Effects of Sex Hormones on the Level of the Messenger RNA for the Rat Hepatic Protein α 2u Globulin*

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α 2u Globulin is a protein synthesized in the liver, secreted into the serum, and excreted in the urine of mature male rats. The effects of androgens and estrogens on the level of the messenger RNA coding for this male rat hepatic protein have been investigated. Castrated male rats have reduced levels of α 2u globulin in serum and liver cytosol, as measured by a radial immunodiffusion assay. The livers from these castrated males were found to contain similarly reduced levels of the mRNA coding for α 2u globulin, as measured in an mRNA-dependent wheat germ cell-free translational system. Administration of dihydrotestosterone to castrated males resulted in increased levels of α 2u globulin in liver and serum, and this increase in the level of the protein following androgen administration was accompanied by a parallel increase in the functional level of α 2u globulin mRNA. Administration of estradiol-17β to intact male rats gradually diminishes the levels of α 2u globulin in liver and serum. The livers from these estrogen-treated males were found to contain α 2u globulin mRNA at similarly reduced levels. The time course of the disappearance of the α 2u globulin mRNA following estrogen treatment parallels the disappearance of the protein in liver cytosol and serum.

These results indicate that sex steroids affect the synthesis of the hepatic protein α 2u globulin by acting pretranslationally, possibly at the level of transcription. Although the liver had not been considered a primary target tissue for sex hormones, these results indicate that sex steroids can affect certain hepatic functions in a manner consistent with the accepted model for the action of steroid hormones on their target tissues.

A rat urinary protein designated α 2u globulin was first described by Roy and Neuhaus (1). It was found to be excreted in the urine of mature male rats and absent from the urine of female rats. Its function is, thus far, unknown. It was first thought to be a prostatic secretion, but liver perfusion and immunofluorescence studies indicated that the liver is the site of α 2u globulin synthesis (2, 3). The hepatic synthesis of α 2u globulin in male rats is under complex hormonal control: androgens, glucocorticoids, thyroid hormones, and pituitary hormones induce the synthesis of α 2u globulin, and estrogens repress the synthesis of this protein (4–6).

The currently accepted model for the action of steroid hormones on their target tissues (7, 8) involves a direct effect of these hormones upon transcription. Reports from various laboratories (9, 10) have shown that steroid hormones can induce in their target tissues the appearance of specific messenger RNAs coding for hormonally inducible secretory proteins. Reports from this laboratory (11, 12) have indicated that glucocorticoids can induce the specific mRNA for liver tryptophan oxygenase.

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MATERIALS AND METHODS

Animals and Hormone Treatment—Male Sprague-Dawley rats, 300 to 350 g, were used in all experiments. Steroids, at the indicated doses, were injected subcutaneously in an emulsion containing (by volume) 89.6% 0.1 M sodium phosphate (pH 7.2)/10% propylene glycol/0.4% Tween 8. PBS-Castrated male rats were purchased from Carworth Ltd., Boston, Mass. Rats were allowed at least 15 days of rest following castration.

Assay of a 2u Globulin in Urine, Serum, and Liver Cytosol—Twenty-four hour urine samples were collected in stainless steel metabolism cages. The urine was frozen and kept at -20°C until use. A liver cytosol fraction (S-100)1 was prepared as described earlier (6). Blood was collected by cardiac puncture, kept at room temperature for 1/2 hour, then placed at 4°C for 2 to 4 hours. The clot was removed by centrifugation, and the serum was collected and stored at -20°C. The α 2u globulin content of urine, serum, and liver S-100 was measured using a radial immunodiffusion assay as described previously (6).

Measurement of Hepatic α 2u Globulin mRNA—The functional level of the hepatic mRNA coding for α 2u globulin was quantitated using in vitro translation of total hepatic mRNA in a wheat germ cell-free translational system (WGSSO) was prepared from raw wheat germ (Niblack Inc., Rochester, N.Y.) using the procedure of Marcu and Dudock (17), with the modifications described previously (6). The complete reaction mixture for protein synthesis contained, in a 500-μl volume: 20 to 25 A260 units (150 to 200 μl) of WGSSO, 20 mM Hepes (pH 7.6), 2 mM dithiothreitol, 2.5 mM magnesium acetate, 100 mM KCl, 110 μCi of [3H]leucine (50 Ci/mmol), 32 μM each of the other 19 natural amino acids, 0.8 mM ATP, 0.08 mM GTP, 0.5 mM CTP, 4.0 mM creatine phosphate, 0.06 mg of creatine phosphokinase, and 100 μg of rat liver mRNA. Following incubation at 30°C for 1 hour, polysomes were removed by centrifugation, and the released polypeptide chains were collected and brought to 2% Triton X-100, 0.01 M sodium phosphate (pH 7.2), 10% glycerol, and subjected to Na dodecyl sulfopolyacrylamide gel electrophoresis (6, 15). Total hepatic RNA was extracted with phenol-chloroform and chromatographed on cellulose (Sigmacel) to isolate the poly(A)-containing RNA (18). An mRNA-dependent cell-free protein-synthesizing system (WGSSO) was prepared from raw wheat germ (Niblack Inc., Rochester, N.Y.) using the procedure of Marcu and Dudock (17), with the modifications described previously (6). Protein markers, including pure α 2u globulin, were run on parallel gels and stained with Coomassie brilliant blue.

