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# THE PATTERNS OF RNA SYNTHESIS AND INDUCTION OF GLYCOGENIC ENZYMES IN RAT LIVER UNDER CONTINUOUS CORTISOL ADMINISTRATION

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## SUMMARY

The synthesis of DNA-dependent RNA and of the enzymes of gluconeogenesis was studied under prolonged cortisol administration. Daily cortisol administration to rats for 15–22 days results in significant decrease and even loss of the capacity of liver cells to react to hormone administration by enhanced synthesis of RNA and enzymes of gluconeogenesis. At the same time the cells retain their capacity to insulin induction. Based on experiments on cellular nuclei *in vitro* it is postulated that because of prolonged cortisol administration changes occur in the chromatin of liver cells leading to the impairment of their capacity to be induced.

## INTRODUCTION

ACCUMULATED evidence indicates that enzyme induction plays an important role in the action of many hormones[1–3] and metabolites[4–5] in animal organisms. The induction of DNA dependent RNA and protein synthesis in animals was previously investigated mainly after short term administration of certain hormones and metabolites. There are some situations, however, in which long-term administration of these substances leads to continuous induction effects. Examples of these situations are diseases characterized by hormonal hyperproduction or prolonged hormone administration for theurapetic purposes.

The present study was designed to investigate the patterns of RNA synthesis and enzyme induction in rat liver under continuous cortisol administration.

## MATERIALS AND METHODS

### *Steroids*

Cortisol acetate and [1,2-$^3$H]-cortisol were purchased from Richter, Hungary and from the Radiochemical Centre, Amersham, England, respectively.

### *Tissue analysis: isolation of nuclei*

Normal male Wistar rats (120–130 g body weight) received daily cortisol acetate intraperitoneally (5 mg per 100 g of body weight) during 1–22 days.

The rats were killed 5 h after the last injection and the activities of tyrosine transaminase (TTA, l-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5), glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) and fructose-1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11)[6] were estimated in the liver tissue.

D-RNA and r-RNA were extracted from rat liver nuclei according to Georgiev and Mantjeva[7] (D-RNA has been introduced by Georgiev *et al.*[8] designation of an enriched by m-RNA fraction of nuclear RNA). In these experiments [8-$^{14}$C]-

adenine (specific activity 30 mCi/g) was injected at a dose of 30 $\mu$Ci per 100 g of body weight.

The influence of cortisol on RNA synthesis in isolated rat liver nuclei was investigated. Rat liver nuclei isolated by the Steiner and King method[9] were incubated with cortisol (25 $\mu$g/ml) for 10 min at 37°C following the Lukacs and Sekeris procedure[10].

After incubation the nuclei were centrifuged at 600 *g* for 5 min. The sediment was suspended in 2·4 M sucrose containing 0·001 M $MgCl_2$ and centrifuged at 90,000 *g* for 60 min.

RNA synthesis in isolated nuclei was measured as described by Lukacs and Sekeris[10] using [8-$^{14}$C]-ATP as the precursor. The incubation medium contained 0·2 ml of nuclear suspension (equivalent to 0·3–0·4 mg of DNA), 0·2 $\mu$moles of each of the following nucleoside triphosphates: GTP, CTP and UTP, 0·2 $\mu$ mole [8-$^{14}$C]-ATP (specific activity 0·1 $\mu$Ci/$\mu$ mole), 5 $\mu$ moles $\beta$-mercaptoethanol, 40 $\mu$ moles Tris-HCl (pH 7·9), 5 $\mu$moles phosphoenol pyruvate and 10 mg of pyruvate kinase, in 0·5 ml. The mixture was incubated for 15 min at 37°C.

DNA content was measured by the diphenylamine method[11]. RNA was isolated for specific activity measurements as described by Nakagava and White [12]. Samples were counted using a dioxan scintillation solution and a scintillation spectrometer (Nuclear Chicago, Mark 1).

To investigate the penetration of isolated rat liver nuclei by cortisol the mixture containing 0·5 ml of the nuclear suspension, 2 $\mu$g [1,2-$^3$H]-cortisol (specific activity: 4·07 mCi/mg) and 2 ml of buffer solution containing 0·05 M Tris-HCl, 0·32 M sucrose, 0·001 M $MgCl_2$, 0·025 M KCl (pH 7·5) was incubated for 15 or 30 min at 37°C.

After incubation the nuclei were centrifuged at 1000 *g* for 10 min and the sediment washed with 5 ml of aqueous solution containing 0·024 M EDTA and 0·075 M NaCl three times, and applied on microfilters RUFS (1·2 $\mu$, Synpor, Chechoslovakia).

