Chromatin-bound and free RNA polymerase A activities in rat thymus cells following glucocorticoid treatment

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Received 10 May 1984

Treatment of rat thymus cell suspensions with dexamethasone resulted in inhibition of engaged RNA polymerase A, without significant change in free pool activity. Studies with the re-initiation inhibitor, rifamycin AF/0-13, and measurements of numbers of RNA polymerase A molecules and of elongation rates showed that the inhibition of pre-rRNA synthesis resulted from a decrease in elongation rate. This effect was selectively abolished by mild proteolysis of nuclei. It is concluded that glucocorticoid treatment of rat thymus cells suppresses 45 S rRNA synthesis primarily by decreasing the polyribonucleotide elongation rate, rather than by effecting a change in enzyme redistribution or concentration.

Glucocorticoid *RNA polymerase A* *Rat thymus* *Transcription* *Ribosomal RNA*

1. INTRODUCTION

Previous studies have shown that treatment of rat thymus cells with glucocorticoids results in the transient stimulation of RNA polymerase B activity within 10 min of steroid addition and is followed by the inhibition of both RNA polymerase A and B activities [1]. The glucocorticoid-specific inhibitory effect on RNA polymerase A activity, unlike that on RNA polymerase B activity, is abolished by prior exposure of the cells to inhibitors of mRNA and protein synthesis and by glucose deprivation [2,3]. These observations have therefore suggested that the effect depends on the steroid-induced production of mRNA and on its translation; evidence for the existence of glucocorticoid-induced proteins, which may regulate rRNA synthesis, has been obtained [4].

Furthermore, the inhibitory action of RNA polymerase A activity is unlikely to reflect changes in the total cellular content of the enzyme, since the effect occurs rapidly whereas the turnover of the polymerase, in comparison, is slow. In liver, for example, enzyme concentrations are not changed in response to short-term hormonal stimulation by glucocorticoids [5].

However, it is conceivable that the steroid effects may be attributable to enzyme redistribution of RNA polymerase A between free and engaged pools. Such a mechanism has been proposed for the modulation of the rate of 45 S rRNA synthesis [6,7].

The possibility that this hypothesis may relate to the inhibitory effects of glucocorticoids on RNA polymerase A activity, and hence rRNA synthesis, in rat thymus cells was therefore investigated.

2. EXPERIMENTAL

2.1. *Reagents*

[5-^{3}H]UTP (2 Ci/mmol) was obtained from Amersham International (Amersham, England). ATP, CTP, GTP, UTP, poly[d(A-T)] and α-amanitin were purchased from Boehringer (Lewes, England). Trypsin, grade TRTPCK, was the product of Worthington (Freehold, NJ). Dexamethasone (9α-fluoro-16α-methyl-11β,17,21-trihydroxypregna-1,4-diene-3,20-dione) and ac-

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Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

tinomycin D were supplied by Sigma (Kingston-upon-Thames, England). Medium 199 was obtained from Gibco Biocult (Paisley, Scotland). Rifamycin AF/0-13 was a generous gift from Dr R. Cricchio, Gruppo Lepetit, Milan, Italy.

2.2. *Preparation of rat thymus cells and nuclei*

Procedures for the preparation of rat thymus cell suspensions, in vitro incubations and the preparation of purified nuclei from cell suspensions have been described [1,3].

2.3. *DNA-dependent RNA polymerase assays*

Assay conditions for the determination of RNA polymerase A activity in purified nuclei have been described [1,3]. 'Free' RNA polymerase A activity was measured by the poly[d(A-T)]–actinomycin D technique [8–10]. Polyribonucleotide elongation rates and numbers of RNA polymerase A molecules active in transcription in intact nuclei were determined as in [11].

