REGULATION OF THE HEPATIC SYNTHESIS OF α_{2u} GLOBULIN AND ITS CORRESPONDING MESSENGER RNA IN MATURING MALE RATS

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1. Introduction

α_{2u} globulin is the principal urinary protein in the mature male rat [1,2]. The protein has been shown to be synthesized and secreted by the hepatic parenchymal cells [3,4]. Studies on the action of various hormones on α_{2u} synthesis have shown that androgens, thyroid hormones, glucocorticoids, pituitary growth hormone and insulin promote, whereas estrogen treatment completely suppresses the synthesis of this protein [5–10]. α_{2u} globulin could be induced in the mature female rat after ovariectomy followed by androgen treatment [5].

Results obtained with various model systems involving reproductive tissues have provided strong support for the concept that circulating steroid hormones regulate cell function by modulating the synthesis of cellular RNA and especially the synthesis of specific messenger RNA [11–18]. However, alternative possibilities such as post-transcriptional and translational regulation of protein synthesis by steroid hormones have not been completely ruled out [19,20]. Our earlier studies have shown that androgen treatment of spayed rats results in the induction of both α_{2u} globulin and its corresponding messenger RNA in the liver [21]. All of the above studies on the steroidal control of gene regulation have been limited to effects of hormone treatment or hormone withdrawal in the experimental system and, to our knowledge, changes in the levels of specific mRNA activity under physiological variations of the steroid hormones have not been investigated. Therefore, in order to expand our knowledge of the mechanism of the androgenic regulation of α_{2u} synthesis and to study the role of physiological variations of the androgen on the regulation of α_{2u} gene activity we undertook the present investigation concerning the role of circulating testosterone on the hepatic synthesis of α_{2u} mRNA in maturing male rats. The results indicate that rising levels of the circulating androgen promote α_{2u} synthesis primarily by increasing the hepatic level of α_{2u} mRNA.

2. Materials and methods

Experiments were carried out on male albino rats of Yale strain obtained from a local supplier. The animals were housed in an air-conditioned animal room with 12 h light and 12 h darkness. Daily urine samples were collected in stainless steel metabolism cages with 0.5 ml preservative solution containing 1% penicillin, 1% streptomycin and thymol to saturation.

Blood samples were collected at the time of sacrifice of the animal for the removal of the liver. Serum testosterone was determined by double antibody radioimmunoassay. A radioimmunoassay kit containing rabbit antiserum against testosterone-3-*O*-carboxymethyloxine–BSA, sheep antiserum to rabbit gamma globulin and [^{125}I]testosterone-3-*O*-carboxymethyloxine-tyrosine-methyl ester were obtained from Serono Laboratories, Inc., Boston, Mass. The assay procedure was as described by the supplier. The lower limit of sensitivity of the assay was found to be 10 pg testosterone/ml.

Liver samples were removed under light ether anesthesia and part of the tissue was used for the extraction of RNA and the rest for the preparation of a high speed supernatant fraction. Procedures for the preparation of soluble protein extracts from the

liver were carried out at 0°–4°C. The hepatic tissue was homogenized in 0.1 M Tris–HCl, pH 8.0 (10 ml/g liver) and was centrifuged in a Sorval refrigerated centrifuge at 27 000 × *g* for 15 min. The 27 000 × *g* supernatant fraction was subjected to ultrasonic treatment for 15 s (setting 80 of Bronwill Biosonik III, Bronwill Scientific, Rochester, N.Y.) and recentrifuged at 160 000 × *g* for 120 min in a Beckman L-5 ultracentrifuge. The supernatant fraction obtained after the last centrifugation was used for the estimation of hepatic α_{2u} globulin. Concentration of α_{2u} globulin in the hepatic extract and in the urine was determined by double antibody radioimmunoassay with ^{125}I-labeled α_{2u} globulin, rabbit antiserum to α_{2u} globulin and goat antiserum to rabbit γ-globulin. The assay procedure was developed by modification of the methods for radioimmunoassay of the pituitary hormones [22,23] and the details of which will be published elsewhere. Total protein concentration was determined according to Lowry et al. [24].

