

EFFECT OF REPEATED INJECTIONS OF HYDROCORTISONE
AND SODIUM RIBONUCLEATE ON MITOCHONDRIAL RNA
CONTENT IN ALBINO RAT ORGANS

Ya. L. Germanyuk and A. G. Minchenko

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Daily injections of hydrocortisone acetate (5 mg/100 g body weight) into albino rats for 14 days lead to an increase in the RNA content of the liver mitochondria and a decrease in the RNA content in the mitochondria of the spleen and skeletal muscles, accompanied by an increased blood glucose concentration. If combined injections of the hormone and yeast RNA (5 mg/100 g body weight) are given, the deviations of these indices from the normal are less marked.

The effect of exogenous glucocorticoid hormones on mitochondrial RNAs has received little study.

The object of the present investigation was to determine the effect of prolonged injections of hydrocortisone alone and combined injections of hydrocortisone and sodium ribonucleate on the mitochondrial RNA content in organs with different physiological functions. Effects of exogenous RNA observed previously were taken into account [1-6, 9].

EXPERIMENTAL METHOD

Experiments were carried out on adult albino rats. The animals of group 1 (control) received injections of 0.9% NaCl solution, those of group 2 received a solution of yeast RNA (Sigma) made up with NaOH to pH 7.3 in a dose of 5 mg RNA/100 g body weight, group 3 received hydrocortisone acetate (Richter) in a dose of 5 mg/100 g body weight, and group 4 received hydrocortisone acetate and RNA in the same doses. All the preparations were injected intraperitoneally in a volume of 0.2 ml daily for 14 days. The animals were decapitated 24 h after the last injection. All subsequent procedures for the isolation of the mitochondrial fraction of the cells were carried out at 0-4°C. Mitochondria were obtained from the liver, spleen, and skeletal muscles by the method of O'Brien and Kalf [10]. Minced tissues of the organs were homogenized in a glass homogenizer with teflon pestle in 0.34 M sucrose solution containing 0.002 mole tris-HCl buffer, pH 7.4. The homogenates were centrifuged for 15 min at 3000 rpm to remove nuclei and cell fragments. The supernatant was centrifuged on a TsVR-1 centrifuge for 10 min at 7000 rpm to sediment the mitochondria. The mitochondria were resuspended in sucrose buffer by means of the homogenizer and centrifuged for 10 min at 5000 rpm. This procedure, with washing of the mitochondria in sucrose buffer, was repeated 5 times. The purity of the mitochondria was verified in the JEM-7 electron microscope: they were free from microsomes and lysosomes. The mitochondria contained no adsorbed cytoplasmic RNAs as impurities, for treatment with ribonuclease had no quantitative effect on the content of mitochondrial RNAs. The RNA content in the mitochondria was determined with a type SF-4A spectrophotometer [7], and the blood glucose concentration with orthotoluidine reagent [8].

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TABLE 1. Effect of Repeated Injections of Hydrocortisone and Sodium Ribonucleate on RNA Content (in $\mu\text{g}/\text{mg}$ protein) in Mitochondria of Organs of Intact Albino Rats ($M \pm m$; $n = 17$)

Organ	Control	Sodium ribonucleate	Hydrocortisone	Hydrocortisone and sodium ribonucleate
Liver	$6,45 \pm 0,26$	$6,54 \pm 0,246$ $P > 0,5$	$11,51 \pm 0,787$ $P < 0,001$	$8,7 \pm 0,24$ $P < 0,001$ $P_1 = 0,002$
Spleen	$23,4 \pm 0,647$	$22,5 \pm 0,885$ $P > 0,5$	$18,5 \pm 0,922$ $P < 0,001$	$20,8 \pm 0,868$ $P = 0,02$ $P_1 = 0,08$
Muscles	$8,3 \pm 0,214$	$8,21 \pm 0,194$ $P > 0,5$	$7,08 \pm 0,252$ $P < 0,001$	$7,94 \pm 0,258$ $P = 0,32$ $P_1 = 0,03$

Note: P relates to comparison of groups 2, 3, and 4 with group 1; P_1 to the comparison of group 4 with group 3.

EXPERIMENTAL RESULTS

Daily injections of hydrocortisone for 2 weeks into albino rats caused an increase in the blood glucose concentration by 34.7% over the normal level (78.1 mg %), an increase in the RNA content in the liver mitochondria by 78.4%, and a decrease in the RNA content in the splenic mitochondria by 21% and in the muscle mitochondria by 14.6% (Table 1). In animals receiving combined injections of hydrocortisone with RNA the difference between these indices and their normal values was less marked. It was 8% for glucose and 34.9, 11.2, and 4.3% respectively for mitochondrial RNAs of the liver, spleen, and muscles.

The results of these experiments show that repeated and prolonged injections of hydrocortisone into animals cause different changes in the content of mitochondrial RNAs in different organs. The RNA content was reduced in the mitochondria of the spleen and muscles, although these organs differ in the levels of their protein-synthesizing and mitotic activities. In this case, the lympholytic and catabolic actions of the glucocorticoid hormone were evidently reflected in these findings. In the liver, an organ with intensive protein synthesis, the content of mitochondrial RNAs was increased. The sharp increase in the RNA/protein ratio together with disturbances in the structure of isolated mitochondria observed in the electron microscope could indicate abnormalities in the ratio between the biopolymers of these organelles. Changes in the mitochondria of the organs studied are significant if it is remembered that, as the authors have shown, an increase in the weight of the liver and a decrease in the weight of the spleen and muscles, especially the quadriceps femoris muscle, took place after repeated and prolonged injections of hydrocortisone.

Exogenous RNA makes an important contribution to the normalization of RNA and, evidently, protein synthesis in the mitochondria of all three organs if the glucocorticoid hormone level in the body is excessively high. The positive effect of sodium ribonucleate has also been described in animals with experimental diabetes mellitus [4, 5], when the action of glucocorticoid hormones is not balanced by insulin because of the impaired function of the β -cells of the pancreatic islets of Langerhans.

LITERATURE CITED

1. Ya. L. Germanyuk, Ukr. Biokhim. Zh., No. 1, 59 (1964).
2. Ya. L. Germanyuk, M. V. Demchuk, and N. I. Stadnik, Vopr. Med. Khimii, No. 6, 34 (1965).
3. Ya. L. Germanyuk, Dopovidi AN Ukrain'sk. RSR, No. 2, 209 (1966).
4. Ya. L. Germanyuk and S. V. Varga, Vopr. Med. Khimii, No. 2, 170 (1969).
5. Ya. L. Germanyuk, V. I. Mironenko, and P. M. Khol'yavko, Probl. Endokrinol., No. 5, 57 (1970).
6. Ya. L. Germanyuk, V. L. Il'ich, and O. L. Dmiterko, in: Problems in Endocrinology and Metabolism [in Russian], No. 2, Kiev (1970), p. 70.
7. R. G. Tsanev and G. G. Markov, Biokhimiya, 25, 151 (1960).
8. A. Hyvärinen and E. Nikila, Clin. Chim. Acta, 7, 140 (1962).
9. E. Kaiser and W. Rindt, Endokrinologie, 49, 69 (1965).
10. T. W. O'Brien and G. F. Kalf, J. Biol. Chem., 242, 2172 (1967).