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THE EFFECT OF TESTOSTERONE ON RAT BONE MARROW NUCLEAR RIBONUCLEIC ACID METABOLISM

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SUMMARY

The effect of testosterone on the metabolism of nuclear RNA (nRNA) of female rat bone marrow was studied. The hormone increased the incorporation of ^{14}C-formate into total nRNA. The sedimentation analysis of nRNA showed that testosterone stimulates preferentially the incorporation of the labelled precursor into species with sedimentation coefficients higher than 30 S. A concomitant effect of the hormone on the stimulation of nuclear ribonuclease activity was also observed. The probable significance of these facts is discussed.

INTRODUCTION

The stimulatory effect of androgens on the metabolism of bone marrow cells has been studied in several laboratories. Current evidence suggests that there are two mechanisms by which the androgens produce their effects: an early and direct one, acting on some particular bone marrow cells, presumably related to the erythropoietic line [1, 2] and a late and indirect one, mediated by an increase in the amount of endogenous erythropoietin [3].

In previous papers we have demonstrated that a significant change is produced in the incorporation of [^{14}C]-formate into total RNA adenine and DNA thymine after the administration of testosterone to normal or transfusion-induced polycythemic rats [2]. The effect reaches a maximum two hours after the injection and is produced by testosterone itself and not be 5α-dihydrotestosterone [4], which is a metabolite of testosterone reported to possess considerable androgenic activity in some androgen-dependent tissues [5].

The main purpose of the present work was to study the effect of testosterone on the metabolism of rat bone marrow nuclear RNA in an attempt to determine early molecular events in nucleic acid metabolism induced by the hormone.

MATERIALS AND METHODS

Strain A × C female rats (140–160 g weight) fasted for 12 h with water *ad libitum*, were used. Two hours before killing, rats were injected intraperitoneally with 0·5 ml 10% ethanol/saline or 0·5 ml of a 10% ethanol/saline solution of testosterone (14 mg/l).

When nRNA was to be analysed, the animals were also injected intravenously 30 min before death with 10 μCi of [^{14}C]-formate (S.A. 35 μCi/μmol; New England Nuclear) in 0·25 ml saline.

I. *Isolation and characterization of bone marrow nRNA from control and testosterone-treated rats*

From pooled femurs and tibias derived from four to six animals, bone marrow cells were removed and washed with ice-cold saline. Cells were suspended in 10 mM Tris–HCl buffer, pH 7·4, containing 5 mM $MgCl_2$ and 5 mM NaCl (TMS buffer). After 10 min swelling, cells were broken using a glass–Teflon homogeniser and the nuclear fraction was separated by centrifugation (at 700 ***g***, for 10 min). The resulting crude nuclear pellet was purified by sequential washing, first with 0·25 M sucrose solution containing 1 mM $MgCl_2$ and 1 g/l Triton X-100, second with 0·25 M sucrose containing 1 mM $MgCl_2$ and finally with TMS buffer. All the above steps were done at 4° C. The overall procedure takes less than 35 min and was easily performed since the bone marrow is not a solid tissue.

The purified nuclear fraction thus obtained was disrupted by suspension in 50 mM sodium acetate buffer, pH 5·2, containing 5 g/l sodium dodecyl sulphate. A volume of 0·5 ml of a 60 g/l bentonite suspension in acetate buffer was added to the mixture. RNA was then extracted using Kirby's method 2 [7] modified as follows: both interphase and aqueous phases obtained

after phenol–chloroform (1:1, v/v) treatment at 54° C, were re-extracted once with an equal volume of phenol: chloroform containing 1% isoamyl alcohol and three times with chloroform containing 1% isoamyl alcohol. In each of these re-extractions a cycle of homogenization, heating (at 54° C for 5 min), cooling and centrifugation (27,000 *g* for 10 min at 2° C) was employed. The final aqueous phase obtained was treated according to Kirby's method 2 [7].

Solutions (3 mg/ml) of the final product were made in 10 mM Tris–HCl buffer, pH 7·4 containing 1 mM EDTA, and 10 mM NaCl and used for RNA, DNA [8], protein [9] estimations and for gradient centrifugation analysis. No protein or DNA contamination was detected in these nRNA preparations.

