# 'PARADOX' EFFECT OF CORTISOL AND ACTINOMYCIN D ON RNA POLYMERASE ACTIVITY OF RAT LIVER NUCLEI

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In the course of our studies on the stimulation of RNA synthesis in rat liver nuclei by cortisol [1–7], we were confronted with the task of identifying the nature of the newly synthesized RNA. Among other experimental tools, we have utilized the property of actinomycin D to inhibit preferentially r-RNA synthesis, as a criterion for the type of RNA synthesized. During these experiments we observed an inhibitory effect of cortisol on the RNA polymerase activity of liver nuclei from rats previously submitted to actinomycin D treatment.

In a series of experiments, four groups of male Wistar BR II rats, 100–200 g, were treated as follows: one group received i.p. 1.5 mg/100 g actinomycin D dissolved in tris-buffer containing 0.25 M sucrose, 0.025 M KCl and 0.01 M $MgCl_2$, pH 7.55 (TSS). Thirty minutes later 2.5 mg/100 g cortisol suspended in TSS was administered i.p. A second group of animals were treated as above, but instead of cortisol the same amount of TSS was injected. A third group received cortisol alone; and a fourth group, acting as control, received only the corresponding amount of buffer. Forty-five minutes after the last injection, the animals were sacrificed by cervical dislocation, the liver perfused with ice-cold TSS, and liver nuclei prepared with Triton X-100 as previously described [5]. RNA polymerase was assayed either in the 'nuclear sediment' obtained after hypotonic lysis of the nuclei [7] or as 'aggregate enzyme' according to Weiss [8]. The assay was performed by measuring the amount of $^{14}$C-UTP incorporated into 5% PCA insoluble material. The results are shown in fig. 1. It is evident that cortisol stimulates RNA polymerase activity of rat liver nuclei as reported previously [2]. In these experiments we found a stimulation of about 20% over the controls. Actinomycin D causes a 44% inhibition of the RNA synthesis in this system, while, contrary to our expectations, the RNA polymerase activity of the animals having received both actinomycin D and cortisol was even lower (75% inhibition). To eliminate uncontrollable factors and secondary effects, we undertook the confirmation of these findings in an *in vitro* system, using concentrations of 20 μg per ml of actinomycin D. Lower concentrations of the antibiotic inhibit RNA polymerase activity to a lower degree (see table 1). Nuclei isolated as described above were incubated at 37°C either with actinomycin D dissolved in TSS or with TSS alone for 5–10 min, and then in the presence or the absence of 5 μg/ml cortisol for another 10 min. The incubation was stopped by adding two volumes of ice-cold TSS and the nuclei recovered by centrifugation. RNA polymerase was assayed either in the 'nuclear sediment' or as 'aggregate enzyme'. The results are shown in table 1.

As in the *in vivo* experiments, cortisol stimulates *in vitro* the RNA polymerase activity of the nuclei [5, 7] while actinomycin alone inhibits it to about 55% in respect to the controls. Addition of cortisol to the actinomycin incubated nuclei results in a still greater inhibition of the RNA polymerase activity which reaches values as low as 30% of the controls.

This paradoxical effect could not be seen if actinomycin D was given simultaneously or after cortisol administration, either *in vivo* or *in vitro* (table 1), suggesting that actinomycin should be present when cortisol begins to act. Since cortisol inhibits RNA synthesis in the thymus gland [9, 10], an explana-

