Interacting Role of Thyroxine and Growth Hormone in the Hepatic Synthesis of \(\alpha_{2u}\)-Globulin and Its Messenger RNA*

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Hypophysectomy completely abolishes thyroidectomy results in a 90% reduction in the hepatic content of \(\alpha_{2u}\)-globulin and its mRNA in the male rat. Thyroid hormone is also known to be required for the synthesis and secretion of pituitary growth hormone. In the hypothyroid rat either thyroxine or growth hormone was found to increase the activity and number of sequences of the mRNA for \(\alpha_{2u}\)-globulin (measured by transcriptional assay and hybridization analysis with a cloned cDNA probe) to the euthyroid level. Treatment of hypophysectomized rats with a hormone combination containing growth hormone but not thyroxine increased the hepatic level of the mRNA for \(\alpha_{2u}\)-globulin to that of normal animals. From these results we conclude that thyroxine indirectly influences the hepatic concentration of the mRNA for \(\alpha_{2u}\)-globulin through its effect on pituitary growth hormone. Although administration of growth hormone to hypothyroid animals raised the hepatic concentration of \(\alpha_{2u}\)-globulin mRNA to the euthyroid level, synthesis of \(\alpha_{2u}\)-globulin remained low (50% of the normal). Complete recovery of \(\alpha_{2u}\)-globulin synthesis required thyroxine. Therefore, in addition to an indirect effect on the hepatic level of \(\alpha_{2u}\)-globulin mRNA, thyroxine also directly influences the synthesis of this protein. This direct effect of thyroxine on \(\alpha_{2u}\)-globulin synthesis seems to be exerted at a step distal to the formation of mature mRNA.

The thyroid hormones, tri- and tetraiodothyronines (T3 and T4), regulate metabolic homeostasis, growth, and differentiation (1). The role of thyroid hormone in the regulation of metabolic processes is exemplified by changes in energy metabolism associated with thyroid abnormalities and the dramatic effect of the hormone on the processes of differentiation and morphogenesis is shown by the thyroxine mediated metamorphic transition of the amphibian tadpole from its aquatic to terrestrial habitat. Most of these effects are thought to be mediated through thyroxine-dependent changes in specific gene expression (2). Chromatin-associated thyroid hormone receptor has also been identified and characterized (3, 4). In addition, thyroxine-dependent changes in the cellular concentrations of the mRNAs for several proteins and enzymes have provided some evidence for a direct role of thyroid hormone in gene transcription (5–11). One of these proteins is \(\alpha_{2u}\)-globulin, the male rat urinary protein of hepatic origin (12). We have previously shown that thyroidectomy causes more than a 90% reduction in the androgen-dependent synthesis of \(\alpha_{2u}\)-globulin and that the normal level of synthesis can be regained with thyroxine treatment (13). Furthermore, these effects are associated with corresponding changes in the hepatic concentration of the mRNA for this protein (10, 11). The hepatic synthesis of \(\alpha_{2u}\)-globulin is also influenced by pituitary growth hormone (13–16). Hervas et al. (17) have shown that thyroidectomy within 24 days causes more than a 99% reduction in the pituitary level of growth hormone. The requirement for thyroxine in the synthesis and secretion of growth hormone has subsequently been confirmed and extended both at a cellular and molecular level (5–7). These observations have led us to examine the interacting influence of thyroxine and growth hormone in the regulation of the hepatic synthesis of \(\alpha_{2u}\)-globulin and its mRNA. The results show that the effect of thyroxine on the mRNA for \(\alpha_{2u}\)-globulin is indirectly mediated through pituitary growth hormone. A preliminary report of this study has appeared as an abstract (18).

EXPERIMENTAL PROCEDURES

**Animals and Hormone Treatment**—Male rats (~300 g) of Sprague-Dawley strain were obtained from Zivic Miller Laboratory (Allison Park, PA). Surgical operations were performed by the supplier. Hypophysectomized rats were given at least 2 weeks of postoperative rest. Thyroidectomized animals were subjected to a single intraperitoneal injection of 3.71 (100 μCi/ml) and the experiments were performed at least 1 month later. Doses of different hormone supplementations per 100 g body weight were: 5α-dihydrotestosterone, 50 μg; thyroxine, 1.5 μg; growth hormone (ovine, 0.6 units/mg), 0.2 units; corticosterone, 2 mg. Earlier studies in our laboratory have established these doses as optimum for the induction of \(\alpha_{2u}\)-globulin. Thyroxine was administered intraperitoneally while all other hormones were given subcutaneously. Unless otherwise mentioned experimental animals received eight daily treatments of the respective hormones before they were killed. Animals were housed in an air-conditioned room (23°C) with 12 h of light and darkness and were given free access to food and water.

