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MORPHOMETRIC ANALYSIS OF CARDIOMYOCYTE MITOCHONDRIA IN NORMAL RATS AND DURING POSTISCHEMIC REPERFUSION

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KEY WORDS: myocardium; ultrastructure; mitochondria; morphometry.

Swelling of the mitochondria (MC) is invariably observed in the cardiomyocytes in the course of their damage during ischemia and reperfusion [1, 2, 5]. However the general principles governing this process are unknown.

The aim of this investigation was a morphometric analysis of the size, shape, and number of MC in cardiomyocytes under normal conditions and during postischemic reperfusion.

EXPERIMENTAL METHOD

Experiments were carried out on eight male Wistar rats weighing 250-300 g. The heart was removed under thiopental anesthesia, the aorta and left atrium were cannulated, and the left side of the heart was perfused with Krebs–Henseleit buffer by the method in [4]. The four rats in the control group were perfused for 90 min. The hearts of four experimental animals, after control perfusion for 10 min, were subjected to total normothermic ischemia for 30 min and reperfusion for 40 min. At the end of perfusion, a piece of myocardium from the apex of the left ventricle was fixed in 2.5% glutaraldehyde solution, postfixed with OsO_4, dehydrated, and embedded in Epon–Araldite. Sections through the myocardium were stained with lead citrate and uranyl acetate and examined in the ÉVM-100L electron microscope under a magnification of 23,000. Using a multipurpose test grid [7] with 270 control points the bulk density of MC (V_V), the relative surface density of MC (S_V), and the number of mitochondrial profiles per square micron area of section (n) were determined on the photographic plates. The results were used to calculate the diameter (D), length (L), and volume (V_{1mc}) of one mitochondrion, the number of MC in 1 μ^3 of sarcoplasma (N), and the surface area of one MC (S_{1mc}) and of all MC in 1 μ^3 in sarcoplasm (S). In longitudinal sections through the cardiomyocytes the length of MC (l_{mc}) and of the sarcomeres (l_{sarc}) was measured, and in sections not cut longitudinally, the diameter of MC (d_{mc}) was measured. The results were subjected to statistical analysis (95% confidence limits).

EXPERIMENTAL RESULTS

During postischemic reperfusion the cardiomyocyte MC had a significantly higher bulk density and lower relative surface density than in the control (Table 1). The number of

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TABLE 1.

Parameter	Control	Postischemic reperfusion	Mean
		A	
$\ell_{mc(c)}$, μ	1,000±0,030	1,021±0,092	1,017±0,034
$\ell_{mc(e\ell)}$, μ	1,271	1,270	1,278±0,028
d_{mc}, μ	0,545±0,031	0,817±0,023	—
V_v, μ^3/μ^3	0,292±0,004	0,386±0,023	—
S_v, μ^2/μ^3	6,991±0,114	5,293±0,101	—
n, $1/\mu^2$	0,617±0,038	0,582±0,021	0,586±0,020
ℓ_{sarc}, μ	1,291±0,112	1,452±0,127	1,412±0,064
		B	
$F(\varepsilon)$	111,76±5,26	79,91±0,48	—
$\varepsilon_{e\ell}$, μ/μ	0,208±0,022	0,860±0,028	—
ε_c, μ/μ	0,222±0,045	—	—
$D_{e\ell}$, μ	0,615±0,011	1,060±0,011*	—
D_c, μ	0,636±0,016*	—	—
$L_{e\ell}$, μ	3,021±0,280*	1,253±0,042	—
L_c, μ	3,044±0,477*	—	—
$V_{1\,mc(e\ell)}$, μ^3	0,834±0,079	0,779±0,048	0,809±0,026
$V_{1\,mc(c)}$, μ^3	0,957±0,128	—	0,850±0,042
$S_{1\,mc(e\ell)}$, μ^2	5,825±0,489	4,113±0,178	—
$S_{1\,mc(c)}$, μ^2	6,679±0,820	—	—
$N_{e\ell}$, $1/\mu^3$	0,357±0,041	0,497±0,026	—
N_c, $1/\mu^3$	0,318±0,052	—	—
$S_{e\ell}$, μ^2/μ^3	2,040±0,053	2,040±0,098	2,019±0,064
S_c, μ^2/μ^3	2,040±0,053	—	2,019±0,064

