CAN EXOGENOUS RNA BE USED TO ANALYZE COMPLEX EFFECTS OF BIOLOGICALLY ACTIVE SUBSTANCES?

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UDC 612.766.1-06:612.398.015.38].014.46:615.357.453

KEY WORDS: exogenous RNA; protein synthesis; hydrocortisone; physical endurance; gluconeogensis.

RNA isolated from organs and tissues in which a process due to activation of protein synthesis at the transcription level is taking place, has been shown, if injected into a recipient, to reproduce that process in the corresponding organs and tissues [4, 12]. The exogenous RNA method provides new opportunities for analyzing complex effects of biologically active substances affecting the whole organism.

In the present investigation the exogenous RNA method was used to analyze a complex effect, namely increased physical endurance in response to administration of glucocorticoid hormones and, in particular, of hydrocortisone [5]. The aim of the investigation was to determine the role of activation of protein synthesis in certain organs, primarily in the liver and kidneys, in the effect of hydrocortisone, because the stimulating action of glucocorticoids on protein synthesis is observed chiefly in these organs, and it consists mainly of an increase in the rate of formation of enzymes of gluconeogenesis de novo [1].

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 160-170 g. RNA was isolated from the organs of donor animals by the method with hot phenol [2], yielding a total fraction of nearly all cellular RNAs, which was used in most experiments, at 65°C. The rats were killed and RNA isolated from the liver, renal cortex, skeletal muscles (thigh), heart, and combined brain and spinal cord 1.5 h after intraperitoneal injection of hydrocortisone acetate in a dose of 50 mg/kg, causing activation of glucose synthesis during gluconeogenesis from all the most important precursors [10]. RNA isolated from a particular organ of each donor rat was dissolved in distilled water, and the solutions obtained from all donors were pooled. The resulting RNA preparation from a particular organ was injected, in a dose of RNA, disregarding differences in weight of the organs from which they were obtained, and the content of RNA in the organs, was justified by our existing experimental data showing that preparations of exogenous RNA were about equally effective over a wide dose range (from 0.02 to 0.5 mg/kg, and probably higher). RNA from liver was injected intraperitoneally, RNA from the other organs subcutaneously, so that its passage into the target organ was facilitated, avoiding the liver with its powerful metabolic systems.

The physical endurance of the recipients (running on a treadmill at a speed of 40 m/min) or (in animals of separate groups) activity of gluconeogenesis in the renal cortex [11] was estamated 1 h 20 min after injection of RNA. Lactate, in a concentration of 10 mM, was used as the substrate of gluconeogenesis. Effects of hydrocortisone itself and of RNA from the organs of control rats were studied in parallel experiments. In some experiments, before RNA or hydrocortisone, the animals were given an additional injection of actinomycin D (0.25 mg/kg), a selective inhibitor of RNA synthesis. Recipients of some groups received RNA preparations pretreated for 30 min at 37°C with pancreatic ribonuclease (0.5 mg/mg RNA) or trypsin (1 mg/mg RNA). In some experiments the effect of two nuclear fractions of RNA, obtained by isolation with hot phenol, was investigated: a fraction enriched with precursors of ribosomal RNAs and a fraction enriched with precursors of template RNAs [2]. In addition, the action of polysomal RNAs, isolated by the method in [3], was estimated.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 81-83, July, 1985. Original article submitted May 29, 1984.

0007-4888/85/0007-0935$09.50

TABLE 1. Effect of Hydrocortisone Acetate (50 mg/kg), Actinomycin D (0.25 mg/kg), and Exogeneous RNAs (0.1 mg/kg) from Organs of Animals Receiving Hydrocortisone Acetate on Physical Endurance

Preparations	Number of experiments	Length of running, min
Control (physiological saline)	8	33.5 ± 2.8
Hydrocortisone acetate	8	62.2 ± 5.9†
Actinomycin D	7	30.6 ± 3.5
Actinomycin D + hydrocortisone acetate	8	28.4 ± 3.1
RNA from liver of control donors ($RNA_{hep.c}$)	8	35.4 ± 3.6
RNA from liver of experimental animals receiving hydrocortisone acetate ($RNA_{hep.h}$)	8	52.1 ± 5.5*
RNA from kidneys of control donors ($RNA_{ren.c}$)	8	32.7 ± 1.9*
RNA from kidneys of experimental donors ($RNA_{ren.h}$)	8	47.0 ± 4.9*
$RNA_{hep.c}$ + $RNA_{ren.c}$	8	31.5 ± 4.0
$RNA_{hep.h}$ + $RNA_{ren.h}$	8	58.2 ± 6.6†
RNA from skeletal muscles of control donors	8	36.2 ± 3.8
RNA from skeletal muscles of experimental donors	8	37.8 ± 5.2
RNA from heart of control donors	7	24.1 ± 4.3
RNA from heart of experimental donors	8	32.0 ± 2.0
RNA from brain and spinal cord of control donors	7	30.4 ± 3.9
RNA from brain and spinal cord of experimental donors	7	33.1 ± 4.7

