ACTION OF DEXAMETHASONE ON RNA SYNTHESIS IN BLOOD LYMPHOCYTES STIMULATED BY PHYTOHEMAGGLUTININ

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Phytohemagglutinin (PHA) stimulates RNA synthesis 6 h after the beginning of incubation with blood lymphocytes *in vitro*. Dexamethasone, in a concentration of 60 µg/ml, inhibits RNA synthesis in PHA-treated lymphocytes from donors' blood 6 h after the beginning of incubation of the cells with the hormone and PHA *in vitro*. Changes in the mechanism of action of the hormone which may lead to changes in the sensitivity of the same cells, at different stages of the cell cycle, to glucocorticoids are analyzed.

KEY WORDS: lymphocytes; RNA synthesis; phytohemagglutinin; dexamethasone.

Most of the model cell systems used to study mechanisms of resistance of cells to glucocorticoids have been obtained by cultivating lymphocytes in medium with increasing concentrations of hormones or their derivatives [8]. Besides the advantages of such models (the simplicity of obtaining resistant strains of cells, the possibility of working with a large quantity of cell material) they also have certain disadvantages. It is not always possible to obtain a resistant strain of cells and the investigator is forced to work with a cell population in which all the changes have taken place [8]. Such a system is static and what are in fact analyzed in it are phenotypical changes. On the basis of data in the literature it can be postulated that mechanisms controlling the development of resistance to various regulatory or harmful factors are encoded in the cell genome. In the course of one cell cycle sensitivity to the same regulatory factors may evidently vary, or competence toward new regulators develops [8].

The study of control mechanisms in the processes of the cell cycle is of undoubted interest.

Changes in the rate of RNA synthesis in the early stages of the action of dexamethasone in a culture of blood lymphocytes after treatment with phytohemagglutinin (PHA) were studied.

EXPERIMENTAL METHOD

Small lymphocytes from donors' blood were separated from the buffy coat by sedimentation on a nylon column by means of the writers' modification of the method of Shapot and Gorozhanskaya [3]. At the beginning of incubation PHA and dexamethasone were added to a final concentration of 2 µg/ml and 60 µg/ml., respectively. Nuclei were isolated from the lymphocytes in a medium of 0.32 M sucrose with 0.01 M $MgCl_2$ and Triton X-100 in a final concentration of 0.5%, after which the cells were disintegrated in a Potter-Elvehjem homogenizer. The purity of the nuclei was verified in the phase-contrast microscope. DNA was determined by Burton's [1] and activity of the DNA-dependent RNA polymerase (ED 2.7.7.-) by measuring the incorporation of labeled precursor into RNA. Partial purification of the DNA-dependent RNA polymerase of the nuclear extracts, determination of the protein concentration, and isolation of the cytoplasmic and nuclear RNA were carried out as described earlier [2, 4, 9]. The RNA preparations were then passed through a column with Biogel P-2. The nuclear RNA samples were fractionated in a linear sucrose density gradient, and

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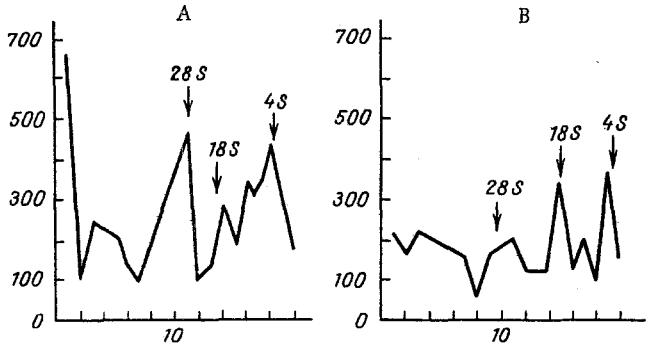

Fig. 1

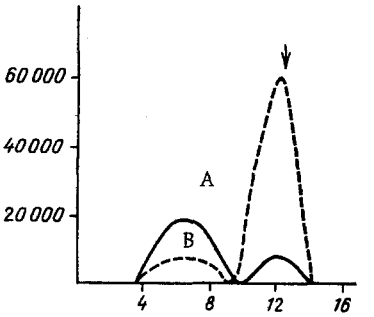

Fig. 2

Fig. 1. Sedimentation profiles of nuclear RNAs of human blood lymphocytes stimulated by PHA. Centrifugation in sucrose density gradient (5-20%) on Spinco L-50 ultracentrifuge in SW-39 rotor at 37,000 rpm for 200 min. PHA (2 μg/ml) and dexamethasone (60 μg/ml) were added simultaneously with the beginning of incubation; uridine-^{3}H (100 μCi/ml) was added after incubation for 45 min. Ordinate, specific radioactivity of RNA (in counts/min); abscissa, No. of fractions. A) Incubation of lymphocytes for 6 h; B) incubation of lymphocytes with dexamethasone for 6 h.

