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CHANGES IN RNA-POLYMERASE ACTIVITY IN HEART AND LIVER CELLS IN IMMOBILIZATION STRESS

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In emotional-painful stress the rate of synthesis of total RNA and of proteins is drastically changed. Soon after the end of exposure to stress the rate of RNA and protein synthesis falls, but later it is restored and exceeds the control values [2]. To study the character of function of the protein-synthesizing system of cells under the influence of stress factors it is important to know how the rate of synthesis of different classes of RNA and, in particular, the rate of synthesis of messenger RNA (mRNA) and ribosomal RNA (rRNA) changes.

In the investigation described below activity of RNA-polymerase I, an enzyme transcribing ribosomal genes, and of RNA-polymerase II, an enzyme responsible for mRNA synthesis, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. The animals were fixed by the limbs in the supine position (immobilization stress) for 6 h. The stress syndrome which develops as a result of this procedure is accompanied by the development of gastric ulcers. The animals were investigated immediately after the end of exposure to stress, and again 12, 24, and 48 h later.

Activity of the RNA-polymerases was studied in isolated nuclei of the heart, liver, and spleen in a cell-free system. The nuclei were isolated by the method in [8] with certain modifications [3]. Activity of RNA-polymerases I and II was determined under optimal conditions for the action of each enzyme, and was tested with α-amantine. RNA-polymerase I is known

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to be insensitive to α-amantine, activity of RNA-polymerase II is inhibited by small doses of it, and activity of RNA-polymerase III, which transcribes genes of transfer RNA (tRNA) and 5S RNA, is inhibited by high doses of α-amanitine. The incubation medium (0.5 ml) for determination of activity of the RNA-polymerases contained, in final concentration, the following components (in μM): for RNA-polymerase I (system 1) Tris-HCl (pH 7.9) 50.0, KCl 25, $MgCl_2$ 6, $(NH_4)_2SO_4$ 50, GTP and CTP 0.6, ATP 2, UTP 0.06, creatine phosphate 5, creatine kinase, and 2 μCi of ^{3}H-UTP. To determine RNA-polymerase II activity the same incubation mixture was used, but instead of $MgCl_2$, 2 μM of $MnCl_2$ was added, and the concentration of ammonium sulfate was 160 μM (system 2). The reaction was started by addition of the nuclear suspension (50 μg DNA). To inhibit RNA-polymerase II activity, α-amanitine (1 μg/ml) was added to system 1, and to inhibit RNA synthesis completely, actinomycin D (50 μg/ml) was added. The samples were incubated at 36°C. After incubation, a cold solution of 10% TCA, containing 0.04 M sodium pyrophosphate, was added to the samples. The residues were washed on Millipore filters of Aufs type with 5% TCA solution containing 0.02 M sodium pyrophosphate, alcohol, and ether [3]. Radioactivity of the specimens was measured in toluene solvent containing POPOP and PPO, on a Mark II counter (Nuclear Chicago, USA).

Changes in activity of the RNA-polymerases were judged from incorporation of radioactive UMP (^{3}H-UMP) into the acid-insoluble fraction of the nuclei. It was shown previously that incorporation of ^{3}H-UMP into nuclei depends on the presence of DNA and of added triphosphates, and is inhibited by 95% by actinomycin D [3]. All these observations prove that incorporation of ^{3}H-UMP into nuclei reflects RNA synthesis. Incorporation of ^{3}H-UMP in system 1 reflects synthesis of rRNA, since activity of RNA-polymerase II in this case was inhibited by small doses of α-amanitine. In system 2, α-amanitine inhibited RNA-polymerase activity by 80%, i.e., mainly DNA-like RNA was synthesized in that system. As regards activity of RNA-polymerase III, it could be disregarded, for it was low, only 10% of the total RNA-polymerase activity [5]. Since mainly rRNA was synthesized in system 1 and mainly DNA-like RNA in system 2, when these systems are described henceforward the term activity of RNA-polymerases I and II will be used.