Legend. *$P < 0.05$, †$P < 0.01$, compared with control.

Incidentally, 24 h before the experiments the animals were selected: in all experiments including the biochemical tests only these rats which could run on the treadmill for 15 min without signs of fatigue were used.

EXPERIMENTAL RESULTS

Hydrocortisone increased the animal's physical endurance significantly (Table 1). The universally known action of glucocorticoids *in vivo*, namely activation of gluconeogenesis, also was observed (Fig. 1). Actinomycin D, in a dose of 0.25 mg/kg, which itself did not affect the working capacity or gluconeogenesis, completely abolished both the above effects of hydrocortisone. Consequently, the increase in endurance was due to activation of synthesis of certain proteins, among which the main role, it can be tentatively suggested, is played by enzymes of gluconeogenesis, the process maintaining working capacity as a result of intensification of glucose resynthesis and lactate utilization.

Direct proof of the key role of the liver and kidneys in realization of the effect of hydrocortisone on working capacity was obtained in experiments with exogenous RNAs. RNAs from liver and renal cortex of donor rats receiving hydrocortisone, if injected together, completely reproduced the effect of the hormone on the duration of running by the animals (Table 1). If given separately, they also caused increased endurance, although the increase was smaller. RNA from other organs (skeletal muscles, heart, brain) of the animals of the experimental group and RNA from all organs of the control donors studied, including the liver and kidneys, had no effect whatever.

The RNA preparation from the renal cortex of the experimental rats was found to have a highly specific activating action on gluconeogenesis, reproducing the effect of hydrocortisone (Fig. 1). A similar effect in the recipients' liver ought to be induced by hepatic RNAs, but by the method used, gluconeogenesis activity could not be determined sufficiently accurately in this organ, possibly because of the presence of large reserves of preformed glycogen. Thus the decisive role of activation of gluconeogenesis in the mechanism of the increase in working capacity produced by hydrocortisone seems to be most likely. However, this role can be unequivocally confirmed only in experiments with exogenous RNA, which code synthesis of only gluconeogenic enzymes.

The fact that specific information is transferred from donors to recipients by RNA molecules is confirmed by loss of the biological activity of the RNA preparations after treatment with ribonuclease and their resistance to trypsin (Fig. 2). Since the effects of exogenous RNAs studied (effect on working capacity and on gluconeogenesis) were abolished by actinomycin

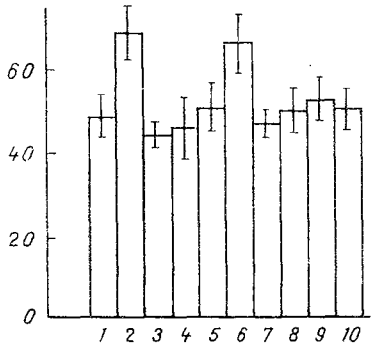

Fig. 1

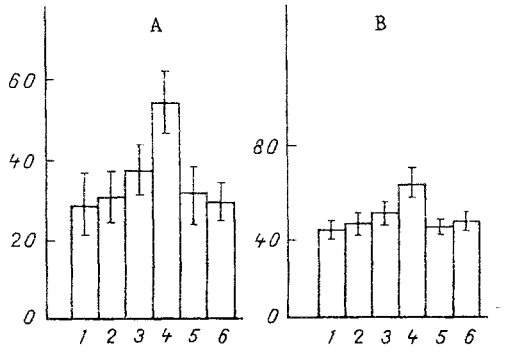

Fig. 2

Fig. 1. Effect of hydrocortisone acetate (50 mg/kg), actinomycin D (0.25 mg/kg), and exogenous RNAs (0.1 mg/kg) from organs of donor animals receiving hydrocortisone acetate on activity of gluconeogenesis in renal cortex of rats. Ordinate, activity of gluconeogenesis (in micromoles glucose/g wet weight of tissue/h); substrate, lactate (10 mM); 1) control; 2) hydrocortisone acetate; 3) actinomycin D; 4) actinomycin D + hydrocortisone acetate; 5, 7, 9) RNA from organs of control donors; 6, 8, 10) RNA from organs of donors receiving hydrocortisone acetate; 5, 6) RNA from renal cortex; 7, 8) RNA from skeletal muscles; 9, 10) RNA from brain and spinal cord (together).

