MECHANISM OF EARLY EFFECT OF HYDROCORTISONE ON THE TRANSCRIPTIONAL PROCESS: STIMULATION OF THE ACTIVITIES OF PURIFIED RAT LIVER NUCLEOLAR RNA POLYMERASES.

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SUMMARY

We have previously demonstrated the presence of four RNA polymerases in rat liver nuclei. Two of these enzymes appear to be of nucleolar origin. In this communication we report that a single injection of hydrocortisone results, after 1½ hours, in the stimulation of both the purified nucleolar RNA polymerases whereas the nucleoplasmic RNA polymerases remain unaffected. Since the enzymes were analyzed using exogenous templates, we conclude that hydrocortisone initially stimulates ribosomal RNA synthesis in the nucleolus mostly by regulating the level or activity of nucleolar RNA polymerases, rather than availability of the template. Evidence is presented to show that hydrocortisone initially induces an allosteric change in nucleolar RNA polymerases, which results in increased activity of the enzymes and consequently in enhanced synthesis of ribosomal RNA.

INTRODUCTION

Ever since Karlson (1) proposed that steroid hormones act at the genetic level, a number of investigators have studied extensively the modes of action of hormones on the transcriptional process. Cortisol, when administered *in vivo*, stimulates nuclear RNA synthesis (2, 3, 4) and RNA polymerase activity *in vitro* (5, 6, 7). Studies on RNA polymerase activity were carried out using an "aggregate" enzyme preparation in which the enzyme is tightly bound to its native deoxyribo-

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nucleoprotein template. Consequently, these investigations do not distinguish changes in the activity and/or level of RNA polymerases from the alterations in the template availability. The exact mechanism by which cortisol stimulates RNA synthesis is thus poorly understood.

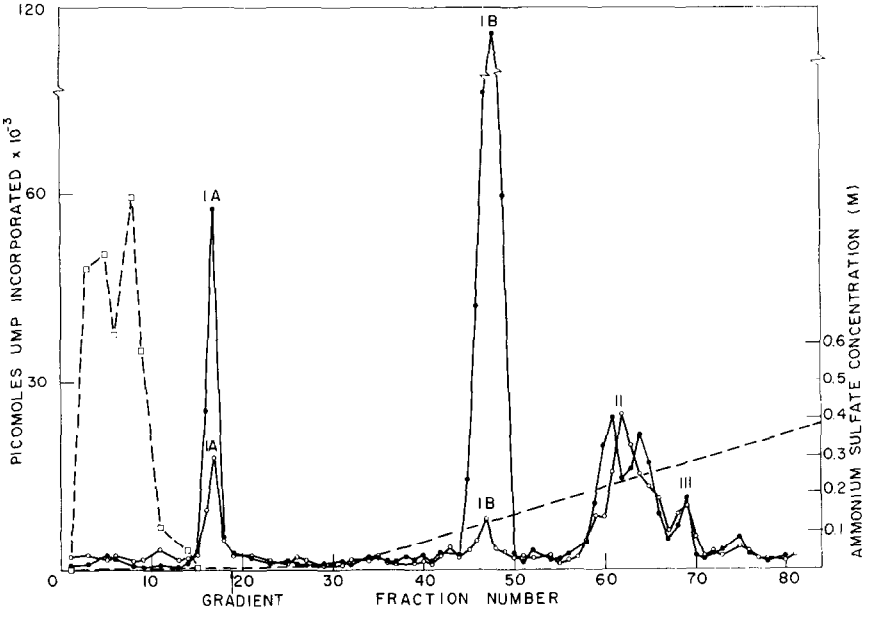
To overcome this problem, we have solubilized quantitatively RNA polymerase from the nuclei and nucleoli of rat liver (8). These studies have revealed at least four RNA polymerases in the rat liver nuclei (9). We (7) have previously demonstrated that the earliest event in RNA synthesis induced by hydrocortisone is the stimulation of nucleolar 45S RNA synthesis and of RNA polymerase activity in whole nucleolar preparations. We now report that cortisol enhances ribosomal RNA synthesis mostly by inducing allosteric changes in the nucleolar RNA polymerases.

MATERIALS AND METHODS

Normal and adrenalectomized male albino rats (160-180 g) fed ad libitum on Purina Chow, were obtained from Carworth Incorporated, New York. Adrenalectomy was performed 3-4 days prior to the experiment. UTP-H^3 (specific activity 16c/m M) was purchased from New England Nuclear Corp. Calbiochem provided nucleotides, Cleland's reagent and spermine. Hydrocortisone-21-phosphate was supplied by Merck, Sharpe and Dohme in vials containing 50 mg of the corticosteroid/ml.