3. RESULTS

3.1. *Effect of dexamethasone on free and engaged pools of RNA polymerase A activity*

Free form RNA polymerase A activity was determined, simultaneously with that of the engaged form, in rat thymus nuclei in the presence of poly[d(A-T)] and actinomycin D (table 1). The concentration of inhibitor used (160 μg/ml) was capable of inhibiting endogenous rRNA synthesis in excess of 90%, while the further addition of a saturating amount of poly[d(A-T)] (50 μg/ml) resulted in increased RNA polymerase A activity which was additive to, and did not inhibit or compete with, transcription of the endogenous template (not shown). In contrast to findings with rat liver nuclei [10], rat thymus nuclei prepared by hypo-osmotic lysis of cells contained a substantial pool of free RNA polymerase A activity. This enzyme pool, however, was capable of diffusing from nuclei with comparative ease under iso- and hypo-osmotic conditions [10]. Hence, free activity was also determined in the cytosol fraction remaining after nuclei preparation. Treatment of rat thymus cells with 1 μM dexamethasone for 3 h resulted in inhibition of the activity of the engaged form of RNA polymerase A by 30.0 ± 5.4%, inhibition of the nuclear free form by 11.2 ± 9.0% and inhibition of free RNA polymerase A activity in the cytosol by 8.3 ± 2.4% (table 1).

Hence, total free activity was inhibited to essentially the same extent as that of nuclear and cytosol fractions. Under control conditions, RNA polymerase A activity of the free form was 2-fold higher than that of the engaged form; dex-

Table 1

Effect of dexamethasone on free and engaged RNA polymerase A activities in rat thymus cells

Enzyme form	RNA polymerase A activity (pmol UMP/mg DNA per 15 min)		Dexamethasone-treated (% of control)	No. of experiments
	Control	+ Dexamethasone		
Nuclear engaged	34.4 ± 12.4	24.3 ± 9.5	70.0 ± 5.4	8
Nuclear free	36.3 ± 9.5	32.4 ± 10.1	88.8 ± 9.0	8
Cytosol free	33.2 ± 4.5	30.5 ± 4.9	91.7 ± 2.4	2
Total free	69.5	62.9	90.5	
Total free/engaged	2.02	2.59		

Rat thymocytes were incubated for 3 h at 37°C with or without 1 μM dexamethasone. Nuclei were then prepared and free activity was determined, simultaneously with that of the engaged form, in the presence of poly[d(A-T)] (50 μg/ml) and actinomycin D (160 μg/ml). The $MgCl_2$ supernatant from the preparation of nuclei was the source of cytosol free enzyme. Activity of the free enzyme was referred to the DNA content of assayed nuclei. Results are mean values ± SD

amethasone increased the ratio through suppressing endogenous UMP incorporation.

In further experiments with solubilized chromatin-associated enzymes from 3-h-incubated rat thymus cells, no decrease in the concentration of engaged RNA polymerase A was detected after dexamethasone treatment; similar levels of the enzyme were present in both control and steroid-treated cells (not shown).

3.2. *Effect of rifamycin AF/0-13 on control and dexamethasone-inhibited RNA polymerase A activity*

The inhibition of endogenous RNA polymerase A activity was not apparently due to enzyme redistribution, and therefore the effect of dexamethasone on the engaged form was investigated under conditions of limited enzyme reinitiation. Rifamycin AF/0-13, a eukaryotic inhibitor of initiation, was used to suppress reinitiation of RNA polymerase A in the assay in vitro. Fig.1 shows the activity of the engaged form of RNA polymerase A (RNA polymerase A II [10]) in nuclei from rat thymus cells incubated for 3 h in the presence or absence of 1 μM dexamethasone. In the absence of rifamycin, the initial UMP incorporation rate in nuclei from steroid-treated cells was 62.5% of that in nuclei from control cells. Rifamycin AF/0-13 did not affect initial synthetic rates, but prevented reinitiation by binding enzyme released after termination of the polynucleotide chains [12]. In the presence of rifamycin (200 μg/ml), sensitivity to the inhibitor developed earlier in nuclei from control cells than in nuclei from steroid-treated cells,

