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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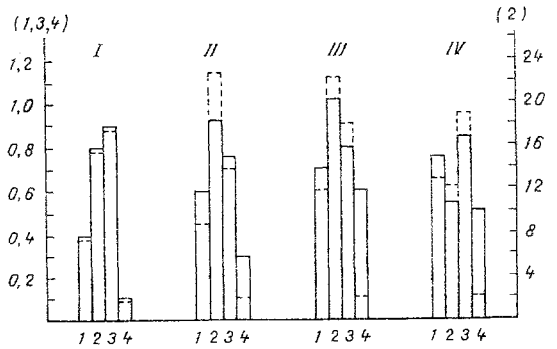

Fig. 1. Changes in vessels of microvascular bed during myocardial ischemia. I) Intact rats; II) ischemia for 10 min, III) for 40 min, IV) for 120 min. I) Average height of endothelium (in μ); 2) density of pinocytosis (in μ^2); 3) mean radius of vessel (in μ); 4) hematocrit number. Continuous line, zone of myocardial ischemia; broken line, distant parts of the heart. Changes in vessels of microcirculatory bed during ischemia.

distant parts of the heart (posterior part of the ventricular septum). Paraffin sections were stained with hematoxylin-eosin, by Lie's method (hematoxylin, basic fuchsine, picric acid) for detecting erythrocyte sludging, and by the PAS reaction. Pieces of myocardium for electron-microscopic investigation were fixed in 1% buffered OsO_4 solution, dehydrated in alcohols of increasing strength, and embedded in a mixture of Epon and Araldite. Semithin sections were cut on an LBK Ultratome, and strained with toluidine blue and with methylene blue—azure II-basic fuchsine. Ultrathin sections were stained with uranyl acetate and lead citrate and studied in the JEM-100B electron microscope. The state of the capillaries was studied quantitatively by noting the following parameters: mean height of the epithelium, density of distribution of pinocytotic vesicles in it, the mean radius of the blood vessels, and the ratio of the area of the blood cells to the area of the lumen of the vessel (the hematocrit number [2]). The results were subjected to statistical analysis by a parametric method.

EXPERIMENTAL RESULTS

Investigation of the ischemic and remote zones of the myocardium at different times of ischemia and reperfusion revealed successive stages of development of injury to the cardiomyocytes from reversible changes in cell ultrastructure after ischemia for 10 min to irreversible changes after 120 min.

After ischemia for 10 min moderate hydration of the cytoplasm and focal hypercontraction of the myofibrils appeared in the cardiomyocytes in the zone of reduced blood flow. After ischemia for 40 min marked dystrophic changes were observed in the cardiomyocytes in this zone, in the form of hydration of their cytoplasm, a reduction in the number of cytogranules, and widening and vacuolation of the sarcoplasmic reticulum; amorphous condensations were found in the matrix of the mitochondria of some cardiomyocytes, and are regarded as a manifestation of irreversible cell damage [12, 14]. After ischemia for 2 h most cardiomyocytes had signs of irreversible alteration. Together with necrotic cells, muscle cells with a relatively well preserved ultrastructure were found.

Changes in the capillaries of the ischemic myocardium were heterogenous. With an increase in the duration of ischemia edema of the endotheliocytes and sludging of the erythrocytes both increased in severity, and this was accomplished morphometrically by an increase in height of the endothelium and in the hematocrit number (Fig. 1).

In the distant parts of the heart the normal cardiomyocyte ultrastructure was largely preserved, but after 40 min of myocardial ischemia focal dilatation of the cisterns of the sarcoplasmic reticulum (SPR) was found in many cardiomyocytes; after 120 min of ischemia the dilatation of the cisterns of SPR was more widespread and, in addition, small foci of hypercontracted myofibrils appeared. Swelling of the endotheliocytes was observed in the capillaries, increasing with an increase in the duration of ischemia; the hematocrit number was virtually unchanged.