**Isolation of Messenger RNA, Its Translation in the Rabbit Reticulocyte Lysate and SDS**—Polyacrylamide Gel Electrophoresis of the Translation Products—Total hepatic nucleic acid was extracted with phenol-SDS (19) and poly(A)-containing mRNA was isolated by affinity chromatography on oligo(T)-cellulose (20). Total mRNA was translated in micrococcal nuclease treated rabbit reticulocyte lysate in the presence of [35S]methionine (21). The translation products were separated by SDS-polyacrylamide slab gel electrophoresis as described (22). After electrophoresis the gel was fixed in methanol-acetic acid/water (40:10:50 v/v), dried, and autoradiographed using a Kodak X-Romat X-ray film. In some cases, the amount of \(\alpha_{2u}\)-globulin synthesized within the total translation products of hepatic mRNA was quantitated by specific immunoprecipitation followed by affinity chromatography on oligo(T)-cellulose. A complementary DNA (cDNA) clone of \(\alpha_{2u}\)-DNA was cloned in Escherichia coli (HB101) using pBR322 as the vector (15). The recombinant plasmid was constructed by annealing the (dG)20 tailed plasmid with the (dC)20 tailed cDNA. The competent HB101 cells are used for transformation.

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1 The abbreviation used is: SDS, sodium dodecyl sulfate.
were transformed by the chimeric plasmid and the \( \alpha_{2u} \)-cDNA containing clones were identified through colony hybridization and specific hybrid selected mRNAs translation (24, 25). The double-stranded cDNA, after its excision from the recombinant plasmid, was labeled to \( 10^6 \) cpm/\( \mu g \) of DNA by nick translation (26) using \( [\alpha-^3P]dCTP \), and a single-stranded probe was prepared from the double-stranded cDNA (27). RNA excess hybridization was carried out at 68 °C using siliconized glass vials and these procedures have also been described before (22).

Agarose Gel Electrophoresis and Blot Hybridization of \( \alpha_{2u} \)-Globulin mRNAs—Samples (20 \( \mu g \)) of poly(A)-enriched hepatic RNA isolated by affinity chromatography on oligo(dT)-cellulose were subjected to electrophoresis on 1.5% agarose slab gel containing 10 mM methylmercury hydroxide. The gel was stained with ethidium bromide and photographed under ultraviolet light. Electrophoretically resolved RNA bands were transferred and covalently bound to diazobenzyloxymethyl-cellulose paper according to Alwine et al. (28). The paper was subsequently hybridized to \( ^3P \)-labeled \( \alpha_{2u} \)-globulin cDNA probe for 12 h at 42 °C in a plastic bag according to Wahl et al. (29). After hybridization the paper was washed, air-dried, and autoradiographed on Kodak X-ray film at -70 °C.

In Vivo Labeling of Total Hepatic Proteins and Quantitation of Newly Synthesized \( \alpha_{2u} \)-Globulin—Animals were given intraperitoneal injections of \( [\text{[35S}] \)methionine (0.8 mCi/rat) and were killed 18 min later. The liver was homogenized in a solution containing 50 mM Tris-HCl, pH 7.5, 5 mM Mg-aceate, 25 mM KCl, 0.25 mM sucrose, 1% Triton X-100, and 0.05% cycloheximide (1 ml/g of liver). The cytosol was prepared from the liver homogenate as described (30). \( \alpha_{2u} \)-Globulin was immunoprecipitated from the cytosol containing \( 0.8 \times 10^6 \) cpm of protein radioactivity and the immunoprecipitate was subjected to SDS-polyacrylamide disc gel electrophoresis. After electrophoresis the gels were fractionated and the ratio of radioactivity under the \( \alpha_{2u} \)-globulin peak to total input radioactivity was used as the index of \( \alpha_{2u} \)-globulin synthesis.