Radioactivity was measured with a liquid scintillation spectrometer using a scintillation solution containing 4g/l PPO and 0·2 g/l dimethyl-POPOP in toluene with an efficiency of 47%.

## RESULTS

### *Glycogenic enzymes activity and RNA synthesis in rat liver under long-term cortisol treatment*

As shown in Fig. 1, 5 h after the first cortisol administration the TTA activity increased five-fold. As had been established earlier[13], it is concerned with DNA dependent RNA synthesis[14].

Daily administration of cortisol during the next 3–5 days maintained the increased TTA activity at the same level. However, more prolonged hormone administration results in the decrease of TTA activity: after 7–10 days, the activity is only 3 times higher than normal and after 20 days of cortisol administration it exceeds the initial level by a factor of 1·4.

Assay of glucose-6-phosphatase and fructose-1,6-diphosphatase activities under continuous cortisol administration revealed similar patterns (Figs. 2 and 3).

It seems that in adult rats under the long-term cortisol treatment the ability of liver cells to react on hormone administration by increase of glycogenic enzymes activity is lost. In young animals such unresponsiveness to cortisol develops

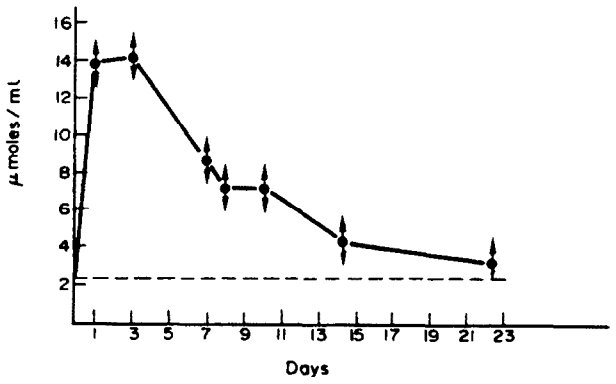


Fig. 1. Decrease of induced TTA activity in rat liver under prolonged cortisol injection ($\mu$ moles *p*-hydroxiphenyl pyruvate per ml of 10%-homogenate for 18 min incubation under 37°C).

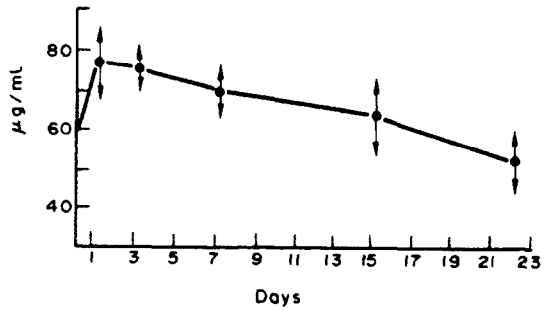


Fig. 2. Activity of glucose-6-phosphatase in rat liver under prolonged cortisol administration ($\mu$g Pi per ml of 10% homogenate for 15 min incubation under 37°C).

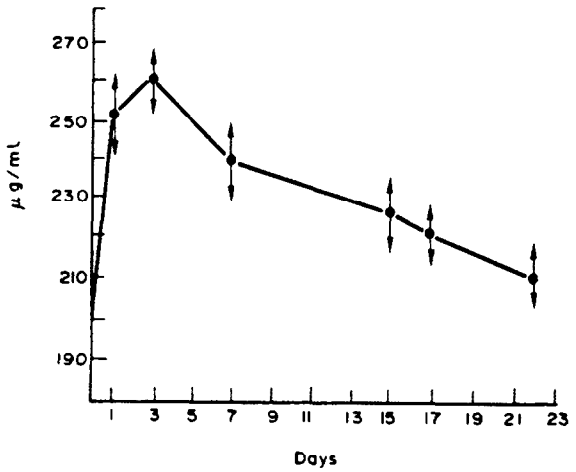


Fig. 3. Activity of fructose-1,6-diphosphatase in rat liver under prolonged cortisol administration ($\mu$g Pi per ml of 10% homogenate for 10 min incubation under 37°C).

during a much shorter period of continuous cortisol administration. Figure 4 shows that in 10–18 day old rats a drastic decrease of TTA induction is attained after 9 days of daily cortisol treatment.

Using TTA activity as a measure of induction efficiency, we have investigated the restoration of the response of rat liver cells to cortisol after cessation of continuous hormone treatment. It was demonstrated that when continuous treatment was stopped it took about a month to restore in adult rats the ability of liver cells to react by TTA induction on single cortisol administration[15].

Considering that transcription processes underlie the increase of the investigated enzyme activities we studied RNA synthesis under the same conditions of prolonged cortisol treatment.

