Pretranslational Regulation of α₂u-Globulin in Rat Liver by Growth Hormone


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Hypophysectomy is known to cause complete suppression of the hepatic synthesis of α₂u-globulin. The effect of hypophysectomy on the synthesis of α₂u-globulin can be reversed by multiple hormone treatment. The role of pituitary growth hormone in the multihormonal regulation of α₂u-globulin in rat liver was examined in the hypophysectomized male rats with and without growth hormone supplementation. Daily treatment of hypophysectomized rats with 5α-dihydrotestosterone, corticosterone, thyroxine, and growth hormone for 8 days caused about 80% recovery in the hepatic content of α₂u-globulin and its corresponding mRNA as determined by radioimmunoassay, in vitro translation, and liquid hybridization with a cloned cDNA probe. However, omission of growth hormone from the treatment regimen failed to raise hepatic α₂u-globulin and its mRNA to more than 5% of the normal control. The possible effect of growth hormone on the translation of the mRNA for α₂u-globulin was examined with cultured hepatocytes derived from growth hormone-deficient rats. Culture of these cells in the presence of growth hormone for 24 h did not turn on the synthesis of α₂u-globulin. These results indicate that growth hormone regulates the synthesis of α₂u-globulin by acting at a step antecedent to mRNA translation.

α₂u-Globulin is a rat urinary protein of hepatic origin (1). In the male rat this protein appears at the time of puberty and its synthesis can be induced in the spayed female with androgen (2). The androgen-dependent synthesis of α₂u-globulin is known to require the simultaneous presence of glucocorticoid, thyroxine, pituitary growth hormone, and insulin (3-6). The multihormonal requirement for the hepatic synthesis of α₂u-globulin can serve as an important model for the study of the hormonal interactions in the regulation of a specific gene expression and this system has been effectively used for investigating the mechanism of action of both steroid and nonsteroidal hormones (6-9). The mechanism of the growth hormone-mediated changes in specific gene expression is not clearly understood and earlier results from our laboratory have indicated pretranslational regulation of α₂u-globulin by growth hormone (10). We have therefore continued and extended our investigations on the mechanism of action of growth hormone in the regulation of α₂u-globulin. The results that follow demonstrate that growth hormone is required for the maintenance of the normal hepatic concentration of the mRNA for this protein.

EXPERIMENTAL PROCEDURES

Animals and Hormone Treatment—Hypophysectomized male Sprague-Dawley rats (~300 g) were obtained from the Zivic-Miller Laboratory (Alison Park, PA). Doses of different hormones that cause complete suppression of α₂u-globulin and were therefore used for this study are: 5α-dihydrotestosterone, 50 μg/100 g; corticosterone, 2 mg/100 g; thyroxine, 1.5 μg/100 g; growth hormone (ovine, 0.6 unit/mg), 0.2 unit/100 g.

Isolation of mRNA and Cell-free Protein Synthesis—Total hepatic RNA was extracted with phenol-sodium dodecyl sulfate as described earlier (8). Poly(A)-containing mRNA was isolated by affinity chromatography on oligo(dT)-cellulose (11). Poly(A)-containing RNA was translated in vitro by micrococcal nuclease-treated rabbit reticulocyte lysate in the presence of [35S]methionine (12).

Cloning of α₂u-Globulin cDNA and Solution Hybridization of α₂u-Globulin mRNA—Total poly(A)-containing RNA from liver of male rats was reverse transcribed using avian myeloblastosis virus reverse transcriptase. Blunt-ended double-stranded cDNA was prepared from single-stranded cDNA with the DNA polymerase I (Klenow fragment) followed by S1 nuclease treatment (13). The cDNAs were tagged at the 3′ end with dCTP using terminal transferase (14). Plasmid pBR222 was linearized with Pst I (Bethesda Research Laboratories) and tagged at the 3′ end with dGTP. (dG)20-tailed plasmid was annealed with equimolar ratio of (dC)20-tailed cDNA. The chimeric plasmid was used to transform Escherichia coli (HB101) according to Dagert and Ehrlich (15). Tetracycline-resistant and ampicillin-sensitive colonies were screened for α₂u-globulin cDNA inserts by colony hybridization (16) with a purified α₂u-globulin cDNA probe (17). The positive clones were further authenticated by specific hybrid-selected mRNA translation (18). α₂u-Globulin cDNA insert (500 base pairs) was excised from a recombinant plasmid by digestion with the restriction endonuclease Hha I. The cDNA insert was cloned into a pBR322 vector and recovered by electrophoresis. Single-stranded cDNA probe, prepared from this fragment according to Tasi et al. (19), was used for hybridization analysis of the α₂u-globulin mRNA.

