REPRODUCTION OF CARDIOMYOCYTE MITOCHONDRIA REVEALED BY SCANNING ELECTRON MICROSCOPY

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KEY WORDS: scanning electron microscopy.

Heart muscle compensates any injury to it entirely through processes of intracellular regeneration, in which reproduction of the mitochondria plays a most important role. It enables the disturbed energetic potential of the cradiomycyte to be restored and to be adapted to the new level of load falling on the myocardium [3]. Mitochondria can reproduce by "self-assembly" from hyaloplasm [1, 5] or by division [4]. To study the mechanisms of this process, electron-microscopic analysis of the cardiomyocyte is essential. However, by means of transmission electron microcscopy, only a two-dimensional picture can be obtained, and it therefore cannot provide adequately based characteristics of spatial relations between cellular ultrastructures, and, consequently, it cannot yield direct proof either of synthesis of mitochondria *de novo* or of their division.

In the investigation described below the state of the cardiomyocyte mitochondria was investigated by scanning electron microscopy, so that not only could the arrangement of the ultrastructures in the cell be estimated, but the state of their surface could also be characterized, an essential matter for determining whether division of the mitochondria takes place.

EXPERIMENTAL METHOD

Experiments were carried out on 20 intact male Chinchilla rabbits weighing 2.5-3.5 kg. Relations between seasonal and circadian changes in cardiomyocyte ultrastructures were studied parallel with changes in reproduction of the mitochondria. The animals were killed by thoractomy under superficial hexobarbital anesthesia at midnight, 6 a.m., noon, and 6 p.m., on June 21, 1984 (five animals in a group). The test object for scanning electron microscopy consisted of pieces of the papillary muscles of the left ventricle, processed by they usual methods,[2]. A piece of papillary muscle was washed in Hanks' solution, frozen in liquid nitrogen, cleaved, and placed in a 2% solution of glutaraldehyde. The material was then dehydrated in acetone, and dried by the critical point method using liquid carbon dioxide (Balzers Union, Lichtenstein), and sprayed with gold-palladium alloy by ionic bombardment, using a cold "Sputter" (Poliron, England). An IsI-60 scanning electron microscope with resolving power of 6 nm (magnification 1000-20,000) was used. Where necessary, the electron micrographs obtained were compared with the results of investigation in the BS-540 transmission electron microscope (Tesla, Czechoslovakia). In these cases the heart was perfused with 2.5% glutaraldehyde solution. After perfusion pieces of papillary muscles from the left ventricle were cut out, fixed in 2.5% glutaraldehyde solution, and postfixed in 1% buffered OsO_4 solution, pH 7.2-7.4. The material was dehydrated and embedded in Araldite. Sections were cut on an "Ultracut" ultramicrotome (Reichert, Austria), stained with lead hydroxide and uranyl acetate, and examined under the microscope (magnification 10,000-40,000). The electron micrographs were subjected to the simplest quantitative analysis: in scanning electron micrographs the volume, and in transmission the area of the mitochondria were calculated by the appropriate equations for two- and three-dimensional geometric shapes similar to mitochondria. The numerical results were subjected to statistical analysis by Student's method. Differences between means were assessed at a level of significance of $P \leq 0.05$.