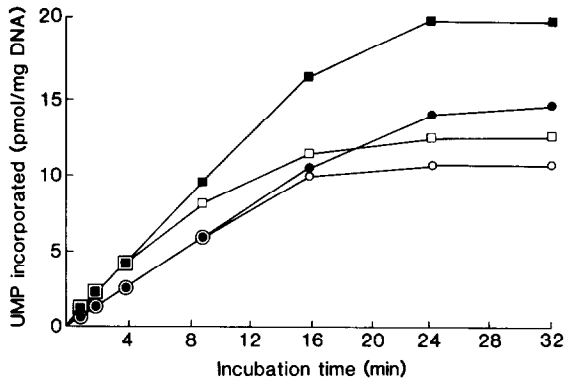

Fig.1. Effects of dexamethasone and rifamycin AF/0-13 on RNA polymerase A activity in rat thymocyte nuclei. Rat thymocytes were incubated for 3 h at 37°C in the absence (■, □) or presence (●, ○) of 1 μM dexamethasone; nuclei were then isolated and assayed for RNA polymerase A activity in the absence (■, ●) or presence (□, ○) of rifamycin AF/0-13 (200 μg/ml).

although similar levels of incorporation of UMP were attained. Furthermore, the effect of dexamethasone on the plateau value of incorporation of UMP in the presence of rifamycin AF/0-13 was much less than the effect on the initial synthetic rate; the mean inhibition by dexamethasone at 15 min was 7.3 ± 1.4% (mean ± SD, n = 3).

3.3. *Effect of dexamethasone on numbers of RNA polymerase A molecules and elongation rates*

To substantiate the previous studies direct measurements were made of numbers of transcribing RNA polymerase A molecules and of their elongation rates (table 2). Glucocorticoid treat-

Table 2

Effect of dexamethasone on number and elongation rate of form A RNA polymerases in rat thymocyte nuclei

Dexamethasone	UMP incorporated (pmol/mg DNA per 7 min) (% control)	10^{-3} × no. of transcribing enzyme molecules/cell (% control)	Polyribonucleotide elongation rate (nucleotides/min) (% control)
−	12.6 ± 0.4 (100)	2.06 ± 0.35 (100)	90.7 ± 1.9 (100)
+	8.8 ± 0.7 (70)	2.02 ± 0.25 (99)	66.8 ± 4.5 (74)

Rat thymocytes were incubated for 3 h at 37°C with or without 1 μM dexamethasone. Nuclei were then prepared and assayed for RNA polymerase A activity. Simultaneously, numbers of transcribing RNA polymerase molecules and their elongation rates were determined. Results are mean values of duplicate experiments ± SD

Table 3

Effect of treatment of nuclei with trypsin on RNA polymerase A activity

Dexamethasone	Trypsin	RNA polymerase A activity (pmol UMP/mg DNA per 15 min) (% control)	
		Expt.1	Expt.2
−	−	28.1 ± 0.2 (100)	23.0 ± 0.1 (100)
−	+	27.3 ± 2.0 (97)	23.0 ± 2.1 (100)
+	−	14.9 ± 0.6 (53)	12.7 ± 0.4 (55)
+	+	27.2 ± 2.1 (97)	20.0 ± 0.2 (87)

Rat thymocytes were incubated for 3 h at 37°C with or without 1 μM dexamethasone. Nuclei were then prepared and incubated for 4 min at 22°C in the presence or absence of trypsin (1.7 μg/ml). TKMS [50 mM Tris–HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, 0.25 M sucrose] buffer (2 ml) was then added and the nuclei centrifuged at 800 × g for 2 min and redispersed in buffer for assay of RNA polymerase A activity. Results are mean values for duplicate nuclear preparations ± SD

ment produced a reduction in the elongation rate, this decrease entirely accounting for the steroid-induced inhibition of nuclear RNA polymerase A activity. No change was observed in the number of transcribing enzyme molecules following dexamethasone treatment.

3.4. *Effect of mild proteolysis on RNA polymerase A activity*

Previous studies of RNA polymerase A activity in rat liver [13] have shown that glucocorticoids increase the catalytic activity of the engaged enzyme, per se rendering this increased activity sensitive to mild proteolysis. Although glucocorticoids inhibit rat thymic RNA polymerase A activity, this experimental approach was used to investigate the sensitivity of the thymic enzyme activity. Following the treatment of rat thymus cells with dexamethasone for 3 h, RNA polymerase A activity was inhibited by 45.9 ± 1.6% (mean ± SD, n = 2) in trypsin-untreated nuclei (table 3). Strikingly, mild treatment of nuclei with trypsin did not affect control enzyme activity, but restored dexamethasone-inhibited activity to a value similar to that of control (91.9 ± 3.3%; mean ± SD, n = 2).

