UC 577,1; 61 ISSN 0354-3447

Jugoslov Med Biohem 22: 243–248, 2003 *Originalni naučni rad*
Original paper

CLINICAL IMPLICATIONS OF BIOCHEMICAL ALTERATIONS INDUCED BY HYPOTHALAMIC THYROTROPHIN HORMONE IN ELDERLY PEOPLE

Snežana Đurica[1], Miroljub Petrović[3], Miodrag Rajić[2], Mladen Davidović[1], Dragoslav P. Milošević[1]

[1]Internal Clinic of Zvezdara University Center, Belgrade,
[2]Division of Haematology, University School of Pharmacy, Belgrade,
[3]Internal Division, University School of Stomatology, Belgrade

Summary: Thyrotrophin releasing hormone (TRH) test has been done in 62 subjects (females, average age 72), in order to analyse the stimulated TSH action, to assess the immediate thyroid reserve and to make the rational parameters of the thyroid function in the elderly. It was concluded that biochemical alterations provoked by application of hypothalamic thyrothropin hormone are very complex, but important for the clinical practise, giving the possibility of assessment of the actual state of the thyroid's function. It is also concluded that the estimation of TRH stimulated TSH in 20th and 60th minute, and T3 and FT4 in 60th minute of the TRH test provides very solid and rational method of thyroid function estimation, as well as the estimation of the thyroid reserve.

Key words: thyrotrophin releasing hormone, thyrotrophin, biochemical alterations, clinical implication

Introduction

Thyrotrophin hypothalamic releasing hormone (TRH) is the first hypothalamic peptide agent that has become available as a powerful, nontoxic diagnostical tool. It has been isolated (1) and produced in sufficient quantity (2), so that its structure could be properly analysed. It consists of three aminoacids/histidine, glutamic acid and prolyne in their levogyre stereochemical types (L-pyroglutamil-L-histidyl-L-prolyne amide). TRH obtained by synthetical means and natural TRH have both the same biological action (3).

TRH is tripeptide with molecular mass of 362 Daltons, which retains its biological activity given orally, as well as by the nasal and parenteral route (4). Under certain conditions, as well as in elderly people, rectal use is also possible and recommanded. Biological half-life of TRH in circulation is about 4 minutes, what makes the analysis most convenient, after the intravenous administration. Thyroid hormones alter the pharmacokinetics of TRH, influencing the elimination rate. In an euthyroid person the average plasma elimination rate of TRH is 22 ± 3.4 mL/kg/min (5). In hyperthyreosis the rate is 22.7 ± 10.7%; in hypothyroidism it is slower (41.1 ± 4.6%), and is in negative correlation with the logarhythm of thyroxine concentration (r=0.91).

TRH is resistant to most of proteolytic agents, but its degradation is not studied enough. Its degradation may caused by peptidases and some amidases (6). Enzyme pyroglutamil peptidase causes degradation of TRH resulting in the cyclic compound named hystidil-prolyne-diketopiperazine, which has numerous biological activities: antagonism of the ethanol-mediated narcotic effect, hypothermia, increase in the cyclic guanosine monophosphate (cGMP) in brain, inhibition of the potasium-sodium pump, ATP-ase and prolactin secretion. The compound has been found in free and bound form in different parts of brain tissue (7), its distribution being different from the distribution of TRH in brain; it is supposed that this dimer is a mediator of TRH activity in the central nervous system. The influence of amidases contributes to TRH-OH form action, whose role has not yet been thoroughly investigated (9, 10). Enzymatic degradation products of TRH are involved in the regulation of TRH action.

The purpose of this study was: a) to analyse the stimulated TSH action; b) to asses the swift, immediate thyroid reserve which has a certain prognostic value in the states of adaptation, different causes of pathological ageing and the states of infection; c) to

Address of correspondence
Miroljub Petrović
Grčića Milenka 1
11000 Belgrade

gain the rational parameters of the thyroid function in the elderly.