Determination of the Hepatic Level of \( \alpha_{2u} \)-Globulin—Preparation of the rat liver cytosol and radioimmunoassay for \( \alpha_{2u} \)-globulin were performed according to Roy (31).

RESULTS

Figure 1 shows that unlike normal (euthyroid) rats, thyroidectomized (hypothyroid) rats contain very low level of translationally active \( \alpha_{2u} \)-globulin mRNA. Treatment of hypothyroid rats with thyroxine resulted in an increase in the mRNA for \( \alpha_{2u} \)-globulin to almost normal level. Furthermore, treatment of thyroidectomized rats with growth hormone also resulted in the elevation of the mRNA for \( \alpha_{2u} \)-globulin which from the intensity of the band appears almost equivalent to the euthyroid control.

Fig. 2 shows the extent of hybridization between a cloned \( \alpha_{2u} \)-globulin cDNA probe and poly(A)-enriched hepatic RNA obtained from animals with different endocrine status. From these results it could be estimated that hypothyroid rats treated with growth hormone contained approximately 89% of the normal euthyroid level of \( \alpha_{2u} \)-globulin mRNA sequences as compared to 94 and 10% respectively, in thyroidectomized animals receiving thyroxine and no hormone supplementation. The hybridization data, therefore, are in agreement with the results of the in vitro translation of the hepatic mRNA from thyroidectomized rats receiving either thyroxine or growth hormone.
growth hormone supplementation. These results show that either thyroxine or growth hormone is effective in increasing the hepatic level of the activity and number of sequences of the mRNA for $\alpha_2\beta$-globulin in the hypothyroid rat.

The possibility of an indirect role of thyroxine in the regulation of the hepatic concentration of mRNA for $\alpha_2\beta$-globulin was further examined in hypophysectomized male rats receiving multiple hormone treatment. Surgical removal of the pituitary gland results in complete inhibition of the hepatic synthesis of $\alpha_2\beta$-globulin and the disappearance of the mRNA for this protein. These effects of hypophysectomy can be reversed by treatment with androgen, glucocorticoid, thyroxine, and growth hormone (13). Fig. 3 shows that hypophysectomized rats which received a three-hormone treatment including androgen, glucocorticoid, and growth hormone contained an almost normal level of $\alpha_2\beta$-globulin mRNA. However, as compared to untreated controls no significant increase in the hepatic content of $\alpha_2\beta$-mRNA was observed in hypophysectomized rats which received a three-hormone combination containing thyroxine, but not growth hormone (i.e. androgen, glucocorticoid, and thyroxine). A higher dose of thyroxine (30 mg/100 g), when used together with the above two steroids, also failed to induce the mRNA for $\alpha_2\beta$-globulin (data not included in the figure). These results indicate that growth hormone and thyroxine do not operate through a common mechanism and substantiate the conclusion that the effect of thyroxine on the hepatic concentration of $\alpha_2\beta$-globulin mRNA may be mediated through pituitary growth hormone.

Determination of $\alpha_2\beta$-globulin in the liver cytosol of thyroidectomized rats after thyroxine or growth hormone supplementation showed that, in spite of a normal hepatic content of $\alpha_2\beta$-globulin mRNA, thyroidectomized rats receiving growth hormone alone contained ~50% less $\alpha_2\beta$-globulin than did the euthyroid rat. A subnormal rate of synthesis of $\alpha_2\beta$-globulin in the liver of thyroidectomized rats receiving growth hormone was also found by in vivo labeling of the hepatic proteins with an 18-min pulse of $[^35]S$-methionine followed by immunoelectrophoretic isolation of $\alpha_2\beta$-globulin. These results are summarized in Fig. 4. As can be seen that treatment of hypothyroid rats with either thyroxine or growth hormone was able to raise the hepatic concentration of $\alpha_2\beta$-globulin mRNA to almost normal. Unlike thyroxine, growth hormone alone could not reverse the hepatic synthesis of $\alpha_2\beta$-globulin to normal. The above observations indicate that growth hormone is required for maintaining the hepatic concentration of the $\alpha_2\beta$-globulin mRNA while the effect of thyroxine is exerted at a step distal to mRNA synthesis.