For the nuclear RNA polymerase experiments, animals in groups of fifteen, each received 3 mg of hydrocortisone/100 g body weight while control rats were administered saline, all the injections being carried out intravenously. For studies on nucleolar RNA polymerase, groups of thirty animals were employed. The animals were sacrificed 1½ hours after treatment with hormone and the nuclei and nucleoli were isolated as described previously

(7). RNA polymerases were extracted from the isolated nuclei and nucleoli as reported earlier (8), with a few modifications. The solubilized enzyme was precipitated with ammonium sulfate (0.4 g/ml), stirred at 4^{o} for 1 hr and centrifuged at 110,000 xg for 50 min. The precipitate was suspended in 0.05 M Tris-HCl buffer (pH 7.9) containing 25% (v/v) glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA and 0.5 mM dithiothreitol (TGMED), dialyzed overnight against the same buffer and subjected to DEAE-Sephadex chromatography. DNA was prepared from liver nuclei by the Murmur's procedure (10). In some experiments, calf thymus DNA obtained commercially was used as the template. RNA polymerase was



Legend to Fig. 1

DEAE-Sephadex chromatography of RNA polymerase solubilized from livers of adrenalectomized and hormone-treated rats. The enzyme was solubilized from liver as described in the text. The dialyzed ammonium sulfate-precipitated enzyme (14-18 mg protein in 3 ml) was layered over a 0.9 x 12 cm. DEAE-Sephadex (A-25) column, washed with 16 ml of the buffer (TGMED) and eluted with 60 ml of linear gradients of ammonium sulfate as indicated. Fractions of 1.0 ml were collected and assayed for RNA polymerases activity as described previously (11). 0-0 adrenalectomized; ●-● hydrocortisone-treated, □-□ protein, as estimated by modified Lowry's method (12).

analyzed as described previously (11). Protein content was estimated by the procedure of Bennett (12).

RESULTS AND DISCUSSION

Figure 1 represents the DEAE-Sephadex chromatographic profile of RNA polymerase solubilized from the nuclei of adrenalectomized rats and of animals treated with hydrocortisone *in vivo*. As already reported by us (9), four forms of RNA polymerases were identified. These enzymes were eluted at 0, 0.12, 0.20, and 0.30 M ammonium sulfate concentrations, and are designated as 1A, 1B, 11 and 111, The fractionation of solubilized nucleolar RNA polymerase by DEAE-Sephadex chromatography (not shown in the figure) gave rise to peaks 1A* and 1B only, suggesting the presence of multiple forms of nucleolar RNA polymerases (Jacob and Sajdel, manuscript in preparation). These enzymes could be related to the two RNA polymerase activities, oneactivated by Mg^{2+} and the other by Mn^{2+}/ammonium sulfate, reported by us in the isolated nucleoli, where the enzyme was assayed with its associated deoxyribonucleoprotein template (13).

In adrenalectomized rats, the enzyme activity in peak 1A is higher than or the same as 1B (Table 1; Fig. 1). Administration of a single dose of hydrocortisone stimulates the activity of enzyme 1A by 150-160%, whereas the activity of enzyme 1B is stimulated usually by 210-370% (Table 1; Fig. 1). In experiments, where younger animals, weighing 100 g, were used, the stimulation of enzyme 1B was of the order of 960% (Fig. 1; Table 1). A similar situation has been observed in studies on the induction of RNA synthesis by dibutyryl cyclic AMP, where younger animals

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* Since this enzyme, previously designated by us as V_0 (9).is now known to be localized in the nucleolus, we redesignate it as 1A in order to conform to the numbering in the literature (14).

Table 1. Effect of hydrocortisone administration in vivo on the activities of RNA polymerases 1A and 1B

Animals	RNA Polymerase Activity (Picomoles Incorporated x 10^{-3})					
	1A			1B		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3*
Adrenalectomized	25	7	35	12	7	25
Adrenalectomized + hydrocortisone	66	18	91	37	33	266
% Stimulation	160	150	160	210	370	960

* In these experiments, rats weighing 100 g were used.

Legend to Table 1

Effect of hydrocortisone administration in vivo on the activities of RNA polymerase 1A and 1B. RNA polymerase was solubilized from the control and hormone-treated liver nuclei as described in the text. The enzyme was fractionated by column chromatography as described in the text and in the legend to the fig. 1. The enzyme activities in the regions of 1A and 1B (Fig. 1) were determined as described previously (11).

