Effects of Vitamin A Administration on Serum Thyrotropin Concentrations in Healthy Human Subjects

G. Ceresini, I. Rebecchi, S. Morganti, M. Maggio, S.B. Solerte, L. Corcione, S. Izzo, P. Mecocci, and G. Valenti

Retinoids play an important role in the regulation of normal growth and development. Their biological action is mediated by a nuclear receptor that belongs to the steroid/thyroid hormone receptors superfamily. Retinoic acid has been shown to inhibit the secretion and synthesis of thyrotropin (TSH); however, little is known on the effects of retinoids on TSH secretion in normal human subjects. In the present study, we evaluated serum TSH concentration following both vitamin A (vit A) and the combined vit A and triiodothyronine (T_3) administration. Basal and thyrotropin-releasing hormone (TRH)-stimulated TSH serum concentratrions were measured in healthy young subjects in the following experimental conditions: (1) after 10 days of treatment with vit A orally administered as retinol at a dose of 50,000 IU/d; (2) after 10 days of oral placebo (PL) treatment; (3) after 1 hour from the administration of 40 mg T_3 at the end of 10 days of PL treatment; and (4) after 1 hour from the administration of 40 mg T_3 at the end of 10 days of vit A treatment. Serum TSH concentrations were also measured during vit A administration in healthy elderly subjects according to the following protocol: (1) after 10 days of treatment with PL; and (2) after 10 days of treatment with vit A at the same dose used for young subjects. In young subjects, basal serum TSH levels were found to be similar in the 4 different treatment conditions. In the same group of subjects, each of the 4 experimental conditions induced an increase in serum TSH, which rose from basal values of 1.80 ± 0.31 to a peak of 11.92 ± 1.75 μIU/mL ($P < .001$) during the PL treatment, from basal values of 1.81 ± 0.22 to a peak of 10.81 ± 1.00 μIU/mL ($P < .001$) during vit A treatment, from basal values of 1.72 ± 0.28 to a peak of 9.92 ± 1.10 μIU/mL ($P < .001$) during PL + T_3 treatment, and from basal values of 1.79 ± 0.30 to a peak of 9.51 ± 1.12 μIU/mL ($P < .001$) during vit A + T_3 treatment. The 2-way repeated measure analysis of variance revealed no significant differences among treatments. In old subjects, basal serum TSH levels were similar in the 2 experimental conditions and were not different from those observed in young subjects. In these subjects, serum TSH levels increased significantly in response to the TRH stimulus from basal values of 2.16 ± 0.3 to a peak of 10.27 ± 0.55 μIU/mL ($P < .001$) during PL treatment and from basal values of 2.10 ± 0.51 to a peak of 7.82 ± 1.4 μIU/mL ($P < .001$) during vit A treatment. No significant effects of treatment were found in this group of subjects on TRH-induced TSH levels; however, TSH responses were somewhat lower during vit A treatment with a difference close to statistical significance. These results suggest that TSH secretion is poorly affected by vit A administration in healthy human subjects; the data also indicate that any cooperation between T_3 and vit A is unlikely to occur in the regulation of TSH secretion.