Patients and Methods

TRH test with a dose of 200 μg/mL given in morning hours was carried out on 62 females, average age 72 years, 65 82 y). Neurological conditions that could influence the dopaminergic system and thus alter the TSH action were excluded (11). All subjects were euthyroid before the test, and none was from the iodine-deficitary areas. Family history concerning thyroid gland goiter, was negative. All subjects willingly submitted to the test.

TRH test was also done in 26 control subjects (average age 40 years, 32 50 y). Blood samples were taken 20, 60 and 90 minutes after TRH injection. All subjects were submitted to the following hormone analyses:

a) Thyreostimulating hormone (TSH) was evaluated by immunoradiometric, ultrasensitive analysis (IRMA). Concentrations in the range of 0.33 to 5.5 mU/L were considered as physiological;

b) Triiodothyronine (T3) and thyroxine (T4) in serum were analysed by radioimmunological (RIA) analysis, and normal values were 1.2 2.8 for T3 and 55 152 nmol/L for T4;

c) Concentrations of free thyroid hormones (FT3 and FT4) were estimated by fluoroimmunometry (FIA). Normal values were 2.39 6.20 pmol/L for FT3 and 10 22 pmol/L for FT4.

d) Values of the swift, immediate thyroid gland reserve were estimated by T3, FT3, T4, FT4 concentration measurements as well as measuring the stimulated TSH activity 60 minutes after TRH injection.

e) The results obtained were analysed by parametrical statistical processing. Hormone concentration values were the result of the mean value of tripple measurement of the very same serum sample ($\bar{x}$) with the exclusion of one standard deviation value. Intrassay variation coefficient (CV) did not exceed 5% and 10% in interassay. Significance of differences within the same group was assessed by one-course variant quotient, and between the groups by the Student's t-test.

Results

The basic value of TSH concentration in normal elderly persons was higher ($p(t) < 0.001$) compared to the control group (3.98 ± 1.20 mU/L versus 1.88 ± 1.64 mU/L). There was a positive correlation between TSH concentration and the subjects age ($r=+0.18$), and between the basic concentration of TSH in the elderly and T4 concentration in sera in the range of +0.23 while the mutual dependence on T3 was very small ($r=+0.07$). These data reveal the importance of the influence of T3 and T4 TSH regulation.

Basal values of T3 and T4 concentrations are lower ($p(t) < 0.01$) than those in the control group (*Table I*).

TSH is stimulated after the intravenous administration of TRH, and its serum concentrations increased in all subjects. Concentrations of hormones in sera were in ranges that were suitable for the methods used (*Table II*).

Table I Basal concentration values ($\bar{x}$ ± SD) of thyroid-hypophyseal hormones in analysed groups

Subjects	Number	TSH, mU/L	T4, nmol/L	FT4, pmol/L	T3, nmol/L	FT3, pmol/L
elderly	62	3.98 ± 1.22	82 ± 18	14.20 ± 2.80	1.62 ± 0.84	3.60 ± 1.48
control	26	1.88 ± 1.64***	122 ± 24**	16.40 ± 3.60	2.20 ± 0.68**	4.46 ± 1.82

*** ($p(t) < 0.001$)
** $p(t) < 0.01$

Table II Relative change (%) of thyroid-hypophyseal axis hormones mean values after the injection of TRH (200 μg/mL, iv)

Subjects	Number	Minutes	Change % TSH	Change % T4	Change % FT4	Change % T3	Change % FT3
elderly	62	20	160	104	104	100	100
		60	182	110	108	106	100
		90	150	108	106	104	104
control	26	20	300***	140	112	118	120
		60	222***	118	110	116	118
		90	126	110	102	112	110

