Regulation of Thyrotropin Biosynthesis

DISCORDANT EFFECT OF THYROID HORMONE ON α AND β SUBUNIT mRNA LEVELS*

(Received for publication, March 14, 1983)

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We have studied the regulation of the biosynthesis of thyrotropin (TSH) and its α and β subunits by thyroid hormone in thyrotropic tumors carried in hypothyroid mice. Treatment with 3,5,3′-triiodothyronine (T₃) (20 μg/100 g, body weight) daily for 4 or 10 days reduced serum TSH to 3 and 0.3% of control, respectively. Serum levels of free α subunit were reduced to 60 and 11% of control at 4 and 10 days, respectively, and serum free TSH-β was undetectable at both time points. There was no significant decrease in tumor TSH content after 4 days of treatment and, after 10 days, TSH content was reduced to 15% of control levels. There was no significant effect of T₃ on tumor α subunit levels, whereas translatable TSH-β mRNA in a rabbit reticulocyte lysate system showed that thyroid hormone decreased translatable TSH-β mRNA to undetectable levels at both 4 and 10 days, whereas translatable α mRNA was reduced strikingly only at 10 days in one of two tumors. RNA blot hybridization with ³²P-labeled plasmid probes containing α or TSH-β cDNAs showed that TSH-β mRNA was reduced to less than 10% of control after both 4 and 10 days of T₃ treatment, whereas, again, α mRNA was only reduced in one of two tumors at 10 days. Our data thus show that thyroid hormone affects α and TSH-β mRNA and protein levels discordantly and suggest that regulation of TSH biosynthesis may occur predominantly at the level of TSH-β mRNA.

TSH is one of the family of glycoprotein hormones, which also includes the pituitary hormones, FSH and LH, and placental CG. Each hormone is composed of two dissimilar, noncovalently bound glycosylated subunits, α and β. In the same species, these hormones share a common α subunit, whereas each β subunit is unique and confers biologic and immunologic specificity to the complete hormone (1). It has been shown that the α and β subunits of each hormone are synthesized from separate mRNAs (2-9).

Thyroid hormones exert a major inhibitory effect on TSH production; using the mouse thyrotropic tumor as a model system, thyroid hormones have been shown to decrease both basal and thyrotropin releasing hormone-stimulated synthesis and secretion of TSH and its free α and β subunits (10-12). Ross et al. (13) have recently reported decreased serum TSH, α, and TSH-β levels in hypothyroid mice after thyroxine treatment; however, although the pituitary content of TSH and TSH-β also decreased, pituitary α content was either unaffected or increased. In a preliminary report (14), we had previously shown that treatment of hypothyroid mice bearing thyrotropic tumors with T₃ caused a marked decrease in tumor TSH and TSH-β content, but increased tumor α subunit levels. Serum TSH and TSH-β were suppressed by T₃, but serum α decreased only minimally.

To determine the molecular basis for the discordant effect of thyroid hormones on TSH subunit biosynthesis, we have investigated the regulation of α and TSH-β mRNA levels by T₃ using mouse α and TSH-β cDNAs, synthesized and cloned in this laboratory (15, 16), as specific probes. We now report that T₃ discordantly affects α and TSH-β mRNA levels, as well as subunit protein concentrations. Our data suggest that the regulation of TSH biosynthesis occurs predominantly at the level of TSH-β mRNA.

EXPERIMENTAL PROCEDURES

Animals and Hormone Treatment—Male LAF mice (Jackson Laboratories), 1-2 months of age, were placed on a low iodine diet for 1 month before radiothyroidectomy by injection with 135 μCi of I¹³¹. Between 1 and 2 months later, all mice in a particular experimental group were injected subcutaneously with mouse thyrotropic tumor tissue from a single donor animal. Tumors were used when they had reached about 5 g in size, typically 6 months after injection. Experimental animals received T₃ (Sigma) at a dose of 20 μg/100 g, body weight, intraperitoneally daily for 4 or 10 days before sacrifice; control animals received saline. There was no change in body weight or tumor size during T₃ treatment. Blood was collected by cardiac puncture under ether anesthesia and serum stored at −20 °C.