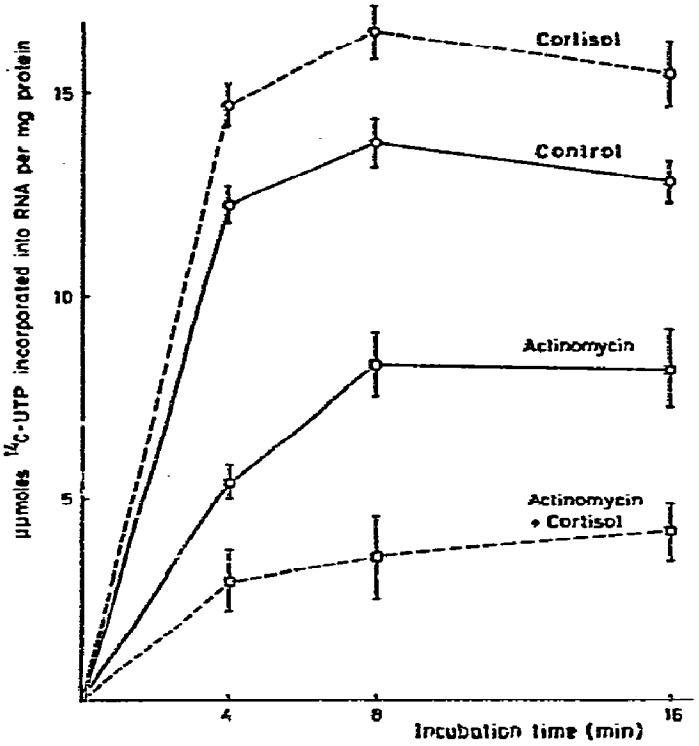


Fig. 1. "In vivo" effects of cortisol and actinomycin D on the RNA polymerase activity of rat liver nuclei. Rat liver nuclei were isolated from animals treated as described in the text and the "agregate enzyme" according to Weiss was prepared. RNA polymerase activity was measured in a system consisting of 0.25 µMol each of ATP, GTP and CTP; 0.1 µC $^{14}$C-UTP, 2.5 µMol creatine phosphate, 5 µg creatine phosphokinase, 1.5 µMol mercaptoethanol, 1 µMol $MnSO_4$ and 12 µMol tris, pH 7.9, in a final volume of 0.150 ml. For each assay amounts of "agregate enzyme" corresponding to 130 µg DNA were added. Aliquots were collected on filter paper discs and the radioactivity incorporated into RNA measured as described in ref. [5]. Each point of the curve is the mean of duplicate determinations from five experiments.

Table 1

*In vitro* effects of cortisol and actinomycin D on the RNA polymerase activity of isolated rat liver nuclei

| | µmol $^{14}$C-UTP incorporated into RNA per mg protein | |
|---|---|---|
| | Incubation time | |
| | 4 min | 8 min |
| Control | 3.90 ± 0.161 | 5.65 ± 0.182 |
| Actinomycin D (2 µg/ml) | 4.01 ± 0.180 | 5.27 ± 0.095 |
| Actinomycin D (10 µg/ml) | 2.56 ± 0.105 | 4.32 ± 0.113 |
| Actinomycin D (20 µg/ml) | 1.96 ± 0.071 | 2.07 ± 0.114 |
| Cortisol | 5.91 ± 0.193 | 7.24 ± 0.204 |
| Preincubation with actinomycin D (20 µg/ml), then cortisol | 1.27 ± 0.088 | 1.61 ± 0.091 |
| Simultaneous incubation with actinomycin D (20 µg/ml) and cortisol | 1.89 ± 0.101 | 2.18 ± 0.142 |
| Preincubation with cortisol, then actinomycin D (20 µg/ml) | 2.01 ± 0.092 | 1.92 ± 0.123 |

Isolated rat liver nuclei were treated as described in the text and then lysed in 0.05 M tris/HCl buffer pH 7.4. The nuclear sediment obtained after centrifugation at 8000 X *g* for 10 min was suspended in 0.065 M tris/HCl buffer pH 7.9, to a final concentration of 10 mg protein/ml, and used for the assay of the RNA polymerase as described in the legend to fig. 1. Each value represents the means and standard deviation of duplicate determinations from five experiments.

tion of the paradoxical effect could be that the hormone not only activates some genes in the liver cell nucleus [7, 11, 12], but also represses actinomycin D insensitive RNA synthesis. Assuming that the newly derepressed fragments of the genome will be rapidly blocked by actinomycin D before RNA polymerase binds to them, only the inhibitory effect of cortisol would appear, resulting in levels of RNA synthesis below the actinomycin values. When actinomycin D is given simultaneously or after cortisol treatment, some RNA polymerase molecules should bind to activated genes making their DNA inaccessible to the antibiotic. We have therefore studied the influence of cortisol on the uptake of labelled actinomycin D by the nuclei. Two series of experiments were performed. In one series rat liver nuclei isolated after the method of Chauveau et al. [13] were incubated for 5 min with different steroids ($3 \times 10^{-5}$ M) and then 20 µg/ml $^{3}$H-actinomycin D (spec. act. 73 µC/mg) was added to the incubation mixture. The incorporated radioactivity was measured by taking aliquots at different time intervals. The results of these experiments are shown in table 2. Preincubation of the nuclei with cortisol or testosterone, which are known to produce an activation of the RNA synthesis [5], results in an increased incorporation of $^{3}$H-actinomycin D. Hor-