Linear sucrose gradients (5 to 20% w/w) were prepared from ribonuclease free sucrose (Serva, West Germany) solutions in 10 mM Tris–HCl buffer, pH 7·4, containing 1 mM EDTA and 10 mM NaCl in nitrocellulose tubes using a Buchler density gradient system. After overnight equilibration, 300 μg of nRNA were layered on top of the gradient, and the charged tubes were centrifuged at 38,000 rev./min for 4 h at 0° C in the SW 39 rotor of the Beckman L2 65B ultracentrifuge. At the end of the run, the tubes were pierced and 40 fractions of 9 drops each (0·12 ml) were collected directly in counting vials to which 10 ml of scintillator (4 g PPO, 400 ml ethanol and 600 ml toluene) were added. A Nuclear Chicago liquid scintillation spectrometer was used for counting at 30% efficiency. For absorbance determination, fractions were collected in tubes, diluted with water to 1 ml and read at 260 nm.

The sedimentation coefficients were calculated using the McEwen tables [10]. The computed values were in good agreement with the sedimentation profile obtained with *E. Coli* 23*S* rRNA (Miles Res. Labs., U.S.A.).

II. *Ribonuclease activity determination*

Purified bone marrow nuclear fractions were prepared from control and from testosterone-treated rats using a previously reported method [6]. RNase activity in each nuclear fraction was measured as follows: 25 μmol Tris–HCl buffer, pH 7·3, 70 μmol NaCl, 5 μmol $MgCl_2$, 2 mg purified yeast RNA and 10^5 nuclei were mixed in a final volume of 1·0 ml. Controls without RNA or nuclei were also run. The mixtures were incubated for 30 min at 37° C with gentle shaking. At the end of the incubation time, 0·50 ml of 0·75% uranyl acetate in 25% perchloric acid were added to each incubation; the resulting suspensions were cooled in an ice bath for 20 min and centrifuged at 2500 *g* for 15 min. After centrifugation, 0·50 ml of supernatant was diluted with water to 3·0 ml and the absorbance of the acid-soluble oligonucleotides was measured at 260 nm. One unit of rat bone marrow nuclear RNase is the quantity of enzyme that releases an amount of acid-soluble oligonucleotides equivalent to 0·100 $O.D._{260}$ units under the conditions described. Determinations were made in duplicate.

Yeast RNA, sodium dodecyl sulphate, testosterone, Triton X-100 and Tris were obtained from Sigma Chem. Co. All other chemicals were analytical grade.

RESULTS AND DISCUSSION

The results of the *in vivo* experiments (Table 1) show that testosterone increases the specific radioactivity of rat bone marrow nRNA up to 50% as compared with controls. This increase, mediated by the hormone, could be due to changes in (a) the permeability of the cell to labelled RNA precursors, (b) the pool size of radioactive precursors, (c) the rate of degradation of polynucleotides or (d) the rate of biosynthesis of some particular species of RNA. We have not yet done experiments to show which, if any, of these possibilities is the correct explanation. We have, however, attempted to characterise the several different species of nRNA labelled in response to the hormone and those labelled in the bone marrow cells of control animals.

The O.D. profiles obtained on centrifugation and shown in Fig. 1 a and b, corresponding to nRNA from control and testosterone-treated rats, are rather similar and suggest that the bulk of RNA preparations are rich in species with low sedimentation coefficient. Whether this represents the actual picture of RNA in the intact nuclei or is the consequence of the action of endogenous ribonuclease is not known. Attempts to inhibit or inactivate nRNase by reagents often used to protect RNA from nuclease degradation during isolation have not been successful.

The specific radioactivity profile of the sedimentogram of control nRNA (Fig. 1 a) shows four labelled peaks: one sedimenting in the region of 30 S and the other three, representing most of the radioactivity, with sedimentation coefficients of less than 20 S.

Table 1. *In vivo* effect of testosterone on the [^{14}C] formate incorporation into rat bone marrow nuclear RNA

nRNA	Specific activity (counts/min/mg RNA)
Control rats (5)*	2241 ± 396†
Testosterone-treated rats (6)	3404 ± 508

For experimental details, see the text.
* Number of experiments.
† Standard error.