Fig. 2. Sedimentation profiles of DNA-dependent RNA polymerase of nuclear extracts of human blood lymphocytes stimulated by PHA. Centrifugation in sucrose density gradient (5-20%) on Spinco L2-65B ultracentrifuge in SW-65 rotor at 60,000 rpm for 24 h. Dexamethasone (60 μg/ml) and PHA (2 μg/ml) added simultaneously with the beginning of incubation. Ordinate, specific radioactivity of DNA (in counts/min/mg); abscissa, No. of fractions. A) Incubation of lymphocytes for 6 h; B) incubation of lymphocytes for 6 h with dexamethasone. Arrow indicates fraction sensitive to L-amanitine.

TABLE 1. Action of Dexamethasone on RNA Synthesis in PHA-Treated Lymphocytes

	Specific radioactivity of RNA, counts/min/mg			
	nucleus		cytoplasm	
	uridine-^{3}H	uridine-^{14}C	uridine-^{3}H	uridine-^{14}C
Control	7 100 000	1 300 000	1 030 000	320 000
Experiment	993 000	1 433 000	710 000	660 000

TABLE 2. Action of Dexamethasone on Adsorption of PolyA-Containing RNA in PHA-Treated Lymphocytes on Sepharose 4B Columns

	Specific radioactivity of RNA, counts/min/mg			
	nucleus		cytoplasm	
	uridine-^{3}H	uridine-^{14}C	uridine-^{3}H	uridine-^{14}C
Control	63 700	24 000	22 800	16 300
Experiment	28 800	16 800	14 400	13 100

TABLE 3. Effect of PHA Stimulation on Binding of Dexamethasone by Lymphocytes

	Association constant ($K_{as} \cdot 10^9$ M^{-1})	Number of binding sites, 10^{-13} moles/mg protein
Control	2,665	0,9
Experiment	4,74	2,835

TABLE 4. Action of Dexamethasone on DNA-Dependent RNA-Polymerase Activity of Nuclei of PHA-Treated Lymphocytes

	Specific radioactivity of RNA, count/min/mg	
	RNA polymerase A	RNA polymerase B
Control	92 000	90 000
Experiment	89 000	140 000

RNA containing polyA sequences were adsorbed on a polyU—Sepharose 4B column [10]. Uridine-^{3}H, added 45 min before the end of incubation, and uridine-^{14}C, added at the beginning of incubation, were used as the radioactive precursors of RNA. The radioactivity of the labeled samples was measured with the Nuclear Chicago Mark II scintillation counter in toluene scintillator (PPO 4 g/liter, POPOP 0.2 g/liter, Triton X-100 330 mg/liter). The receptor apparatus of the lymphocytes for dexamethasone was analyzed by the method described in [5].

EXPERIMENTAL RESULTS

As Table 1 shows, after 6 h the synthesis of short-living total RNAs in lymphocytes treated with dexamethasone was inhibited. The specific radioactivity of the long-living ^{14}C-RNA was increased in the cytoplasm but not significantly changed in the nucleus.

The results of analysis of cytoplasmic RNAs on columns with Sepharose 4B and polyU fixed on it are given in Table 2. Slowing of the synthesis of RNAs enriched with polyA sequences in the lymphocytes treated with the hormone was evidently observed. Under the influence of dexamethasone incorporation of ^{3}H-uridine was reduced into all fractions of nuclear RNA obtained by ultracentrifugation in a sucrose density gradient (Fig. 1).

The results of analysis of RNA described above thus show that dexamethasone, in the early stages of blast transformation, inhibits chiefly the synthesis of short-living RNAs.

Analysis of the receptor apparatus of the PHA-stimulated lymphocytes showed that in such cells only the number of binding sites of the hormone was increased, whereas the association constant was unchanged (Table 3).