EXPERIMENTAL RESULTS

Activity of RNA-polymerases at different times after the end of exposure to stress was changed unequally (Fig. 1A, B). Immediately after exposure to the stress factor, activity of RNA-polymerases I and II in nuclei of heart and liver cells was reduced. The greatest decrease was observed in activity of RNA-polymerase I – by 25%, whereas activity of RNA-polymerase II was reduced by only 10-12%. Activity of RNA-polymerases I and II 12 h after the end of exposure to stress was completely restored, and activity of RNA-polymerase I was a little higher than the control level. Later activity of RNA-polymerase II lay within the control limits, whereas activity of RNA-polymerase I, in both liver and heart cells, continued to rise. For instance, 24 h after exposure to stress activity of RNA-polymerase I in the liver nuclei was on average 39% higher than in the control, and 35% higher in heart nuclei. Activity of RNA-polymerase I in heart and liver nuclei 48 h after removal of the stress factor began to fall, although the values of this parameter still exceeded those in the control (by 18-20%). The period of poststress increase in RNA-polymerase I activity in both heart and liver nuclei thus lasted much longer than the period of its inhibition. Under these circumstances the increase in this activity reached a peak 24 h after the end of exposure to stress.

Changes in RNA-polymerase activity also were found in nuclei of the spleen (Fig. 1C). Inhibition of RNA-polymerase activity in the spleen was less marked, whereas the poststress increase in activity of RNA-polymerase I 24 h after exposure to stress was quite distinct in character and amounted on average to 20%. It will be recalled that the great majority of spleen cells undergo rapid and continuous migration, which makes it difficult to determine the general effect of stress on the metabolism of the spleen.

The response of the organism to stress thus includes sharp changes in the rate of rRNA synthesis, and less marked changes in the rate of mRNA synthesis. The initial effect of the stress factor in both liver and heart cells is slowing of the rate of synthesis of rRNA and mRNA. In the period of poststress intensification of synthesis of high-molecular-weight compounds activation of rRNA synthesis takes place, whereas the rate of mRNA synthesis is unchanged.

When the possible mechanisms of inhibition of RNA-polymerase activity by stress are evaluated, it should be noted that stress causes breaks in the polynucleotide structure of DNA in the heart [1]. The nuclear enzyme poly(adenine diphosphate ribose)polymerase [poly(ADPR)polymerase] takes part in DNA repair, and its activity rises sharply in the presence of DNA injur-

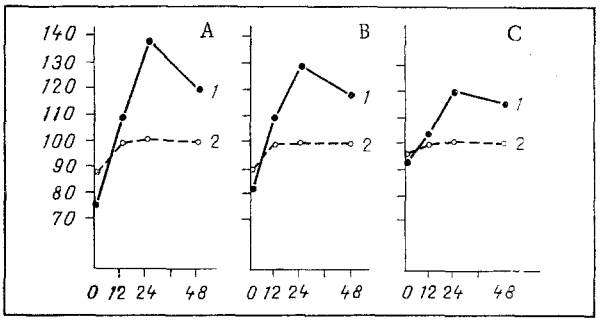

Fig. 1. Changes in RNA-polymerase activity under the influence of immobilization stress. Abscissa, time after exposure to stress (in h); ordinate, activity (in % of control). A) Liver, B) heart, C) spleen. 1) RNA-polymerase I; 2) RNA-polymerase II.

ies [4, 6]. Factors lowering poly(ADPR)-polymerase activity in the cardiocytes increase activity of RNA-polymerases [7]. It can be postulated that DNA repair under conditions of competition for common sources of energy and common precursors will be accompanied by slowing of transcription.

The poststress increase in activity of RNA-polymerase I is a phenomenon that is evidently due to hormones and, in particular, to corticosteroids, production of which rises sharply during stress [9]. Cortisone and hydrocortisone increase RNA-polymerase I activity in liver cells [10, 11]. It has been stated that cortisone modifies this enzyme and converts it into a catalytically more active form [10]. The poststress increase in RNA-polymerase I activity is not accompanied by changes in activity of RNA-polymerase II, which transcribes structural genes. A similar situation has been described during exposure to various physiological factors, including administration of cortisone to animals [11, 12]. Meanwhile we know that glucocorticoids regulate transcription of individual genes. Possibly during stress, hypersecretion of glucocorticoids also leads to changes in expression of various genes and, correspondingly, to a change in the rate of synthesis of individual mRNA. Changes in the rate of mRNA synthesis under these circumstances could fail to be found. It can also be assumed that the concentration of mRNA molecules is regulated after transcription — at the level of their degradation rate. These are matters for special investigation.

The main result of this investigation as a whole is that stress induces different changes in activity of RNA-polymerases I and II. At the beginning of the poststress reaction activity of both enzymes is depressed; activity of RNA-polymerase I is reduced more than that of RNA-polymerase II. Later, activity of RNA-polymerase I rises significantly above the control level, whereas activity of RNA-polymerase II is restored only to the original values.

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