VITAMIN A (VIT A) and its biologically active derivatives, such as all-*trans* retinoic acid (atRA) and 9-cis-RA play an important role on normal growth and development.[1,2] The biologic action of retinoids is mediated by a nuclear receptors that belongs to the steroid/thyroid hormone receptors superfamily.[3,4] In fact, RA receptors (RAR) regulate gene transcription by binding to the specific DNA element called RA-responsive element (RARE), located within the promoter region of target genes.[3] The RARE consists of a direct repeat of the hexamer sequence (AGGTCA), which is identical to the consensus half-site sequence for the binding site for thyroid hormone receptor (TR).[5,6] RAR binds to RARE as a heterodimer with 9-cis-RA receptor and with TR.[3,7] RAR and TR activate gene transcription through a common hormone-responsive element, such as the palindromic thyroid hormone response element (TRE).[8] It is well known that thyroid hormones (eg, triiodothyronine [T_3]) are the major regulators of pituitary thyrotropin (TSH) production[9,10] and negatively affect pituitary TSH secretion.[11,12] All the above-described findings raised the possibility that both retinoids and T_3 cooperate in the regulation of both TSH β-gene transcription and pituitary TSH secretion. In fact, elevated pituitary TSH levels were found in vit A-deficient rats as compared with vit A-sufficient animals,[13] and vit A deficiency has been shown to determine an increase in rat pituitary TSH β-messenger RNA levels.[14] Moreover, an in vivo study demonstrated that atRA inhibits both basal and thyrotropin-releasing hormone (TRH)-stimulated TSH secretion in both euthyroid and hypothyroid rats.[15] Central hypothyroidism has been reported in patients treated with high doses of a retinoid X receptor-selective ligand for cutaneous T-cell limphoma[16]; nevertheless, little is known of the role played by retinoids on TSH secretion in normal human subjects. Of particular interest is the study of the role played by retinoids in elderly subjects in whom a deficiency in vit A has been reported.[17] For this reason in the present experiment, we studied the effects of vit A administration on serum TSH levels both in basal conditions and following a challenge with TRH stimulation in both young and old healthy human subjects.

MATERIALS AND METHODS

Subjects

Eleven healthy young subjects, 6 males and 5 females, aged 28 ± 0.9 years, with a mean body mass index (BMI) of 23.1% ± 0.7% and 9 elderly subjects, 3 males and 6 females, aged 75.4 ± 2.1 years and with a BMI of 26.1% ± 1.1% were studied. All subjects were nonsmokers, had a low-caffeine intake, and took no medication. The presence of any

From the Departments of Internal Medicine and Biomedical Sciences and Pathology, Section of Geriatrics, University of Parma, Parma; Department of Geriatrics, University of Perugia, Perugia; and the Department of Geriatrics, University of Pavia, Pavia, Italy.

Submitted March 29, 2001; accepted December 31, 2001.

Address reprint requests to G. Ceresini, MD, Department of Internal Medicine and Biomedical Sciences, Section of Geriatrics, University of Parma, Via Don Bosco, 2, 43100 Parma, Italy.

0026-0495/02/5106-0006$35.00/0
doi:10.1053/meta.2002.32724

concomitant endocrine disease was excluded in all subjects. The subjects gave informed consent to the study, and the protocol was approved by the local ethical committee of the University of Parma.

Study Design in Young Subjects

All subjects were submitted to a challenge with TRH (2-minute intravenous [IV] bolus injection of 200 μg TRH dissolved in 4 cc of 0.9% NaCl) with blood samples at –15, 0, 15, 30, 45, and 60 minutes from the beginning of the injection. In each subject, the test was performed on 4 occasions: (1) after 10 days of treatment with vit A orally administered as retinol at a dose of 50,000 IU/d; (2) after 10 days of oral placebo (PL) treatment; (3) after 1 hour from the administration of 40 mg T_3 at the end of 10 days of PL treatment; and (4) after 1 hour from the administration of 40 mg T_3 at the end of 10 days of vit A treatment. The order of the test to be performed in each subject was established according to a randomized, double-blind design. After collection, blood was centrifuged, and serum was stored at –20°C for TSH evaluation. Plasma was collected both before and after vit A treatment and stored at –80°C for the evaluation of circulating vit A levels.

Study Design in Old Subjects

All subjects were submitted to a TRH test according to the same experimental procedures for young subjects. The tests were performed according to a randomized, double-blind design on 2 different occasions: (1) after 10 days of treatment with PL; and (2) after 10 days of treatment with vit A at the same dose used for young subjects. Serum and plasma samples were stored as for young subjects for the evaluation of the same parameters.

Measurements of Circulating Levels of TSH and Vit A

Serum levels of human (h)TSH were measured with a sensitive immunoradiometric assay with a sensitivity of 0.013 μIU/mL, defined as the minimum concentration of hTSH, which can be distinguished from the 0 μIU/mL standard. Plasma concentrations of vit A were measured after extraction with ethanol and exane, by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 280 nm[18] with a Waters Simmetry C8 column (150 mm× 4.6 id) (Waters Co, Milford, MA).

