SYNTHESIS AND SOME CHARACTERISTICS OF INTERNAL MEMBRANE PROTEINS OF MYOCARDIAL MITOCHONDRIA OF RATS WITH DISSEMINATED NECROSIS OF THE HEART

L. A. Kopteva, L. I. Zhevlolyukova, and E. A. Grigor'eva

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Two fractions of the inner mitochondrial membranes were isolated from the rat myocardium: a combination of inner membranes and matrix (fraction A) and aggregates of inner membranes without matrix (fraction B). Both membrane fractions were shown to incorporate labeled amino acids with equal activity. Experiments with anodic electrophoresis in polyacrylamide gel showed that the number of protein components in fractions A and B varies from 20-30 and their molecular weights from 10,000 to 200,000. Disseminated necroses in the myocardium produced by Novodrin (isoproterenol sulfate), based on an ischemic state of the heart, led after 2 days to a marked decrease in the protein-synthesizing activity of fractions A and B. The protein profiles and molecular weights of the components of the membrane proteins were not appreciably altered.

KEY WORDS: inner mitochondrial membranes; protein synthesis; protein spectrum; isoproterenol sulfate; disseminated necrosis of the myocardium.

A key stage in the pathogenesis of ischemic disease consists of primary metabolic changes in the myocardium, expressed primarily as inability to satisfy the increased oxygen demand of the myocardium [5]. The question naturally arises why the energy system of the myocardial cell becomes unable to control respiration of the injured cell, whereas the intensity of the processes of energy metabolism in the myocardium is 15-20 times higher than in other organs and tissues [6]. Some help in solving this problem would evidently be obtained from an explanation of the conversions in the metabolism of myocardial cells that take place not only at the level of the mitochondria (the structures responsible for the respiratory function of the cell), but also at the level of their inner membranes, in which all components of the respiratory system of the cell are located.

The object of this investigation was to determine the composition and protein-synthesizing activity of the inner mitochondrial membrane proteins of the myocardium of intact animals and of animals with disseminated necrosis of the heart.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 250-280 g. Necrosis of the myocardium was produced by two subcutaneous injections of Novodrin (isoproterenol sulfate), each of 80 mg/kg body weight, at an interval of 24 h. This dose was known to produce necrosis [1, 7]. The ECG was recorded in the three classical leads (AVR, AVL, AVF) on the 6NEK-3 apparatus 24 h after the first and second injections of Novodrin, using needle electrodes (without fixation of the rat).

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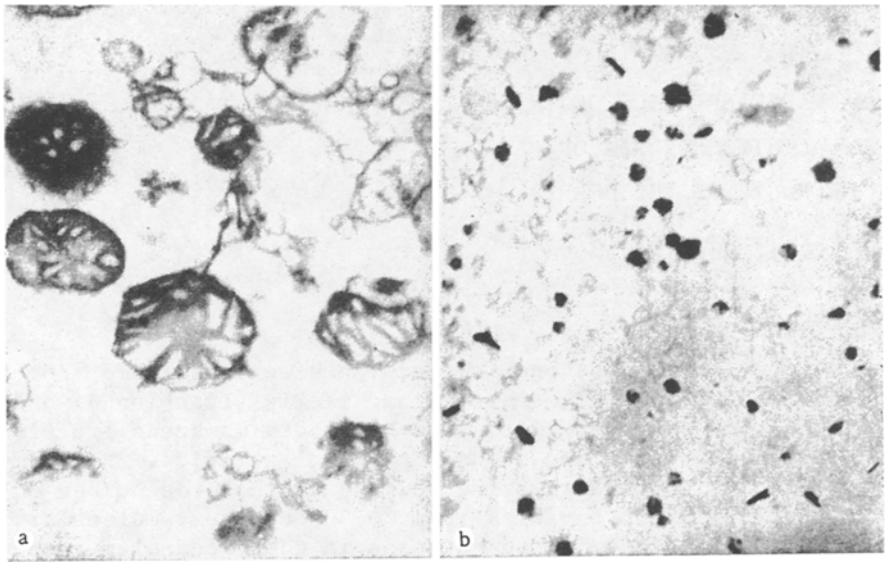

Fig. 1. Electron-microscopic control of inner membranes of myocardial mitochondria of rate (25,000×): a) combination of inner membranes and matrix (fraction A); b) aggregates of inner membranes without matrix (fraction B).

TABLE 1. Incorporation of C^{14}-Amino Acids *in vitro* into Inner Membranes of Myocardial Mitochondria of Intact Rats and Rats with Disseminated Necrosis of the Myocardium (M ± m)

Statistical number	Specific activity (counts/min/mg protein)			
	intact animals		animals with myocardial necrosis	
	fraction A	fraction B	fraction A	fraction B
M	1004	943	743	554
± *m*	29	29	14	22
n (number of expts.)	18	18	15	15