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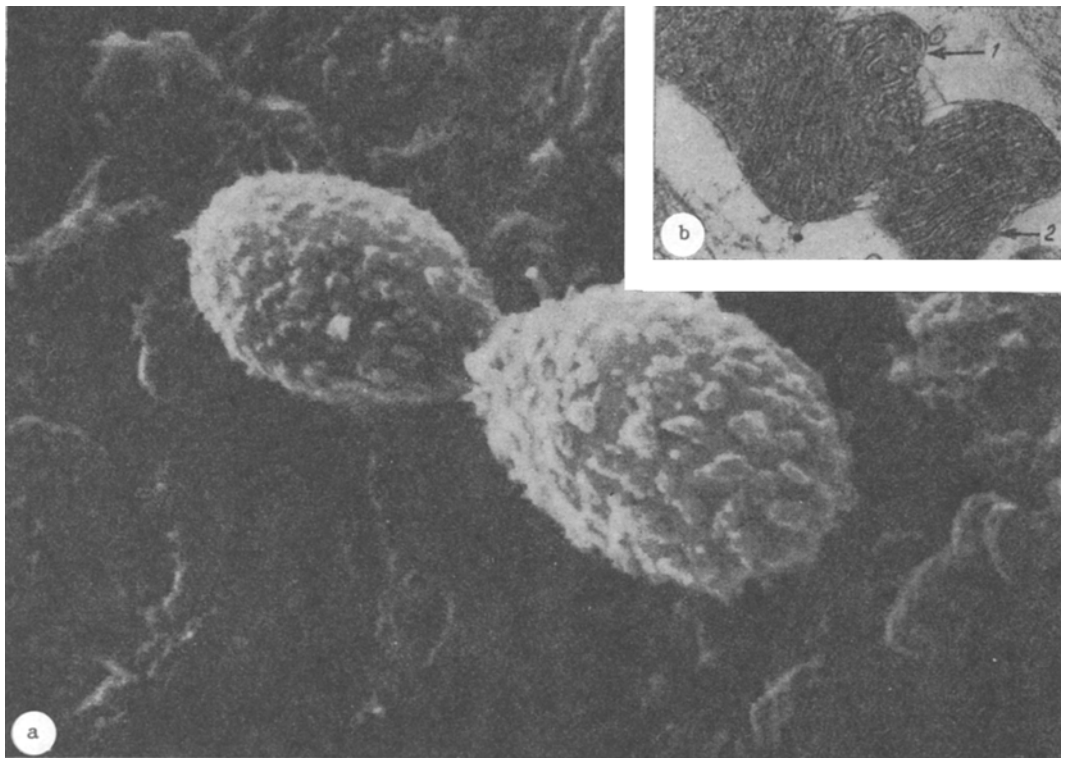

Fig. 1. Formation of new mitochondria from outgrowths of their outer membrane: a) scanning electron micrograph of left ventricular myocardium of rabbit heart: outgrowths on outer membrane of mitochondria. 10,000×; b) transmission electron micrograph of left ventricle of rabbit heart: separation of new mitochondria — small (1) and large (2) — by budding. 40,000×.

EXPERIMENTAL RESULTS

The volume of the mitochondria, which were basically shaped like a sphere or an ellipsoid of rotation, varied in the course of the 24-h period from 0.01 ± 0.001 μ^3 at midnight to 0.33 ± 0.07 μ^3 at 6 a.m. (difference between means significant). Similar variations in the area of the mitochondria also were observed during analysis of electron micrographs obtained with the transmission electron microscope. At different times of the 24-h period some interesting features of the mitochondria were discovered (chiefly at midnight and 6 p.m.), and these will be described below.

In some scanning electron micrographs we observed very large mitochondria in the cardiomyocytes (up to 0.6 μ^3 in volume), covered by oval or irregularly shaped outgrowths, the volume of which varied between 0.002 and 0.004 μ^3 (Fig. 1a). Besides this feature, the connection of two neighboring organelles with one another must also be mentioned (Figs. 1 and 2). A transmission electron micrograph showing separation of two mitochondria from another large mitochondrion, is given in Fig. 1b: one new mitochondrion is small, the other large, and the volume of the small mitochondrion, separated after budding, must be about 0.002 μ^3 in view of its radius. It can be thus postulated that in this case two processes could be observed on the scanning electron micrograph: division of mitochondria (formation of a constriction ring in a large organelle) and the formation of new mitochondria by budding. The process of division of mitochrondria also is illustrated in Fig. 2, where both organelles are covered with buds identical with those seen in Fig. 1 (these mitochondria are a little smaller in volume: 0.28 and 0.16 μ^3 respectively). It will be noted that on the larger mitochondrion there are several outgrowths, consisting of distinctive pedicles, tapering toward the periphery, and which merge with an amorphous mass which is present in large quantities in the cytoplasm. This fusion, like the presence of quite clearly outlined mitochondria in individual conglomerates of this mass (Fig. 2, arrow), suggests that in this case we are seeing the final stage of synthesis of new mitochondria from the hyaloplasm.

