STIMULATION OF RNA POLYMERASE ACTIVITY OF RAT LIVER NUCLEI BY CORTISOL *IN VITRO* INDEPENDENT OF EFFECTS ON THE ACETYLATION AND METHYLATION OF HISTONES *

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1. Introduction

In a previous paper of this series we have shown that addition of cortisol to isolated rat liver nuclei leads to a rapid stimulation of RNA polymerase activity and RNA synthesis [1,2] due mainly to increased template activity of chromatin [3]. One means of regulation of gene activity could be by modification of histones by acetylation, methylation or phosphorylation [4,5,6]. We have therefore studied the action of cortisol on the acetylation and methylation of histones of isolated rat liver nuclei.

2. Material and methods

Male Wistar BR II rats, weighing 120–180 g were used. Cortisol was kindly provided by Schering AG, Berlin, Triton X-100 by Rohm and Haas, Philadelphia. ^{3}H(methyl)-S-adenosylmethionine (sp. act. 4 C/mM) and ^{14}C-UTP(sp. act. 30μC/mM) were purchased from Amersham Radiochemical Centre. 1–^{14}C-acetyl-CoA(sp. atc. 54.5μC/mM) from the New England Nuclear Corporation. UTP, GTP, CTP, creatine phosphate and creatine phosphokinase were obtained from Boehringer, Mannheim, ATP from SIGMA.

2.1. *Preparation of nuclei:* Rat liver nuclei were prepared from adrenalectomized rats either by homogenization in isotonic sucrose solution containing 0.025 M KCl, 0.01 M $MgCl_2$, 0.05 M tris/HCl, pH 7.55 (TSS), differential centrifugation and treatment with the detergent Triton X-100 as described in ref. [7] or by a slightly modified Chauveau procedure (see ref. [8]).

2.2. *Incubation of nuclei:* Rat liver nuclei suspended in TSS were incubated in capped tubes at 37° in a shaking water bath. When appropriate cortisol dissolved in amounts of ethanol not exceeding 10 μl/ml suspension was added. The controls received ethanol alone.

2.3. *Measurement of RNA polymerase activity:* As enzyme preparation either nuclei or the nuclear sediment obtained by centrifuging nuclei lysed in 0.05 M tris/HCl buffer, pH 7.4 at 8000 *g* for 5 minutes was used. For details of the method see ref. [1].

2.4 *Measurement of the rate of methylation and acetylation of histones:* The nuclear suspension was incubated at 37° in TSS containing either 1–^{14}C-acetyl-CoA (0.1 μC/ml) or ^{3}H-S-adenosyl-methionine (4μC/ml). At different time intervals aliquots were pipetted in cold buffer and the suspension centrifuged at 800 *g* for 5 minutes. After washing the nuclei with ice cold 0.14 M NaCl either total histones were extracted with 0.25 N H_2SO_4 or the histones fractionated into F1, F2a, F2b and F3 fractions according to Johns [6] (see ref. [7]). The incorporated radioactivity was measured as described in ref. [7]. Protein was determined according to Lowry et al. [10], DNA according to Ceriotti [11].

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3. Results

Addition of cortisol to isolated nuclei leads to a rapid stimulation of RNA polymerase activity over the controls [1]. There is however no effect on the rate of histone acetylation during the whole period of the experiment (see fig. 1). Histone methylation is also not affected during the first 6 minutes of incubation while at 8 and 12 minutes there is an inhibition of methyl uptake by the histones of the cortisol treated nuclei. However, this inhibition is statistically not significant. In another series of experiments we measured the effect of cortisol on the acetylation and methylation of the histone fractions obtained according to Johns. In this way possible changes in the individual fractions could be found which otherwise would elude detection. The results are shown in table 1. As regards acetylation there is a slight stimulation of acetate uptake in fraction F1 and a slight inhibition in fraction F2a which are not statistically significant while methyl uptake is inhibited in all histone fractions.

4. Discussion

Isolated rat liver nuclei incorporate acetate and methyl from acetyl-CoA and S-adenosylmethionine