Legend. Mean value calculated for values not differing significantly. Asterisk indicates that calculated value differs significantly from measured value. el) Ellipsoid, c) cylinder.

mitochondrial profiles per square micron area of section of the cardiomyocytes did not differ significantly between the groups. Measured values of the length of the sarcomeres and MC in cardiomyocytes from the control and damaged myocardium were similar. The diameter of MC of the cardiomyocytes was significantly greater in the course of postischemic reperfusion than in the control. Assuming that the MC is cylindrical in shape, its length must be 1.017 μ. If the shape of MC is close to an ellipsoid of rotation, progressive swelling of MC, with no change in length, ought to lead to the MC becoming spherical in shape, and the diameter of the sphere can be determined by the equation

$$D_S = \frac{4ds}{\pi(1+c^2)},$$

where d_S (the diameter of sections of the sphere) = 1.017 μ and c is the coefficient of variation. The diameter of the sphere and, consequently, the length of the ellipsoid MC being analyzed (with some approximation) are thus 1.278 μ.

Direct mathematical analysis of the cross section of MC (the latter vary widely in shape and size) could not be undertaken and a population of abstract MC, which we described as "standard," was analyzed; for these standard MC: 1) measured values (V_V, S_V, and n) are valid; 2) the shape and size are equal; 3) the shape can be described by elementary stereologic equations.

For cylindrical MC a method of indirect determination of their dimensions has been developed [3]. The authors cited suggest using the equations

$$\left(\sqrt{\frac{V_v}{n}}\cdot S_v\right)^3 = \frac{2\pi(\varepsilon+2)^3}{\beta\varepsilon}, \quad (1)$$

$$\varepsilon = \frac{D}{L}, \quad (2)$$

where β is the coefficient of configuration of MC. The value of the left side of the equation can easily be determined. Knowing the ratio between β and ε for a cylinder, it is easy to calculate the value of ε from the formula on the right side of the equation and, consequently, to determine the dimensions of MC and their number in 1 μ^3 of cytoplasm.

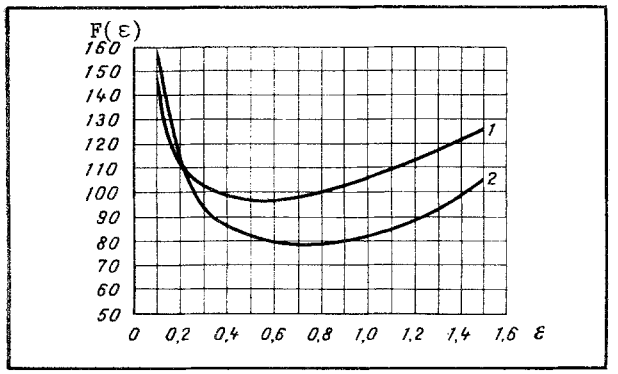

Fig. 1. Graph of the function $F(\varepsilon)$ for cylinders and ellipsoid. 1) $F(\varepsilon)_c = \frac{2\pi(\varepsilon+2)^3}{\beta\varepsilon}$; 2) $F(\varepsilon)_{el} = \frac{144\pi}{\beta\varepsilon(3-\varepsilon)^2}$.

