SUCCINATE DEHYDROGENASE ACTIVITY OF ISOLATED MYOCARDIAL MITOCHONDRIA IN CHRONIC HEART FAILURE

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UDC 616.12-008.46-036.12-07:616.12-008.931:577.152.121

Succinate dehydrogenase (SD) activity of mitochondria isolated from the muscle cells of the chronically failing heart was studied. Highest SD activity was found in mitochondria of condensed type. Low enzyme activity was observed in orthodox mitochondria. SD activity in mitochondria of intermediate type was midway between activity of the enzyme in orthodox and condensed mitochondria. Orthodox and intermediate forms of mitochondria were predominant in the fraction tested, reflecting lowered SD activity in the heart muscle tissue. Biochemical investigation of the mitochondrial fraction revealed a state of mild uncoupling of respiration and oxidative phosphorylation. The observed decrease in SD activity evidently characterizes a state of overstrain of the energy-producing structures.

KEY WORDS: mitochondria; myocardium; chronic heart failure.

It was shown previously that mitochondria isolated from tissue differ in the degree of condensation of the matrix and in the size of the space between the cristae. Two main types of mitochondria have been distinguished: condensed and orthodox [7]. Some workers have described the functional characteristics of mitochondria of a particular type [1, 5, 6]. There is no general agreement regarding the assessment of the energy-producing state of mitochondria with one or another configuration.

The object of this investigation was to determine succinate dehydrogenase activity of mitochondria isolated from the muscle cells of the chronically failing heart, as an indicator of their functional state.

EXPERIMENTAL METHOD

Chronic heart failure was produced by measured injury to the posterior wall of the mitral valve. The myocardium from 10 dogs with chronic heart failure from 6 to 12 months in duration was studied. For electron-microscopic study the tissue of the ventricles and atria and the mitochondrial fraction were fixed in 1% OsO_4 solution by Caulfield's method and embedded in Araldite. The mitochondrial fraction was obtained from the myocardium of the left ventricle by differential centrifuation in 0.32 M sucrose solution. The functional state of the mitochondria was assessed as succinate dehydrogenase (SD) activity, determined by an electron-histochemical method [8]. The rate of oxygen uptake and of oxidative phosphorylation was determined concurrently with a closed Clark's polarographic electrode, and expressed per milligram protein of the mitochondrial fraction. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

Electron-microscopic study of the myocardium of the ventricles and atria showed a high glycogen content in the muscle cells. Accumulation of glyogen granules was most marked in the atria muscle cells (Fig. 1a). The mitochondria were circular in shape, 0.8-1.5 μ in diameter, with a fine-grained matrix of average electron density. The mitochondrial cristae were curved in configuration and they frequently

Department of Pathological Anatomy, Faculty of Stomatology, Moscow Medical Stomatological Institute. Central Research Laboratory, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 1, pp. 37-40, January, 1976. Original article submitted April 7, 1975.

