EFFECT OF ENERGY INHIBITORS ON THE SYNTHESIS OF MITOCHONDRIAL RNA

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2,4-Dinitrophenol (DNF) (5×10^{-4} M), oligomycin (10 μg/ml), rotenone (10^{-5} M), cyanide (10^{-3} M), and azide (2×10^{-3} M), inhibitors of energy metabolism, completely suppress the synthesis of mitochondrial RNA in whole cells of an ascites tumor of the rat ovary. In a system in vitro oligomycin and rotenone had no effect on RNA synthesis in the mitochondria, whereas DNP, cyanide, and azide inhibited it only 15 min after their addition to the incubation medium.

KEY WORDS: mitochondrial RNA and its synthesis; inhibitors of energy metabolism; ascites tumor of the rat ovary.

Processes of RNA metabolism in animal cells have been shown to depend on the integrity of the mechanisms of energy metabolism [10]. The mitochondria are organelles whose chief function is to supply the cell with energy. However, the mitochondria contain DNA and can synthesize RNA and protein independently [4, 9]. The processes of biosynthesis of DNA, RNA, and protein in the mitochondria are localized on the mitochondrial membrane [2], i.e., in the immediate vicinity of the enzyme systems of the respiratory chain.

In the investigation described below the effect of inhibitors of energy metabolism, completely suppressing the synthesis of total cell RNA, on RNA synthesis in the mitochondria was studied.

EXPERIMENTAL METHOD

Experiments were carried out on cells of an ascites tumor of the rat ovary (ATO) and on rat liver. The ATO cells were isolated on the 8th-10th day after inoculation and were incubated as described earlier [10]. Mitochondria of the ATO cells were isolated by Dounce's method [5], and from rat liver by the method of Schneider and Hogeboom [14] with certain modifications: 1 mM EDTA was added to the solution for isolating the mitochondria. RNA synthesis in the cells was determined from the incorporation of uridine-C^{14} in a dose of 1 μCi/ml (specific activity 40 μCi/mmole) and RNA synthesis in the mitochondria was determined in a system in vitro [11] from the incorporation of uridine-C^{14} (1 μCi/ml) or UTP-5-H^3 in a dose of 1 μCi/ml (specific activity 1 Ci/mmole) into the acid-insoluble fraction. The mitochondria were incubated for 1 h with constant stirring at 30°C. The reaction was stopped by the addition of cold 5% TCA and the mixture applied to AUFS membrane filters. The filters were washed with 5% TCA and 70% ethanol. Radioactivity was measured in a scintillation counter (Intertechnique SL-30, Franch). The media for isolation and incubation of the mitochondria, the apparatus, and the instruments were all sterile. Protein was determined by Lowry's method [8].

EXPERIMENTAL RESULTS AND DISCUSSION

The effect of inhibitors of energy metabolism on the synthesis of total and mitochondrial RNA in rat ATO cells was studied. The results given in Table 1 show that oligomycin, rotenone, azide, and 2,4-

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TABLE 1. Effect of Energy Inhibitors on Synthesis of Total and Mitochondrial RNA of Rat ATO (mean results of 10 experiments)

Inhibitors	Concentration	% of control	
		cells	mitochondria
Control	—	100	100
Oligomycin	10 μg/ml	10	8
Rotenone	10^{-5} M	12	6
Na azide	$2 \cdot 10^{-3}$ M	10	6
2,4-DNP	$5 \cdot 10^{-4}$ M	10	10

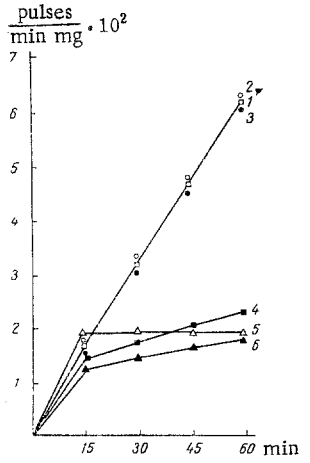

Fig. 1. Effect of inhibitors of energy metabolism on RNA synthesis by rat liver mitochondria in a system in vitro: 1) control; 2) oligomycin; 3) rotenone; 4) cyanide; 5) DNP; 6) azide. Mean results of 10 experiments shown. Abscissa, time (in min); ordinate, specific radioactivity (pulses/min/mg protein $\times 10^2$).

