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EFFECT OF DISSEMINATED NECROSIS OF THE HEART ON UPTAKE OF RADIOACTIVE PRECURSORS INTO RNA OF INTERNAL MITOCHONDRIAL MEMBRANES OF THE RAT MYOCARDIUM

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The role of the internal mitochondrial membranes of the rat myocardium as the site of uptake of radioactive precursors into mitochondrial RNA was studied. The kinetics of the changes in specific RNA activity of the internal mitochondrial membranes of the myocardium was found to differ in vivo and in vitro. Necrosis of the myocardium, in experiments in vivo and in vitro, caused significant changes in the specific RNA activity of the mitochondrial membranes in the same direction.

KEY WORDS: disseminated necrosis of the heart; RNA synthesis; internal mitochondrial membranes; isoproterenol.

The internal membranes of the mitochondria are the site of incorporation of radioactive precursors of nucleic acids and proteins [6, 9]. Necrosis of the heart has been shown to have a considerable effect on protein synthesis in the internal mitochondrial membranes of the rat myocardium [3, 4]. It is not known, however, whether this effect extends only to the activity of the protein molecule already synthesized or whether the resulting ischemia is capable of producing changes in metabolism at a lower level of integration of the organelle, in the reaction sequence DNA—RNA—mitochondrial protein.

To investigate this problem RNA synthesis was studied in the internal mitochondrial membranes of the myocardium in intact rats and in rats with disseminated necrosis of the heart.

EXPERIMENTAL METHOD

Male Wistar rats weighing 250-280 g were used. Myocardial necrosis was induced by subcutaneous injection of the sympathomimetic agent isoproterenol sulfate [1, 3, 4]. The appearance of necrotic foci in the myocardium was monitored by periodic recording of the ECG. Investigations were carried out both in vitro and in vivo.

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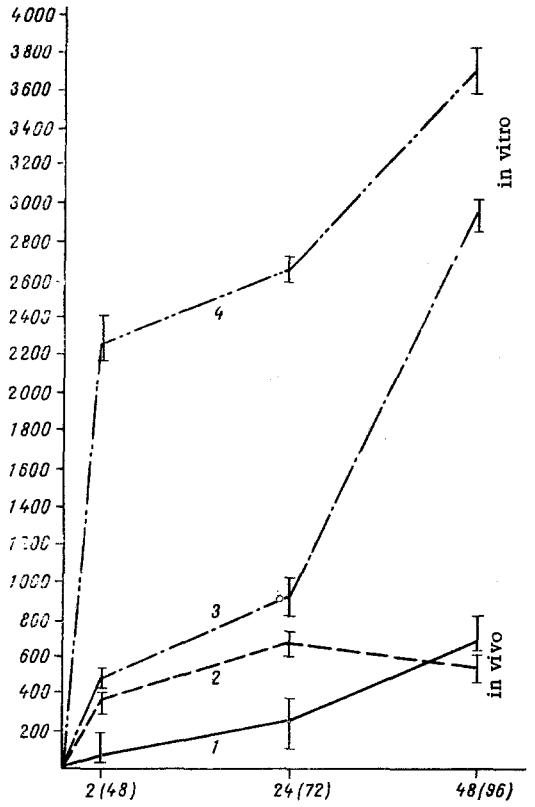

Fig. 1. Changes in specific activity (in cpm/mg protein) of RNA of internal mitochondrial membranes of myocardium of rats with disseminated necrosis of the heart in vivo and in vitro. Horizontal axis: numbers denote time (in h) from beginning of formation of myocardial necrosis (in parentheses) and from time of injection of radioactive isotope (without parentheses). 1, 4) Intact animals; 2, 3) animals with myocardial necrosis.