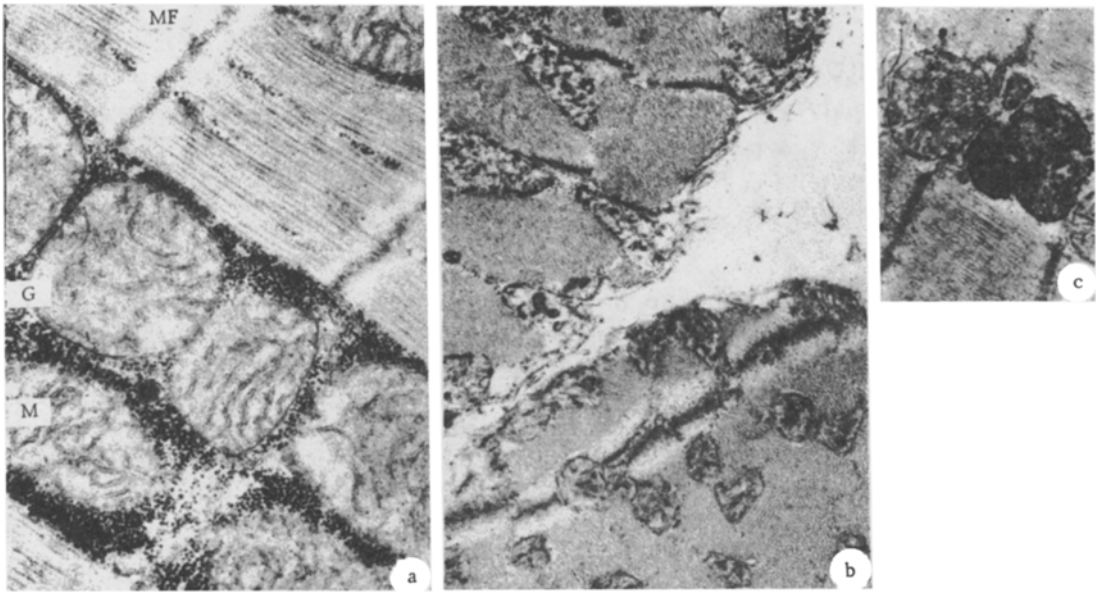

Fig. 1. Myocardium of left atrium: a) accumulation of glycogen (G) granules between mitochondria (M) and myofibrils (MF), 50,000×; b) muscle cells with varied succinate dehydrogenase (SD) activity. 15,000×; c) mitochondria of a myocyte with varied SD activity, 30,000 ×.

anastomosed with each other. Sometimes the membranes forming the cristae lost their clearness of outline and seemed to separate into layers. The structure of the A disks was indistinguishable from normal but the I disks in some cells were destroyed.

The electron-histochemical study of the myocardial muscle cells revealed lower tissue SD activity than normally. This decrease in enzyme activity was reflected in fewer muscle cells with high enzyme activity and lower SD activity in each mitochondrion of the myocyte. Meanwhile, heterogeneity of SD distribution was observed in individual cells and mitochondria. Two cells lying side by side often differed sharply in enzyme activity (Fig. 1b), indicating differences in their functional state. Mitochondria even in the same cell possessed different SD activity (Fig. 1c). In some mitochondria, for instance, the chelate granules, indicating SD activity, were concentrated on the cristae. In other mitochondria, despite their structural integrity, enzyme activity was weak.

As a result of the electron-microscopic study the isolated mitochondria were divided into three structural types: condensed, orthodox, and intermediate forms. Mitochondria of the orthodox type had the lowest SD activity (Fig. 2a). The mitochondria were large, their matrix was electron-transparent, and their narrow cristae were arranged parallel to each other. Dense granules of chelate, measuring 150 to 250 Å, were located on the membranes of the cristae and also between the outer and inner membranes of the mitochondria. The number of granules was small.

Mitochondria of the condensed type had the highest SD activity (Fig. 2b). These mitochondria were smaller than the orthodox, and their fine-grained matrix possessed considerable electron density. The chelate granules were very large (300-450 Å) and filled large areas of the space between the cristae frequently.

SD activity in mitochondria of intermediate type was midway between the enzyme activity in the orthodox and condensed mitochondria (Fig. 2c).

In chronic heart failure, intermediate and orthodox types of mitochondria predominated in the fraction of isolated mitochondria. All three types of mitochondria, with their different configurations and SD activities, could lie side by side (Fig. 2d).

Biochemical investigation of the mitochondrial fraction showed that the rate of oxygen uptake by the mitochondria in metabolic state 3 (with the addition of ADP) in the atrium fell to 1.5 μA O_2/g/sec, and in the ventricle it fell to 2.0, from normal values of 2.86 and 3.9 μA O_2/g/ sec respectively (Table 1). Comparison of the respiratory activity of the mitochondria in metabolic state 3 with that in state 4 (V_4, without ADP),