Radioimmunoassays—Radioimmunoassays for TSH and its free α and β subunits were carried out using a double antibody technique, as previously described (17), using antibodies prepared in this laboratory against bovine TSH, TSH-α, and TSH-β (kindly provided by Dr. J. G. Pierce, Los Angeles, CA). Rat LH-α, TSH, and TSH-β, used for iodination and as standards, were provided by the Hormone Distribution Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. Mouse TSH and TSH-β were found to be immunologically distinct from standard rat TSH and TSH-β, as shown by the nonparallelism of TSH-α, TSH-β, and TSH-β in the TSH and TSH-β assays, respectively. Therefore, a single mouse tumor extract was used as a standard, and the results converted from microliter equivalents of tumor extract into nanogram equivalents of rat TSH or TSH-β by using the cross-reactivity of 50% displacement. Mouse and rat α showed parallel displacement in the same assay. In the α assay, rat TSH-β did not cross-react significantly, and rat TSH showed a 15% cross-reaction. In the TSH assay, rat LH-α did not cross-react, and rat TSH-β showed a 40% cross-

* This work was supported by United States Public Health Service Grant CA-25385 and by Grant 1-682 from the March of Dimes Birth Defects Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipient of United States Public Health Service Research Career Development Award AM-00679.

‡ The abbreviations used are: TSH, thyrotropin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; CG, chorionic gonadotropin; T₃, 3,5,3′-triiodothyronine; T₄, 3,5,3′,5′-tetraiodothyronine; SSC, 0.15 M NaCl, 0.015 M Na citrate.
reaction. Rat LH-α showed no cross-reactivity in the TSH-β assay, and rat TSH showed a 16% cross-reaction. The statistical significance of differences was determined using Student's two-tailed t test.

RNA Extraction, Cell-free Translation, and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis—Total RNA was extracted from tumor tissue by a phenol/chloroform method (8), and poly(A) mRNA was selected by two cycles of oligo(dT)-cellulose chromatography (18).

Poly(A) mRNA was translated in a cell-free reticulocyte lysate system (19) containing [35S]methionine (1000 Ci/mmol, New England Nuclear) and supplemented with dog pancreatic microsomal membranes (8) (2.0 Aeq/ml; kindly supplied by Dr. Dennis Shields, Bronx, NY). Synthesized peptides were identified by indirect immunoprecipitation using purified IgG fractions of rabbit anti-ovine LH-α, anti-bovine TSH-β, and nonimmune serum, as previously described (8). Peptides were separated by electrophoresis on 12–20% polyacrylamide slab gels containing 0.1% sodium dodecyl sulfate (20). After treatment for fluorography (21), the dried gels were exposed at -70 °C to Kodak XAR-2 x-ray film.

Blot Hybridization of α and TSH-β mRNA—For RNA gel blot hybridization, poly(A) mRNA was denatured with glyoxal (22), fractionated by electrophoresis on 2% agarose gels in 10 mM sodium phosphate, pH 7.0, (22) and transferred to nitrocellulose, as described by Thomas (23). The blots were then hybridized (24) with a plasmid probe containing mouse α (pTSH-α) or TSH-β (pTSH-β) cDNA insert, labeled with [α-35P]dCTP by nick translation (25) to a specific activity of 1–4 × 106 cpn/μg of DNA. After washing (23), the blots were exposed to Kodak XAR-2 x-ray film at -70 °C with intensifying screens.

Quantitation—The intensity of bands on autoradiograms was quantitated by scanning in a densitometer with a peak area integrator (Quick Scan Jr., Helena Laboratories).

**RESULTS**

Regulation of T3 of TSH and Free α and TSH-β Protein Levels—Treatment of hypothyroid mice bearing the thyrotropic tumor TtT 108B with T3 (20 μg/100 g, body weight) daily for 4 or 10 days decreased the level of intact TSH in pooled serum to 3 and 0.5% of control, respectively (Fig. 1). After 4 and 10 days of T3 treatment, serum levels of free α subunit were reduced to 60 and 11% of control, respectively; free TSH-β subunit was reduced to undetectable levels (<0.2% of control) at both 4 and 10 days. Although the decrease in the tumor mean TSH content was not statistically significant after 4 days of T3 treatment, after 10 days the TSH content was reduced to 15% of the level in untreated animals (p < 0.002). There was no significant change in tumor free α content after either 4 or 10 days. In contrast, tumor free TSH-β content was reduced to 29% (p < 0.01) and 10% (p < 0.001) of control levels after 4 and 10 days of T3 treatment, respectively.

