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TRANSCORTIN AND α_{2u}-GLOBULIN MESSENGER RNA ACTIVITIES DURING TURPENTINE-INDUCED INFLAMMATION IN THE RAT

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Summary—Previously we have shown that the serum concentration of transcortin and α_{2u}-globulin markedly decreases during turpentine-induced inflammation. In the present study transcortin and α_{2u}-globulin mRNA from healthy rats and from animals with inflammation was translated in a rabbit reticulocyte lysate system. Female rats had higher levels of translatable transcortin mRNA than male animals and the level of mRNA for transcortin and α_{2u}-globulin decreased rapidly during inflammation. These results indicate that the sex difference in the serum level of transcortin and the changes in serum transcortin and α_{2u}-globulin during inflammation are mainly determined by differences in the mRNAs in the liver.

INTRODUCTION

Acute inflammation is associated with major changes in the concentration of several proteins [1]. In a previous paper [2] we have demonstrated that serum transcortin and α_{2u}-globulin, two proteins synthesized by the rat liver and secreted into the blood, markedly decrease during turpentine-induced inflammation. In the present report we studied the mechanisms of this decrease by measuring the levels of translatable liver mRNAs of both proteins, before and after turpentine injection in male and female rats.

EXPERIMENTAL

Animals

Male and female Wistar rats, 10-weeks old, received oil of turpentine at 9.00 a.m. as described previously [2]. Control animals were not injected. Eighteen and 36 h after the administration of turpentine, 3 or 4 animals of each sex were anesthetized with ether, blood was taken from the arteria carotis and the livers were excised and stored in liquid nitrogen.

Measurement of transcortin and α_{2u}-globulin

Both proteins were measured by radial immunodiffusion with monospecific antisera [2].

Isolation of RNA

RNA was extracted as described by Rosen *et al.*[3], with modifications. The frozen livers were ground, transferred into a Waring blendor and homogenized for 1 min in a mixture of 6 vol (v/w) of aqueous phase (0.075 M NaCl, 0.5% sodium dodecyl sulfate, 0.025 M EDTA, pH 8.0) and 6 vol of buffer-saturated phenol. Extraction was performed at 4°C for 30 min. After centrifugation (3500 ***g***, 20 min) the aqueous phase was reextracted with an equal volume of phenol–chloroform (1:1 v/v). The resulting aqueous phase was incubated with Proteinase K (20 μg/ml; Boehringer) at 37°C for 20 min and extracted again with phenol–chloroform. Then the aqueous phase was made 0.2 M in NaCl and the nucleic acids were precipitated with 2 vol of ethanol at −20°C. The following day the pellet was collected, dissolved in water and precipitated in 3 M sodium acetate, pH 6.0 at 4°C. After 2 h the precipitate was suspended in water, made 0.2 M in NaCl and precipitated with ethanol. Poly(A^+) RNA was isolated by oligo (dT)cellulose chromatography [4]. A 0.1 M NaCl washing step was introduced before the final elution.

Translation and immunoprecipitation

Poly(A^+) RNA was translated in a rabbit reticulocyte system [5]. The reaction mixture, containing 333 μl reticulocyte lysate (Amersham International), 0.66 mCi [^{35}S]methionine (New England Nuclear), and 24 μg RNA in a final vol of 440 μl, was incubated for 90 min at 30°C. Five microliters of the translation mixture were precipitated with trichloroacetic acid. The precipitates were collected on filters, solubilized in Soluene-350 (Packard Instrument Company) and the radioactivity was determined in a liquid scintillation spectrometer.

Immunoprecipitation [6] with antisera to rat transcortin, α_{2u}-globulin and non-immune serum were performed on aliquots of translation mixture, containing 1.2 10^6 cpm trichloroacetic acid-insoluble material (usually about 40 μl). The immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide (10%) slab gel electrophoresis and autoradiography [7]. The radioactive bands were excised from the dried gels, solubilized in Soluene-350 and counted. Data were corrected for non-specific binding of radioactive material to the gel.