Table 2

Effect of different steroids on the uptake of $^3$H-actinomycin D by isolated rat liver nuclei.

| | $^3$H-actinomycin D incorporated cpm per mg DNA ($\times 10^{-3}$) | |
|---|---|---|
| | Incubation time | |
| | 10 min | 20 min |
| Control | 128.40 ± 2.18 | 156.44 ± 4.02 |
| Cortisol | 148.60 ± 5.66 | 170.72 ± 7.12 |
| Testosterone | 151.88 ± 9.73 | 168.60 ± 8.33 |
| Androstendione | 135.33 ± 9.30 | 148.80 ± 12.51 |
| Pregnenolone | 129.61 ± 4.26 | 147.14 ± 7.55 |
| Control sonicated | 143.60 ± 4.07 | 156.34 ± 3.82 |
| Cortisol sonicated | 176.34 ± 11.90 | 181.96 ± 13.15 |

Rat liver nuclei were incubated in TSS (2 mg protein/ml) in the presence of different steroids (in concentrations of $3 \times 10^{-5}$ M) for 10 min at 37°C and then $^3$H-actinomycin D (20 µg/ml) was added. The incorporated radioactivity was measured in aliquotes taken at 10 and 20 min after washing the nuclei three times with TSS. The final pellet was taken in Vol. 5 of Nuclear Chicago Solubilizer (NCS) and shaken at 37°C for 4 hr. Scintillation counting was performed in Bray's solution using a Mark I liquid scintillation counter with a final efficiency of 18% as calculated by the channel ratio method. Each value represents the means and standard deviation of triplicate determination from two experiments.

monally inactive steroids like androstendione and pregnenolone do not influence $^3$H-actinomycin D binding. Since the effect persists when the nuclei are sonicated prior to the addition of $^3$H-actinomycin D we can assume that the stimulation in the uptake of radioactivity by the nuclei is independent of the integrity of the nuclear membrane, and is probably a direct consequence of the derepression caused by the hormone. Moreover a similar stimulation of $^3$H-actinomycin D incorporation was observed using nuclei isolated by treatment with detergent, when cortisol was added 5–10 min after preincubation of the nuclei with labelled antibiotic (table 3). This finding supports the idea that newly derepressed fragments of the chromatin are rapidly blocked by actinomycin D and therefore cannot be transcribed by the RNA polymerase. Another possibility to explain the paradoxical phenomenon described above could be an additive effect of actinomycin D and cortisol on the breakdown of newly synthesized RNA by the nuclei. We

Table 3

Effect of cortisol on the uptake of $^3$H-actinomycin D by isolated rat liver nuclei.

| | $^3$H-actinomycin D incorporated cpm per mg DNA ($\times 10^{-3}$) | |
|---|---|---|
| | Incubation time (after addition of cortisol) | |
| | 5 min | 10 min |
| Control | 181.02 ± 3.92 | 189.35 ± 5.66 |
| Cortisol | 199.45 ± 8.15 | 217.98 ± 10.12 |

Rat liver nuclei isolated by treatment with detergent were incubated in TSS (2 mg protein/ml) in the presence of $^3$H-actinomycin D (20 µg/ml) for 10 min at 37°C. Aliquots were then further incubated at 37°C in the presence or in the absence of cortisol ($3 \times 10^{-5}$ M). The incorporated radioactivity was measured in aliquots taken 5 and 10 min after the addition of cortisol, as described in table 2. Each value represents the means and standard deviation of duplicate determinations for two experiments.

have therefore measured the RNase activity of the nuclei in the presence or in the absence of actinomycin D and cortisol. The result of *in vivo* experiments were greatly variable and did not allow definite conclusions. Using isolated nuclei we found also some degree of variation, but an additive effect of actinomycin D and cortisol on the RNase activity could be excluded.

In experiments to be published, an inhibitory effect of actinomycin D on the uptake of 1–2 $^3$H-cortisol by isolated rat liver nuclei could be demonstrated. Since cortisol only binds to the protein components of the chromatin and not to DNA or RNA [14], the inhibitory effect of actinomycin D on the binding of cortisol by the nuclei is possibly the consequence of structural modification of the chromosomal proteins. Actinomycin D has often been used to inhibit hormonal metabolic effects dependent on RNA synthesis. Our experiments focus attention on the importance of the time sequence of administration of hormone and actinomycin D.

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