We did similar calculations for a shape approximating to an ellipsoid of rotation, the volume and surface area of which are close to values calculated by equations for a cylinder with two hemispheres at its ends. For MC of the shape described

$$S_v = \frac{12}{D(3-\varepsilon)} \tag{3}$$

and, consequently

$$D = \frac{12}{S_v(3-\varepsilon)}, \tag{4}$$

$$V_{1MC} = \frac{144\pi}{\varepsilon S_v^3(3-\varepsilon)^2}, \tag{5}$$

$$N = \frac{V_v}{V_{1MC}} = \frac{V_v \varepsilon S_v^3(3-\varepsilon)^2}{144\pi} \tag{6}$$

and at the same time

$$N = \frac{n^{3/2}}{\beta V_v^{1/2}} \tag{7}$$

Equating (6) and (7) and grouping the parameters V_V, S_V, and n on one side of the equation, we obtain:

$$\left(\sqrt{\frac{V_v}{n}}\cdot S_v\right)^3 = \frac{144\pi}{\beta\varepsilon(3-\varepsilon)^2}. \tag{8}$$

Knowing the relationship between β and ε for a cylinder and ellipsoid [6], values of $F(\varepsilon)$ were calculated by the formulas on the right side of equations (1) and (8), and graphs of them were plotted (Fig. 1). For cardiomyocyte MC in the control and during postischemic reperfusion, the value of $F(\varepsilon) = \left(\sqrt{\frac{V_v}{n}}\cdot S_v\right)^3$ was calculated on the basis of the measured parameters V_V, S_V and n and the value ε was determined graphically. It turned out that the "standard" MC shape of the damaged cardiomyocytes was close to an ellipsoid. For every $F(\varepsilon)$ point, ε had two values along both sides of the bending in the graph. The higher values of ε were taken for it was when they were used that the calculated values of S_V agreed with those measured. In the control cardiomyocytes the "standard" MC had a shape which could be described both as a cylinder and as an ellipsoid of rotation. The diameter, length, and volume of MC of ellipsoid shape were calculated by equations (4), (2), and (5), and the other parameters by the equations:

$$N = \frac{V_v}{V_{1MC}}, \tag{9}$$

$$S_{1MC} = S_v \cdot V_{1MC}, \tag{10}$$

$$S = S_{1MC} \cdot N. \tag{11}$$

Parameters of the "standard" MC of cylindrical shape were calculated by equations:

$$D = \frac{2(2+\varepsilon)}{S_v}, \tag{12}$$

$$V_{1\,MC} = \frac{\pi D^3}{4\varepsilon} \qquad (13)$$

and also by equations (2), (9), (10), and (11).

The calculated length of "standard" cardiomyocyte MC in the course of postischemic reperfusion did not differ significantly from the measured length of MC and of the sarcomeres. It was accordingly concluded that the shape of these MC can be described, with some degree of approximation, as an ellipsoid of rotation, whose length is commensurate with the length of the sarcomeres (Fig. 2). MC of cardiomyocytes of the control myocardium had the same length in the sections as MC during postischemic reperfusion, but the length of the control MC, calculated by the equations, was significantly greater. In our view, the cardiomyocyte MC of the control myocardium are long, curved structures, forming shapes whose longitudinal sections correspond in length to the length of MC during postischemic reperfusion.

Analysis of the parameters of "standard" MC showed that the volume of a short MC in a damaged cardiomyocyte does not differ significantly from the volume of the original MC. In this case the surface area of each short MC is reduced, but the number of MC in 1 μ^3 of sarcoplasm is greater than in the control. The total volume of all MC in 1 μ^3 of sarcoplasm is increased during postischemic reperfusion proportionally to the increase in their number, but the total surface area of MC in 1 μ^3 of sarcoplasm was not significantly altered.

We suggest that the increase in the number and total volume of MC takes place as a result of constriction band formation in the long curved MC, giving rise to "daughter" MC, the volume of which increases up to that of the original MC.

It can be concluded that by analyzing the parameters of "standard" MC it is possible to judge the general principles governing changes in true MC. However, changes taking place in real MC, which deviate in shape from a resular cylinder and ellipsoid, are much more complex. These differences are manifested in the significantly lower values of the measured diameters of MC compared with the calculated diameters of "standard" MC.