*** ($p(t) < 0.001$)
** $p(t) < 0.01$

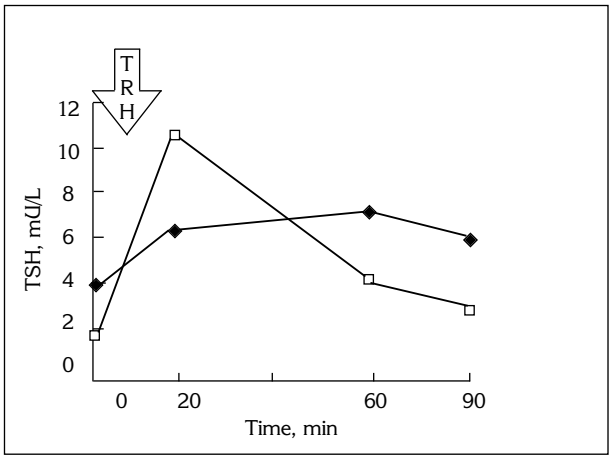

Figure 1. Stimulated TSH response in elderly people (–◆–) comparated to the control group (–□–) during TRH test

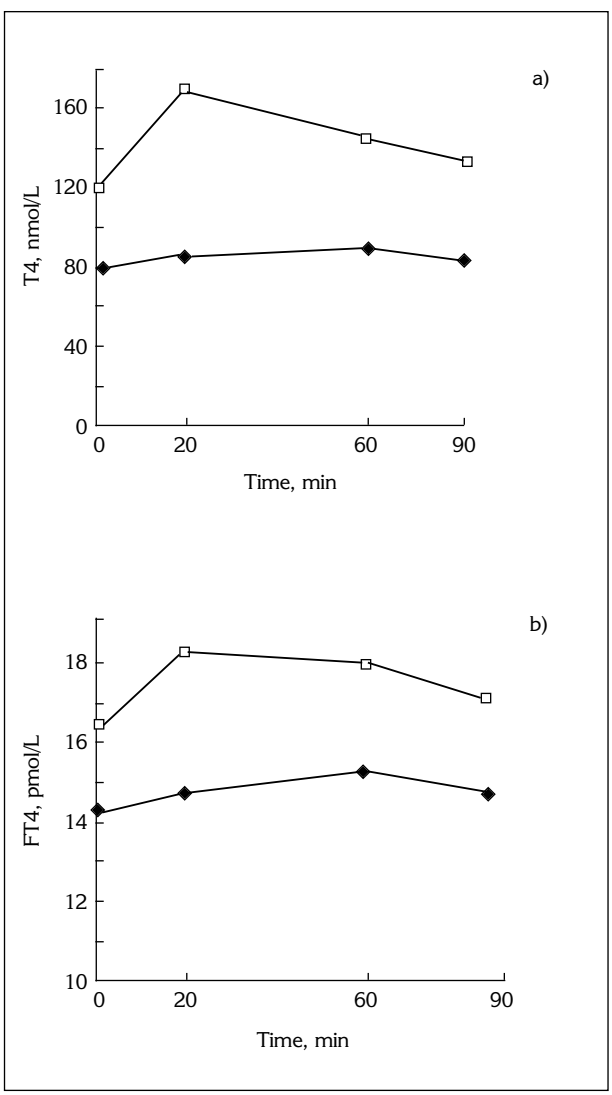

Figure 2. Stimulated T4 (a) and FT4 (b) response is lower in elderly people (–◆–), then in control group (–□–) during TRH test