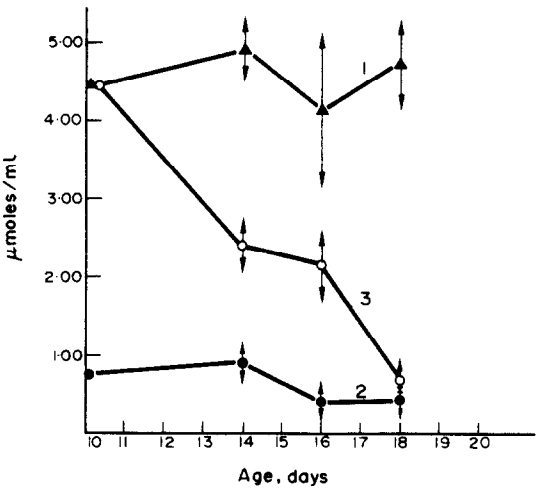


Fig. 4. TTA activity in liver of young rats under cortisol treatment (1-single cortisol administration, 2-control, 3-continuous cortisol administration).

The data presented in Tables 1 and 2 indicate that the incorporation of $^{14}C$-adenine into D-RNA and r-RNA increases during the first days of cortisol administration, but drops to the initial level after 20 days of hormone treatment.

The results obtained suggest that under prolonged cortisol administration rat liver cells lose their capacity to respond to the administration of this hormone by specific enzyme induction.

It was interesting to find out whether this peculiar unresponsiveness was limited to cortisol or whether it was more generally exhibited by various inductors.

It is known that insulin increases RNA and protein synthesis in liver cells inducing, particularly, some glycolitic enzymes[16]. When insulin was injected into rats previously subjected to repeated cortisol treatment the inductive action of insulin was exhibited by the increase of RNA synthesis (Tables 2 and 3) and was as effective as in control rats[17].

*RNA synthesis induced by cortisol in isolated rat liver nuclei*

Lukacs and Sekeris[10] have shown that isolated rat liver nuclei are capable of responding to cortisol *in vitro* by increased rate of RNA synthesis. Similar results were obtained in our experiments (Table 4). However, nuclei isolated from

Table 1. D-RNA synthesis in rat liver during cortisol administration for 1–21 days

| Control | Duration of cortisol administration (days) | | |
|---|---|---|---|
| | 1 | 7–11 | 21 |
| 1495 | 3551 | 1554 | |
| 1339 | 1889 | 1291 | |
| 1333 | 1860 | | 945 |
| 668 | 985 | | 717 |
| 519 | 1646 | | 697 |

In each experiment RNA was extracted from the liver of 3–4 animals. Radioactivity was expressed in counts/min/mg RNA.

Table 2. R-RNA synthesis in rat liver under prolonged cortisol administration and subsequent insulin injection

| Control | Cortisol, day 1 | Cortisol, day 21 | Cortisol, day 21 + insulin (3 days) |
|---|---|---|---|
| 640 | 1570 | 297 | 447 |
| 513 | 1076 | 304 | 408 |

Radioactivity was expressed in counts/min/mg RNA.

Table 3. D-RNA synthesis in rat liver during insulin administration after cortisol injection for 21 days

| Cortisol, day 21 | Cortisol, day 21 + insulin (3 days) |
|---|---|
| 1021 | 1346 |
| 645 | 1760 |
| 1055 | 1593 |
| 1110 | 1309 |

Radioactivity was expressed in counts/min/mg RNA.

Table 4. Incorporation of AMP-$^{14}$C into isolated nuclei of rat liver after their incubation with cortisol in vitro ($\mu\mu$moles/mg DNA for 15 min incubation at 37°C)

| Experimental conditions NN | Control | Incubation with cortisol *in vitro* | Prolonged induction *in vivo* | Prolonged induction *in vivo* and incubation with cortisol *in vitro* |
|---|---|---|---|---|
| 1 | 2220 | 3810 | 1242 | 1330 |
| 2 | 1136 | 2660 | 1306 | 1432 |
| 3 | 1683 | 2691 | 1911 | 1872 |
| 4 | 1221 | 1859 | 1115 | 812 |
| 5 | 2559 | 3655 | – | 2178 |

the liver of rats subjected previously to long-term cortisol treatment were found unresponsive to incubation with this hormone *in vitro*.

*The penetrance of cortisol into rat liver nuclei*

The differences in the response to cortisol between liver nuclei isolated from long-term cortisol treated and normal rats could be due to different penetration of this hormone through nuclear membranes.

The data presented in Fig. 5 demonstrate that [1,2-$^3$H]-cortisol penetrates to about the same extent into intact and long-term treated rat liver nuclei. Also, the penetration into these different nuclei *in vitro* was similar.

