calculated with a calibration coefficient derived from cross-reactivity as follows. $T = T' - \alpha\alpha$, $\alpha = \alpha' - tT$, $\alpha = (\alpha' - tT)/(1 - \alpha t)$, where α : true value of free TSH- α ; T : true value of TSH; α' : observed value of free TSH- α in the free TSH- α RIA; T' : observed value of TSH in the TSH RIA; α : TSH- α cross-reactivity in the TSH RIA (0.69%); t : TSH cross-reactivity in the TSH- α RIA (11.3%).

Free-TSH- β was determined by sandwich enzyme immunoassay as described previously (Iijima *et al.*, 1988). Briefly, one piece of silicone rubber rod coated with mouse anti-human TSH- β monoclonal antibody (Chem-Sero-Therapeutic Institute, Japan) was added to a mixture of 100 μ l of serum to be tested or standard human TSH- β

(UCB), 50 μ l of mouse anti-human TSH- β monoclonal antibody- β -D-galactosidase conjugate and 150 μ l of buffer. The mixture was incubated overnight at room temperature, and the enzyme activity in the washed rod was measured using 4-methylumbelliferyl- β -D-galactopyranoside as a substrate. The TSH cross-reactivity in this assay was negligible.

Chromosome analysis

Chromosome 1 of the patients was analyzed by the high-resolution banding technique according to standard procedure (Yunis 1976) and assessed according to an international system for human cytogenetic nomenclature.

Genomic DNA Southern blot hybridization

Genomic DNA Southern blot hybridization was performed by the method of Southern (Southern 1975). Genomic DNAs extracted from peripheral leukocytes were digested with restriction enzymes *Eco* RI, *Pvu* II or *Hind* III. The digests were subjected to electrophoresis and transferred to Gene Screen Plus nylon membrane. The hybridization was performed using a ^{32}P -labeled DNA fragment carrying exon 3 of the human TSH- β as a probe (Hayashizaki *et al.*, 1985).

Results and Discussion

Our previous report revealed that the patients' congenital hypothyroidism was due to TSH deficiency (Miyai *et al.*, 1971). In the present study, serum TSH determined by a sensitive IRMA was not detected (less than 0.1 $\mu\text{U/ml}$), even in the hypothyroid state and after administration of TRH as shown in Fig. 1. These findings confirmed our previous observation (Miyai *et al.* 1971, 1976), and exclude the possibility of TRH deficiency in the patients. Deficiency of TSH, which is heterodimer of α and β subunits, may result from an absence of thyrotrophs or impairment of synthesis and/or secretion of TSH in the pituitary gland.

As shown in Fig. 1, free TSH- α concentrations in the sera of the patients were kept at high level in the hypothyroid state

Fig. 1. Serum concentrations of TSH, free TSH- α and - β subunits in patients with congenital isolated TSH deficiency ($\circ$, case 1; $\square$, case 2) in the euthyroid states during displacement therapy with thyroid hormone and before and after TRH administration in the hypothyroid state. < indicates undetectable level.

(8.0 ng/ml in case 1 and 17.8 ng/ml in case 2), and significantly increased following TRH administration (peak values were 14.6 and 25.5 ng/ml, respectively). In the euthyroid state during displacement therapy with thyroid hormone, the free TSH- α decreased to undetectable levels (less than 2 ng/ml). These findings indicate that "thyrotrophs" which produce TSH- α and respond to TRH as well as to negative feedback regulation with thyroid hormone, exist in the pituitary gland.

The occurrence of congenital isolated TSH

deficiency accords with an autosomal recessive inheritance pattern. Their parents had no signs and symptoms and no abnormal secretion of TSH (data not shown).

The common α subunit gene of the patients seems to be normal judging from the present findings that free TSH- α could be detected, and also from previous findings (Miyai *et al.*, 1976) that the secretion of gonadotropins which share the same α subunit precursor with TSH (Fiddes and Goodman 1981) were normal.

In control experiments, the free TSH- β

was detected in 8 of 23 cases before TRH and in all 5 cases after TRH administration in patients with primary hypothyroidism with a high serum TSH levels (Iijima *et al.*, 1988). On the contrary, the serum free TSH- β could not be detected in patients with isolated TSH deficiency even after TRH stimulation in the hypothyroid state. These observations suggest that patients with TSH deficiency apparently cannot produce active TSH- β subunit.

To examine whether or not gross rearrangements are present, high-resolution banding analysis of chromosome 1, on which the human TSH- β gene is located (Fukushige *et al.*, 1986), and the genomic Southern hybridization (using TSH- β gene as a probe) were performed. However, no abnormal patterns were seen in the region so far analyzed (data not shown). Therefore it is possible that the mutation(s) must be in a small region even if abnormality is present in or near the TSH- β gene.

Our present and previous studies (Miyai *et al.*, 1976) demonstrated that TRH administration resulted in abnormal increased response of free TSH- α and growth hormone (GH) in the patients with congenital isolated TSH deficiency. Recently Nelson *et al.*, reported that a common positive tissue specific transcription factor appears to bind to all the cell-specific elements in each gene and to be required for the activation of both the prolactin and GH genes (Nelson *et al.*, 1988). Fiddes and Goodman (1981) reported that there is a very marked homology of nucleotide sequence in the 5' untranslated regions of the common α subunit and the human GH genes. It is interesting to speculate that a "transactivator" induced by TRH binds to the "transcription regulatory elements" on the common α subunit gene and the GH gene, and activates both genes. On the other hand, since CRH also stimulated GH secretion in the patients (Hayashizaki *et al.*, 1986), abnormal secretion of free TSH- α by CRH is a possibility.

Our preliminary experiment, however, demonstrated that no free TSH- α was detected CRH administration (data not shown).

Further studies are required on the mechanism of TSH deficiency and abnormal secretion of GH.

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