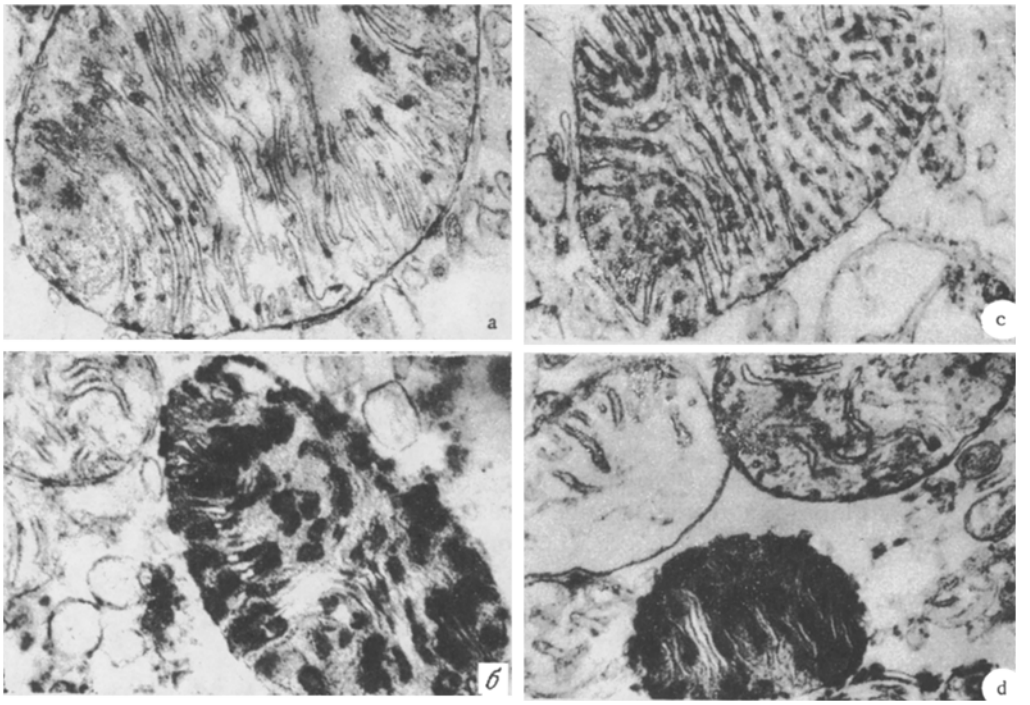

Fig. 2. Succinate dehydrogenase activity of isolated mitochondria: a) low enzyme activitiy in mitochondria of orthodox type, 140,000×; b) high SD activity in mitochondria of condensed type, 140,000×; c) enzyme activity in intermediate mitochondria midway between SD activity in condensed and orthodox mitochondria, 140,000 ×; d) condensed and orthodox mitochondria lying side by side, 120,000×.

TABLE 1. Respiratory Activity of Myocardial Mitochondria (in $\mu A\ O_2/g/sec$)

Experimental conditions	Control (10 dogs)		Chronic failure (10 dogs)	
	ventricle	atrium	ventricle	atrium
Active respiration of mitochondria (V_3)	3,9±0,02	2,86±0,07	2,01±0,04	1,56±0,04
Respiration of mitochondria without acceptor (V_4)	1,0±0,01	0,83±0,02	0,9±0,03	0,83±0,01
Respiratory control (after Chance)	2,9±0,05	2,07±0,04	2,00±0,03	1,86±0,02

by Chance's method [4], revealed a significant decrease in the respiratory control in the left atrium and ventricle (Table 1). A decrease in the respiratory control of this sort indicates a disturbance of the coupling of oxidation with oxidative phosphorylation.

The unequal SD activity observed in mitochondria of different configuration agrees with the results of investigations showing a close dependence of mitochondrial structure on function. For instance, Kozyreva and Mityushin [3] consider that the structure of isolated mitochondria depends on the degree of coupling of oxidation with phosphorylation and on the presence of ATP of endogenous type in the mitochondria. Bakeeva [1] showed that the structural features of mitochondria depend on the conditions of their energy metabolism, and explained the difference between the morphological types of both isolated mitochondria and mitochondria in the tissue by the osmotic properties of the inner mitochondrial membrane and the energy-dependent distribution of ions between the space of the matrix and the surrounding medium. Beketova [2] considers that condensed mitochondria are in a state of energy accumulation, whereas orthodox mitochondria reflect functional overstrain of the mitochondria and they are characterized by the uncoupling of oxidation and phosphorylation. Consequently, the decrease in myocardial SD activity and the lower efficiency of the energy-forming processes observed in the present experiments evidently reflect maximal stress and some degree of exhaustion of energy-producing processes in the heart muscle cells during the development of chronic failure.

The relationship discovered between the degree of SD activity, the intensity of oxidative processes, and the configuration of the mitochondria is a qualitative characteristic of the latter and is determined by the

functional state of mitochondria of different types. This confirms once again the well-known views of Packer [10] and Lehninger [9], according to whom respiration, coupled with oxidative phosphorylation, shows definite correlation with conformational changes in the mitochondrial membranes.

The results showing differences in SD activity of mitochondria of different configuration, the predominance of orthodox and intermediate forms in the fractions studied, the general lowering of SD activity in the heart muscle tissue, depression of the oxidative metabolism of the mitochondria, and uncoupling of oxidation and phosphorylation all characterize a state of functional overstrain of the energy-producing structures.

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