Isolation and Culture of Rat Hepatocytes—Hepatocytes were isolated from rat liver by the collagenase perfusion method (20). Falcon tissue culture flasks (25 cm²) were seeded with 5-10 X 10⁶ cells in Dulbecco’s modified Eagle’s medium containing 10% human male serum, insulin (1 μg/ml), glucagon (1 μg/ml), corticosterone (5 X 10⁻⁹ M), 5α-dihydrotestosterone (1 X 10⁻⁹ M), triiodothyronine (1 X 10⁻⁹ M), and ovine growth hormone (5 μg/ml).

After 3-h incubation in the above medium, cells were placed in a serum-free medium containing the same hormone combinations plus epidermal growth factor (10 ng/ml). For protein labeling the cells were incubated under serum-free conditions for another 20 h and subsequently changed to methionine-free medium for the labeling experiments. The hepatocytes were pulse-labeled for 2 h with 200 μCi of [35S]methionine in a total volume of 1.5 ml of medium. The medium was discarded, the cells were washed three times with 0.01 M phosphate-buffered saline, and used for two-dimensional gel electrophoresis. For RNA labeling, after attachment cells were incubated under serum-free conditions for 20 h and subsequently labeled with [3H]orotic acid (1 μCi/flask in a total volume of 1.5 ml) for 3 h. The medium was discarded, the cells were washed three times with 0.01 M phosphate-buffered saline and the total cellular RNA was extracted with phenol-sodium dodecyl sulfate (8). The labeled cellular RNA was separated on a 5–20% sucrose density gradient (Beckman SW28.1 rotor, 48,000 rpm, for 3 h) and fractions were assayed for radioactivity (21).

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RESULTS AND DISCUSSION

Earlier studies from our laboratory have shown that the normal level of $\alpha_2\beta$-globulin synthesis can be induced in hypophysectomized male rats by simultaneous administration of androgen, glucocorticoid, thyroxine, and growth hormone (5). Therefore, hypophysectomized male rats receiving a combination of the first three of the above hormones with and without growth hormone can serve as an in vitro model for the exploration of the specific role of growth hormone in the regulation of $\alpha_2\beta$-globulin. Fig. 1 shows an autoradiogram of the in vitro translation products of total hepatic mRNA separated by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. This autoradiogram shows that for normal male rats, the translationally active $\alpha_2\beta$-globulin mRNA is present in hypophysectomized rats that received a combination of androgen, glucocorticoid, thyroxine, and growth hormone for 8 days. However, for hypophysectomized animals that received only three hormones (androgen, glucocorticoid, and thyroxine but not growth hormone), there was an almost undetectable level of $\alpha_2\beta$-globulin mRNA activity within the total population of hepatic mRNA. These results indicate that growth hormone is essential for maintaining the concentration of translationally active mRNA for $\alpha_2\beta$-globulin in the liver.