Each mesurement was performed in duplicate. The intra- and inter-assay coefficients of variation were 3.4% and 5.6%, respectively, for TSH evaluation and 5.6% and 6.7%, respectively, for vit A determination.

Statistical Analyses

A preliminary analysis with the Shapiro-Wilk W statistic test was performed to determine whether the data conformed to a normal distribution, and the homogeneity of variance was computed by Bartlett's test. Data were analyzed using a 2-way repeated measure analysis of variance in which the effects of both time and treatment were evaluated. If an F value was significant ($P < .05$), Student's t test was used to compare the mean values. Since data on vit A levels were found to be not normally distributed, the effects of retinol administration on this parameter were evaluated by Wilcoxon signed rank test.

All statistical calculations were made using SPSS software (SPSS, Chicago, IL).[19] Values are expressed as mean ± SE.

RESULTS

Results Obtained in Young Subjects

Plasma vit A levels were significantly increased in young subjects after retinol treatment from a mean basal level of 2.48 ± 0.05 to a mean treatment level of 2.61 ± 0.07 μmol/L, $P < .05$ by Wilcoxon test (data not shown).

Basal serum TSH levels were similar in the 4 different treatment. In each of the experimental conditions, serum TSH increased significantly in response to the TRH test (Fig 1). Serum TSH increased from a basal value of 1.80 ± 0.31 to a peak of 11.92 ± 1.75 μIU/mL ($P < .001$) during the PL treatment and from a basal value of 1.81 ± 0.22 to a peak of 10.81 ± 1.00 μIU/mL ($P < .001$) during vit A treatment. When the TRH-induced TSH response was evaluated after 1 hour from the administration of T_3, the levels increased from a basal value of 1.72 ± 0.28 to a peak of 9.92 ± 1.10 μIU/mL ($P < .001$) during PL treatment and from a basal value of 1.79 ± 0.30 to a peak of 9.51 ± 1.12 μIU/mL ($P < .001$) during vit A treatment. The 2-way repeated measure anlaysis of variance revealed, however, no differences among treatments, although lower TRH-induced TSH responses were found after T_3 administration with or without concomitant vit A treatment.

The TRH-induced TSH responses were somewhat higher in females as compared with male subjects, although the difference did not reach statistical significance. No significant effects of sex were found in the responses of TSH to the different treatments.

Results Obtained in Old Subjects

Plasma vit A levels were significantly increased in old subjects after retinol treatment from a mean basal level of 2.46 ±

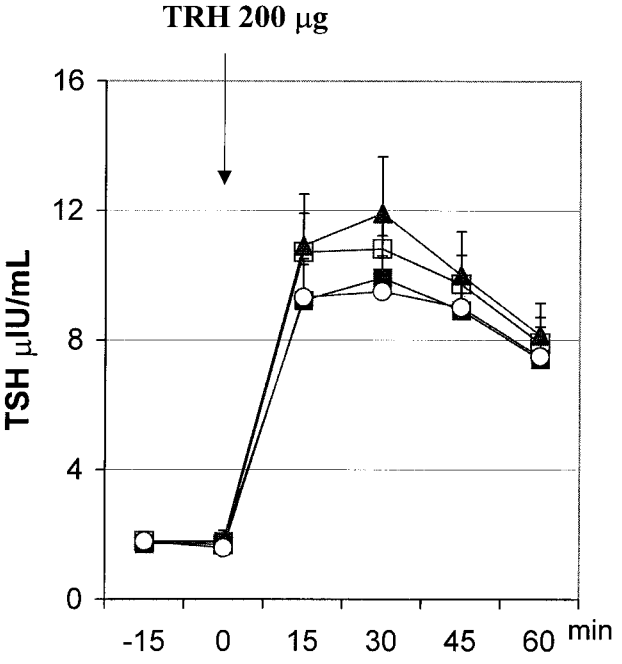

Fig 1. Serum TRH-induced TSH concentrations evaluated in young subjects after 10 days of treatment with either PL (▲) or vit A (□) and after 1 hour from the administration of 40 mg T_3 at the end of 10 days of treatment with either PL (■) or vit A (○). In each treatment, TSH values were significantly ($P < .001$) increased above baseline at each time point. The 2-way repeated measure analysis of variance revealed no differences among treatments.

