Androgenic repression of the messenger RNA for a 26.3-kDa hepatic protein in the rat

B. Chatterjee, C.V. Ramana Murty and A.K. Roy*

*Departments of Chemistry and *Biological Sciences, Oakland University, Rochester, MI 48063, USA*

Received 14 March 1984

Abstract not received

Androgenic repression *Hormone action* *Steroid hormone* *5α-Dihydrotestosterone* *Gene regulation* *Hepatic mRNA*

1. INTRODUCTION

Sex-steroids are known to influence the synthesis of several enzymes and non-enzyme proteins in the liver [1,2]. Earlier studies in our laboratory have shown that steady state levels of the mRNAs for three hepatic proteins (18 kDa, 26.3 kDa and 28.5 kDa) change markedly during ageing of the male rate [3]. One of these mRNAs codes for α_{2u} globulin (18 kDa), the male specific rat urinary protein. The mRNAs for both α_{2u} globulin and the 28.5-kDa protein are absent in the prepubertal male, appear after puberty and disappear at senescence (>800 days). The mRNA for 26.3-kDa protein, however, is present in the prepubertal male, its hepatic concentration is greatly reduced after puberty and increases during senescence. Since ageing in the male rat is associated with a general decline in androgen responsiveness, and a high level of the mRNA for the 26.3-kDa liver protein (LP-26) is also found in the female rat [4], we have studied the specific role of the androgen and estrogen in the regulation of this mRNA. The results presented here show that the synthesis of LP-26 is primarily regulated through the repressive influence of the androgen.

2. MATERIALS AND METHODS

Albino rats of Sprague-Dawley strain were used. 5α-Dihydrotestosterone (Sigma) was injected subcutaneously at a daily dose of 50 μg/100 g body wt according to the protocol in [5]. [^{35}S]Methionine (1000 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Chemicals for polyacrylamide gel electrophoresis were obtained from BioRad (Richmond, CA).

Poly(A) containing hepatic mRNA was extracted as in [3,6]. The mRNA was translated in the micrococcal nuclease treated rabbit reticulocyte cell-free system (with subsaturation levels of mRNA) in the presence of [^{35}S]methionine [7]. Typically, a 30-μl reaction mixture contained 750 ng of Poly(A) containing mRNA and was incubated for 60 min at 30°C.

[^{35}S]Methionine-labeled in vitro translation products of the hepatic mRNA were electrophoretically separated on a 16% polyacrylamide slab gel in the presence of SDS [8]. Additional details for sample preparation and gel electrophoresis have been described in [3]. After electrophoresis, the gels were fixed in methanol:acetic acid:water (40:10:50, by vol.), dried and autoradiographed on Kodak X-ray films.

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V.

3. RESULTS AND DISCUSSION

Since mRNA for LP-26 was detected in the liver of both prepubertal male and adult female rats, it was of interest to examine the effect of ovariectomy on the steady state level of the mRNA for LP-26. Results presented in fig.1 show that the hepatic concentration of the mRNA for this protein did not show any detectable decrease at 30 days post-ovariectomy. This result suggests that the presence of estrogen is not required for maintaining the hepatic level of LP-26 mRNA.

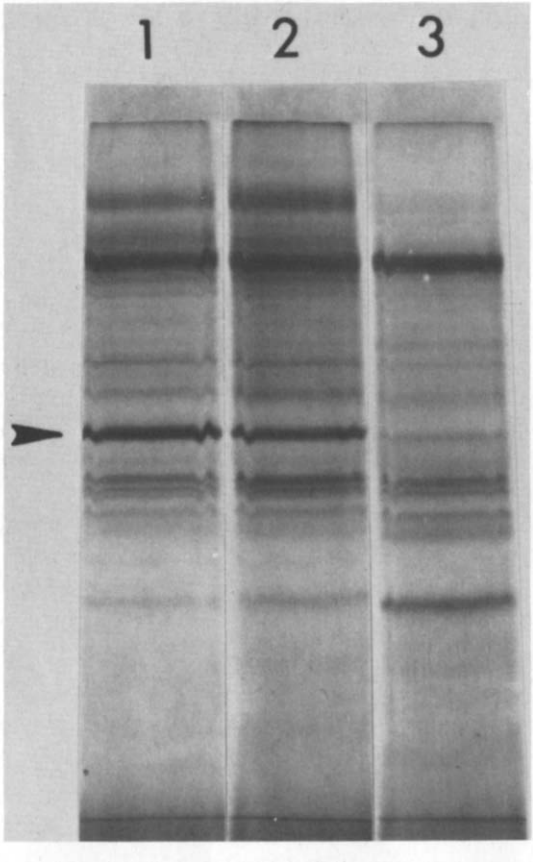

Fig.1. Effect of androgen on the hepatic level of the mRNA for the 26.3-kDa protein. The electrophoretically separated in vitro translation products in various lanes are labeled as follows: (1) 150-day-old normal female; (2) 150-day-old female ovariectomized at 120 days of age; (3) 150-day-old female ovariectomized at 120 days of age and treated daily for 15 days with 5α-dihydrotestosterone before sacrifice. The position of the LP-26 band is shown with an arrow.

