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Structural and functional changes of mitochondria in rat cardiomyocytes in subacute administration of sydnocarb

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Summary. Structural and functional changes of mitochondria in rat cardiomyocytes in subacute administration of sydnocarb. Kharaponova E.B., Drozdov O.L., Silkina Yu.V.

We described three types of mitochondria, differing in shape, size and density of cristae. During subacute administration, sydnocarb has cardiotoxic effect in form of degradation of mitochondria and mitochondrial cristae. Due to sydnocarb-induced vasodilatation and swelling of cardiomyocytes, the most sensitive to hypoxia were type-1 mitochondria. It was reported not only about destruction of cristae, but also mitochondrial degradation in some areas of cardiomyocytes. The least sensitive to increasing hypoxia were type-3 mitochondria.

Key words: mitochondrion, cardiomyocyte, sydnocarb, cardiotoxic effect.

It is considered that a structure is a reflection of the function. This thesis can be applied to a whole organ, as well as to the subcellular unit. Hence, it can be assumed that, mitochondria, for example, being at different phases of energy synthesis cycle have specific features of ultrastructure. But whether only a level of functional tension dictates polymorphism? A number of researchers who studied mitochondria as a part of heart muscle cells, suggested that functional differences of mitochondria were caused not only by cyclical functional changes, but also their

location in cardiomyocytes [8, 1918; 10, 412; 5, 9]. Thus, researcher Frolov V.A. et al. [6, 23-56] presented data showing significant differences of subsarcolemmal, intermyofibrillar and paranuclear subpopulations of mitochondria. Researches of rat cardiomyocytes let to observe increased density of mitochondria in those parts of sarcoplasm, directly adjacent to the exchange vessel.

Mitochondrial cristae are described as many-sided wavy membranes or grid-type structures that contribute to maintenance of energy potential of myocardium and prevent hypoxia development [3, 849]. Under action of pathogenic factors, mitochondria react quickly to changing conditions due to high sensitivity of oxidative phosphorylation process primarily to oxygen deficit. This was confirmed by numerous works, which identified changes in structural and functional state of mitochondria under conditions of toxic substances associated with restructuring of multienzyme membrane complex [9, 1563-1565]. In this regard, it should be assumed that sydnocarb cardiotoxic effect, which we described in several studies, also manifests with a high probability as structural and functional mitochondrial disorders. However, we could not find published data to confirm or contradict this assumption.

Therefore, the *aim* of this work was to establish structural and functional mitochondrial characteristics of rat cardiomyocytes in condition of subacute administration of sydnocarb.

Materials and methods. Administration of sydnocarb into Wistar rats (n = 96) were provided for the purpose of experimental simulation of psychosis. The drug was administered at 5 mg/kg/dose twice a day for 14 days. Control animals (n = 15) were administered isotonic sodium chloride solution at 1 ml/kg/dose. Average body weight of rats was 160-180 g. Animals were kept in standard conditions with standard diet.

Collection and processing of the material were performed as follows. Myocardial samples of 1 × 1 mm in size were fixed for 3-4 hours at 2° C in 2.5% glutaraldehyde solution, made with 0.2M phosphate buffer. Then it was fixed up in 1% buffered osmium oxide for 1 hour. It was treated with alcohols of increasing

concentrations and propylene oxide. Epon-Araldite was used to manufacture epoxy blocks. Manufacture of ultrathin slices was performed with ultramicrotome UMTP-6M placing them on supporting grids [4, 24]. The study of mitochondria was performed using transmission electron microscope PEM-100-01 at magnifications of 2000 – 50 000 times according to G.G.Avtandilov's procedure (1990) [1, 25-34]. Statistical processing was performed according to standard procedure [2, 12-20].

Results and discussion. Conducted ultrastructural studies showed that mitochondrial polymorphism is typical for contractile cardiomyocytes of rats of the control group. Classification of mitochondria was done depending on the packing density of the cristae. Evaluation of integral parameters allowed to find 3 types of mitochondria, their ultrastructural characteristics are presented in the table.