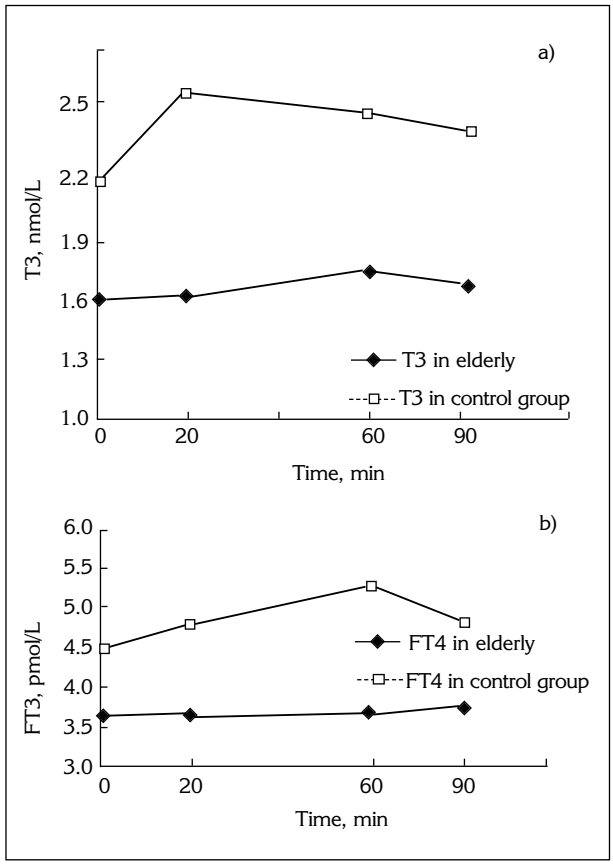

Figure 3. Stimulated T3 (a) and FT3 (b) response is lower in elderly people (–◆–), then in control group (–□–) during TRH test

In healthy elderly persons mean values of concentrations of total and free thyroid hormones are significantly lower ($p(t) < 0.001$) than those in the control group of middle-aged persons (*Table II*). In elderly, the increase of thyroid hormones concentrations is slower and reaches its maximum in the sixtieth minute of the test. However, thyroxine and triiodothyronine, as well as their free fractions, rapidly increase in control group after the use of TRH, reaching maximal values in the twentieth minute of TRH test (*Table II*).

Concentrations of free thyroid hormones (FT3, FT4) showed no statistically significant change in the elderly during TRH test ($p(F) < 0.1$.

On the basis of the obtained data regardine the changes of TSH and thyroid hormones and the time of reaching the maximal values, the swift, immediate thyroid reserve was estimated. this was obtained by the analysis of TSH concentration in the 20th and the 60th minute of the test and T3 and FT4 in the 60th minute of the test. In 94% (58/62) of healthy elderly subjects, euthyreosis was confirmed. In 4.84% (3/62) of elderly subjects subclinical hypothyreosis was found. Initial TSH in these individuals (4.28 ± 1.44 mU/L) in the 20th minute of TRH test increased the concentration (10.88 ± 4.22 mU/L) reaching its maximum in the 60th minute of the test (12.6 ± 3.80 mU/L).

Triiodothyronine and free, metabolically active fraction of thyroxine (FT4) reach their maximal values in the 60th minute of the test (1.64 ± 1.20 pmol/L and 10.80 ± 1.62 pmol/L, respectively).

In 1/62 patients (1.6%), on the basis of TRH test, primary hypothyreosis with a very low thyroid reserve was diagnosed. Basic, initial values of T4 (74.40 ± 1.20 nmol/L), T3 (1.42 ± 0.24 nmol/L), TSH (4.48 ± 1.68 mU/L) as well as FT3 (2.90 ± 0.46) and FT4 (9.80 ± 0.26 pmol/L) did not affect the clinical status of the subject. Undoubted confirmation of hypothyreosis were the elevation of the stimulated TSH in the 60th minute (22 ± 6.20 mU/L for three measurements of the same serum sample); T3 in the 60th minute 1.24 ± 0.80 nmol/L and the low value of FT4 in the 60th minute of the test (8.64 ± 2.42 pmol/L); this was the reason for introduction a substitutional therapy.

In all subjects of the control group TRH test revealed an euthyroid function and a satisfactory thyroid reserve.