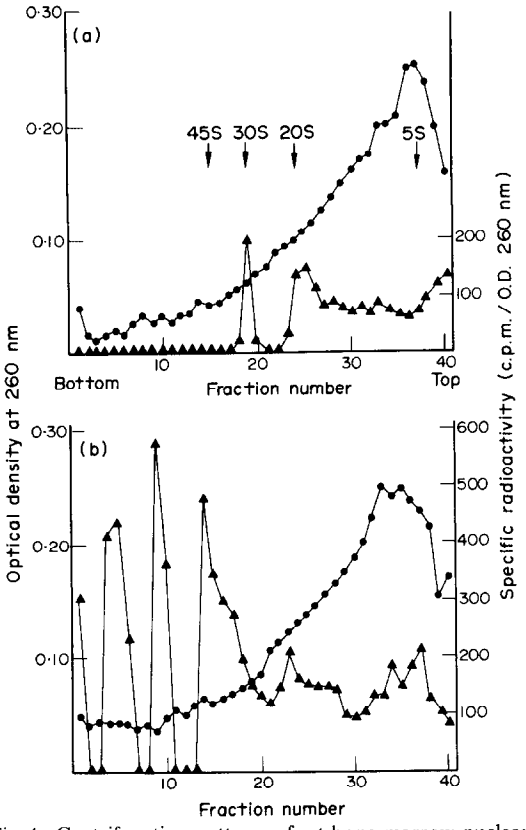

Fig. 1. Centrifugation patterns of rat bone marrow nuclear RNA from control (a) and testosterone-treated (b) rats. Absorbance at 260 nm. Specific activity expressed as counts/min/O.D.$_{260}$ nm. The arrows show calculated sedimentation coefficients[9]. For experimental details, see the text.

Significant differences from control nRNA could be established when the sedimentation pattern of nRNA from testosterone-treated rats was studied. As shown in Fig. 1 b, six labelled peaks are present. Of these, three show sedimentation coefficients higher than 30 S, have a high specific radioactivity and represent 10 to 15% of the radioactivity present in the gradient. A fourth peak appears in the middle of the gradient, sedimenting approximately at 20 S and with a low specific radioactivity. The last two peaks show sedimentation coefficients near 5 S.

When nRNA from both control and testosterone-treated animals was heated at 80° C [11], no changes in the sedimentation patterns were observed. This fact strongly suggests that the high molecular weight species detected consist of long continuous polynucleotide strands.

The above results show that a different sedimentation pattern is obtained with material from testosterone-treated animals compared with controls. The most pronounced difference in the sedimentation patterns of nRNA from control and testosterone-treated rats is in the markedly increased labelling of species of RNA having sedimentation coefficients higher than 30 S. Among these species, the ribosomal RNA precursors, with sedimentation coefficients in the range of 45 S and bearing a nucleolar origin, should be present [12]. In those species with sedimentation coefficients higher than 45 S, mRNA precursors described in several tissues are likely to be present [13]. It is worth mentioning that in erythropoietic cells, the presence of RNA molecules of large molecular weight has been described and attempts have been made to correlate the appearance of these RNA species with the effect of hormones. It seems that these RNA molecules contain a portion providing information for globin biosynthesis that could be released, after a specific processing step controlled by nucleases. [14].

In RNA fractions with sedimentation coefficients lower than 30 S it was found that the specific activity shown by nRNA species derived from testosterone-treated rats is markedly higher than in equivalent fractions from control nRNA. This could be a consequence of: a. the stimulatory effect of testosterone on the labelling of nRNA species, b. a concomitant effect of the hormone on the processing events of those species with sedimentation coefficients higher than 30 S.

The last possibility looks attractive not only because of the existence of a highly specific ribonuclease present in the nuclear fraction of rat bone marrow cells [6], but also because the activity of this enzyme is hormone dependent, as shown in Table 2. It can be seen that after the administration of testosterone to female rats, a 30% increase in the free nuclear RNase activity was achieved. This effect was observed between one and a half and two hours after the testosterone injection. Two hours is the time required for a marked increase in the incorporation of the labelled precursor into nRNA (Table 1).

The increased enzymatic activity induced by the hormone is the result of a modification in the relationship between free and inhibitor-bound nuclear ribonuclease of bone marrow cells (unpublished results). We can speculate that free RNase is necessary for an acceleration of the processing rate of those RNA

Table 2. The effect of testosterone on bone marrow nuclear ribonuclease activity

	Ribonuclease activity units/mg DNA
Control rats	1316 ± 76
Testosterone-treated rats	1724 ± 54

The reported results are the mean (± S.D.) of 4 experiments.

precursors into functional RNA's. An alternative explanation for the increase in the free ribonuclease activity could be the need of a higher capacity for degradation of the non-informative portions released in the course of the maturation events of some nRNA precursor species [15].

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