0.10 to a mean treatment level of 2.57 ± 0.12 μmol/L, $P < .05$ by Wilcoxon test (data not shown).

Basal serum TSH levels were similar in the 2 experimental conditions and were not different from those observed in young subjects. Serum TSH levels increased significantly in response to the TRH stimulus (Fig 2) from a basal value of 2.16 ± 0.31 to a peak of 10.27 ± 0.55 μIU/mL ($P < .01$) during PL treatment and from a basal value of 2.10 ± 0.51 to a peak of 7.82 ± 1.40 μIU/mL ($P < .001$) during vit A treatment. No significant differences between young and old subjects were demonstrated in TRH-induced TSH responses obtained after PL treatment. No significant effects of treatment on TRH-induced TSH levels were demonstrated in old subjects, although TSH responses were somewhat lower during vit A treatment with a difference in the comparison with PL treatment close to the statistical significance.

Side Effects

No significant side effects were noted in either group of subjects during vit A administration, although 3 subjects in the young group and 1 in the old group, respectively, experienced a mild headache in the last 2 days of treatment.

DISCUSSION

The results of the present study demonstrate that vit A administration does not significantly affect either basal or TRH-induced serum TSH levels in healthy human subjects.

The data also exclude any synergistic effect between vit A and T_3 in the inhibition of the TSH response to TRH. It has been demonstrated that a single dose of T_3 is able to moderately, but significantly, inhibit TRH-induced serum TSH levels in humans within 1 hour of its oral administration.[12] When, in our study, T_3 was administered at the end of 10 days of PL treatment, only a moderate, nonsignificant decrease in the TSH response to TRH was detected. Analogously, no significant changes in TRH-induced TSH levels were demonstrated following T_3 administration at the end of 10 days of vit A treatment. We believe that if vit A had any synergistic effect with T_3, a significant reduction in serum TSH should have been demonstrated when T_3 was administered at the end of vit A treatment. On the contrary, our data suggest that no cooperation occurs between vit A and T_3 in the regulation of circulating TSH levels in humans, although this hypothesis was tested in our study only in young volunteers, due to the possibility that T_3 administration could be harmful to old subjects.

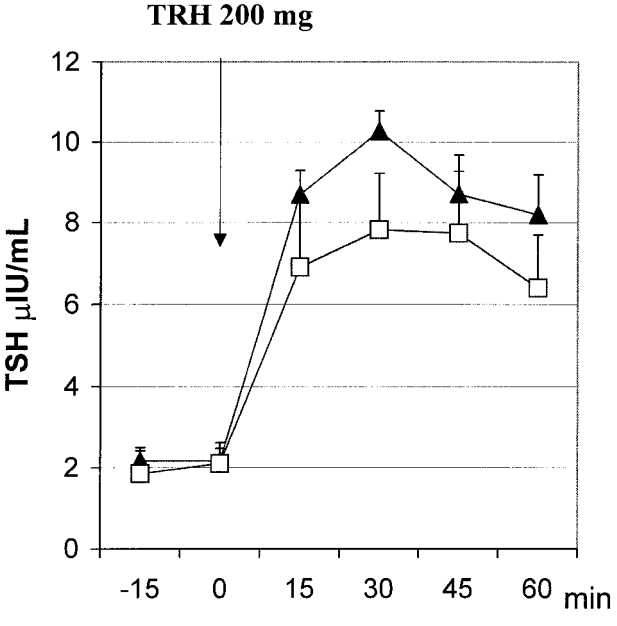

Fig 2. Serum TRH-induced TSH concentrations evaluated in old subjects after 10 days of treatment with either PL (▲) or vit A (□). In each treatment, TSH values were significantly ($P < .001$) increased above baseline at each time point. The 2-way repeated measure analysis of variance revealed no differences between treatments.