Total hepatic RNA was extracted with phenol and sodium dodecyl sulfate (SDS) according to Rosenfeld et al. [25]. Poly(A) containing RNA fraction was separated by chromatography of the total RNA on oligo-(dT)-cellulose according to Aviv and Leder [26]. Poly(A) containing hepatic RNA was translated in a heterologous cell-free system derived from wheat germ [27]. The conditions and protocol for the translational system have been described earlier [28]. Aliquots of 80 – 200 μl of the post-ribosomal supernatant containing 2 × 10^6 cpm of released peptide chains were used for immunoprecipitation of α_{2u} globulin and subsequent SDS-polyacrylamide gel electrophoresis of the immunoprecipitate. After electrophoresis, gels were mechanically fractionated in a Gilson gel fractionator and fractions obtained from 2 mm portions of the gel were used for the determination of radioactivity. Details of these procedures have been published earlier [28].

3. Results and discussion

Results presented in fig.1 summarize the relationship between the serum levels of testosterone, urinary levels of α_{2u} globulin and the hepatic levels of both α_{2u} globulin and its corresponding mRNA in male rats of various ages ranging from pre- to post-puberty. In male rats of 42 days of age and younger (data for 30- and 35-day-old rats are not shown in fig.1) the level of serum testosterone was found to be below 2 ng/ml. These animals did not produce any detectable amount of α_{2u} globulin and no mRNA activity for α_{2u} globulin could be detected within their total hepatic mRNA. The levels of serum testosterone showed a slow rise from 48–66 days of age, which was followed by a sharp rise after 66 days of age. A biphasic rise in the level of serum testosterone in the maturing male rat has also been reported by Dohler and Wuttke [29]. Unlike the biphasic rise of serum testosterone the daily urinary output of α_{2u} globulin and hepatic levels of the mRNA for α_{2u} globulin showed an almost linear rise from 48–70 days of age. The rising levels of α_{2u} globulin within the hepatic tissue however, plateaued beyond 66 days of age.

Daily treatments to male rats with either testosterone (250 μg/100 g body wt.) or 5α-dihydrotestosterone (50 μg/100 g body wt.) between the ages of 25 and 35 days failed to induce α_{2u} globulin and its corresponding mRNA in the liver (data not shown in the figure). The above doses of these androgens were found to result in maximum induction of α_{2u} globulin in spayed female rats (A. K. Roy, unpublished). These results are in conformity with our earlier observation regarding the androgen insensitiveness for α_{2u} synthesis in the prepubertal rats [30].

The hepatic level of α_{2u} mRNA at different ages showed a more striking correlation with the daily urinary output of α_{2u} globulin than the concentration of the protein in the liver. This may be explained by the fact that the hepatic level of any secretory protein reflects primarily the balance between its rate of synthesis and rate of secretion. If the increased rate of synthesis is associated with a rise in the rate of its secretion, the hepatic level of the protein may not show any change. Therefore, the daily urinary output of α_{2u} globulin may be a better indication of its rate of synthesis than the hepatic level of the protein. In spite of a lack of increase in the level of serum testosterone in 66-day-old rats over the group of 52-day-old animals, the rate of synthesis of α_{2u} globulin as indicated by both the hepatic level of the protein and its daily urinary output, showed a steady increase (about 100% rise). A similar degree of elevation of the hepatic level of mRNA for α_{2u} globulin in the 66-day-old rats was also observed as