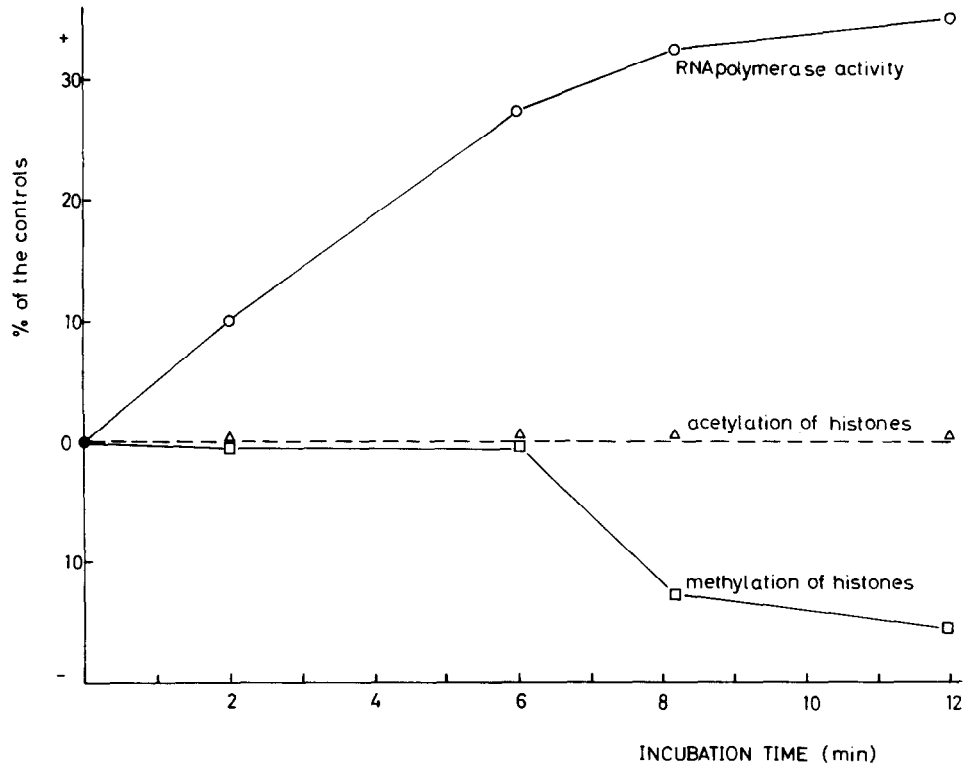

Fig. 1. Time course of the effect of cortisol on RNA polymerase activity and on histone methylation and acetylation of isolated rat liver nuclei. Rat liver nuclei (15–25 mg protein) suspended in 5 ml TSS were incubated in the presence or absence of 10 μg/ml cortisol at 37° for 12 min. Three parallel series of incubations were performed one for the measurement of RNA polymerase activity, one for the measurement of histone acetylation and one for the determination of the rate of methylation. In the last two cases the nuclear suspension contained 1 μC/ml ^{14}C-acetyl-CoA or 4 μC/ml ^{3}H-adenosylmethionine respectively. The measurements were performed as described in Methods. Each value represents the mean of double determinations from 4–6 experiments. The results are expressed as % values of the cortisol-treated nuclei in comparison to the controls.

Table 1
Effect of cortisol on the acetylation and methylation of histone fractions obtained according to Johns.

Histone	*acetylation* cts/min ^{14}C-acetyl incorp. per mg prot			*methylation* cts/min ^{3}H-methyl incorp. per mg prot		
	Control	Cortisol	% of control	Control	Cortisol	% of control
F1	408	468	+ 14.5	634	601	− 5.5
F2a	5555	5037	− 9.5	2174	1815	− 16.5
F2b	1165	1196	+ 2.5	1525	1230	− 19.5
F3	8110	8423	+ 4	6421	5760	− 10.5

Rat liver nuclei suspended in TSS were incubated at 37° for 8 min in the presence of 1 μC/ml ^{14}C-acetyl CoA or 4 μC/ml ^{3}H-S-adenosylmethionine for the determination of acetylation or methylation respectively. Cortisol was present when appropriate in amounts of 10 μg/ml. Fractionation of histones was performed according to Johns. Other experimental conditions as described in Methods. The values are means of 6 experiments.

respectively into histones. The incorporation is linear in the first 10 minutes and reaches a plateau after 20–30 min. At the pH of our experiments both acetylation and methylation are of enzymic nature and the distribution of label into the individual histone fractions is similar as to that seen in *in vivo* experiments (for details see refs. [7,8]).

The results presented above show that there is no correlation between histone acetylation and methylation and the cortisol stimulation of RNA polymerase activity of isolated rat liver nuclei. The rate of histone acetylation is not affected at all. A slight inhibition of histone methylation is seen 8 minutes after addition of cortisol which shows no statistical significance. Since at this time the RNA polymerase activity of the cortisol treated nuclei is already significantly stimulated, no causal relationship between histone modification and RNA polymerase stimulation can be postulated. The possibility that slight modifications of the histones, not detectable by our methods, may have taken place during the first minutes of hormone action, should be left open.

Our results are in disagreement with the observations of Allfrey [12] who studied the effect of cortisol on the *in vivo* acetylation of histones of rat liver. There are several explanations to this discrepancy. One possibility could be that cortisol stimulates intermediary reactions leading to increased production of acetyl-CoA and does not act directly on the histone acetylation step which we are studying *in vitro*.

It could also be possible that the nuclei have been damaged during the isolation procedure and do not react to the hormone. However, due to the fact that the nuclei respond to the hormone with increased RNA synthesis, this possibility can be excluded.

Recent studies by Libby [13] have shown stimulation of histone acetylation in a cell free system from rat uterus by estradiol-17β. The connection of these findings to the estradiol stimulation of uterine RNA synthesis remains to be seen.

In conclusion the increased activity of RNA polymerase seen after addition of cortisol to isolated rat liver nuclei does not seem to be related to changes in histone methylation or acetylation. The primary effects of hormones leading to stimulation of RNA synthesis still await elucidation.

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