It is well known that both atRA and 9-cis-RA exert a suppressive effect on the activity of the thyrotroph-specific TSH β-subunit gene promoter,[14,20,21] and although some reports have demonstrated the lack of effects of in vivo vit A administration on the hypothalamo-pituitary-thyroid axis in experimental animals,[22] vit A deficiency has been shown to determine an increase in both TSH β-messenger RNA, as well as TSH content in rat pituitary.[13,14] Recent studies have shown that the parenteral administration of atRA in rats produces a blunting effect on both basal and TRH-stimulated TSH levels.[15] In 1999, Sherman et al[16] demonstrated that retinoid X receptor-selective ligands lead to central hypothyroidism when administered at high doses in a cohort of patients with cutaneous T-cell limphoma, although no effects were found in patients affected by other malignancies.[23] Our results suggest that a reduction in circulating TSH levels is unlikely to occur following oral vit A administration in normal human subjects. Based on literature reports, the possibility could be hypothesized that RA, but not retinol, is able to reduce serum TSH levels. However, RA represents a biologically active metabolite of retinol; therefore, an exposure to the effects of RA is likely to be hypothesized in our subjects. As in our study, vit A was given for 10 days, one could hypothesize that the treatment period was too short for any detection of retinol-induced hormonal effect. However, the RA-mediated inhibition of TSH release was reported to occur in rats after 14 days of treatment, a period only a few days longer than that established in our protocol; moreover, the dose of retinol used in our study was higher than the mean dose usually needed for chronic vit A replacement in vit A-depleted human subjects.

Literature reports demonstrate that TSH responses to TRH administration are slightly, although significantly, higher in women than in men.[24] However, we found no differences between sexes in TRH-stimulated TSH levels in any of the different treatments, probably due, at least in part, to the variability of the data.

We did not find any significat difference in either basal or TRH-stimulated TSH levels between young and old subjects. Although contrasting data have been shown on the effects of age on TSH secretion, our results are in agreement with previous reports.[25,26] Interestingly enough, we detected a somewhat blunting effect of vit A on TRH-induced TSH responses in old subjects. These results, however, did not reach statistical significance, probably due, at least in part, to the variability of the data observed in this group of subjects.

An age-related reduction in circulating levels of vit A have been reported.[17,27,28] In the present study, plasma levels of vit A were found to be lower in old subjects, but without any statistically significant difference in comparison with the young group. However, the possibility of an upregulation of RA receptors in old subjects, secondary to the lower circulating vit A levels, and hence higher effects of vit A administration in these subjects, cannot be ruled out.

In conclusion, the present study demonstrates the lack of effects of vit A on TSH circulating levels in normal human subjects. Also, these results suggest that no cooperation occurs between retinol and T_3 in the regulation of serum TSH concentrations.