Discussion

The thyroid reserve contributes to the maintain of the levels of metabolic processes in healthy elderly and middle-aged individuals as well as during hypothyroidism. It is of vital importance for the clinical practice, because it has a certain role in pathogenesis, clinical course and onset of complications. It is easy to be estimated by hypothalamic thyrotrophin factor (TRH) and analysis of changes of serum TSH concentration in the 20th and 60th minutes of the test, and also by analysis of thyroglobulin proteolysis of free, metabolically active T4 and T3 in the 60th minute after TRH administration. In middle-aged subjects T3 analysis in the 60th minute of the test is not crucial, but in the elderly it is. It is well known that all thyroxine (100%) in the organism is produced in thyroid cells. As for T3 30% is produced in thyroid gland cells. The rest of 70% is produced after the catalytic influence of selenium-dependent (12) heterogenous enzymatic complexes of 5-deiodinase in external fenolic ring of the thyroxine molecule especially in the liver (40%), sceletal muscles (5%), kidneys (17%) and the thyroid gland (20%). Ageing makes these enzymes less active leading to a possible syndrome of the low T3 in the elderly. Our previous studied revealed lower concentrations of serum T3 in 19% of subjects aged over 65. This finding refers to a very important detection of serum T3 sixty minutes after the beginning of TRH test. These parameters are the »golden standard« in the estimation of the swift, immediate thyroid gland reserve.

Essential biological action of TRH lies in the stimulation of TSH secretion from adenohypophysis. TRH also has numerous other effects: it stimulates the prolactine liberation from lactotrophic cells of adenohypophysis, can cause hypothermia (13), decreases the need for nutrition not altering the need for water intake. Influence of TRH on the nutritional needs can be diminished by antagonists of dopamine receptors (14). TRH has an influence on the motor activity, it changes the electroencephalographic activity similarly to the effect of amphetamine and tricyclic antidepressants (15) and has an originally antidepressive effect.

In order to express its action, TRH first has to react with specific receptors on the external side of the anterior hypophysis cells membrane (16). This bimollecular process is reversible, with the dissociation constant of 10^{-8} mol/L. The new complex dissociates with the biological half-life ($T_{1/2}$) of 14 minutes. In a complex interaction between TRH and receptor place at the cell membrane, phospholipids of the membrane have an important role. Guanosine triphosphate (GTP) and adenosine triphosphate (ATP) may inhibit the linkage of TRH to the receptor. Another condition for TRH activity is the activation of adenylcyclase with the increase of cells cAMP (3). Similar action has dibutyril cyclic adenosin monophosphate (dbcAMP), although its action is more intensive towards the growth hormone (GH) than towards TSH. TSH secretion stimulated by TRH is a very complex process and it depends on the concentration of calcium ione at both sides of the cell membrane (17), so that all substances that alter the calcium concentration simultaneously alter TRH stimulation of adenophypophyseal TSH secretion (18).

There is a dependence between the number of receptor places at the external side of adenophypophyseal basophil cells and TRH; the level of their interaction is influenced by this dependence. It is experimentally confirmed (19) that the number of receptors is greatest on the first day after birth, and it gradually decreases, and by the 16 h day it has the same value that is characteristic for an adult animal. It is also experimentally confirmed that there are TRH-like substances in the anterior and posterior hypophyseal lobes (20), but also in other tissues (21). Tissues that contain TRH-like substances are more sensible to the effect of thyroxine.

TSH stimulates all aspects of the growth and the function of thyrocytes. This finding has confirmed the fact that elderly persons have higher initial values of TSH concentrations, but the biological answer to TSH is not strong as expected. Our previous studies (22) confirmed the decrease in the high affinity TSH receptors in thyrocytes membranes in the elderly as compared to the middle-aged subjects. TSH very quickly dissociates from the complex with the receptor, i.e. complex TSH-receptor energetically does not last enough to activate adenyl-cyclase system and cell G-regulatory proteins, so that the expected postregulatory biological answer (synthesis of enzymes necessary for oxydative cell processes) does not happen.