In the experiments in vivo [2-^{14}C]orotic acid with a specific activity of 54 mCi/mmole was injected intraperitoneally into the dogs in a dose of 100 μCi per animal. The period of exposure was 2, 24, and 48 h after injection of the radioactive products (48, 72, and 96 h after the appearance of myocardial necrosis). The animals were decapitated at these times, and the heart was removed and placed in an ice-cold solution of 0.14 M KCl. Mitochondria were isolated as described previously [2]. The suspension of mitochondria was treated with digitonin (Merck, West Germany) in a dose of 1.5 mg digitonin per 10 mg mitochondrial protein [5], which caused the formation of an internal membranes—matrix complex. The purity of the preparations was verified electron-microscopically [3, 4]. Preparations of the internal membranes were solubilized in 8% Triton, made up to 100 volumes (final concentration 1% in the sample), and incubated for 30 min at 30°C. The radioactivity of the liquid samples was determined in a Mark II scintillation counter (Nuclear Chicago, USA).

In the experiments in vitro, [^{3}H]uridine triphosphate in a dose of 5 μCi/ml (specific activity 55 mCi/mmole) was incorporated into the internal mitochondrial membranes obtained as described above and the final protein concentration of the sample was 4-6 mg. The composition of the incubation medium (in mM) was: sucrose 33.4, KCl 4, $MgCl_2$ 7, KH_2PO_4 5, EDTA 0.27, phosphoenolpyruvate 7.5, Na_2ATP 0.8, Na_4GTP 0.8, and Na_4CTP 0.8, pyruvate kinase 2 Federal units; final volume of the incubation mixture 1 ml (the triphosphates and pyruvate kinase were from Sigma, USA). Incubation continued for 20 min at 37°C with constant agitation. The reaction was stopped by the addition of 20% TCA to a final concentration of 5%. The residues were washed on membrane filters with a pore diameter of 1.5 μ (Czechoslovakia). The residues were repeatedly washed with 5% TCA and absolute ethanol. The radioactivity was determined in the scintillation counter. Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS AND DISCUSSION

Incorporation of [2-^{14}C]orotic acid into RNA of the internal mitochondrial membranes of the intact animals reached a maximum 24 h after injection of the radioactive label into the animal, and after 48 h the specific activity was reduced (Fig. 1). In animals with myocardial necrosis, a reduction in the specific activity of RNA was observed during the first 48 h of the disease, whereas after 72 h it was 42.7% higher than the specific activity of RNA in the intact animals at the same times. A marked increase in the specific activity of RNA in the internal mitochondrial membranes of the animals with myocardial necrosis was observed 96 h after the beginning of necrosis. Characteristically 5 days after injection of isoproterenol the T wave of the ECG was

depressed and the Q wave reduced [2, 5]. The reason was evidently that at this time the formation of new necrotic foci in the myocardium had ceased and the ensuing hyperfunction of the myocardium led to intensification of the protein-synthesizing system not only of the myocardial cell as a whole, but also of certain intracellular structures, in this case the mitochondria.

In the experiments in vitro (Fig. 1) the specific activity of RNA of the internal mitochondrial membranes of the myocardium of the intact animals was much higher in absolute values than in the experiments in vivo. Specific activity of RNA of the internal mitochondrial membranes of the intact animals 48 h after injection of the label had still not reached the maximum. In the animals with myocardial necrosis the change in specific activity of RNA in the internal mitochondrial membranes of the myocardium depended on the time and, consequently, the degree of development of the pathological process. The direction of these changes was the same in vivo and in vitro. Specific activity of RNA of the mitochondrial membranes fell considerably in the first 2 days after the onset of necrosis, to reach 19% of the initial activity. In the stage of formation of myocardial hypertrophy an increase in the specific activity of RNA in the internal mitochondrial membranes was observed, and by the end of the period of investigation it had reached 84%.

Hence it follows that myocardial necrosis causes marked changes in the specific activity of RNA in the internal mitochondrial membranes both in vivo and in vitro.

It can be concluded from these results that the system of in vitro incorporation reflects changes taking place in the body as a whole. This conclusion reinforces the validity of results obtained on isolated mitochondria and enables a stricter interpretation to be given of the results of experiments in vivo.

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