Effect of RNA synthesis on the binding of ^3H -cortisol to nuclear ribonucleoprotein particles from rat liver carrying DNA-like RNA in vivo.

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SUMMARY

^3H -cortisol was found to associate with rat liver nuclear 30S ribonucleoprotein particles carrying D-RNA* in vivo. No interaction was detectable when RNA synthesis was inhibited by α -amanitine. The association appears to be specifically for RNP carrying RNA synthesized after the administration of cortisol to adrenalectomized rats. The DNA/protein/RNA ratio of rat liver nuclei was not effected by α -amanitine under our conditions. However, the drug caused a 5-10 fold decrease in nuclear uptake of cortisol. The results are discussed in relation to a supposed transfer of cortisol-receptor complexes from the chromatin template to the nascent RNA chains.

INTRODUCTION

Evidence has been accumulated that cortisol^{1,2} as well as other steroid hormones^{3,4} are bound to cytoplasmic receptor proteins in the target cells. Thus, the enhancement of nuclear RNA synthesis induced by cortisol seems to be due to the action of a cortisol-receptor complex entering the nucleus rather than to the action of unbound cortisol⁵. The biochemical mechanism(s) underlying the cortisol mediated stimulation of nuclear RNA synthesis in vivo is still unknown. The binding of the steroid hormones to chromatin seems to be essential for the stimulation of RNA synthesis^{6,7}. On the other hand, it has been shown recently that the estradiol-receptor complex from calf uterus associates with RNP from uterine nuclei in vitro⁸. The same authors could

* Abbreviations used are: D-RNA, DNA-like RNA; rRNA, ribosomal RNA; RNP, ribonucleoprotein

demonstrate the binding of estradiol in vivo with RNP released by DNase digestion from uterine nuclei⁹. However, little is known about the RNA moiety of these RNP.

We have looked for possible interaction of cortisol in vivo with the relatively well characterized nuclear 30S RNP from rat liver nuclei carrying D-RNA^{10,11}. The binding of ³H-cortisol by rat liver nuclei and nuclear 30S RNP was compared in vivo under conditions of ongoing and inhibited RNA synthesis.

MATERIAL AND METHODS

³H-Cortisol (40 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, 2-¹⁴C-orotic acid (45,7 mCi/mmol) from the Institute of Isotopes, Hungary, Budapest. α -Amanitine was obtained from Boehringer, Mannheim. All other chemicals were of analytical grade.

Femal wistar rats weighing 150-180 g were adrenalectomized one week before each experiment. The rats were maintained on a standard laboratory diet and given 1 % NaCl to drink.

50-60 μ g α -amanitine in 0.9 % NaCl per animal was administered intraperitoneally to groups of three rats. Control animals were injected with solvent only. One hour later the animals were given an intraperitoneal injection of 50-75 μ Ci ³H-cortisol, 15-20 μ Ci ¹⁴C-orotic acid, and further 25-35 μ g α -amanitine or NaCl solution correspondingly. 30 minutes after administration of radioactivity the animals were decapitated.

Nuclei were isolated by a modification of Chaveaus method¹². Livers were perfused via the portal vein with ice cold saline, rapidly excised, minced well and homogenized with 3 volumes of 2.2 M sucrose - 3 mM MgCl₂ and centrifuged for 1 hour at 17 000 rpm in a Spinco-SW27 rotor at 0°C. The nuclear pellet was washed by centrifugation once with 0,25 M sucrose - 3 mM MgCl₂ containing 0.25 % triton X-100 and twice with 0.25 M sucrose - 3 mM MgCl₂. All procedures were carried out at 0-4°C.

Nuclear RNP was extracted following the procedure of Samari-na et al.¹⁰. The nuclear pellet was resuspended in 1.5 mM

STM8 (100 mM NaCl; 10 mM Tris-HCl, pH 8.0; 1 mM MgCl₂) and extracted for 20 min at 0°C by continuous stirring. After centrifugation at 15 000 g for 10 min the supernatant was used as the nuclear extract. The extraction procedure was repeated twice and the extracts were pooled. The nuclear residue was resuspended in 0.25 M sucrose - 3 mM MgCl₂. Nuclear RNP particles were purified by sucrose gradient centrifugation. 2 ml of the nuclear extracts were layered on a 15-30 % sucrose gradient containing 100 mM NaCl; 10 mM Tris-HCl, pH 7.4; 1 mM MgCl₂, and centrifuged for 15 hours at 75 000 g in a Spinco SW41 rotor at 4°C. The gradients were fractionated and each fraction measured for absorbance and radioactivity. ³H-labelled rRNA from rat liver was run in a parallel tube as a marker.