However, treatment of ovariectomized animals with 5α-dihydrotestosterone for 15 days resulted in almost complete disappearance of LP-26 mRNA from the liver of these animals. The latter finding indicates that the hepatic synthesis of LP-26 mRNA is inhibited by the androgen.

Further evidence for the androgenic repression of LP-26 mRNA was obtained from male rats that were castrated before puberty (fig.2, lane 1). These animals continue to synthesize LP-26 mRNA at post-pubertal life. Results presented in fig.2 also show that in the normal male rat sexual maturation (~40 days) is associated with a marked decrease in the hepatic concentration of LP-26 mRNA (lane 2). Thus, increased androgen action after puberty may be the primary reason for the decreased hepatic synthesis of LP-26 mRNA in the post-pubertal male rat. Presence of a high level of LP-26 mRNA in the female rat (lane 3) and its continued synthesis beyond puberty in the prepubertally castrated rat again show that the expression of LP-26 gene is favored in the absence of the androgen.

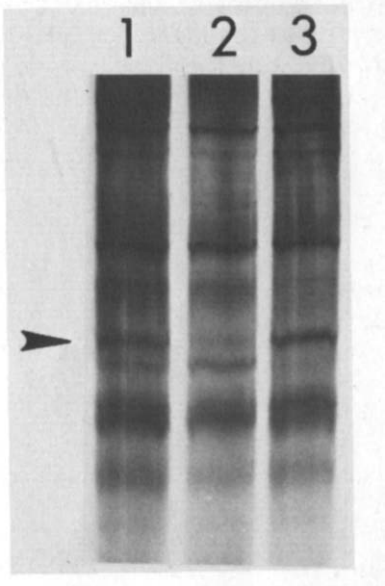

Fig.2. Effect of castration on the hepatic level of LP-26 mRNA. The picture shows a portion of an autoradiogram of the electrophoretically separated in vitro translation products of the hepatic mRNA. The lanes are labeled as follows: (1) male rat castrated at 24 days of age and sacrificed at day 76; (2) 76-day-old normal male rat; (3) 76-day-old normal female rat. The arrow marks the position of in vitro translated LP-26.

High concentrations of LP-26 mRNA both in the livers of castrated male and ovariectomized female rats, and its decrease after sexual maturation in the male suggest that the hepatic concentration of this mRNA is regulated primarily through the repressive influence of the androgen rather than the inductive influence of the estrogen. This contention is also supported by the observation of a direct correlation between hepatic androgen-insensitivity in the prepubertal and the senescent male rat and the presence of high levels of LP-26 mRNA in their livers. Earlier studies in our laboratory have established that the hepatic tissue of these animals shows almost complete androgen insensitivity possibly because of the lack of a cytoplasmic androgen binding protein in their liver [4,9]. Therefore, these animals are expected to be spared from the repressive effect of the androgen. Inability of the androgen to repress the hepatic concentration of LP-26 mRNA in these animals may thus be due to the lack of androgen receptivity of the liver. To our knowledge mRNA for LP-26 represents the only known hepatic mRNA whose synthesis is repressed by the androgen and thus could serve as an important molecular marker for the study of androgen action.

ACKNOWLEDGEMENT

This study was supported by NIH research grants AG-03527 (to B.C.) and AM-14744 (to A.K.R.).

REFERENCES

[1] Roy, A.K. and Chatterjee, B. (1983) Annu. Rev. Physiol. 45, 37–50.
[2] Bardin, C.W. and Catterall, J.F. (1981) Science 211, 1285–1294.
[3] Chatterjee, B., Nath, T.S. and Roy, A.K. (1981) J. Biol. Chem. 256, 5939–5941.
[4] Roy, A.K., Chatterjee, B., Demyan, W.F., Milin, B.S., Motwani, N.M., Nath, T.S. and Schiop, M.J. (1983) Rec. Prog. Horm. Res. 39, 425–461.
[5] Roy, A.K. (1977) Eur. J. Biochem. 73, 537–543.
[6] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
[7] Pelham, H.R.B. and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247–256.
[8] Laemmli, U.K. (1970) Nature 227, 680–685.
[9] Roy, A.K., Nath, T.S., Motwani, N.M. and Chatterjee, B. (1983) J. Biol. Chem. 258, 10123–10127.