Quantitation of Hepatic Cytosol Androgen Receptor—The measurement of specific binding of [3H]5α-dihydrotestosterone cytosol protein was done using the method of Besto et al. (20). Liver cytosols prepared by differential centrifugation were treated with 1/2 volume of a suspension of dextran-treated charcoal (3.75 g of activated charcoal plus 0.375 g of Dextran 500 in 100 ml of Tris-HCl (pH 8.0)/1 mM dithiothreitol) for 10 min at 0°C. The charcoal was removed by centrifugation, and the steroid-free cytosols were then used for the binding assays.

Hybridization of a 2u globulin, preparation of rabbit anti-α 2u globulin IgG, and determination of antigen-antibody equivalence were done as described previously (6, 15). Protein determinations were done with the method of Lowry et al. (21), using bovine serum albumin as a standard.

RESULTS

Effect of Estradiol-17β on a 2u Globulin Synthesis—As reported by Roy and Neuhaus (4), administration of estradiol-17β to an adult male rat gradually depresses the hepatic synthesis of α 2u globulin (Fig. 1). After 4 days of estradiol-17β, the α 2u globulin content of the liver S-100 fell to approximately 30% of the control value, and the urinary output of α 2u globulin was similarly decreased. After 8 days of estradiol treatment, no α 2u globulin was detectable in the liver S-100 or urine. Fig. 2 shows the effects of increasing doses of estradiol-17β on α 2u globulin synthesis in intact males. Inhibition was apparent at the lowest dose used (0.25 mg/kg/day), and increasing doses of estradiol-17β brought about a greater depression of a 2u globulin synthesis.

Effect of Estradiol-17β on a 2u Globulin mRNA—To determine if this estrogen-mediated depression of α 2u globulin synthesis was due to a depression of the level of the hepatic mRNA coding for this protein, the poly(A)-containing RNA was extracted from the livers of these estrogen-treated males and translated in the wheat germ system. The amount of in vitro α 2u globulin synthesis directed by these mRNAs was determined as described under “Materials and Methods.” It was found that [3H]leucine incorporation into α 2u globulin in vitro, as a percentage of [3H]leucine incorporation into total trichloroacetic acid-precipitable protein, was diminished when the wheat germ translational system was driven by hepatic mRNA from males treated with estradiol-17β (Fig. 3). Thus, administration of estradiol-17β to male rats depressed the functional level of hepatic α 2u globulin mRNA. The time course of this depression of the level of the mRNA closely paralleled the decrease of the level of the protein in liver S-100 and urine (Fig. 1). After 8 days of estrogen treatment, no α 2u globulin mRNA was detectable. Increasing doses of estradiol were increasingly effective in diminishing the level of α 2u globulin mRNA, and the decreased mRNA levels correlated well with the decreased levels of the protein in liver S-100 (Fig. 2).

Effect of Castration and Androgen Administration on a 2u Globulin Synthesis—As reported (4), castration of male rats diminishes hepatic α 2u globulin synthesis. Fifteen days...
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Fig. 2. Dose response of estradiol-mediated inhibition of α 2u globulin synthesis. Liver S-100 α 2u globulin was measured immunologically. α 2u Globulin mRNA was quantitated as described under "Materials and Methods."

Fig. 3. Na dodecyl SO₄-polyacrylamide gel electrophoretic profiles of α 2u globulin synthesized in vitro by hepatic mRNA from male rats treated with estradiol-17β. Protein synthesis performed as described under "Materials and Methods." α 2u Globulin immunoprecipitated from 400 μl of the released chain fraction of the WGS30 system containing 100 μg of mRNA from livers of male rats treated with estradiol-17β (0.5/mg/kg/day) for: A, 1 day; B, 2 days; C, 4 days; D, 6 days; E, 8 days; or F, 8 days mock injected. Arrows mark position of authentic α 2u globulin.

Following castration, α 2u globulin levels in urine, serum, and liver S-100 were approximately 10% of control values. This low but detectable level of α 2u globulin synthesis following castration seems to be maintained indefinitely. α 2u Globulin synthesis could be induced in castrated males by administration of dihydrotestosterone (Fig. 4). An increase in α 2u globulin synthesis could be seen after 1 day of androgen treatment, and the levels of this protein in urine and serum reached 75 to 80% of control values after 8 days of treatment with dihydrotestosterone. Androstenedione and androsterone, both weakly androgenic, could also induce α 2u globulin synthesis in castrated males (Fig. 5), but to a lesser extent than could dihydrotestosterone.