Type-1 Mitochondria had an elongated shape, a larger volume and surface area of outer membrane, and developed apparatus of cristae; size and structure of type -1 mitochondria were typical for most muscle cells of myocardium. Type- 2 organelles approached to spherical shape, having a smaller volume and surface area of outer membrane. They conceded type-1 mitochondria in density and the number of cristae. Type-3 mitochondria had a spherical shape, extremely small volume, area of the outer membrane and non-uniform electron density of intermembrane area. Their cristae density was two times more compared with that of type -1 mitochondria. Mean values of mitochondrial ultrametric parameters obtained in myocardium slices of left ventricles in rats, are shown in the table.

Table

Ultrastructural parameters of mitochondria of contractile cardiomyocyte of left ventricle of heart in rats (M ± m)

Parameter	Control			After administration of sydnocarb		
	types of mitochondria			types of mitochondria		
	1	2	3	1	2	3
Volume of mitochondrion, μm^3	0,94±0,11	0,68±0,07	0,43±0,06	1,21±0,10	0,74±0,08	0,62±0,06

Surface area of outer membrane, $\mu м^2$	5,22±0,63	5,02±0,53	2,12±0,23	7,14±0,69	5,78±0,64	3,10±0,33
Density of cristae, $\mu м^{-2}$	8,14±0,95	5,34±0,63	17,14±1,30	3,68±0,32	4,87±0,50	15,51±1,9
A number of cristae in mitochondrion	16,3±3,20	7,71±1,60	14,92±2,46	8,92±0,75	6,21±0,65	11,32±1,4
Surface area of the inner membrane, $\mu м^2$	10,26±1,12	4,37±0,51	9,15±3,53	4,38±0,46	3,12±0,35	7,41±0,80

It should be noted that the area of inner mitochondrial membrane was in average 8.43 μm^2 in mature ventricular myocytes of the control group of rats; Quite often there were organelles within 3-5 μm^2 and 10-12 μm^2 ($p < 0.05$). A similar pattern of statistical distributions was set for numerical density and the number of mitochondrial cristae, area of the outer membrane and volume of mitochondria. Analyzing these data, it is possible to draw a conclusion about sustainable heterogeneity of mitochondrial apparatus of mature cardiomyocytes.

In the group of rats treated with sydnocarb, mitochondria of contractile cardiomyocytes were also characterized by polymorphism and heterogeneity of distribution in the sarcoplasm. Type-2 mitochondria were characterized by the smallest differences in ultrastructural and quantitative characteristics, compared with the control group. Dynamic of changes of integral parameters in comparison with the control was most expressed in type-1 mitochondria, which are the main "working" mitochondria in cardiomyocytes and have intermyofibrillar localization. A decrease in the area of the inner membrane (average option was 4.97 μm^2 , which is 41.0% less than in the control group ($p < 0.05$)) and an increase of mitochondrial volume were observed, that is likely associated with symptoms of organelle swelling; phenomenon of cristae destruction as well as signs of degradation of entire parts of mitochondrial zones were present in significant number of organelles.

These described changes had an expressed focal character, which depended on the localization of ischemic areas. At the same time, mitochondria of all three

types (more or less) had signs of matrix vacuolization, fragmentation of mitochondrial membranes, cristolysis. In our opinion, mitochondrial degradation in a whole and in their structural components in particular is associated with the phenomena of hypoxia observed during subacute administration of sydnocarb. The drug is a potent stimulator of sympathetic-adrenal system, increases the need of heart muscle for oxygen with parallel vasotoxic effects manifested in paradoxical vasodilatation, starting with a 3-day experiment. Expressed vascular reaction to sydnocarb due to neurotransmitter exhaustion was caused by growth of hypoxic effects. Under these conditions, mitochondrial functional deficit, accompanied in parallel by their hydration and change of matrix electrolyte composition, led to their structural degradation and growth of energy deficit in cardiomyocytes in general.

Conclusion. Thus, we found that phenomena of mitochondrial degradation as a part of contractile cardiomyocytes were observed under the conditions of cardiotoxicity effect of sydnocarb. The most sensitive to increasing hypoxic effects were type-1 mitochondria, which were characterized not only by cristae destruction, but also by degradation of entire mitochondrial zones in some cardiomyocytes. The least sensitive to hypoxia were type-2 mitochondria.

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