Regulation by T3 of Translatable α and TSH-β mRNA—We wished to determine whether the divergent effects of T3 on tumor α and TSH-β protein content might be due to similarly divergent changes in the tumor content of translatable α and TSH-β mRNAs. Therefore, poly(A) mRNA was isolated from TtT 108B tumors from individual untreated mice (Fig. 2, A and B) and from individual animals treated with T3 for 4 days (Fig. 2, C and D) and for 10 days (Fig. 2, E and F). The RNAs were electrophoresed in a rabbit reticulocyte cell-free translation system which was supplemented with microsomal membranes to ensure cotranslational processing of pre-α and pre-β subunits to their mature, glycosylated, immunoprecipitable forms. Immunoprecipitation of translation products with TSH-β specific antibody (Fig. 2, bottom) showed that T3 treatment for either 4 or 10 days reduced the synthesis of TSH-β protein directed by tumor mRNA to undetectable levels. Mature TSH-β subunit migrates with a Mr = 18,000; the background protein bands are unrelated to TSH-β since they are also precipitated by nonimmune serum (Fig. 2, bottom, lane NI). In contrast, T3 treatment did not decrease the translatable α mRNA level at 4 days, and at 10 days translatable tumor α mRNA was only markedly decreased in one of the two animals (Fig. 2, top). Thus, in spite of the variation in translatable α mRNA between individual animals, the discordance of the effect of T3 on α and TSH-β mRNA was striking.

Regulation by T3 of Hybridizable α and TSH-β mRNA—To investigate whether T3 treatment caused parallel changes in tumor α and TSH-β mRNA content and α and TSH-β mRNA translatability, we determined α and TSH-β mRNA content by blot hybridization with plasmid probes containing α and TSH-β-specific cDNA inserts (15, 16). Glyoxal-denatured poly(A) mRNA from untreated and T3-treated tumors was fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized to 32P-labeled α and TSH-β plasmid probes. Under these conditions, α mRNA was seen as a single species approximately 850 nucleotides in length and TSH-β as a single band of about 750 nucleotides (Fig. 3). It was found that after both 4 and 10 days of T3 treatment there was a dramatic decrease in the tumor content of TSH-β.
tumors have demonstrated qualitatively normal responses to regulators of TSH production, such as thyroid hormones and thyrotropin releasing hormone (10–12).

We have shown that treatment of mice bearing thyrotropic tumors with T3 daily for 4 or 10 days suppressed the serum levels of TSH, free TSH-α, and less markedly, free α subunit. The responses of tumor α and TSH-β protein content to T3 were discordant; there was a marked decrease in free TSH-β, with a parallel decrease in complete TSH, but no significant change in tumor free α content after either 4 or 10 days of T3 treatment. Such a divergent response is similar to that reported by Ross et al. (13), for α and TSH-β subunits in the pituitaries of hypothyroid mice in response to thyroxine. The nature of the response is consistent with the hypothesis that the availability of TSH-β subunits limits the production of complete TSH in the pituitary and in thyrotropic tumors. The fact that serum TSH, α, and TSH-β levels were suppressed to a greater extent than were tumor TSH, α, and TSH-β protein content suggests that T3 may have a rapid post-translational effect on subunit secretion which is distinct from any effects on subunit synthesis.

Treatment with T3 caused a marked reduction in TSH-β mRNA, as estimated both by hybridization with the TSH-β-specific probe and by in vitro translation. These data suggest that T3 acts on TSH-β biosynthesis at a pretranslational level. The effects of T3 on translatable and hybridizable α mRNA were more variable between individual animals, but it is clear that the reductions in α mRNA were less pronounced and independent of those in TSH-β mRNA. The regulation of specific proteins by thyroid hormones has been correlated in several cases with modulation of mRNA levels (29–31) and the binding of a T3-receptor complex to specific nuclear sites (32). Moreover, it has recently been directly demonstrated that T3 increases the level of growth hormone gene transcription (33, 34). Our results are consistent with a model in which T3 regulates TSH-β mRNA levels by inhibition of TSH-β gene transcription. However, with our present data, we cannot rule out mechanisms involving changes in specific cytoplasmic mRNA turnover or in the processing of nuclear RNA precursors. If the availability of the TSH-β subunit were limiting the formation of complete TSH, then T3 would modulate TSH biosynthesis by decreasing TSH-β mRNA levels via the inhibition of TSH-β gene transcription. The considerably less marked effect of T3 treatment on α mRNA content shows that the discordant effect of T3 on α and TSH-β protein levels is paralleled by similar divergent effects on α and TSH-β mRNA levels.

Acknowledgment—We thank Jo Ann Gili for preparation of the manuscript.

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