In order to obtain additional evidence for the enhanced utilization of $\alpha_2\beta$-globulin mRNA by thyroxine, we studied the effect of this hormone on the hepatic level of $\alpha_2\beta$-globulin and
its mRNA in thyroidectomized rats pretreated with growth hormone. In this set of experiments, a group of thyroidectomized rats were initially treated for 8 days with daily injections of growth hormone. On the 9th day, the animals ceased to receive growth hormone and were started on a daily treatment with thyroxine. Hepatic concentrations of a2-globulin and its mRNA at various days after thyroxine treatment are presented in Fig. 5. The results show that thyroxine did not cause any increase in the concentration of a2-globulin mRNA over the level reached after pretreatment with growth hormone. However, it caused an almost linear increase in the level of a2-globulin, resulting in more than a 2-fold increase at day 7 over the day 1 value. These data indicate that the effect of thyroxine on the hepatic content of a2-globulin mRNA is not additive over growth hormone and also substantiate the conclusion that thyroxine may enhance the utilization of a2-globulin mRNA induced by growth hormone.

**DISCUSSION**

Both thyroxine and growth hormone exert a considerable influence on protein synthesis in the liver but their mechanisms of action have not been clearly established. It has been suggested that thyroxine acts at a pretranslational level to stimulate the synthesis of the mRNA for a2-globulin (10, 11). However, the present results clearly show that the thyroxine-mediated increase in the hepatic concentration of the mRNA for a2-globulin is brought about indirectly via growth hormone. A thyroidectomized rat is, in fact, deficient in both thyroxine and growth hormone, and supplementation of thyroxine and other secretory proteins. Coordinated proliferation of the endoplasmic reticulum and topographic redistribution of the hormone may impair the postsynthetic processing of albumin for a2-globulin is brought about indirectly via growth hormone.

These investigators suggested that a deficiency in thyroid hormone on target cells, and (b) a delayed secondary effect due to a gradual rise in the circulating level of the growth hormone. The indirect nature of thyroxine action can also account for the long lag period (about 4 days) in the thyroxine-dependent stimulation of a2-globulin and its mRNA in the thyroidectomized rat (10, 13). No such lag is observed when thyroidectomized rats are pretreated with growth hormone.

Thyroxine also exerts a direct effect on the hepatic utilization of the mRNA for a2-globulin. This is indicated by the fact that despite a normal level of a2-globulin mRNA, hypothyroid rats treated with growth hormone can only synthesize about 50% of the normal level of a2-globulin. This is also true for hypophysectomized rats receiving a three-hormone treatment which contains growth hormone but not thyroxine. It is of interest to note that Peavy et al. (32) recently reported that thyroidec
tomy in the rat results in a 50% decrease in the hepatic secretion of albumin, without causing any concomitant decrease in the hepatic concentration of albumin mRNA. These investigators suggested that a deficiency in thyroid hormone may impair the posttranslational processing of albumin and other secretory proteins. Coordinated proliferation of the endoplasmic reticulum and topographic redistribution of the hepatic polyribosomes from free to the bound form during thyroxine induced metamorphosis of the bullfrog tadpole have been reported (2). These results support a possible role of thyroxine in the regulation of translation and processing of secretory proteins. A paucity of the rough endoplasmic reticulum in the liver of hypothyroid rats and its correction after thyroxine administration have also been observed (33). However, recent preliminary results from our laboratory have failed to provide conclusive evidence for an altered distribution of a2-globulin mRNA between free and the membrane-bound state in hypothyroid rats which received either thyroxine or growth hormone. 2 Therefore, the mechanism of the thyroxine mediated increase in the utilization of the mRNA for a2-globulin at this point remains unclear and its elucidation will require further investigation.

The results presented in this article clearly demonstrate a dominant role of growth hormone in the regulation of the hepatic concentration of a2-globulin mRNA in the hypothyroid rat and also an important role of thyroxine in the utilization of the mRNA for this protein. However, two important points need to be emphasized. First, considerable caution should be exerted for any inference concerning translational regulation of a2-globulin by thyroxine. Second, it should be pointed out that studies on the thyroxine mediated expression of the growth hormone gene in GH1 cells and identification of chromatin associated receptors for thyroid hormone provide strong support for the concept of a direct effect of the thyroid hormone on gene expression (3–7). The observations reported here and elsewhere (2, 32) only highlight the pleiotropic nature of the action of thyroid hormone on protein synthesis and gene expression in target cells.

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**REFERENCES**


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Thyroxine, Growth Hormone, and α2u-Globulin