Cytosol was prepared from the 50 000 g supernatant of the nuclear pellet. The supernatant was diluted with 2 volumes of a Tris-HCl buffer (25 mM, pH 7.5) containing 50 mM KCl and 5 mM MgCl₂, and centrifuged for 15 hours at 100 000 g in a Spinco SW27 rotor at 4°C. The resulting supernatant was used as cytosol.

Measuring of radioactivity: 0.4 ml of a dioxan-based scintillation mixture containing 30 % soluene were added to the samples. After 15-20 hours 10 ml of the scintillation mixture was added and radioactivity both ³H and ¹⁴C was counted in a Mark II liquid scintillation spectrometer.

Protein was determined by the Lowry method¹³, DNA according to Burton¹⁴ and RNA with the orcinol reagent¹⁵.

RESULTS

α-Amanitine is known to inhibit specifically the nucleoplasmic RNA polymerase B^{16,17}. Under the conditions of application employed in our experiments nuclear RNA synthesis was inhibited for nearly 85 % as indicated by incorporation of ¹⁴C-orotic acid in total nuclear RNA (see Tab. 3). Determination of the DNA/protein/RNA ratio of the nuclei from control and α-amanitine treated animals revealed no significant differences in the composition of the nuclei (Tab. 1). The effect of α-amanitine treatment on the uptake of ³H-

cortisol by the nuclei is shown in Fig. 1. Though the homogenate of drug treated rats contained approximately the same amount of ^3H -cortisol uptake by the nuclei and content of ^3H -cortisol in nuclear extracts was 8-10 fold lower.

Tab. 2 shows the extractability of ^3H -cortisol from nuclei of control and drug treated animals. Approximately 30 % of the total nuclear ^3H -pool could be extracted both from control and α -amanitine nuclei though nuclei of drug treated animals contained an amount of ^3H -cortisol significantly lower (5-10 fold) than in the control. It can be concluded that the extractability of cortisol over a wide range does not depend upon the total amount of ^3H -cortisol in the nuclei. Thus, α -amanitine treatment did not yield significant quantitative differences in the extractability of cortisol from the nuclei.

If the dosage of ^3H -cortisol administered to α -amanitine treated animals was increased (Tab. 3) the nuclei of drug treated rats showed an increase in the uptake of the hormone.

Tab. 1 DNA/protein/RNA ratio of liver nuclei from adrenalectomized rats (control) and adrenalectomized rats treated with α -amanitine in vivo

		DNA	protein	RNA
control	(n=9)	1	2.54 ± 0.24	0.24 ± 0.10
α -amanitine	(n=5)	1	2.45 ± 0.47	0.20 ± 0.20

Tab. 2 Extractability of ^3H -cortisol from liver nuclei of adrenalectomized rats (control) and adrenalectomized rats treated with α -amanitine

Dosage of ^3H -cortisol administered: $40 \mu\text{Ci} / 100 \text{g}$ body weight.

	nuclei	nuclear extracts	nuclear residues
control (n=4)	100 %	$32.1 \pm 4.9 \%$	$65.5 \pm 6.3 \%$
α -amanitine (n=4)	100 %	$29.6 \pm 7.8 \%$	$69.4 \pm 5.5 \%$

Nearly the same specific activities (DPM/ mg DNA) were found in the nuclei both of control and α -amanitine treated rats. Comparing the nuclear extracts an approximately 15 % lower specific activity of ^3H -cortisol was measured in the α -amanitine group (Tab. 3) whereas a 5-10 fold lower specific activity was found when both groups of animals was injected the same amount of ^3H -cortisol. Yet these data provide no evidence in which form ^3H -cortisol is extracted from the nuclei. Nuclear extracts obtained with STMS contain RNP carrying D-RNA. It was of interest to look for a possible binding of ^3H -cortisol to these structures since cortisol

Fig. 1 Distribution of ^3H -cortisol in nuclear fractions of adrenalectomized rats. A - control, B - animals treated with α -amanitine. Values on top of the bars represent specific activities (DPM/mg protein $\times 10^{-3}$). H - homogenate, N - nucleus, NE - nuclear extract, NR - nuclear residue.

