IN VITRO STIMULATION OF RNA SYNTHESIS IN ISOLATED RAT LIVER NUCLEI BY GLUCOCORTICOIDS

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Received April 23, 1969

Summary. Cortisol, 21-dehydrocortisol and cortisol 21-phosphate were added *in vitro* to isolated intact, and to fragmented liver nuclei obtained from adrenalectomized rats and the DNA-dependent RNA polymerase activities were measured. 21-dehydrocortisol possessed the greatest capacity to stimulate RNA synthesis of fragmented rat liver nuclei. It was effective at a concentration of 10^{-8} M which was comparable to the rate stimulated by cortisol at 10^{-4} M. The results suggest that the stimulation of RNA synthesis in rat liver nuclei by cortisol may be dependent upon its transformation to 21-dehydrocortisol in trace amounts and that this metabolite mediates the action.

The changes induced in the metabolism of cells by steroid hormones are attributed to the biologically active form of the hormones. Many studies attempting to elucidate the action of hormones have been undertaken with this premise as a basic principle. However, there are reports suggesting that a metabolite of the hormone may be the active agent. When hormones are administered *in vivo* to animals or added *in vitro* to tissue cultures, marked stimulation of RNA synthesis was observed (1). In contrast, the stimulatory effects were significantly lower or none when hormones were added *in vitro* e.g., cortisol, to subcellular fractions such as isolated nuclei, fragmented nuclei or chromatin (2-8). The inability or diminished ability of hormones to stimulate RNA synthesis in subcellular fractions when added *in vitro* in contrast to their dramatic effects when administered *in vivo* may be dependent upon an initial transformation of the hormone to an active metabolite, a deficiency of essential cofactor(s), or a requirement of a definite cytostructure.

In this study the ability of cortisol, 21-dehydrocortisol and cortisol

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21-phosphate to stimulate DNA-dependent RNA polymerase activity of isolated nuclei and fragmented nuclei of rat liver was measured to determine whether cortisol or one of the other derivatives is the active agent.

Materials and Methods. Cortisol was purchased from Ikapharm, Israel; sodium cortisol 21-phosphate from Sigma Chemical Co., St. Louis, Mo., Uridine-5 $-^{3}$H-5'-triphosphate, tetrasodium (28.2 Ci/mmole) from New England Nuclear Corp., Boston, Mass. 21-dehydrocortisol was prepared by the oxidation of cortisol in methanolic copper acetate by the method of Lewbart and Mattox (9). After recrystallization from acetone-water solution the final product contained no cortisol when analyzed on silica gel chromatogram sheet with indicator (chromagram 6061 Eastman Kodak Co., Rochester, N.Y.) as described by Ohtsuka and Koide (10). Cortisol was dissolved in ethanol, 21-dehydrocortisol in ethanol; water (1:1, ^{v}/v) and cortisol 21-phosphate in water.

Liver nuclei were isolated from adrenalectomized rats according to the method of Blobel and Potter (11). From 20 g of rat liver (wet wt.) 8 ml of nuclei preparation was obtained which contained an average of 1.6 mg of DNA per ml. DNA was determined by the diphenylamine reaction described by Schneider (12). The nuclei preparation was centrifuged at 600 x g for 30 min. The sediment was resuspended in 0.05 M Tris-HCl buffer, pH 7.4. This suspension was designated as the fragmented nuclei preparation. The procedures for the incubation of nuclei and assay for RNA polymerase activity are modifications of the methods described by Weiss (13) and Sekeris and co-workers (2,6).

Results. Tables 1 and 2 show the stimulatory influence of cortisol, 21-dehydrocortisol and cortisol 21-phosphate added in vitro on the DNA-dependent RNA polymerase activities of intact and fragmented nuclei isolated from rat liver, respectively. Cortisol at a given concentration stimulated polymerase activities of intact rat liver nuclei to the greatest extent (Table 1). On the other hand, 21-dehydrocortisol possessed the great-

Table 1

Effect of Corticosteroids on DNA-dependent RNA Polymerase Activities in Isolated Rat Liver Nuclei

Steroid	Concentration (M)	Activity cpm/mg DNA/min	Per Cent of Control	p
Control	---	154.1 ± 12.8 (10)	100 ± 8.3	---
Cortisol	10^{-4}	230.5 ± 26.2 (6)	149.6 ± 11.4	< 0.001
	10^{-6}	183.7 ± 12.3 (6)	119.2 ± 8.0	< 0.002
	10^{-8}	173.2 ± 11.5 (6)	112.7 ± 8.3	< 0.02
21-dehydro-cortisol	10^{-4}	186.6 ± 11.5 (6)	121.2 ± 7.3	< 0.001
	10^{-6}	172.6 ± 11.2 (6)	112.1 ± 7.3	< 0.01
	10^{-8}	166.4 ± 11.8 (6)	108.3 ± 7.6	< 0.1
Cortisol 21-phosphate	10^{-4}	218.8 ± 17.5 (4)	125.0 ± 7.0	< 0.01
	10^{-6}	174.0 ± 11.3 (4)	113.1 ± 6.9	< 0.05
	10^{-8}	169.1 ± 11.1 (4)	109.9 ± 6.5	< 0.1