4. DISCUSSION

The mechanism by which glucocorticoids regulate RNA polymerase A activity in rat thymus cells has been under investigation for some time in this laboratory. In view of the extended half-lives of eukaryotic RNA polymerases, and the observations that enzyme concentrations do not change in response to short-term stimulation by glucocorticoids [5], the effect of dexamethasone was studied on enzyme pool redistribution.

Previous investigations of the control of ribosomal RNA synthesis have revealed the presence of two discrete pools of RNA polymerase A in eukaryotic cell nuclei [9]. One pool of enzyme exists as a tightly bound transcription complex (engaged form), while the other is 'free' with respect to its ability to transcribe an exogenous poly[d(A-T)] template in the presence of actinomycin D. Studies on the intranuclear distribution of RNA polymerase A revealed that the steroid-mediated inhibition of the chromatin-bound form of the enzyme was not accompanied by significant increases or changes in free pool activity. However, since solubilized forms of free and engaged enzymes retain a similar ability to transcribe purified DNA and synthetic templates [14,15], these results suggested that the glucocorticoid-induced inhibition of RNA polymerase A activity was not simply a consequence of a decrease in number of initiated transcribing enzymes.

Support for this conclusion was obtained from experiments using rifamycin AF/0-13 to suppress

re-initiation of RNA polymerase A in the assay in vitro (fig.1). Clearly, the earlier onset of sensitivity to the inhibitor in nuclei from control cells than in nuclei from steroid-treated cells indicated the earlier release of active enzyme from ribosomal genes in control nuclei. Furthermore, since similar extents of incorporation of UMP were attained in the presence of rifamycin, these results are consistent with the conclusion that glucocorticoid treatment of rat thymus cells does not significantly affect average numbers of RNA polymerase A molecules transcribing, but inhibits rather 45 S rRNA synthesis by decreasing the enzyme elongation rate. Direct measurements of the numbers of transcribing RNA polymerase A molecules and of elongation rates have subsequently confirmed these conclusions (table 2).

Similarly, in liver, stimulated rRNA synthesis after in vivo glucocorticoid administration was observed to be due largely to increased engaged RNA polymerase A activity per se [14,16], rather than to increased availability of the ribosomal RNA genome [6,17]. This higher enzyme activity was reflected in the synthesis of longer RNA chains in vitro [18,19], presumably as a consequence of an increased polyribonucleotide elongation rate.

Furthermore, modification of liver RNA polymerase A per se to a more active species has been observed [13]. Further experiments, using a similar experimental approach, were performed with thymus cells. Treatment of thymocyte nuclei with low levels of trypsin resulted in the restoration of dexamethasone-inhibited polymerase A activity to a value similar to that of control. No significant effect of proteolysis was observed on control enzyme activity. These results are essentially similar to those obtained for rat liver cells, although in this tissue glucocorticoids stimulate ribosomal RNA synthesis [13]. Nevertheless, conversion of existing RNA polymerase A to a form with different catalytic activity and altered sensitivity to proteolysis appears to be a common property of this mechanism in both tissues. The enzyme activity may be regulated by a glucocorticoid-induced protein labile to mild proteolysis.

From these results, we conclude that glucocorticoid treatment of rat thymus cells inhibits 45 S rRNA synthesis primarily by decreasing the polynucleotide elongation rate of RNA polymerase A, probably by modification of the enzyme. Whether this mechanism requires the cooperation of RNA polymerase B activity to mediate the hormonal effect [3,20], or whether glucocorticoids can directly affect the synthesis of ribosomal RNA [19] in thymus cells, remains to be resolved.

ACKNOWLEDGEMENT

The author is grateful to the Tenovus organization for financial support.

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