Liberated T3, T4 after thyroglobuline molecule proteolyse depend on lysosome energetic potential,

but also on the degree of thyroglobuline molecule iodination. Elderly persons exert lower basal and stimulated concentrations of thyroid hormones as compared with the middle-aged persons. There are also differences connected with the sex; female elderly persons (23) have higher basal values of T4 p(t) < 0.01) than males. On the other hand, in males basal T3 is higher than in elderly females (24).

Biochemical alterations provoked by the administration of hypothalamic thyrotrophin hormone (TRH) are very complex and they appear in a very short period of time. They are important for the clinical practice, giving the possibility of assessment of the real state of the thyroid gland function. They can also predict the possible thyroid activity by measuring the value of the thyroid reserve. The value of the immediate thyroid reserve in elderly female subjects is lower as compared to the niddle-aged control group of subjects.

Estimation of TRH stimulated TSH in the 20th and the 60th minute and T3 and FT4 in the 60th minute of TRH test gives a very solid and rational method of the thyroid gland function estimation, as well as the estimation of the thyroid gland reserve.

KLINIČKE IMPLIKACIJE BIOHEMIJSKIH PROMENA PRI DELOVANJU HIPOTALAMUSNOG TIREOTROPNOG HORMONA KOD STARIJIH OSOBA

Snežana Đurica[1], Miroljub Petrović[3], Miodrag Rajić[2], Mladen Davidović[1], Dragoslav P. Milošević[1]

[1]Katedra za internu medicinu Medicinskog fakulteta, Beograd
[2]Katedra za hematologiju Farmaceutskog fakulteta, Beograd
[3]Katedra za internu medicinu Stomatološkog fakulteta, Beograd

Kratak sadržaj: Tireotropnim hipotalamusnim hormonom (TRH) je obavljen test kod 62 osobe ženskog pola, prosečnog životnog doba od 72 godine, s ciljem da se analizira stimulisano dejstvo TSH, da se oceni neposredna tireoidna rezerva i da se odrede racionalni parametri koji pouzdano odražavaju moguću funkciju štitaste žlezde starijih osoba. Zaključeno je da su biohemijske promene izazvane davanjem hipotalamusnog tireotropnog hormona veoma kompleksne, ali važne za kliničku praksu, jer omogućavaju procenu aktuelnog stanja tireoidne funkcije. Takođe je zaključeno da procena TSH odgovara po TRH stimulaciji u 20-tom i 60-tom minutu testa, kao i procena T3 i FT4 u 60-tom minutu testa predstavljaju pouzdan i racionalan metod procene tireoidne funkcije i rezerve.

Ključne reči: tireotropni hipotalamusni hormon, tireotropin, biohemijske promene, klinički značaj

References

1. Guillemin RE, Yamazaki F, Gard DA, Jutisz M, Sakiz E. In vitro secretion of thyrotropin (TSH) stimulation by a hypothalamic peptide (TRH). Endocrinology 1963; 73: 564 67.
2. Schally AV, Armura A, Kastin AJ. Hypothalamic regulatory hormones. Science 1973; 179: 341 45.
3. Naor Z, Snyder G, Fawcett CP, McCann SM. Pituitary cyclic nucleotides and thyrotropin-releasing hormone action: the relationship of adenosine 3'-5'-monophosphate and guanosine 3'-5'-monophosphate to the release of thyrotropin and prolactin. Endocrinology 1980; 106: 1304 7.
4. Szabolcs I, Ploenes C, Bernard W, Herrmann J. Thyrotropin-releasing hormone in geriatric patients: intravenous versus intranasal application. Acta Endocrinol 1989; 120 (2): 149 153.
5. Iversen E. Pharmacokinetics of thyrotrophin-releasing hormone in patients in different thyroid states. J Endocrinol 1991; 128: 152 6.
6. Rupnow JH, Raylor WI, Dion JE. Purification and characterisation of thyrotropin-releasing hormone deamidase from rat brain. Biochemistry 1979; 18: 1206 9.
7. Yanagisawa T, Prasad C, Peterkofsky A. The subcellular and organ distribution and nature form of histidyl proline diketopiperazine in rat brain determined by a special radioimmune assay. J. Biol Chem 1980; 225: 19290 6.
8. Mori M, Prasad C, Wilber JF. Regional dissociation of hystidil-proline diketopiperazine and thyrotropin-releasing hormone in the rat brain. Brain Res 1982; 231: 451 57.
9. Morley JE, Levine AS, Prasad C. Histidylproline diketopiperazine food intake in rats. Brain Res 1981; 210 (1 2): 457 483.
10. Dekin MS, Richerson GB, Getting PA. Thyrotropin-releasing hormone induces rhytmic bursting in neurons of the nucleus tractus solitarius. USA-Science 1985; 229 (4708): 67 72.