Fig. 2. Effects of exogenous RNAs (0.1 mg/kg) from kidneys of donor rats receiving hydrocortisone acetate (50 mg/kg) after treatment of RNA with ribonuclease (0.5 mg/mg RNA) or trypsin (1 mg/mg RNA) or after preliminary injection of actinomycin D (0.25 mg/kg) into recipients. Ordinate: A) duration of running (in min); B) activity of gluconeogenesis (in micromoles glucose/g wet weight of tissue/h). 1, 3, 5) RNA from renal cortex of control donors; 2, 4, 6) RNA from renal cortex of donors receiving hydrocortisone acetate; 1, 2) RNAs treated with ribonuclease; 3, 4) RNAs treated with trypsin; 5, 6) actinomycin D + RNA.

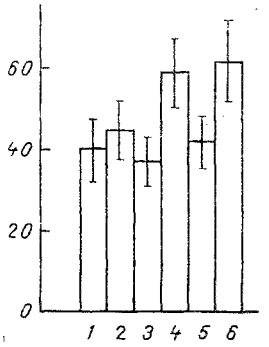

Fig. 3. Effect of individual fractions of exogenous RNAs (0.1 mg/kg) from liver of donor rats receiving hydrocortisone acetate (50 mg/kg) on physical endurance of recipients. Ordinate, duration of running (in min). 1, 3, 5) RNAs from liver of control donors; 2, 4, 6) RNAs from liver of donors receiving hydrocortisone acetate; 1, 2) fraction enriched with template RNAs; 5, 6) fraction of polysomal RNAs.

D, the mechanism of their action must evidently be based on activation of synthesis, in the recipient's target organs, of their own RNAs, functionally identical to those being intensively synthesized in the donor. Other workers also have mentioned a similar mechanism of action, possibly due to selective derepression of genes [6].

According to the results of a series of investigations, specific effects of preparations of exogenous RNAs, connected with selective activation of protein synthesis, are produced by the action of the fraction of template RNAs [8, 9]. Our own data are evidence in support of this conclusion. For instance, the increase in working capacity of the recipients after receiving an injection of the total RNA preparation from the liver of donors receiving hydrocortisone, was reproduced by the fraction of precursors of template RNAs, but not of ribosomal RNAs (Fig. 3). Possibly the precursors are converted in the recipient's body into ready-made template RNAs, which exert a specific action. Such a possibility is confirmed by reproduction of the effect of the total RNA preparation by the polysomal RNA fraction, which contains ready-made templates.

We used the exogenous RNA method to analyze effects not only of glucocorticoids, but also of other substrances [7]. This method appears to be very valuable, for it enables components of complex effects of biologically active substances, due to activation of protein synthesis in particular organs, to be studied separately.

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MORPHOLOGY AND FUNCTION OF THE THYMUS IN GUINEA PIGS RECEIVING THYMUS AND BONE MARROW POLYPEPTIDES

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UDC 612.438.014.46:/615.
362.438+615.361.419

KEY WORDS: thymus; thymalin; hemalin.

In the modern view substances participating in regulation of the immunocompetent system are produced in the thymus and bone marrow [2, 3, 5]. In a previous investigation on mice the writers showed that the polypeptide preparation from the thymus (thymalin) stimulated thymocyte function [4].

The aim of the present investigation was to compare the effects of polypeptide preparations of thymus and bone marrow on morphology and function of the thymus gland in guinea pigs.

EXPERIMENTAL METHOD

Polypeptide preparations were isolated by acetic acid extraction followed by ion-exchange chromatography: thymalin from calf thymus, hemalin from bone marrow [1, 2].

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(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 83-86, July, 1985. Original article submitted September 14, 1985.

0007-4888/85/0007-0938$09.50