Dosage of ^3H -cortisol administered: $50 \mu\text{Ci} / 100\text{g}$ body weight.

Tab.3 Distribution of ^3H -cortisol and incorporation of ^{14}C -cyotic acid into total RNA in cell fractions of rat liver. Specific activities for ^{14}C are given in dpm of acid-insoluble fraction per protein or DNA respectively. Control animals were administered $28\mu\text{Ci } ^3\text{H}$ -cortisol, drug treated animals $47\mu\text{Ci } ^3\text{H}$ -cortisol per 100g body weight. Values represent means - 3 to 5 measurements with standard errors

adrenalectomized rats (control)										
fraction	DNA (mg)	protein (mg)	RNA (mg)	¹⁴ C (dpm × 10 ⁻⁵)		³ H (dpm × 10 ⁻⁵)	specific activities			
				total	acid-in soluble		dpm × 10 ⁻³ / mg protein		dpm × 10 ⁻⁵ / mg DNA	
							¹⁴ C	³ H	¹⁴ C	³ H
nuclei	7.6 ± 0.05	18.7 ± 1.6	1.95 ± 0.16	10.4 ± 0.1	7.3 ± 0.07	1.36 ± 0.31	38.0	7.29	0.95	0.18
nuclear extract	-	3.4 ± 0.13	0.2 ± 0.02	3.1 ± 0.26	0.93 ± 0.08	0.51 ± 0.06	27.3	15.0	-	-
nuclear residue	6.7 ± 0.46	14.0 ± 3.9	1.6 ± 0.4	5.4 ± 0.11	4.33 ± 0.10	30.88 ± 0.01	30.9	6.35	0.64	0.13
cytosol	-	521.0 ± 0.1	33.0 ± 0.8	116.0 ± 0.22	13.2 ± 0.03	83.0 ± 0.35	2.5	35.0	-	-
adrenalectomized rats treated with α-amanitine										
nuclei	4.1 ± 0.15	11.9 ± 2.4	1.38 ± 0.3	1.27 ± 0.48	0.92 ± 0.26	0.74 ± 0.1	7.76	6.2	0.23	0.18
nuclear extract	-	1.5 ± 0.3	0.1 ± 0.05	0.12 ± 0.001	0.04 ± 0.001	0.19 ± 0.02	2.62	12.7	-	-
nuclear residue	3.9 ± 0.14	11.8 ± 0.58	1.35 ± 0.2	1.08 ± 0.04	0.90 ± 0.03	0.48 ± 0.05	7.59	4.1	0.23	0.12
cytosol	-	300.0 ± 7.8	18.6 ± 2.5	124.0 ± 0.56	14.2 ± 0.07	180.0 ± 0.32	4.74	60.0	-	-

has been shown to effect the synthesis of D-RNA contained in 30S RNP from rat liver nuclei¹⁸. To prove possible association of ^3H -cortisol with nucleolar RNP nucleolar extracts were analyzed in a sucrose gradient (Fig. 2).

Nuclear RNP carrying D-RNA sediments with a sedimentation coefficient of 30S¹⁰. From Fig. 2 it can be seen that both gradients contain a 30S peak indicated by absorbance at 260 nm. If RNA synthesis was not inhibited significant amounts of ^3H and ^{14}C were found in the 30S region of the gradient. Newly synthesized RNA is present in form of 30S particles while another part of the ^{14}C -label is found in the light zone of the gradient possibly referring to degraded material. ^3H -cortisol migrates together with the 30S material indicating a binding of the hormone to the RNP complexes. Another part of ^3H -cortisol sediments in the 4-5S fraction of the gradient presumably representing "free" cortisol receptor complexes. Under conditions of α -amanitine treatment no significant amounts of radioactivity were found in

the 30S zone of the gradient. Thus, if no RNA is synthesized under the influence of the hormone ^3H -cortisol does not bind to RNP despite the fact that approximately the same amounts of 30S particles as in the control sediment in the gradient.