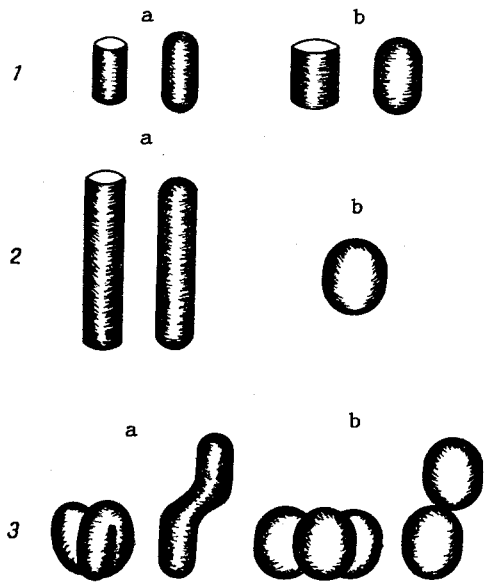

Fig. 2. Shape of cardiomyocyte MC in control (a) and during postischemic reperfusion (b). 1) According to data of direct measurements of parameters of MC in sections; 2) according to results of indirect calculations of parameters of "standard" MC; 3) according to results of direct measurements of parameters of MC in sections and indirect calculations of parameters of "standard" MC.

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POLYMORPHISM OF SMOOTH-MUSCLE CELLS OF AN ATHEROMATOUS PLAQUE OF THE HUMAN AORTA

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KEY WORDS: atherosclerosis; cytoskeleton of intimal cells; myosin; desmin; vimentin.

Some investigators consider [7] that the smooth-muscle cells (SMC) of blood vessels play the key role in the pathogenesis of atherosclerosis. The overwhelming majority of intimal cells of human blood vessels are known to be SMC [11]. During the development of atherosclerosis, the relative numbers and structure of the cells change [9]. The SMC actively produce collagen, which leads to the formation of a vascular fibrous plaque [4]. It has been suggested [5] that two phenotypes of SMC exists in the wall of a blood vessel affected by atherosclerosis, one consisting of cells with an abundance of myofilaments, the other of cells transformed from the usual "contractile" state into a "synthetic" state [7]. Indirect evidence in support of this possibility is given by the increase in the proportion of altered or "modified" SMC in the human atherosclerotic plaque [14]. The cytoplasm of such cells contains few myofilaments and is packed with endoplasmic reticulum and a lamellar apparatus, evidence of active synthesis and secretion of protein [9, 14]. Meanwhile the absence of definite morphological criteria or markers of the modified SMC makes it difficult to study these cells and to determine their precise localization.

In this paper we described the results of analysis of expression of a number of cytoskeletal proteins in cells of an atheromatous plaque of the human aorta.

EXPERIMENTAL METHOD

A double immunofluorescence method, enabling the localization of two proteins to be studied in the same cells, was used. Autopsy material was obtained from 12 persons dying at the age of 28-72 years as a result of trauma, and was used 3-6 h after death. Unchanged segments of the vessel and atheromatous plaques, with a finely granular amorphous mass in their central part, were excised from the thoracic aorta. Frozen sections of the vessel 5 μ thick were cut and kept at −20°C for not more than 1 week. Antiserum to smooth-muscle myosin [3], monoclonal antibodies to vimentin [15] and desmin [6], and also monoclonal antibodies to the surface of human SMC, not reacting with macrophages and endothelial cells, but specifically bound with SMC and fibroblasts in culture [10], and with SMC and pericytes from human tissue sections, were used in the investigation. The sections were immersed for 5 min in phosphate-buffered saline (PBS), containing 10 mM phosphate buffer (pH 7.4) and 2 mg/ml bovine serum albumin, and subsequently incubated with monoclonal antibodies to vimentin, desmin, or SMC (diluted 1:20, 1:100, and 1:50 with PBS respectively), and then with antiserum to myosin (dilution 1:20) for 1 h at 25°C. Antibodies bound with the tissue were revealed by subsequent

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