Effect of Androgens on α 2u Globulin mRNA—To determine if this induction of α 2u globulin synthesis by androgens occurred via an induction of its hepatic mRNA, the α 2u globulin mRNA content of livers from castrated and androgen-treated males was measured using the in vitro translation assay. Livers from castrated males contained α 2u globulin mRNA at a level which was approximately 10% of the level in control males (Fig. 6). Administration of dihydrotestosterone to castrated males resulted in an increase in the level of translatable α 2u globulin mRNA (Fig. 6). The time course of induction of the message paralleled the appearance of the protein in liver, serum, and urine (Fig. 4). Androstenedione and androsterone could also induce α 2u globulin mRNA, but to a lesser extent than could dihydrotestosterone, and the
induction of the mRNA by these weaker androgens also paralleled the induction of the protein in liver S-100 (Fig. 5).

**Effects of Sex Hormones on Hepatic Cytosol Dihydrotestosterone Receptor**—It was reported by Roy et al. (22), that sex hormones affect the level of the hepatic cytosol dihydrotestosterone receptor. Those studies determined the amount of labeled dihydrotestosterone bound to the 3.5 S protein peak in a linear sucrose gradient. Using a charcoal-dextran assay for steroid receptors, we found that male rat cytosol contained a protein with high affinity for [3H]dihydrotestosterone (Table I). Livers from castrated males contained this protein, the putative androgen receptor, at a level approximately 20% that of control livers. Administration of dihydrotestosterone to castrated males increased the level of this dihydrotestosterone receptor in liver cytosol (Fig. 4). The receptor level reached the control value after 8 days of treatment with dihydrotestosterone.

It was reported by Roy et al. (22), and, using our assay, we also found that administration of estradiol-17β to male rats resulted in a decrease in the level of the hepatic dihydrotestosterone receptor (Fig. 1). After 8 days of estrogen treatment, the level of the hepatic dihydrotestosterone receptor fell to approximately 10% of the control value. Estradiol-17β does not bind to the dihydrotestosterone-binding site on the receptor, since unlabeled excess estradiol-17β could not compete with [3H]dihydrotestosterone binding (Table I). Unlabeled androstenedione or androsterone were also ineffective in competing with [3H]dihydrotestosterone (data not shown), but unlabeled cyproterone acetate, an antiandrogen known to compete with dihydrotestosterone for prostate receptor (23), was effective in competing with labeled dihydrotestosterone for receptor in liver cytosol (Table I).

**DISCUSSION**

These findings indicate that androgens induce and estrogens decrease the synthesis of α 2u globulin by modulating the functional level of the specific hepatic mRNA coding for this protein. The striking correlation between the hepatic level of the α 2u globulin mRNA and the tissue level of the protein under the various endocrine manipulations precludes translational control as an important mechanism in the modulation of α 2u globulin synthesis by the sex hormones. The modulation of the level of α 2u globulin mRNA is compatible with hormonal modulation of the transcription of this gene; however, present data cannot exclude the possibility of selective effects upon processing and/or transport of this mRNA.

Previous reports from this laboratory (6, 15) also indicated such pretranslational control of a 2u globulin synthesis by glucocorticoids and thyroid hormones. Thus, the primary mechanism by which the synthesis of α 2u globulin is controlled in rat liver is through control of its mRNA level. An early effect of dihydrotestosterone on the livers of castrated males seems to be the induction of its own receptor. This receptor is required for the steroid to exert its effects on RNA and protein synthesis. This is analogous to the action of glucocorticoids administered to adrenalectomized animals: the glucocorticoid receptor level in liver falls gradually following adrenalectomy, and an early effect of glucocorticoid hormones is the reinduction of the receptor for these hormones (24).

Androgens are required to maintain normal levels of α 2u globulin synthesis in liver. The antagonistic effect of estradiol-17β on the synthesis of this protein in males could, therefore, be the result of indirect processes which result in a decrease in circulating androgen levels following estrogen administration. Estrogens are known to act on the pituitary gland and reduce circulating levels of luteinizing hormone (25) which is required for the synthesis of androgens in the Leydig cells. It has also been shown (96) that estradiol-17β can prevent the synthesis of testosterone from its precursors in testicular tissue. Either of these effects would reduce the circulating levels of androgens. The observed depression in the level of the hepatic dihydrotestosterone receptor following estrogen administration could thus be explained, since maintenance of normal dihydrotestosterone receptor levels seems to require dihydrotestosterone. By lowering circulating androgen levels, estradiol-17β would gradually decrease α 2u globulin synthesis.

The findings herein reported indicate that the sex steroids influence α 2u globulin synthesis via a mechanism consistent
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with the accepted model for steroid hormone action (7, 8). These results are, to our knowledge, the first demonstration that sex hormones can modulate the level of a specific hepatic gene product, and suggest that these steroids may influence transcriptional events in the liver.

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