It was established that after injection to rats of labelled cortisol unmetabolized hormone can be found in liver nuclei of long-term treated rats as well as of control ones[18].

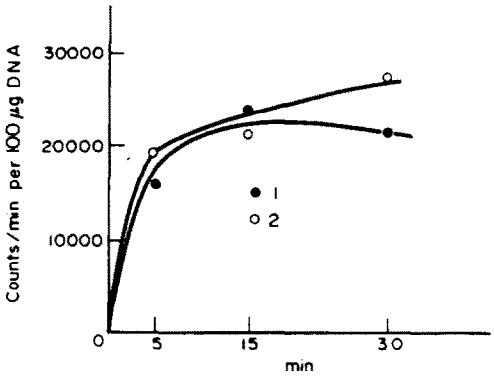


Fig. 5. [$^3$H] cortisol incorporation into nuclei isolated from livers of untreated rats subjected to prolonged cortisol treatment. (1-control, 2-continuous cortisol administration).

## DISCUSSION

The pattern of long-term induction revealed by experiments with cortisol is in line with other studies made in this laboratory. Long-term treatment with insulin made rat liver cells unresponsive to this hormone. Insulin became incapable of inducing the synthesis of RNA, hexokinase and pyruvate kinase[19]. The restriction of the inductive action was observed in rats after long-term galactose treatment. It was seen that the first days of administration induces in rat liver DNA-dependent synthesis of RNA and increase of galactokinase, galactose-1-phosphate UDP-transferase and galactoepimerase activities[20].

What mechanisms underlie the impairment of such induction? The nuclei isolated from rat liver subjected to prolonged cortisol induction *in vivo*, unlike normal ones, cease to display an increased rate of RNA synthesis in response to the hormone when incubated *in vitro* (Table 4). This is not due to a decrease of nucleoid permeability to cortisol (Fig. 5). Presumably prolonged cortisol induction *in vivo* results in changes of the state of the chromatin of liver cells preventing the enhancement of transcription when inductor is administrated.

We have found earlier that the inactivation and elimination of cortisol in rats is enhanced after prolonged administration of hormone[19]. This factor along

with the change of the state of liver nuclei may also contribute to the "cortisol unresponsiveness" of rat liver that develops under continuous cortisol treatment.

## REFERENCES


1. Weber G., Singhal R. I., Stamm N. B. and Srivastava S. K.: *Fed. Proc.* **24** (1965) 745.
2. Kenney F. T.: *Ann. N.Y. Acad. Sci.* **103** (1963) 1083.
3. Williams-Ashman H. G.: *J. cell. Physiol.* **66** Suppl. 1 (1965) 111.
4. Leloir L. F. and Munoz J. M.: *Biochem. J.* **32** (1938) 299.
5. Schimke R. T.: *J. biol. Chem.* **239** (1964) 1783.
6. Salganik R. I., Argutinskaya S. V., Mertvetsov N. P. and Khristoljubova N. B.: *Molek. biologija* **2** (1968) 721.
7. Georgiev G. P. and Mantjeva V. L.: *Biokhimija (Moscow)* **27** (1962) 949.
8. Georgiev G. P. and Mantieva V. L.: *Biochim. biophys. Acta* **61** (1962) 153.
9. Steiner D. and King J.: *Biochim. biophys. Acta* **119** (1966) 510.
10. Lukacs J. and Sekeris C. E.: *Biochim. biophys. Acta* **134** (1967) 85.
11. Burton J.: *Biochem. Jl* **62** (1956) 315.
12. Nakagawa S. and White A.: *Proc. Natn. Acad. Sci. USA* **55** (1966) 900.
13. Goldstein L. and Knox W. E.: *J. biol. Chem.* **237** (1962) 1729.
14. Kenney F. T. and Kull F. J.: *Proc. Natn. Acad. Sci. USA* **50** (1963) 493.
15. Argutinskaya S. V., Mertvetsov N. P., Knorre V. L. *et al.*, 2th Vsesojusn. biokhim. sjezd, Thesis, Thashkent, 1969, Sec. 6, p. 11.
16. Weber G., Singhal R. L. and Srivastava S. K.: In *Advances in Enzyme Regulation*, **3** (1965) 43.
17. Salganik R. I., Morozova T. M., Grjasnova I. M. *et al.*: In *Nucleic Acids*. Moscow 1966, p. 142.
18. Argutinskaya S. V., Salganik R. I. *et al.*, *Molek. biologiya* in press.
19. Mertvetsov N. P.: Dissert. thesis, Novosibirsk 1971.
20. Salganik R., Solovyeva N. and Drevich V.: 7th FEBS meeting, Varna 1971, p. 129.