Mean values ± SD; number of experiments is given in parenthesis. Probabilities are by t test between control and stimulated means.

est potency in stimulating polymerase activity of fragmented nuclei preparation (Table 2).

Discussion. The results of the present study are in accord with the results of Sekeris and co-workers (2,6). Cortisol added in vitro to isolated rat liver nuclei stimulated the DNA-dependent RNA polymerase activity. The finding, however, that 21-dehydrocortisol possessed the greatest potency in stimulating RNA polymerase activity of the fragmented rat liver nuclei preparation (Table 2) suggests that this derivative may be the active agent mediating the effects of cortisol. Furthermore, the observation that the stimulation of the polymerase activity with 21-dehydrocortisol at 10^{-8}M concentration was equivalent to that with cortisol at 10^{-4}M (Table 2) indicates that a transformation of cortisol to 21-dehydrocortisol in trace amounts can account for the stimulatory effects of cortisol. On the other hand, 21-dehydro-

Table 2

Influence of Corticosteroids on DNA-dependent RNA Polymerase Activities in Fragmented Rat Liver Nuclei

Steroid used	Concentration (M)	Activity cpm/mg DNA/min	Per Cent of Control	p
Control	---	243.2 ± 22.4 (10)	100 ± 9.2	---
Cortisol	10^{-4}	356.3 ± 34.4 (6)	136.2 ± 13.1	< 0.002
	10^{-6}	288.2 ± 22.2 (6)	118.2 ± 9.0	< 0.01
	10^{-8}	261.8 ± 23.4 (6)	107.5 ± 9.9	< 0.1
21-dehydro-cortisol	10^{-4}	509.4 ± 41.7 (6)	208.9 ± 17.8	< 0.001
	10^{-6}	401.5 ± 36.7 (6)	164.7 ± 15.0	< 0.001
	10^{-8}	327.3 ± 31.2 (6)	134.2 ± 12.8	< 0.002
	10^{-10}	278.2 ± 18.1 (4)	114.1 ± 6.5	< 0.05
Cortisol 21-phosphate	10^{-4}	314.7 ± 23.6 (4)	128.8 ± 7.0	< 0.01
	10^{-6}	282.9 ± 20.6 (4)	116.4 ± 7.3	< 0.05
	10^{-8}	259.9 ± 20.0 (4)	106.6 ± 7.6	< 0.1

Mean values ± SD; number of experiments is given in parenthesis.

cortisol was less effective than cortisol in stimulating the polymerase activity of intact rat liver nuclei (Table 1). This finding may be attributed to the presence of a nuclear membrane which may hinder the transport or permeability of 21-dehydrocortisol since this derivative interacts with amino groups to form Schiff bases (14).

The mechanism of the stimulation of RNA polymerase activity of fragmented rat liver nuclei by 21-dehydrocortisol has not been elucidated. Our previous finding that 21-dehydrocortisol interacts with the arginine-rich fraction of calf thymus histones (15) and the report of Sluyser (16) that labeled cortisol administered *in vivo* to rats was localized in the arginine-rich fraction of liver histones suggest that the action of 21-dehydrocortisol may result from its interaction with arginine-rich histones of the ribonucleoprotein complex of rat liver nuclei. Since histones suppress DNA-dependent RNA

polymerase activity (17), the interaction of 21-dehydrocortisol with histones may affect the DNA-template activity resulting in a stimulation of RNA synthesis. In what manner the interaction of 21-dehydrocortisol with histones increases the template activity is not known.

The capacity of cortisol 21-phosphate to stimulate DNA-dependent RNA polymerase system of intact and fragmented rat liver nuclei (Tables 1 and 2) was comparable to or slightly lower than that of cortisol. Its stimulatory effect is probably dependent upon its conversion to cortisol since it is readily hydrolyzed to cortisol by various alkaline and acid phosphatases (18).

Acknowledgement: We wish to express our appreciation to Dr. Sheldon J. Segal for his interest, support and encouragement which greatly facilitated this study. The technical assistance of Mrs. M.T. Elvira and C. Avila is gratefully acknowledged.

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