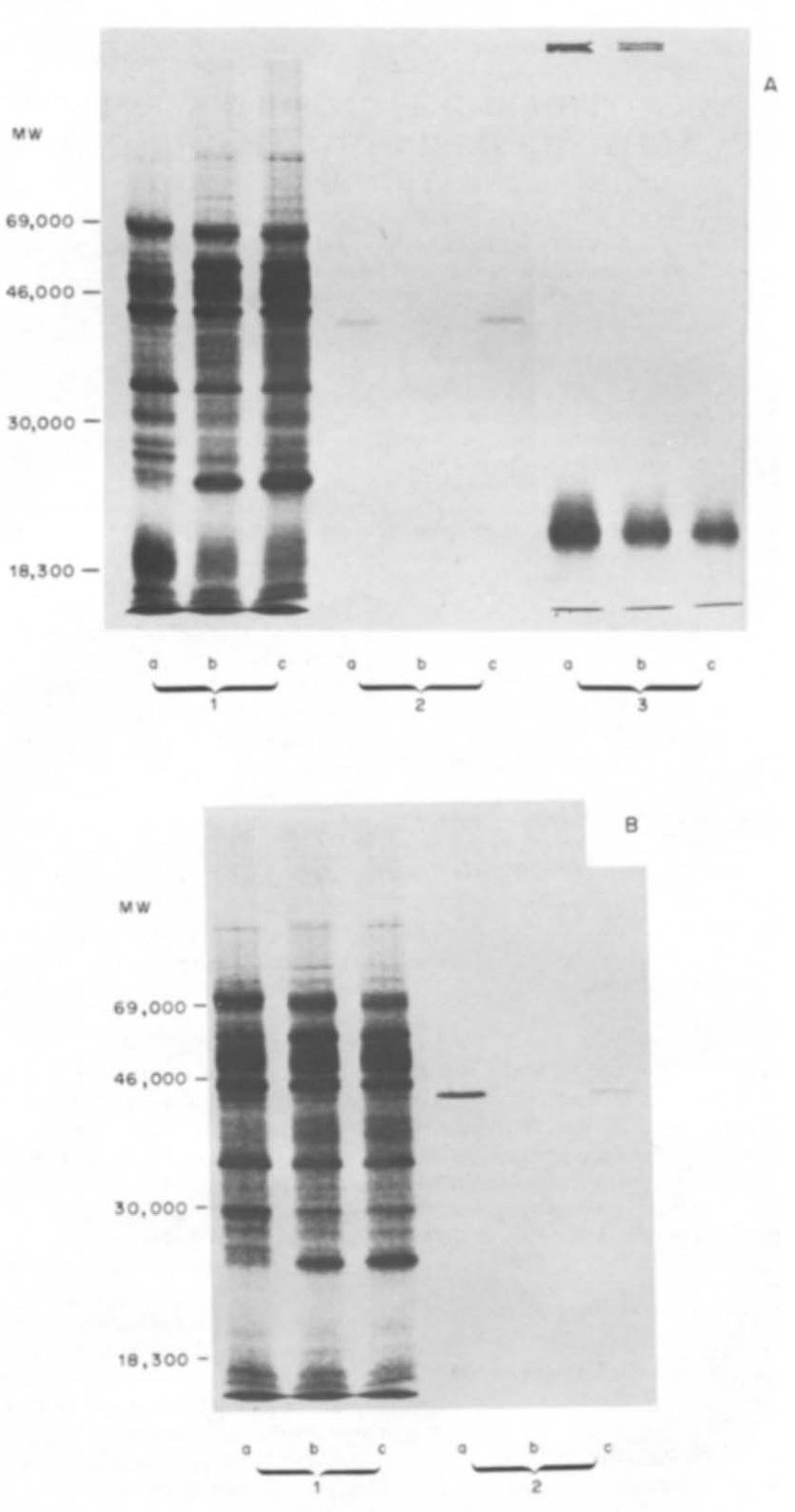

Fig. 1. Influence of acute inflammation on translatable liver mRNA. Poly(A^+) RNA was prepared from livers of male (A) and female (B) rats before (a), 18 h (b) and 36 h (c) after turpentine injection and was translated *in vitro*. The incorporation of [^{35}S]methionine in the complete translation mixtures (1) and in immunoprecipitates obtained with a transcortin (2) or an α_{2u}-globulin antiserum (3) was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers were bovine serum albumin, ovalbumin, carbonic anhydrase and lactoglobin A.

Dot blot hybridization

α_{2u}-Globulin-specific sequences in poly(A^+) RNA were analysed by dot blot hybridization using the method described by White and Bancroft[8]. Poly(A^+) RNA preparations were denatured by heating at 70°C for 5 min, cooled in melting ice, suspended in buffer (0.225 M sodium citrate, pH 7.4 containing 2.25 M NaCl) and diluted serially ($\frac{1}{2}$) in the same buffer. With the help of a dot blot template (Manifold, Schleicher and Schuell), aliquots (100 μl) of the dilutions were transferred onto nitrocellulose paper (BA 85, Schleicher and Schuell), previously soaked in buffer. Then the paper was baked at 80°C for 2 h. Prehybridization and hybridization with an excess of nick-translated [9] α_{2u}-globulin cDNA were performed as described by Dobner *et al.*[10], but glycine was omitted in the hybridization mixture. The α_{2u}-globulin probe used has been described previously [11]. Approximately 10^6 cpm of the cDNA was allowed to hybridize with each filter for 90 h at 42°C. "Dots" were excised, solubilized in Soluene-350 and counted.