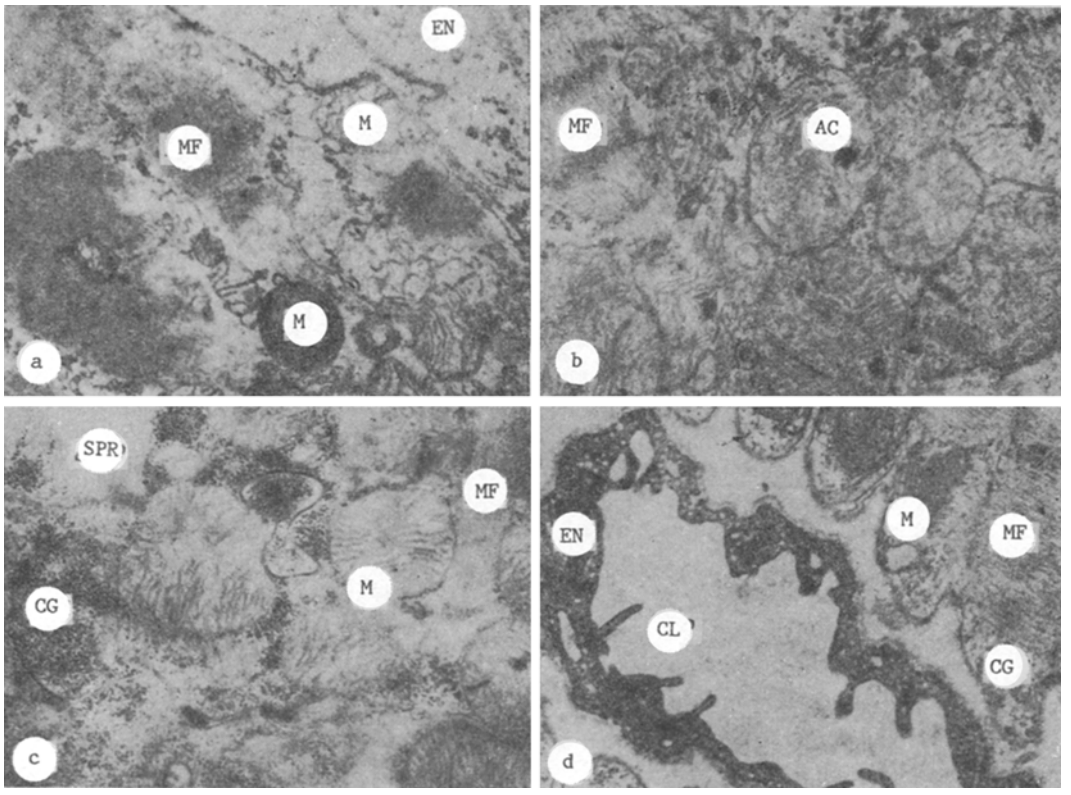

Fig. 2. Changes in cardiomyocyte ultrastructure after reperfusion without (a, b) and with administration of myophedrine (c, d). a, b) Myocardial ischemia for 120 min + reperfusion for 40 min: a) myofibrils (MF) in cardiomyocytes are grossly hypercontracted and lysed, endotheliocytes (EN) are swollen. Magnification 8000×; b) amorphous condensation (AC) present in mitochondrial matrix. 10,000×; c, d) myocardial ischemia for 120 min + reperfusion for 40 min: c) number of cytogranules in cardiomyocytes is increased, mitochondria rather translucent, their structure preserved, tubules of sarcoplasmic reticulum (SPR) dilated. 10,000×; d) capillary lumen (CL) wide, endotheliocytes have dense cytoplasm and contain many pinocytotic vesicles. 10,000×.

Investigation of the myocardial ultrastructure after restoration of the blood flow showed that the effect of reperfusion in the ischemic and distant zones was formed by formation of its injurious and reparative actions [10, 11]. The injurious action of reperfusion was manifested as aggravation of injuries to the cardiomyocytes and microvessels, discovered during ischemia, and it depended directly on the times of ischemia and inversely with the times of perfusion. Moderate hydration of the cardiomyocytes after ischemia for 10 min became severe after 10 min of reperfusion, and at this stage of restoration of the blood flow an increase in the number of foci of hypercontraction of the myofibrils was observed. After 10 min of reperfusion, after 40 min and, especially, after 120 min of ischemia, an increase in hydration, destruction, and hypercontraction of the myofibrils and necrosis of the cardiomyocytes were observed. Changes in the vessels of the microcirculatory bed after reperfusion took the form of more severe swelling of the endotheliocytes, widening of the lumen, and increased stasis of blood.

