SEQUENTIAL STIMULATION OF EXTRANUCLEOLAR AND NUCLEOLAR RNA SYNTHESIS IN RAT LIVER BY CORTISOL

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1. Introduction

Cortisol administration to intact or adrenalectomized rats leads to stimulation of RNA synthesis in the liver [1]. The newly synthesized RNA has been extensively characterized and encompasses DNA-like as well as ribosomal and transfer RNA's [2, 3]. In previous *in vitro* experiments [4, 5] we have indicated that the earliest action of cortisol is the stimulation of formation of AU-rich RNA, a finding corroborated by Yu and Feigelson [6] in *in vivo* studies. We had postulated that stimulation of nucleolar RNA must be a later step in hormone action. In order to directly prove this assumption we have fractionated nuclei from normal and cortisol treated rats into nucleoli and extranucleolar fractions and tested in an *in vitro* system their RNA synthetic capacity. The results presented below clearly show the sequential stimulation first of extranucleolar and then of nucleolar RNA synthesis.

2. Methods

2.1. *Animals and chemicals*

Male Wistar BR II rats (120–200 g) kept under standard conditions were obtained commercially. [^{3}H]UTP (specific activity 1 Ci/mmole) was purchased from Radiochemical Centre (Amersham), nucleoside triphosphates from Boehringer Mannheim, and the other chemicals from Merck Darmstadt. Cortisol was a gift of Schering AG Berlin, α-amanitin was generously supplied by Prof. Th. Wieland (Max-Planck-Institut, Heidelberg).

2.2. *Application of the hormone and preparation of the nuclei*

Groups of two or three animals received intraperitoneally 2 mg/100 g body weight cortisol suspended in isotonic sucrose solution and were sacrificed after 30, 60 and 120 min by cervical dislocation. Controls received the same buffer and were sacrificed immediately and after 30 or 60 min. No difference in any parameter was found within the control group.

Chauveau nuclei were prepared as described previously [7, 8] with the exception that the Mg^{2+}-concentration in the TSS-buffer was reduced to 1 mM instead of 10 mM. All buffers contained 1 mM 2-mercaptoethanol. Nuclei were lysed and homogenized in 0.067 M Tris buffer pH 7.9 and assayed for RNA polymerase activity as described earlier [9].

2.3. *Preparation of nucleoli and extranucleolar chromatin fractions*

The preparation of nucleoli was as described by Busch et al. [10] with the addition that all buffers contained 1 mM 2-mercaptoethanol. Nuclei were sonicated with 6–8 pulses of 1 sec with a Branson sonifier and disruption of nuclei meticulously controlled by phase contrast microscopy. The supernatant after centrifugation of the nucleoli was made 0.14 M NaCl with a 1.4 M NaCl solution and the resulting precipitate was spun down at 50 000 *g* for 20 min. More than 95% of the DNA was pelleted under these conditions. The pellet was homogenized in 0.067 M Tris buffer pH 7.9 and assayed for RNA polymerase activity. The 0.14 M supernatant showed also RNA polymerase activity which, when corrected for DNA content, reached magnitudes similar to the nucleolar RNA polymerase activity.

3. Results and discussion

Nuclei, nucleoli and extranucleolar fractions were isolated from the liver of control and cortisol treated rats after different time periods and tested for RNA synthetic capacity in an *in vitro* system as described under Methods. The results are depicted in fig. 1. As already reported [2] nuclear RNA synthesis is stimulated within 30 min after cortisol administration (fig. 1a). The results of fractionation of the nuclei demonstrate a significant increase of transcription in the extranucleolar fractions (fig. 1c and 1d) 30 min after hormone administration whereas at the same time period nucleolar RNA synthesis is inhibited (fig. 1b). After 60 min transcription by the 0.14 M NACl precipitable fraction reaches a plateau (fig. 1c) and that of the salt soluble fraction increases further (fig. 1d). At 2 hr after hormone application RNA synthesis in both extranucleolar fractions decreases whereas transcription by the nucleoli is highly stimulated. The purity of the various fractions has been checked morphologically by phase contrast microscopy and biochemically by the action of α-amanitin [12] on RNA synthesis. As well known α-amanitin inhibits *in vitro* RNA polymerase B which presumably synthesizes DNA-like RNA on chromatin whereas polymerase A, the enzyme localized in the nucleolus

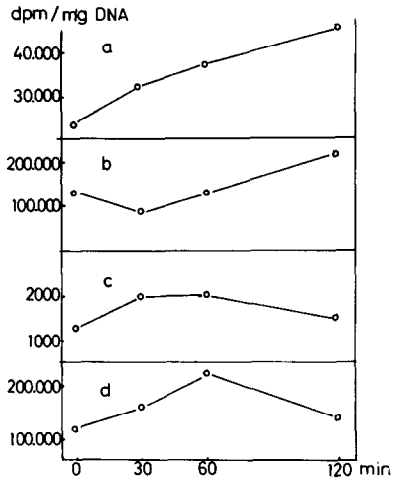

Fig. 1. Effect of cortisol on RNA synthesis of rat liver nuclei and nuclear fractions. Rats received 2 mg/100 g body weight cortisol intraperitoneally and were sacrified after various time intervals. Preparation of fractions and measurement of RNA synthesis *in vitro* is described in Methods. Values are given as dpm/mg DNA. a) nuclei, b) nucleoli, c) 0.14 M NaCl precipitable chromatin, d) 0.14 M NaCl supernatant.

Table 1
Effect of α-amanitin on RNA synthesis by isolated rat liver nuclei and nuclear fractions.