The possibility that translationally inactive $\alpha_2\beta$-globulin mRNA sequences were present within the total mRNA population of the growth-hormone-deficient animals (9) was then investigated by hybridization analysis with a cloned cDNA probe complementary to the mRNA for $\alpha_2\beta$-globulin. Fig. 2 shows the kinetics of hybridization between the cloned cDNA probe and total poly(A)-containing hepatic mRNA. As determined from $R_0$ values, treatment of the hypophysectomized animals with all four hormones (androgen, glucocorticoid, thyroxine, and growth hormone) for 8 days resulted in the reappearance of 78% of the normal control of $\alpha_2\beta$-globulin mRNA sequences, whereas omission of growth hormone from the hormone combination failed to raise the hepatic concentration of $\alpha_2\beta$-globulin mRNA to more than 5% of the normal control. These results are in general agreement with those obtained by qualitative analysis of the translationally active $\alpha_2\beta$-globulin mRNA by in vitro translation followed by sodium dodecyl sulfate-slab gel electrophoresis and autoradiography. We have also quantitated the translationally active $\alpha_2\beta$-globulin mRNA content of the total hepatic mRNA isolated from both growth hormone-deficient and growth hormone-deficient animals by in vitro translation, specific immunoprecipitation followed by disc gel electrophoresis and gel fractionation according to the procedure described earlier (22). The hepatic content of $\alpha_2\beta$-globulin mRNA sequences as determined by hybridization analysis with the cloned cDNA probe, translatable $\alpha_2\beta$-globulin mRNA as estimated by immunoprecipitation of $\alpha_2\beta$-globulin from the in vitro translation product followed by sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis, and the hepatic concentration of $\alpha_2\beta$-globulin as determined by specific radioimmunoassay are shown in Fig. 3. The results show that the low level of hepatic $\alpha_2\beta$-globulin found in the absence of growth hormone correlates with both the translatable and the hybridizable $\alpha_2\beta$-globulin mRNA in rat liver.

In order to examine the effect of growth hormone on the synthesis of $\alpha_2\beta$-globulin under a more well defined environment, we performed an in vitro labeling experiment of the total cellular proteins in isolated hepatocytes. Hepatocytes derived from normal male rats and cultured in the medium described under “Experimental Procedures” maintain normal morphologic appearance and continue to secrete $\alpha_2\beta$-globulin (Fig. 4). Earlier studies have shown that the hepatic synthesis of ribosomal RNA is highly sensitive to growth hormone (25, 26). In order to examine the growth hormone responsiveness of the cultured hepatocytes we have determined the extent of labeled orotic acid incorporation into the ribosomal RNA by the liver cells obtained from hypophysectomized rats and cultured in the presence and absence of growth hormone. Hepatocytes isolated from hypophysectomized rats were cul-
Growth Hormone and $\alpha_2$-Synthesis

Fig. 2. Kinetics of hybridization of the cloned $\alpha_2$-globulin cDNA probe with poly(A)-containing hepatic RNA from rats of different endocrine status. ■■■ hypophysectomized male with no hormone supplementation; □□□ hypophysectomized male that received 8 daily treatments of 5a-dihydrotestosterone, corticosterone, and thyroxine; ○○○ hypophysectomized male that received 8 daily treatments of 5a-dihydrotestosterone, corticosterone, thyroxine, and growth hormone; ●●● normal male.

Fig. 3. Hybridizable $\alpha_2$ mRNA sequence, translatable $\alpha_2$ mRNA, and cytoplasmic $\alpha_2$-globulin in the hypophysectomized rats with and without growth hormone supplementation. Hatched bars, $\alpha_2$-globulin mRNA sequences as determined by liquid hybridization with cloned $\alpha_2$-globulin cDNA probe; dotted bars, translatable $\alpha_2$-globulin mRNA as determined by in vitro translation followed by specific immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis; open bars, cytoplasmic $\alpha_2$-globulin determined by radioimmunoassay. Animals received 8 daily treatments of either 5a-dihydrotestosterone, corticosterone, thyroxine, and growth hormone (DCTG) or 5a-dihydrotestosterone, corticosterone, and thyroxine (DCT). The results are mean of those obtained for 6 animals ± standard error.

Fig. 4. Synthesis and secretion of $\alpha_2$-globulin by monolayer cultures of normal rat hepatocytes. At the top is a photomicrograph of a 24-h hepatocyte culture taken under phase contrast illumination. The graph at the bottom shows linear rate of secretion of $\alpha_2$-globulin by the cultured hepatocytes within the experimental period.