REFERENCES

1. Giguere V: Retinoic acid receptor and cellular retinoid binding protein complex interplay in retinoid signaling. Endocr Rev 15:61-79, 1994
2. Thaller C, Eichele G: Identification and spatial distribution of retinoids in the developing chick limb bud. Nature 327:625-628, 1987
3. Glass CK: Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. Endocr Rev 15:391-407, 1994
4. Evans RM: The steroid and thyroid hormone receptor superfamily. Science 240:889-895, 1988
5. de The H, del Mar Vianco-Ruiz M, Tiollais P, et al: Identification of a retinoic acid responsive element in the retinoid acid receptor β gene. Nature 343:177-180, 1990
6. Umesono K, Murakami KK, Thompson CC, et al: Direct repeats as selective response element for the thyroid hormone, retinoic acid, and vitamin D3 receptors. Cell 65:1255-1266, 1991
7. Yen PM, Sugawara A, Chin WW: Triiodothyronine differentially affects T3-receptor/retinoic acid receptor and T3-receptor/retinoic X receptor heterodimer binding to DNA. J Biol Chem 267:23248-23252, 1992
8. Macchia E, Canale D: Thyroid state-dependent effect of retinoic acid on thyroid hormone and retinoic acid receptors. Eur J Endocrinol 131:375-376, 1994
9. Shupnik MA, Ridgway EC, Chin WW: Molecular biology of thyrotropin. Endocr Rev 10:459-475, 1989
10. Chin WW, Carr FE, Burnside J, et al: Thyroid hormone regulation of gene expression. Recent Prog Horm Res 48:393-414, 1992
11. Surks MI, Lifschitz BM: Biphasic thyrotropin suppression in euthyroid and hypothyroid rats. Endocrinology 101:769-773, 1977
12. Azizi F, Vagenakis AG, Portnay GI, et al: Effect of a single oral dose of triiodothyronine on the subsequent response to TRH in normal individuals. J Clin Endocrinol Metab 40:157-159, 1975
13. Morley JE, Damassa DA, Gordon J, et al: Thyroid function and vitamin A deficiency. Life Sci 22:1901-1906, 1978
14. Breen JJ, Matsuura T, Ross C, et al: Regulation of thyroid-stimulating hormone β-subunit and growth hormone messenger ribonucleic acid levels in the rat: Effect of vitamin A status. Endocrinology 136:543-549, 1995
15. Coya R, Carro E, Mallo F, et al: Retinoic acid inhibits in vivo thyroid-stimulating hormone secretion. Life Sci 60:247-250, 1997
16. Sherman SI, Gopal J, Chiu AC, et al: Central hypothyroidism associated with retinoid X receptor selective ligands. N Engl J Med 340:1075-1079, 1999
17. Mecocci P, Polidori MC, Troiano L, et al: Plasma antioxidant and longevity: A study on healthy centenarians. Free Radic Biol Med 28:1243-1248, 2000
18. Nierenberg DW, Nann SL: A method for determining concentrations of retinol, tocopherol, and five carotenoids in human plasma and tissue samples. Am J Clin Nutr 56:417-426, 1992
19. Norusis MJ: SPSS-X Advanced Statistic Guide. New York, NY, McGraw-Hill, 1985
20. Haugen BR, Brown NS, Wood WM, et al: The thyrotrope-restricted isoform of the retinoid-X receptor-γl mediates 9-cis-retinoic acid suppression of thyrotropin-β promoter activity. Mol Endocrinol 11:481-489, 1997
21. Breen JJ, Hickok NJ, Gurr JA: The rat TSH beta gene contains distinct response elements for regulation by retinoids and thyroid hormone. Mol Cell Endocrinol 131:137-146, 1997
22. Morley JE, Melmed S, Reed A, et al: Effect of vitamin A on the hypothalamo-pituitary-thyroid axis. Am J Physiol 238:E174-E179, 1980
23. Miller VA, Benedetti FM, Rigas JR, et al. Initial clinical trial of a selective retinoid X receptor ligand, LGD1069. J Clin Oncol 15:790-795, 1977
24. Azizi F, Vagenakis AG, Portnay GI, et al.: Pituitary-thyroid responsiveness to intramuscular thyrotropin-releasing hormone based on analyses of serum thyroxine, triiodothyronine and thyrotropin concentrations. N Engl J Med 292:292-297, 1975
25. Harman SM, Wehmann RE, Blackman MR: Pituitary-thyroid hormone economy in healthy aging men: Basal indices of thyroid function and thyrotropin response to constant infusions of thyrotropin-releasing hormone. J Clin Endocrinol Metab 58:320-326, 1984
26. Kabadi UM, Rosman P: Lack of influence of aging on hypothalamic pituitary thyroid axis in normal subjects. J Clin Exp Gerontol 9:189-199, 1987
27. Succari M, Garric B, Ponteziere C, et al: Influence of sex and age on vitamin A and E status. Age Ageing 20:413-416, 1991
28. Stauber PM, Sherry B, Vanderjagt DJ, et al: A longitudinal study of the relationship between vitamin A supplementation and plasma retinol, retinyl esters, and liver enzyme activities in a healthy elderly population. Am J Clin Nutr 54:878-883, 1991