Structures resembling cylinders 0.25 ± 0.01 μ in diameter, with rounded ends, and arranged both singly and in groups, were found in some cardiomyocytes (Fig. 3a). These formations were

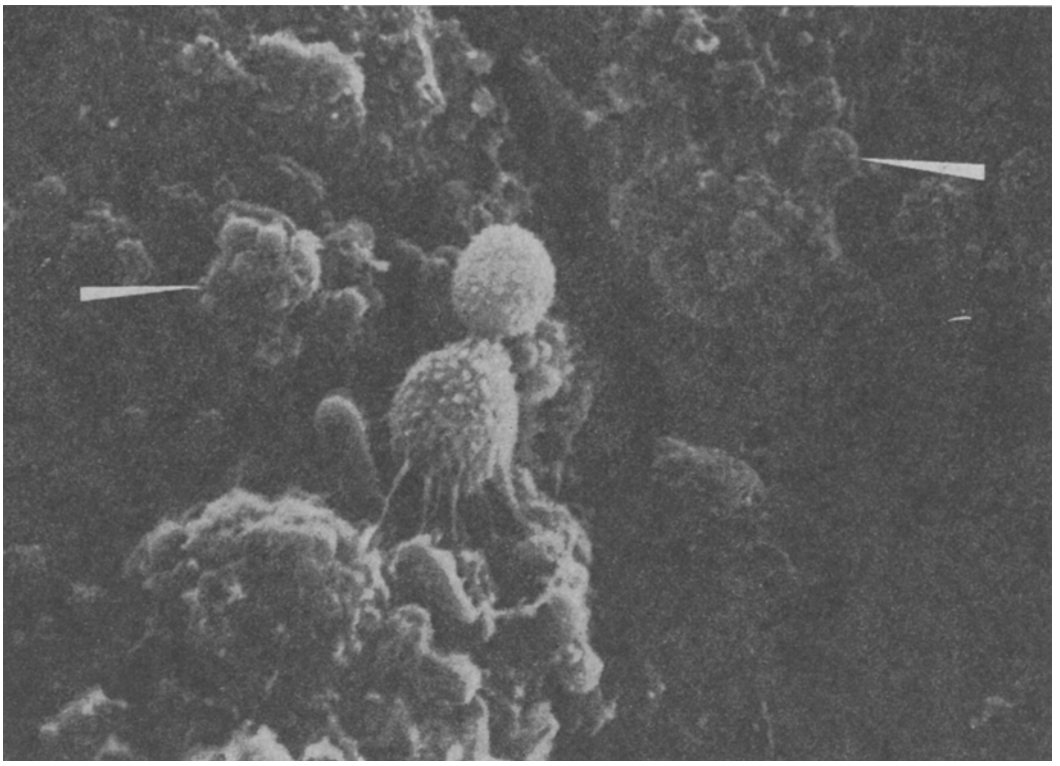

Fig. 2. Scanning electron micrograph of left ventricular myocardium of rabbit heart, 5000×. Explanation in text.