DISCUSSION

The effect of cortisol in the rat liver culminates in the induction of glucoegenic enzymes¹⁹ which is known to be inhibited by actinomycin D or α -amanitine²⁰. The influence of cortisol on nuclear RNA synthesis has been investigated only at relatively long times (1.5-4 hours) after administration of the hormone^{21,22}. It has been found that at this time preferentially rRNA synthesis was being increased. It was supposed that, before enhanced rRNA synthesis can occur, synthesis of specific proteins possibly acting as effectors of nucleolar RNA polymerase A is required²¹. We have in-

Fig. 2 Sucrose gradient centrifugation of nuclear extracts from adrenalectomized rats (A) and adrenalectomized rats treated with α -amanitine (B). Conditions of centrifugation as described in Materials and Methods.

Dosage of ^3H -cortisol administered: 28 μCi /100g body weight (A);
47 μCi /100g body weight (B).

investigated the action of cortisol at an early "preribosomal" phase. α -Amanitine as a specific inhibitor of nucleoplasmic RNA polymerase B was employed in vivo to prove the influence of RNA synthesis on the binding of cortisol to nuclear fractions. Our results indicate no significant effect of α -amanitine on the composition of the nuclei in respect to their DNA/protein/RNA ratio. But it was found that the nuclear uptake of the hormone was significantly diminished in the α -amanitine treated animals. We suggest that this effect is related to the inhibition of RNA synthesis since the action of α -amanitine is known to be highly specific. However, it cannot be excluded that the drug influences the amount of available active cortisol-receptor molecules transporting the hormone to the nucleus. To answer this question further experiments are required. In spite of the differences in the amounts of cortisol found in the nuclei of control and drug treated rats the extractability by STM8-solution was widely independent of the content of the hormone in the nuclei. In both cases approximately 30 % of the total nuclear ^3H -pool could be extracted. Further 30 % could be released from the preextracted nuclei by partial digestion with DNase I in form of slowly sedimenting material (results not shown). From this we suggest that also in the presence of α -amanitine at least a part of the cortisol found in the nuclei is associated with chromatin. Sucrose gradient analysis of the nuclear extracts revealed significant differences of the cortisol distribution pattern in the extracts. Only under conditions of ongoing RNA synthesis was cortisol found to sediment with the 30S RNP particles. If RNA synthesis was inhibited by α -amanitine neither ^3H -cortisol nor rapidly labelled RNA sedimented with the 30S particles fraction, indicating that nuclear RNP from drug treated animals containing no newly synthesized RNA were unable to bind the hormone. Thus, cortisol specifically associated with RNP carrying newly synthesized RNA, presumably the hormone induced RNA.

Newly synthesized RNA is known to be released from the chromatin template in form of high molecular weight RNP^{10, 23}. Under the extraction conditions employed in the present

study these complexes are cleaved by endogenous nucleases to a homogenous population of 30S particles¹⁰. Evidence has been accumulated that nuclear RNP contain a polydisperse protein moiety^{24,25}. The protein pattern has been demonstrated to be tissue specific²⁵. Our results suggest that the cortisol-receptor complex associates with the 30S particles. On this basis we suppose that there exists a "switch-over" mechanism for the hormone-receptor complex. According to this working hypothesis the cortisol-receptor complex is transferred from chromatin to the nascent RNA in the process of transcription. If RNA synthesis is inhibited by α -amanitine the hormone-receptor cannot be detached from chromatin by the supposed "switch-over" process and is partially extractable only in a non-particle bound form. Fig. 3 illustrates the model proposed here.

Fig. 3 Proposed model of cortisol action in the rat liver at an early phase.
 1 - Recognition of specific genes by the cortisol-receptor complex. 2 - Hormone induced transcription and "switch-over" of the cortisol-receptor complex. 3 - Release of the RNP-cortisol-receptor complex.
 (P: DNA-dependent RNA-polymerase B; NHP: non-histone protein
 C-R: cortisol-receptor complex)

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