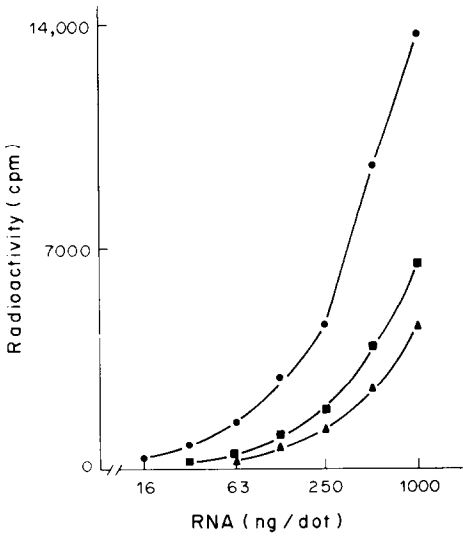

Fig. 2. Influence of turpentine injection on the abundance of α_{2u}-globulin RNA in liver. Total α_{2u}-globulin RNA was measured by dot blot hybridization in liver poly(A^+) RNA preparations from intact rats (●) and from animals injected with turpentine 18 h (■) or 36 h (▲) before they were killed. From the figure a 2.6-fold decrease between 0 and 18 h and a further 1.5-fold decrease between 18 and 36 h after turpentine injection was calculated.

RESULTS

Figure 1 (A, B; 1) shows that the injection of turpentine results in major changes in the level of translatable liver mRNA for several proteins. In both male and female rats a marked increase may be observed in the synthesis of two proteins with a mol. wt of 22,000 and 55,000. Based on their molecular weight and their response as positive acute phase reactants, these proteins can be tentatively identified as α_1-acid glycoprotein and α_1-major acute phase protein [12, 13]. Immunoprecipitation with an antiserum to α_{2u}-globulin shows that the translatable mRNA for this male specific protein decreases 2.3-fold between 0 and 18 h and 1.7-fold between 18 and 36 h after turpentine injection (Fig. 1A-3; Table 1). This corresponds well with the changes in the total α_{2u}-globulin RNA, measured by dot blot hybridization (Fig. 2).