Table 2. Effect of hydrocortisone administration in vivo to adrenalectomized rats on the combined activities of RNA polymerases II and III

Animal	RNA Polymerase Activity (Picomoles Incorporated x 10^{-3})		
	II + III		
	Expt. 1	Expt. 2	Expt. 3
Adrenalectomized	98	70	125
Adrenalectomized + hydrocortisone	110	62	140
% Change	+12	-11	+12

Legend to Table 2

The details of the experiment are identical to those in the legend to Table 1. The enzyme activities in the regions of 11 and 111 (Fig. 1) were determined as described previously (11).

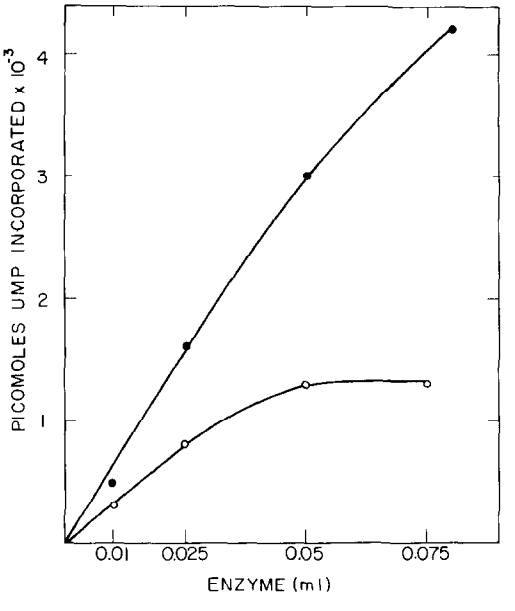
were more responsive to induction (15).

The activities of enzymes 11 and 111 remained unaltered under these conditions (Table 2; Fig. 1). In all the experiments, two peaks of activity were observed in the region of enzyme 11 after treatment with hormone, even though the total activity in this region remained unaltered after hormone treatment. An identical situation exists in the case of enzyme preparations from normal liver (9). The significance of this finding is at present unknown. The possibility of an interconversion between the nucleoplasmic enzymes cannot be excluded.

We have previously shown that hydrocortisone stimulates RNA polymerase activity by about 200% in the nucleolar preparations where the enzyme was analyzed with its associated deoxyribonucleoprotein template. The results of the experiments reported

in this communication show usually a 150-370% stimulation of the activity of purified RNA polymerases 1A and 1B (Table 1). These data therefore suggest a new type of cellular regulation, namely the regulation of RNA polymerase by hormones, independent of template activity. These experiments, however, do not rule out an additional, but less significant stimulation of template activity, or a stimulation of enzymes 11 and 111 at a later time period after hormone treatment.

In order to differentiate allosteric changes in RNA polymerase from changes in the level of the enzymes, the following experiment was performed. The RNA polymerase activity was measured using varying amounts of the total nucleolar RNA



Legend to Fig. 2

Effect of addition of increasing amounts of nucleolar RNA polymerase on the enzyme activity at a fixed DNA concentration. The enzyme fractions corresponding to peaks 1A and 1B (Fig. 1) were pooled and the activity was determined using varying concentrations of the enzyme and a fixed DNA (calf thymus) concentration (5 µg). The lower rate of incorporation in this experiment is mostly due to lesser quantity of DNA used for complete saturation with the enzyme. 0-0 Control; ●-● Hormone.

polymerase solubilized from adrenalectomized and hormone-treated livers and a fixed quantity of DNA. As shown in fig. 2, the enzyme activity was increased with increasing amounts of the enzymes, the difference between the two enzymes being most pronounced at higher enzyme concentrations where DNA was saturated with the enzyme. If the decreased ribosomal RNA synthesis in the livers of adrenalectomized animals is due to a diminished amount of the enzymes, addition of more nucleolar enzymes from control rats would have brought the enzyme activity to the level obtained with the enzymes from hormone-treated animals. The differences in the RNA polymerase activity persisted even after adequate amounts of the control enzyme were added. This experiment also rules out the possibility of preferential extraction of nucleolar enzymes from hydrocortisone-treated livers.

In conclusion these data suggest that the early effect of hydrocortisone on RNA synthesis is to induce an allosteric change in nucleolar RNA polymerases, thereby resulting in increased activity of the enzymes, which leads to enhanced synthesis of ribosomal RNA. The activation of the enzymes could be caused by an increase in the synthesis of some cytoplasmic factor(s) involved in the control of ribosomal RNA synthesis (16, 17).

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