Fig. 5. Effect of growth hormone on the incorporation of $[^3]$H)arotic acid into RNA by cultured hepatocytes isolated from hypophysectomized rats. Total cellular RNA extracted from hepatocytes cultured in the presence (●●●) or in the absence (○○○) of growth hormone was fractionated on 5-20% linear sucrose gradient. For each of the experiments four flasks with a total of 20 X $10^6$ cells were used. Yeast tRNA (100 µg) was used as carrier. The arrows point to the positions of the unlabeled marker RNA in the gradient.
Two-dimensional gel electrophoresis. A, proteins synthesized by testosterone, corticosterone, and thyroxine, and growth hormone. The position of pure α2-globulin in the two-dimensional gel is circled. From hypophysectomized rats that received hepatocytes derived from hypophysectomized rats that received daily treatments of 5α-dihydrotestosterone, corticosterone, and thyroxine. In both cases the synthesis of other radiolabeled proteins by hepatocytes derived by growth hormone depends on the synthesis of α2-globulin mRNA. These observations also indicate that the regulatory influence of growth hormone on the synthesis of α2-globulin is exerted at a level antecedent to the step of mRNA translation. A similar conclusion has also been reached by Keller and Taylor (27) who have studied the growth hormone-dependent regulation of the synthesis of serum albumin. Studies with various steroid hormones provide a general consensus for the action of these hormones at the level of gene transcription (28). However, progress in the exploration of the peptide hormone-mediated regulation of specific gene expression has only been modest and no such definite consensus has yet been reached. Guyette et al. (29) have reported that prolactin (a close relative of growth hormone) may regulate casein synthesis in the mammary gland by selective stabilization of the casein mRNA. Whether growth hormone acts through a similar post-transcriptional mechanism by regulating α2-globulin gene transcription remains to be established.

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Addendum—While this paper was under editorial review, Lynch et al. (30) published a paper describing the effect of growth hormone on α2-globulin synthesis. This article essentially presents a re-examination of the results of Kurtz et al. (9) concerning translational regulation of α2-globulin synthesis by growth hormone and concludes that growth hormone regulates the hepatic concentration of the mRNA for α2-globulin.

REFERENCES


FIG. 6. Autoradiogram of the [35S]methionine-labeled proteins synthesized by isolated rat hepatocytes and separated by two-dimensional gel electrophoresis. A, proteins synthesized by hepatocytes derived from hypophysectomized rats that received 8 daily treatments of 5α-dihydrotestosterone, corticosterone, thyroxine, and growth hormone. B, proteins synthesized by hepatocytes derived from hypophysectomized rats that received 8 daily treatments of 5α-dihydrotestosterone, corticosterone, and thyroxine. In both cases the hepatocytes were cultured in vitro in the presence of 5α-dihydrotestosterone, corticosterone, and thyroxine, and growth hormone. The position of pure α2-globulin in the two-dimensional gel is circled. Non-equilibrium pH gradient gel electrophoresis (NEPGE) was first performed from left (acidic) to right (basic). Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (SDS-PAGE) was then run from top to bottom. Positions of the molecular weight marker proteins are indicated on the left.

labeled α2-globulin mRNA had accumulated in the liver cells of the growth hormone-deficient rats and α2-globulin mRNA translation was activated by growth hormone then the hepatocytes obtained from the growth hormone-deprived rats, when cultured in the presence of growth hormone should synthesize α2-globulin. However, only hepatocytes derived from rats that received growth hormone in vivo synthesized 35S-labeled α2-globulin. In vitro exposure of hepatocytes, derived from the three hormone-treated rats, to growth hormone failed to initiate the synthesis of α2-globulin. The synthesis of other 35S-labeled proteins by hepatocytes derived from growth hormone-deprived and growth hormone-supplemented rats as shown in Fig. 6 was found to be strikingly similar. This indicates the degree of selectivity in the growth hormone-dependent regulation of α2-globulin synthesis.

All of the above results provide strong evidence for growth hormone-mediated changed in the hepatic concentration of the mRNA for α2-globulin. These observations also indicate that the regulatory influence of growth hormone on the synthesis of α2-globulin is exerted at a level antecedent to the step of mRNA translation. A similar conclusion has also been reached by Keller and Taylor (27) who have studied the growth hormone-dependent regulation of the synthesis of serum albumin. Studies with various steroid hormones provide a general consensus for the action of these hormones at the level of gene transcription (28). However, progress in the exploration of the peptide hormone-mediated regulation of specific gene expression has only been modest and no such definite consensus has yet been reached. Guyette et al. (29) have reported that prolactin (a close relative of growth hormone) may regulate casein synthesis in the mammary gland by selective stabilization of the casein mRNA. Whether growth hormone acts through a similar post-transcriptional mechanism or by regulating α2-globulin gene transcription remains to be established.
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