Dinitrophenol (DNP), in the concentrations used, when acting on the whole cell completely suppressed the synthesis of both total and mitochondrial RNA.

The action of the above inhibitors was then studied on RNA synthesis in mitochondria of the rat liver and ATO cells in a system in vitro. Identical results were obtained with the two objects, and for that reason the data obtained for rat liver mitochondria are given.

Mitochondria isolated from rat liver incorporated uridine-C^{14} and UTP-5-H^3 at a constant rate for 1 h. The degree of incorporation was almost identical when different sources of energy were used: succinate, glutamic acid, or phosphoenolpyruvate + pyruvate kinase. Preincubation for 30 min with 0.1 M K-phosphate buffer at 30°C, with the object of increasing the permeability of the mitochondria [13], likewise did not affect the degree of incorporation.

Incorporation of the labeled precursor took place into mitochondrial RNA. On the addition of an excess of nonradioactive UTP to the reaction mixture no incorporation took place. Incorporation of the label into isolated mitochondria was not inhibited by the addition of ribonuclease, in agreement with data in the literature [7], evidence that incorporation took place in a zone restricted to the mitochondrial membrane.

It will be clear from Fig. 1 that oligomycin (10 μg/ml) and rotenone (10^{-5} M) had virtually no effect on RNA synthesis in the mitochondria in vitro. Also, during the first 15 min after addition of 2,4-DNP (5×10^{-4} M), azide (2×10^{-3} M), and cyanide (10^{-3} M), the incorporation of the labeled precursor did not depend on the presence of inhibitors in the medium, and during further incubation RNA synthesis was quickly suppressed.

The action of energy inhibitors on the synthesis of mitochondrial RNA thus depends on whether they act on mitochondria in whole cells or on isolated mitochondria. The first conclusion that can be drawn from these results is that integrity of the energy producing system of the cell is not necessary for the normal course of biosynthesis, and it also follows that a certain regulatory mechanism may exist and is disturbed by isolation of the mitochondria.

It is difficult to explain the inhibitory effect of DNP, for in addition to its hydrolytic effect on the intermediate unphosphorylated high-energy compound, this inhibitor also stimulates mitochondrial ATPase. The absence of an inhibitory effect of oligomycin may point to the possible utilization of intermediate high-energy compounds. Rotenone, cyanide, and azide are inhibitors of electron transport along the respiratory chain, but with different points of application [15]. Rotenone blocks electron transfer to coenzyme Q. Cyanide and azide block the terminal site of the respiratory chain (cytochrome oxidase). By blocking electron transport, these inhibitors at the same time inhibit oxidative phosphorylation; i.e., inhibition of RNA synthesis might be expected as a result of ATP deficiency, although when the synthesis of total RNA in the whole cell is suppressed the ATP level is known to remain unchanged [6]. The results show that rotenone had no effect on the synthesis of mitochondrial RNA whereas azide and cyanide inhibited incorporation only 15 min after their addition to the incubation medium.

Presumably a certain reserve of ATP exists to maintain the incorporation of the labeled precursor into RNA during this period of delay of inhibition, or else oxidative phosphorylation continues throughout this period. It has recently been shown that there is a small rapidly metabolized reserve of ATP in the cell membrane of a culture of fibroblasts [12]. Moreover, the preparations of mitochondria obtained in

these experiments were always contaminated by the microsomal fraction, and the possibility of a discharge of electrons from the terminal site of the respiratory chain of the endoplasmic reticulum to cytochrome oxidase has been shown to exist for such preparations; i.e., respiration of the mitochondria and oxidative phosphorylation are totally suppressed by cytochrome oxidase inhibitors, such as azide and cyanide, as occurred in the experiments described above.

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