The animals 25-30 rats were decapitated and the heart quickly removed. Mitochondria were isolated from the myocardium as described earlier [2]. The mitochondrial fraction was treated with digitonin (E. Merck, West Germany), and subsequent treatment was as described by Hoppell and Cooper [4] with several modifications. Two fractions of the inner membranes of the myocardial mitochondria were isolated: fraction A, a combination of the inner membranes and matrix, and fraction B, aggregates of inner membranes without matrix. The purity of the preparations was verified electron-microscopically. Protein was determined by Lowry's method [5]. The composition of the membrane proteins of the myocardial mitochondria was determined electrophoretically in 10% polyacrylamide gel, in phosphate buffer with 0.1% sodium dodecylsulfate, pH 7.1. The conditions of electrophoresis were as follows: duration 6 h, 50 V, 8 A to the gel. The molecular weight was calculated by the method of Weber and Osborn [8]. Incorporation of C^{14}-amino acids into fractions A and B was carried out *in vitro* in a medium (0.5 ml) of the following composition (in mM): sucrose 330, KCl 25, $MgCL_2$ 1, tris-HCl (pH 7.4) 50, protein 4-5 mg. A mixture of C^{14}-amino acids was used: proline, threonine, serine, and arginine (specific radioactivity 1.6 μCi per sample). After incubation (30 min, 37°C) the reaction was stopped with 10% TCA (1:1). The precipitate of proteins was washed on membrane filters (1.5 μ). The radioactivity was measured (with an adsorption control) in the Mark II scintillation counter (Nuclear Chicago, USA).

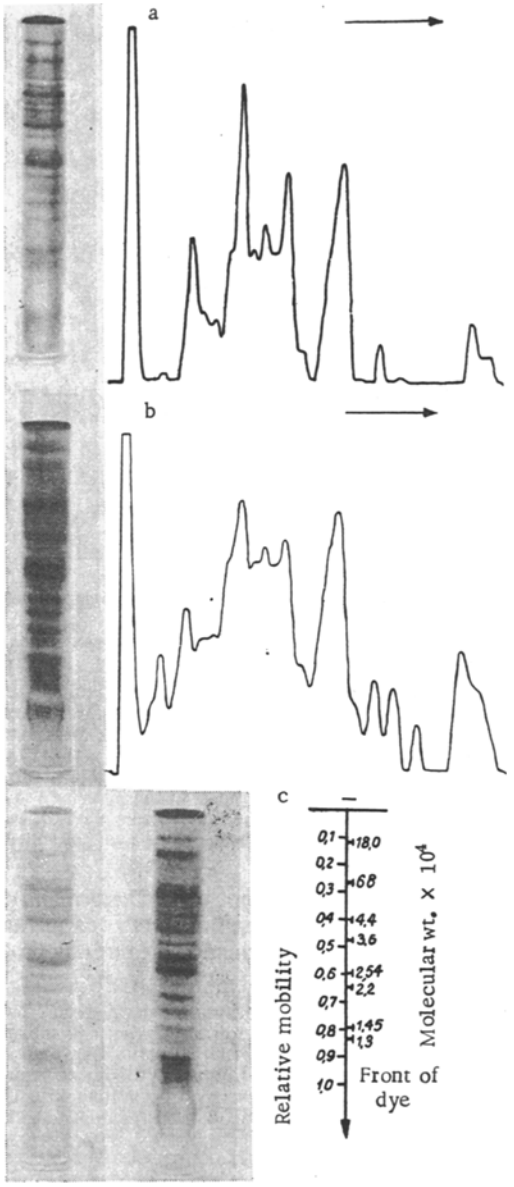

Fig. 2. Electrophoresis of mitochondrial membranes of rat myocardium (intact animals) in 10% polyacrylamide gel: a and b) protein profile of fraction A and fraction B, respectively; c) molecular weights of protein components of fractions A and B reflected in their mobility.

EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 shows, proteins of the inner membranes of the mitochondria, consisting of a combination of inner membranes and matrix (Fig. 1a) and aggregates of inner membranes without matrix (Fig. 1b), incorporated C^{14}-amino acids practically equally. The protein-synthesizing system of mitochondria is known to be located mainly in the inner membranes of the mitochondria.

The protein profile of the inner mitochondrial membrane proteins was fairly heterogeneous (Fig. 2a, b). The molecular weights of the proteins studied varied from 10,000 to 200,000 (Fig. 2c). However, the molecular weights of most proteins were between 33,000 and 170,000.

Injection of Novodrin led to the appearance of necrosis in the myocardium, chiefly in its posterior wall.

A study of the effect of experimental myocardial necrosis on the activity of the protein-synthesizing system of the mitochondrial membranes showed that 24 h after the second injection of Novodrin there was a significant decrease in renewal of the proteins of both fractions (Table 1) — by 32.5 and 37.1% respectively in fractions A and B.

The electrophoretic mobility and molecular weights of the membrane proteins of fractions A and B in rats with experimental myocardial necrosis were unchanged.

Myocardial necrosis produced by injection of the sympathomimetic drug Novodrin thus led to a decrease in the protein-synthesizing activity of the inner mitochondrial membranes 2 days after its onset without any change in their protein spectrum.

LITERATURE CITED

1. O. A. Gomazkov and M. V. Shimkevich, Kardiologiya, No. 4, 127 (1970).
2. L. A. Kopteva and V. I. Biryuzova, Byull. Éksp. Biol. Med., No. 7, 19 (1968).
3. G. Rona, C. Chappel, T. Balazs, et al., Arch. Path., 67, 99 (1959).
4. C. Hoppell and C. Cooper, Arch. Biochem., 135, 173 (1969).
5. O. Lowry, N. Rosebrough, A. Farr, et al., J. Biol. Chem., 193, 265 (1951).
6. F. Pendl, in: Advances in Cardiology [Russian translation], Moscow (1959), pp. 232-280.
7. G. Rona, D. Kahn, and C. Chappel, Rev. Canad. Biol., 22, 245 (1963).
8. K. Weber and M. Osborn, J. Biol. Chem., 244, 4406 (1969).