The effect of dexamethasone on activity of DNA-dependent RNA polymerase was investigated at the same time in the nuclei and nuclear extracts of the lymphocytes (Table 4, Fig. 2). The results of this analysis contradicted to some extent the findings described above. In this case, in fact, an increase in the activity of α-amanitine-sensitive DNA-dependent RNA polymerase, catalyzing the synthesis of nonribosomal RNA, was observed under the influence of the hormone. The specific radioactivity of the short-living RNAs was, however, reduced in analogous samples. An alternative explanation could be that during isolation of the nuclei and purification of the RNA polymerase, certain factors inhibiting the activity of transcription enzymes were lost. It could also be postulated that the changes observed in RNA polymerase activity only partially reflected complex processes determining changes in RNA synthesis not capable of being analyzed by the methods used.

The development of the action of a hormone takes place in several successive stages; it was therefore necessary to discover whether resistance arises at any particular stage or whether key areas exist and it is only in them that the reactions responsible for resistance to the particular hormonal preparation develop. From this point of view it was important to discover how sensitivity to the test hormone changes in the course of one cell cycle of the differentiated cells. The results show that sensitivity to the hormone changes sharply in the early stages of blast formation. These changes can be studied at different stages of RNA synthesis, which they inhibit appreciably. Since cells sensitive to dexamethasone were not selected, it can only be suggested that the changes arising under the influence of the transforming agent and preparing the cells for mitosis significantly affect the threshold of sensitivity of the lymphocytes to dexamethasone.

The results of these experiments still do not answer the question whether these changes in sensitivity are characteristic of all cells prepared for mitosis or for lymphocytes only. Another unexplained fact is that an increase of only twice in the number of binding sites of the hormone can lead to changes of sensitivity to the steroid in the opposite direction. Similar results have been obtained by a group of Japanese workers who showed that strains of lymphoma sensitive and resistant to glucocorticoids differ only in the number of binding sites of the hormones [6]. There were only half as many such sites in the resistant strain of lymphoma. Meanwhile these workers, and also Rosenau et al. [7], have shown that the number of binding sites of the hormone in the nucleus is the same for both sensitive and resistant cells.

Two types of action of glucocorticoids capable of determining sensitivity to them can now be distinguished: cytoplasmic reception and intranuclear interaction of the hormone—cytoplasmic receptor complex with chromatin [5]. However, the problem of whether these stages are the only key areas in the action of the hormone requires further experimental investigation.

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MOLECULAR ORGANIZATION OF THE VAGUS NERVES AND CARDIAC MUSCLE IN VARIOUS FUNCTIONAL STATES

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The mitogenetic radiation of the rabbit vagus nerve was investigated during electrical stimulation of the opposite trunk. The radiation characterizes the structural states of the neuroplasm of the nerve and sarcoplasm of the heart. It was shown that during subthreshold stimulation the number of unbalanced molecular constellations rises. The physiological importance of this phenomenon is discussed.

KEY WORDS: mitogenetic radiation; unbalanced molecular constellations.

Spectral analysis of the mitogenetic radiation of the rabbit heart suggests that in response to weak stimulation of the vagus nerve the number of unbalanced molecular constellations (UMCs) in the sarcoplasm of the heart increases, but to threshold stimulation their number decreases [1]. Structural changes of this sort are interesting. Their further study was based on determination of degradation radiation. However, cooling of the heart (as a degrading factor) is impracticable because of the animal's reaction. One vagus nerve trunk was therefore cooled while the opposite trunk was stimulated, the argument being that chain processes arising in the heart muscle during stimulation of the nerve would in turn induce molecular structural changes in the second, unstimulated nerve [2]. This in no way ruled out, of course, the possibility of conduction of the processes through the brain centers.

EXPERIMENTAL METHOD

Short segments of the vagus nerves were exposed in the neck of an unanesthetized rabbit. Electrodes connected to a stimulator, generating pulses 1 msec in duration, with a frequency of 40 Hz, and of varied voltage, were fixed to one nerve. For the period of exposure a biological radiation detector was placed a few millimeters away from the unstimulated nerve, which was flooded with warm (37-38°C) or cold (5-6°C) physiological saline. Stimuli of threshold strength, slowing the heart beat (palpation) through the chest wall and of subthreshold strength were applied. The amplitude of the threshold stimulation was sometimes reduced during the experiments and in these cases the subthreshold stimuli were reduced correspondingly.

EXPERIMENTAL RESULTS

As Fig. 1 shows, in the resting state the nerve produced weak radiation, which remained at the same level both at the normal temperature and during cooling. During subthreshold

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