ORIGINAL ARTICLE

Increased alpha-subunit response coexist with increased TSH response to TRH test in healthy individuals

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Abstract

OBJECTIVE: The alpha-subunit (Alpha-SU) response to TRH was studied to examine the response of alpha-SU under TRH stimulation in normal situation.
METHODS: Thirty seven healthy individuals were included in the study. They were divided into two groups according to the data taken: A with TSH equal or more than seven fold the basal values (0′: 3.14 ± 1.2 μU/ml., 30′ after TRH: 20.22 ± 4.6 μU/ml) and B with TSH 2 to 6 fold the basal values (0′: 0.5 ± 0.1 μU/ml., 30′ after TRH: 2.9 ± 0.5 μU/ml). Both groups exhibited similar FT3 and FT4 levels.
RESULTS: In group A, prolactin displayed a 3 fold increase from the basal values, whereas alpha-SU increased 4 fold with a difference being statistically significant ($p<0.001$). In contrast, in group B individuals neither prolactin nor alpha-SU exhibited any significant difference from the basal values.
CONCLUSION: The expression of alpha-SU upon TRH stimulation is dependent on the TSH basal values.

Introduction

The alpha subunit (alpha-SU), is common to pituitary glycoproteins (LH, FSH, TSH), whereas the β-subunit of the above hormones determines the hormonal activity and species specificity [21]. Initially, both units are synthesized separately to form after non-covalent bonding heterodimer molecules [14,7], which confer the biological action through the signal transduction pathway. Although the mode and mechanisms of hormonal regulation in alpha-SU expression has been investigated [11], conflicting results have been presented in the literature concerning the basal and TRH-stimulated alpha-SU levels in patients with growth hormone – prolactin – and ACTH – secreting adenomas or nonfunctional pituitary tumors [22]. Also, little is known for the expression of alpha-SU under TRH stimulation in normal situation. In that context, we conducted a study of healthy individuals, visitors of the day care outpatient clinic in Henry Dunant hospital, who were asked for a TRH test, in parallel with their yearly check up.

Material and methods

Twelve males (45.1 ± 4.83 years old) and 25 premenopausal females (39.2 ± 2.27 years old) participated in the study after providing informed consent. The selection was done on the basis of the absence of any haematologic, immune, hepatic, renal or endocrine dysfunction. Moreover none individual received any medication.

All patients were admitted at 8:00 am after an overnight fast in the day care outpatient clinic. An indwelling catheter was inserted at the jugular vein and all subjects remained at the supine position for 20 minutes when basal samples were collected (time 0′). After that, TRH 200 μg was given IV as bolus and blood samples were again obtained after 30 minutes

Alpha-SU was measured by an immunoradiometric method (IRMA) (Immunotech, Marseille, France), with an estimated sensitivity at 0.02 mIU/ml. The intra and inter assay CV were at 6.8 and 8.5% correspondingly.

Prolactin was determined by an IRMA method (Dia Sorin, Saluggio, Italy), as described previously [18]. The sensitivity of the assay has been calculated at 0.5 ng/ml at 95% confidence limit.

For the quantitative determination of TSH, the gamma coat [^{125}I] 3rd generation immunoradiometric assay (Dia Sorin, Minnesota, USA) was used. The sensitivity of the assay was found to be 0.013 μU/ml, whereas the CV of the intra and inter assay were at <3.3 and 5.7% correspondingly. For the quantitative determination of FT3 and FT4 radioimmunoassays by Zen Tech, SA, Belgium were used. For FT4, the sensitivity of the assay was 0.13 ng/dl, whereas the CV of the intra and inter assay was at 3.7 and 4.5% correspondingly. For FT3, the sensitivity of the assay was 0.35 pg/ml whereas the CV of the intra and inter assay were at <2.7 and 8.3% correspondingly.

All measurements were done in duplicate and the average values were used for the determination of means ± SD. Statistical evaluation of the results was performed using ANOVA and student t-test. Values of $p<0.05$ were considered significant.

Results

According to the TSH response after the TRH stimulation test we divided our population into two groups (Table 1): A (16 females, 7 males) with TSH response seven fold or more the basal values (0′: 3.14 ± 1.2 μU/ml, 30′ after TRH: 20.22 ± 4.6 μU/ml) and B (9 females, 5 males) with TSH response 2 to 6 fold the basal values (0′: 0.5 ± 0.1 μU/ml, 30′ after TRH: 2.9 ± 0.5 μU/ml). In group A, FT3 was 2.5 ± 0.4 pg/ml and FT4 1.1 ± 0.2 ng/dl whereas and in group B FT3 was 2.4 ± 1.08 pg/ml and FT4 1.02 ± 0.3 ng/dl.