	RNA synthesis (dpm/mg DNA)		
	Without α-amanitin	With α-amanitin (0.2 μg/ml)	Inhibition (%)
Nuclei	22000	112000	49
Nucleoli	494000	496000	0
0.14 M NaCl precipitable fraction	2200	690	35
0.14 M NaCl soluble fraction	110000	9600	9

Nuclei and nuclear fractions were isolated as described in Methods and tested for RNA synthetic capcacity in the presence or absence of α-amanitin.

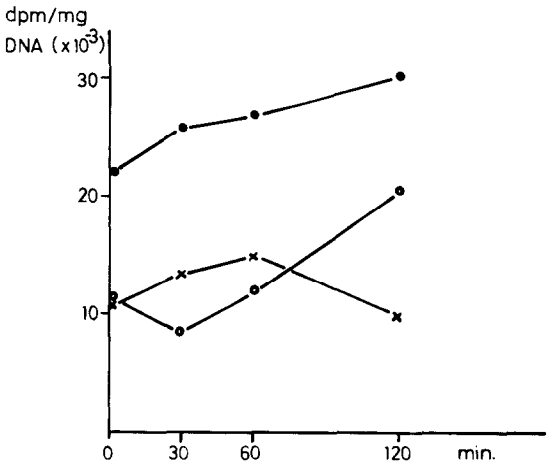

Fig. 2. Effect of cortisol on RNA synthesis of rat liver nuclei in the presence and absence of α-amanitin. Rats received 2 mg cortisol/100 g body weight intraperitoneally and were sacrificed after 30, 60 and 120 min. Nuclei were prepared and tested for RNA synthetic capacity as described in Methods in the absence (●—●—●) or presence (○—○—○) of amanitin (0.2 μg/ml). Amanitin sensitive RNA synthesis (x—x—x) was calculated as the difference between RNA synthesis in the absence and presence of α-amanitin. Values are expressed as dpm/mg DNA.

and transcribing ribosomal RNA, is not affected at all by the toxin.

The inhibitory effects of α-amanitin on RNA synthesis by the various fractions is depicted in table 1. Nucleolar RNA synthesis was completely insensitive to α-amanitin, which confirms the purity of the preparations. The salt soluble fraction was almost completely inhibited by the toxin showing that polymerase B is the enzyme actively engaged in RNA synthesis in this fraction. The chromatin fraction precipitated with salt was inhibited up to 35%. This fraction contains polymerase B as well as amanitin insensitive enzyme, probably involved in synthesis of transfer RNA [13]. Part of the amanitin insensitive enzyme may be RNA polymerase A released from nucleoli damaged during the preparation. The results obtained on the basis of the nuclear fractionation have been corroborated in experiments using α-amanitin. Nuclei isolated from the liver of cortisol treated and control rats were incubated under conditions of RNA synthesis in the presence and absence of α-amanitin. As seen in fig. 2 the increased RNA synthesis induced by cortisol in the first 30 min is abolished in the presence of the toxin. This experiment clearly shows that the early induced RNA is amanitin sensitive, very probably DNA-like RNA, which is synthesized by the polymerase B in the extranucleolar compartment of the nucleus. As in the experiments shown in fig. 1 the α-amanitin resistant RNA is stimulated at a later time period (fig. 2), during which the synthesis of α-amanitin sensitive RNA has declined.

The results presented demonstrate that the earliest effect of cortisol on transcription in rat liver is exerted on the extranucleolar part of the cell nucleus and is most pronounced in the supernatant remaining after 0.14 M NaCl precipitation of the bulk of the chromatin. This soluble fraction seems to be similar to the chromatin fraction recently described by Marushige and Bonner [19]. About 60–80% of the total nuclear RNA synthesis observed takes place in this fraction, whereas the bulk of the chromatin precipitated by 0.14 M NaCl is much less active in RNA synthesis. The response of nucleolar RNA synthesis in later periods seems to be a secondary effect of the hormone, perhaps underlying the control of species of RNA synthesized under the primary influence of cortisol.

Experiments with α-amanitin [13, 15–17] suggest a tight coupling between synthesis of DNA-like and ribosomal RNA. As shown recently α-amanitin, which *in vitro* is a specific inhibitor of RNA polymerase B leads, *in vivo*, also to impairment of ribosomal RNA formation [13, 15] and nucleolar segregation [18, 19] perhaps due to the inhibition of specific protein synthesis dependent of newly synthesized DNA-like RNA (for a discussion of α-amanitin action see Sekeris and Schmid [16, 17]).

Yu and Feigelson [6] using pulse labelling with RNA precursors *in vivo* showed that between 10 and 40 min after cortisol stimulation a preferentially U-rich RNA is made whereas at the same time G-rich, probably ribosomal, RNA is inhibited. An increased synthesis of DNA-like RNA was also demonstrated by Doenecke et al. [5] after application of cortisol to isolated rat liver nuclei *in vitro*. Beato et al. [20] have shown that stimulation of RNA synthesis under these conditions is brought about by an increased template activity of chromatin. The rapid stimulation of DNA-like RNA has also been suggested on the basis of electron microscopy by Petrov and Bernhard [21]. All these findings suggest that the first action of cortisol on transcription is exerted on the extranucleolar part of the chromatin.

The initial highly reproducible depression of nucleolar RNA synthesis after hormone administration is of interest. It is known that cortisol, parallel to its stimulatory effects on transcription, depresses RNA synthesis as shown for the rat thymus [22] and also described for rat liver [23]. At the present moment we cannot offer an explanation as to the mechanism and functional significance of this initial depression of nucleolar RNA synthesis. Work on these lines is in progress.

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