11. Mitsuma TT, Tsuyoshi N. Changes in plasma thyrotrophin-releasing hormone, thyrotropin, prolactin and thyroid hormone levels after intravenous, intranasal or rectal administration of syntetic thyrotrophin-releasing hormone in man. Acta Endocrinologica 1984; 107: 207 212.

12. Freake HC. Molecular biological approach to studyng trace minerals. Why should clinician care. J Am College of Nutrition 1993; 12: 294 9.

13. Tsay LB, Lin MT, Effects of intracerebrovascular administration of thyrotrophic releasing hormone on cardiovascular function in the rat. Neuroendocrinology 1982; 35: 173 9.

14. Lin MT, Ping CC, Shu YL. Effects of TSH, TRH, LH and LHRH on termoregulation and food intake in the rats. Neuroendocrinology 1983; 37: 206 211.

15. Gold MS, Pottash ALC, Extein I. The TRH test in diagnosis of major and minor depression. USA-Psychoneuroendocrinology 1981; 6 (2): 159 165.

16. Labrie F, Lean A, Borgeat P, Borden N, Poirier G, Drouin J. Polypeptide receptor mechanism in anterior pituitary gland. Anatomical Neuroendocrinology. Int Conf Neurobiology of CNS-hormone Interactions, ed. Karger, Basel 1975; 60 7.

17. Kolesnik R, Gershengorn M. Thyrotropin-releasing hormone and pituitary. Am J Med 1985; 79: 729 736.

18. Gershengorn M, Thaw C. Thyrotropin-releasing hormone (TRH) stimulates byphasic elevation of cytoplasmic free calcium in GH cells. Further evidence that TRH mobilizes Endocrinology 1985; 116: 591 9.

19. Bakerji A, Prasad C. The postnatal development of the pituitary thyrotrophin-releasing hormone receptor in male and female rats. Endocrinology 1982; 110 (2): 663 8.

20. Ashworth RJ, Morrell JM, Aitken A, Patel I, Cocla SM. Pyroglutamyl prolineamide is present in rat anterior and posterior pituitary gland. J Endocrinol 1991; 129: R1.

21. Bilek R, Gkonos PJ, Tavianini MA, Smyth DG, Rose BA. The thyrotrophin-releasing hormone (TRH)-like peptides in rat prostate are not formed by expression of the TRH gene but are suppressed by thyroid hormone. J Endocrinol 1992; 132: 177 184.

22. Đurica S, Žakula Z, Isenović E, Ribarac-Stepić N. Age-related changes of TSH receptors in thyroid tissues obtained from euthyroid patients. Arch of Gerontology and Geriatrics 1993; 17 (3): 203 9.

23. Đurica S, Davidović M, Milošević PD. Štitasta žlezda i starenje 1996; 1 291: ed. Nauka, Beograd.

24. Feit H, Thyroid gland in the elderly. USA Clin Geriatr Med 1988; 4 (1): 151 9.

Received: October 10, 2002

Accepted: February 19, 2003