In group A prolactin displayed a 3 fold increase from the basal values after the TRH stimulation (0′: 18.1 ± 3.7 ng/ml., 30′ after TRH: 59.4 ± 8.2 ng/ml), whereas alpha-SU increased 4 fold (0′: 0.3 ± 0.06 mIU/ml, 30′ after TRH: 1.13 ± 0.21 mIU/ml) (Table 2) with a difference being statistically significant ($p<0.001$). In contrast, in group B individuals neither prolactin (0′: 16.9 ± 8.9 ng/ml, 30′ after TRH: 39.2 ± 17.1 ng/ml) nor alpha-SU (0′: 0.5 ± 0.2 mIU/ml, 30′ after TRH: 1.5 ± 0.6 mIU/ml) (Table 2) exhibited any statistically significant difference from the basal values.

Discussion

Previous investigations have suggested that the synthesis of alpha and β subunits of the glycoprotein hormones are independently regulated. The normal pituitary gland contains more free alpha-SUs subunits than all pituitary β subunits [16,13]. Isolated or unbalanced subunit secretion has been demonstrated in pituitary tumors [17,2]. In respect to TSH alpha-SU, β subunit, Kourides et al in 1979 [15] have presented extensive data indicating that each subunit appear to be synthesized as a pre subunit, concluding that alpha-SU and β subunit of TSH are most likely independently [15] synthesized from separate mRNAs. Additionally, divergent response was reported by Doss et al for alpha and TSH β-subunits in the pituitaries of hypothyroid mice in response to T4 [24]. Moreover it has been shown that treatment of mice bearing thyrotropic tumors with T3 suppressed the serum TSH levels of free TSH – β subunit and less markedly of free alpha-SU [10], a finding suggesting that the regulation of TSH biosynthesis occurs predominantly at the level of TSH – β mRNA.

Experimental studies using pituitary cell culture [9] showed that intrapituitary free alpha-SU and intact TSH have divergent response to TRH and/or dopamine challenge, findings suggesting the existence of essential differences in the signal transduction pathways activated by TRH in the regulation of the above hormones. Moreover, patients with microprolactinoma exhibit a marked reduction of alpha-SU response after TRH stimulation as compared to controls [20]. Also, patients with hypogonadotropic hypogonadism had measurable levels of alpha-SU even when TSH secretion was completely suppressed by L-T_4 treatment, a finding proposing that production of gonadotropin subunits are differentially regulated [29]. More recently clinical studies in patients with glycoprotein adenomas [4] and growth hormone producing adenomas [28] showed significant elevation of alpha-SU levels although other studies [25] using TRH challenge in patients with various types of pituitary tumors suggest that abnormal serum alpha-SU levels are mainly due to hypersecretion by the tumor itself. Contributing further to the physiology of alpha-SU metabolism, we found that in healthy individuals, alpha-SU prolactin and TSH responses to TRH stimulation are TSH basal values associated. Increasing amounts of data have been presented in the literature on the biological significance of subclinical thyroid disease for a number of organs [23,6,8]. Minor changes in serum T3 and T4 levels affect predominantly the production of TSH from the pituitary [3]. When thyroid function is abnormal, the association, between serum TSH and both T3 and T4 is log linear [19,26]. This amplified response of serum

Table 1. Classification of normal individuals according to TSH response after TRH stimulation

	TSH (μU/ml)			
	Basal values	**30´ after**	**FT3 (pg/ml)**	**FT4 (ng/dl)**
Group A	3.14 ± 1.2	20.22 ± 4.6	2.5 ± 0.24	1.1 ± 0.2
Group B	0.5 ± 0.1	2.9 ± 0.5	2.4 ± 1.08	1.02 ± 0.3

Table 2. Response of alpha subunit and prolactin after TRH stimulation in high (A) and low TSH (B) groups of normal individuals

	Alpha-SU		Prolactin	
	Basal values	**30´ after**	**Basal values**	**30´after**
Group A	0.3 ± 0.6	1.13 ± 0.21	18.1 ± 3.7	59.7 ± 8.2
Group B	0.5 ± 0.2	1.5 ± 0.6	16.9 ± 8.9	39.2 ± 17.1

TSH to changes in serum T3 and T4 may cause serum TSH to leave the population-based reference range when serum T3 and T4 are outside the individual reference range, even when they are still within the population based reference range [1]. Moreover, Spencer et al [27] have shown a considerable inter individual variability to TRH stimulatory signal in euthyroid subjects suggesting that these differences in fold response most likely reflect alterations in pituitary thyrotropin sensitivity to TRH, modulated at or beyond the TRH receptor.

In our study an exaggerated TSH response (7 fold) was seen with basal TSH levels at the upper normal limits with similar FT4 and FT3 levels. The issue of subclinical hypothyroidism is in question for this group of individuals. As stated previously [5,23,31] the above disease is defined by elevated TSH secretion, though the level of circulating thyroid hormones is normal. Interestingly, recent data [12] showed that the incidence of overt hypothyroidism in patients with grade I subclinical hypothyroidism was 0% on the basis of TSH values that ranged from 4 to 6 μU/ml. Nevertheless, a longitudinal study may be more appropriate to obtain more meaningful results. In conclusion our data, clearly, demonstrate that minor alterations in the function of the pituitary-thyroid axis, but still in the normal range, affect the production of alpha-SU secretion upon TRH stimulation and that the TSH basal values must be taking into account when interpreting alpha-SU results to TRH stimulation test.

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