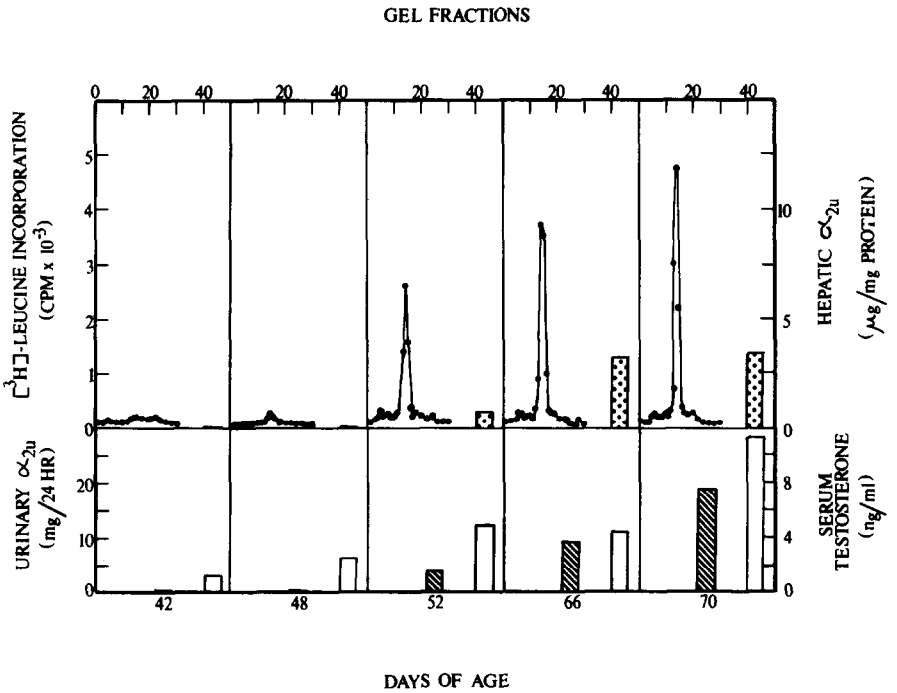

Fig.1. Relationship between the levels of serum testosterone, hepatic messenger RNA for α_{2u} globulin and both hepatic and urinary levels of α_{2u} globulin in the maturing male rats. Data in each vertical frame represent average values from same three animals. Equal amount of the hepatic tissue from each of these animals was blended together for the extraction of RNA. Other values represent mean of three separate assays. The messenger RNA activity for α_{2u} globulin (•——•) is represented as the pattern of radioactivity on SDS-polyacrylamide gel electrophoresis of α_{2u}–anti α_{2u} immunoprecipitate obtained from 2×10^6 cpm of released peptide chians synthesized in vitro under the direction of hepatic mRNA. Key to other symbols: dotted bars, hepatic concentration of α_{2u} globulin; hatched bars, 24 h urinary output of α_{2u} globulin; open bars, serum testosterone.

compared to the 52-day-old group. The above observation indicates that factors other than the levels of circulating androgens may also be involved in the regulation of the synthesis of α_{2u} globulin and its mRNA in the liver. Earlier studies from this laboratory have shown the presence of a cytosol androgen binding protein in the hepatic tissue of rats which produce α_{2u} globulin under androgenic stimulation [31]. Studies on the regulation of the cytosol androgen binding protein of rat liver have shown that it is absent in the prepubescent rats and that its level rises with sexual maturation. Androgen treatment of adult animals leads to elevation of the hepatic level of the cytosol androgen binding protein, while castration has an opposite effect [31]. Some of these results have recently been confirmed by Kurtz et al. [32]. The absence of the cytosol androgen binding protein in the hepatic tissue of the prepubescent male rats may explain the inability of the androgen to induce α_{2u} mRNA in these animals. Similarly, the linear rise in the hepatic level of α_{2u} mRNA between 52 and 66 days of age, in spite of any rise in the level of circulating testosterone, may be due to the higher level of the cytosol androgen binding protein in the hepatic tissue of the 66-day-old animals. Therefore, it seems that in the maturing male rats, although the rising levels of circulating androgen leads to increased level of hepatic mRNA for α_{2u} globulin leading to increased α_{2u} output, the degree of androgen response may be modulated by the degree of androgen receptivity of the hepatic tissue. These results lend important support to the concept of transcriptional regulation of genetic activity by the steroid hormones under physiological conditions.

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