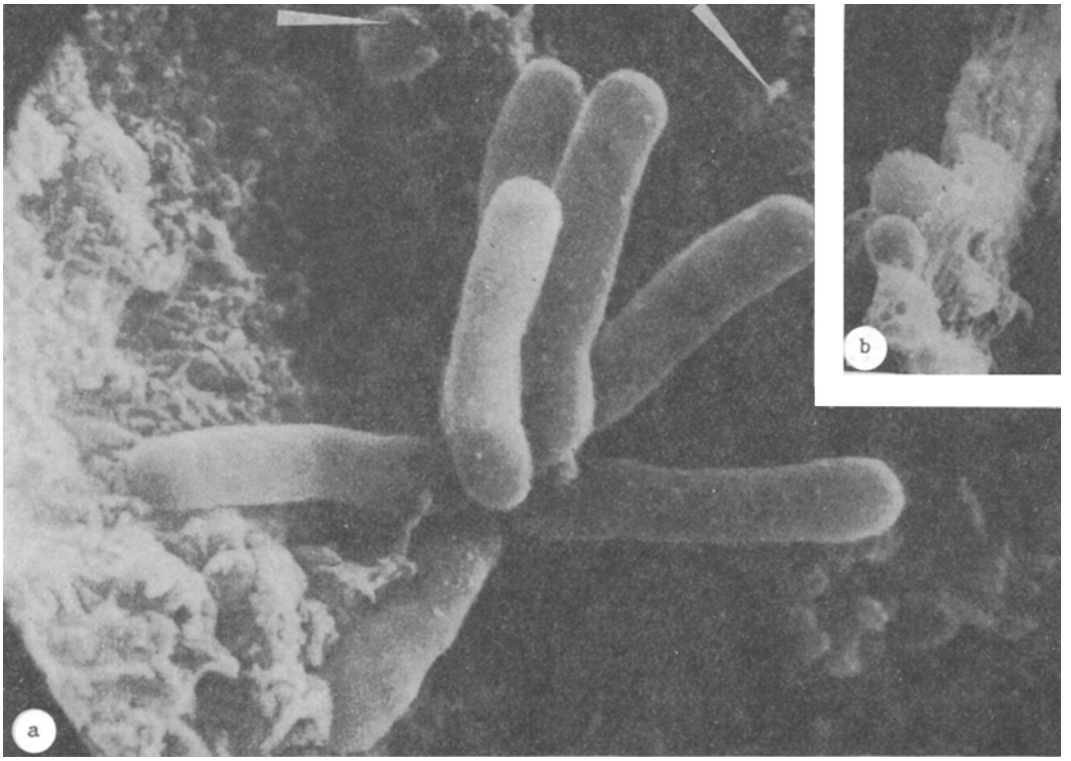

Fig. 3. Scanning electron micrograph of left ventricular myocardium of rabbit heart: a) group of cylindrical formations in a cardiomyocyte (10,000×); b) tubule of sarcoplasmic reticulum with mitochondria budding from it (15,000×). Explanation in text.

commensurate in diameter with the tubules of the sarcoplasmic reticulum in both transmission and scanning electron micrographs. Meanwhile, small mitochondria (arrows) can be seen in Fig. 2, and these also are commensurate in diameter with the cylindrical formations. A region of the sarcoplasmic reticulum with mitochondria budding from it is illustrated in Fig. 3b. The whole of this description suggests that mitochondria may be formed from tubules of the sarcoplasmic reticulum, a suggestion which does not contradict modern views on the role of the sarcotubular apparatus in the biosynthesis of some intracellular organelles and, in particular, in the "assembly" of lysosomes.

Four ways of reproduction of mitochondria in the myocardial cell are thus possible: division, separation by budding, synthesis *de novo* from hyaloplasm, and by constriction ring formation from tubules of the sarcoplasmic reticulum.

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CARDIAC REPERFUSION INJURIES AFTER ACUTE TRANSIENT CORONARY INSUFFICIENCY AND THEIR PREVENTION WITH MYOPHEDRINE

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Acute transient coronary insufficiency (ATCI) is characterized by a temporary regional decrease in the coronary blood flow in the heart followed by its renewal. Injury to the heart during ATCI arises in response not only to myocardial ischemia, but also to subsequent reperfusion [1, 6, 8, 11, 13]. Among the leading factors causing ischemic and reperfusion changes in the heart are catecholamines, whose concentrations in the myocardium are significantly raised in ATCI [3, 6]. It has been shown that under ACTI condtiions the cardiotoxic action of a high concentration of catecholamines can be prevented by the cardiotropic drug myophedrine, which has affinity for the adrenoreceptors of the heart [7, 9, 13].

The aim of this investigation was to study the morphogenesis of perfusion-induced changes in the myocardium after ischemia of varied duration, with and without treatment with myophedrine.

EXPERIMENTAL METHOD

Experiments were carried out on 56 noninbred male albino rats weighing 200 ± 10 g. ATCI was produced under urethane anesthesia (1200 mg/kg) and with artifical ventilation of the lungs with atmospheric air by temporary ligation and subsequent removal of the ligature from the descending branch of the left coronary artery [5]. Myophedrine (DL-methoxypropiophenone hydrochloride) was injected intraperitoneally 10 min before production of ATCI, in a dose of 0.5 mg/kg. Myocardial tissue for histologic and electron-microscopic investigations was taken 10, 40, and 120 min after application of the ligature to the artery and also at the 10th and 40th minutes of reperfusion, from the zone of injury (the anterior wall of the left ventricle) and from

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