Immunoprecipitation of the translation mixtures with a transcortin antiserum yields a radioactive product with a mol. wt of 42,000, both in males and females. Females, however, show higher levels of translatable transcortin mRNA (Table 1), reflecting the sex difference in plasma transcortin. During

Table 1. Effect of turpentine injection on serum transcortin and α_{2u}-globulin and on the corresponding liver mRNAs

Time (hours)	Serum concentration		Translatable liver mRNA	
	mg/l (1)	Normalized, % (2)	cpm (3)	Normalized, % (2)
		Transcortin		
Males				
0	69.5 ± 3.6 (4)	100	800 ± 60	100
18	39.9 ± 3.7 (3)	57	261 ± 169	32
36	24.0 ± 2.5 (3)	34	410 ± 109	51
Females				
0	153.9 ± 13.4 (4)	100	1515 ± 174	100
18	98.7 ± 10.9 (4)	64	232 ± 27	15
36	65.1 ± 10.6 (3)	42	241 ± 102	16
		α_{2u}-Globulin		
Males				
0	29.8 ± 1.8 (4)	100	13687 ± 2132	100
18	18.2 ± 2.4 (3)	61	6022 ± 562	44
36	14.9 ± 1.4 (3)	50	3565 ± 747	26

Serum transcortin and α_{2u}-globulin and the corresponding translatable liver mRNAs were measured in male and female rats before, 18 h and 36 h after injection of turpentine. Translatable liver mRNAs were measured by *in vitro* translation and immunoprecipitation. The immunoprecipitates were subjected to electrophoresis, the radioactive bands were localized by autoradiography, excised and counted in a liquid scintillation spectrometer. Results were corrected for the radioactivity of the corresponding spots on control gels. (1) Results are expressed as means ± SEM of the number of animals given in brackets. (2) Values normalized taking untreated animals as 100%. (3) Mean ± SEM of 4 pairs of gels.

turpentine-induced inflammation both the mRNA and the plasma level of transcortin markedly decrease. Previously we have shown that the patterns of the decrease of serum transcortin and α_{2u}-globulin differ: transcortin reaches its minimum 36 h after turpentine injection and increases thereafter, whereas α_{2u}-globulin remains low for a longer period [2]. The present results show that the observed changes in plasma transcortin and α_{2u}-globulin are preceded by similar changes in the respective mRNAs. For α_{2u}-globulin the mRNA level shows a further decrease between 18 and 36 h after turpentine injection. For transcortin, however, the 36 h level of translatable mRNA already tends to be higher than the 18 h level.

DISCUSSION

In the present study the *in vitro* translation of transcortin mRNA from male and female rat liver was compared. As expected [14] a translation product with a mol. wt of 42,000 was obtained with RNA from female animals. A similar product was obtained with RNA from male liver. However, male animals had a much lower level of translatable transcortin mRNA than female rats. This corresponded with the hormonally-induced sex difference in serum transcortin concentration. In conjunction with data from Perrot-Applanat and Milgrom [15] our results suggest that hormone-induced differences in serum transcortin levels are related to a difference in functional transcortin mRNA in the liver.

During experimental inflammation the fall in the concentration of serum transcortin and α_{2u}-globulin was accompanied by a reduction in their translatable mRNA levels. A decrease in the total hybridizable α_{2u}-globulin RNA paralleled the decrease in the translatable α_{2u}-globulin RNA, suggesting that turpentine injection lowered the transcription of the corresponding genes. For both proteins the time course of the reduction in the RNA concentrations reflected the time course of the decrease of the corresponding protein in the serum. Taken together with observations for other acute phase reactants [12, 13, 16] these results indicate that the changes in serum concentration of transcortin, α_{2u}-globulin and many other proteins during acute inflammation are mainly caused by changes in the corresponding mRNA in the liver.

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