The restorative effect of reperfusion was found after 40 min, to an extent which depended inversely on the duration of ischemia: signs of damage to myocardial cells caused by ischemia for 10 min disappeared almost completely, and large numbers of polyribosomes and much glycogen appeared in the sarcoplasm of the cells. After 40 min of ischemia followed by 40 min of reperfusion only slight improvement of the ultrastructure of most cardiomyocytes was observed. Reperfusion after 120 min of ischemia did not lead to restoration of cardiomyocyte ultrastructure but, on the contrary, caused an increase in the number of necrotic cells with multiple amorphous cendensations in the mitochondria (Fig. 2a, b).

In the distant zone of myocardium structural features of activation of compensatory and adaptive processes were observed during reperfusion: Most cardiomyocytes of this zone appeared

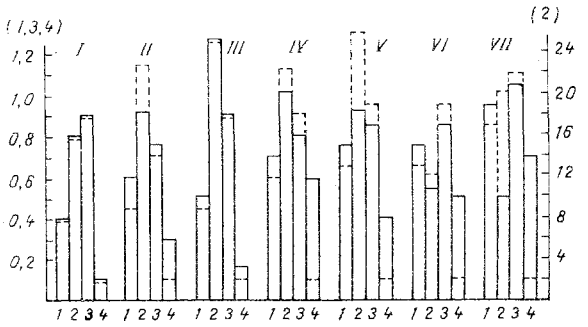

Fig. 3. Changes in vessels of microcirculatory bed during myocardial ischemia and subsequent reperfusion. I) Intact rat; II) ischemia for 10 min; III) ischemia for 10 min + reperfusion for 40 min; IV) ischemia for 40 min; V) ischemia for 40 min + reperfusion for 40 min; VI) ischemia for 120 min; VII) ischemia for 120 min + reperfusion for 40 min. Remainder of legend as to Fig. 1.

dark and contained large numbers of cytogranules, including ribosomes; the nuclei were large and the mitochondria had a dark matrix and blurred cristae. At the same time individual cardiomyocytes with damage to their ultrastructures characteristic of hypoxia were found. The results of morphometry of the microvessels in the distant zone showed that changes in the morphometric parameters at all stages of myocardial ischemia and reperfusion reflected the presence of hypoxia in this zone of the myocardium (Fig. 3).

Investigation of the myocardial ultrastructure at different times of ischemia and reperfusion, after preliminary administration of myophedrine revealed structural evidence of a protective effect of the drug, namely a decrease in the degree of hydration of the cardiomyocytes and injury to vessels of the microcirculatory bed in the ischemic and remote zones of the myocardium. When myophedrine was used, even after prolonged ischemia (40 and 120 min) followed by reperfusion, besides injured cells many cardiomyocytes with intact ultrastructure were present in the zone of ischemia, and they contained an increased number of cytogranules. The fact will be noted that after administration of myophedrine the cell membranes of the cardiomyocytes and endotheliocytes were more osmiophilic, both in ischemia and, in particular, after restoration of the blood flow (Fig. 2c, d). In the distant zone after ischemia for 40 min and

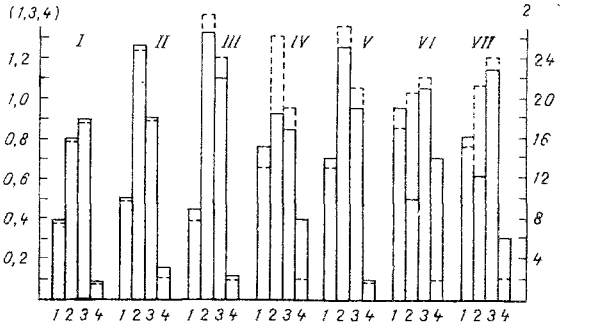

Fig. 4. Changes in vessels of microcirculatory bed in myocardial ischemia followed by reperfusion after preliminary administration of myophedrine. I) Intact rats; II) ischemia for 10 min + reperfusion for 40 min; III) myophedrine + ischemia for 10 min + reperfusion for 40 min; IV) ischemia for 40 min and reperfusion for 40 min; V) myophedrine + ischemia for 40 min + reperfusion for 40 min; VI) ischemia for 120 min + reperfusion for 40 min; VIII) myophedrine + ischemia for 120 min + reperfusion for 40 min. Remainder of legend as to Fig. 1.

120 min, under conditions of myophedrine premedication, changes reflecting, in our view, the so-called metabolic effect of this drug on the myocardium, were discovered. This was manifested by signs of intensified biosynthesis: concentrations of large glycongen granules were found in the cardiomyocytes alongside dilated cisterns of SPR, many ribosomes and polysomes, and also some condensation of the mitochondrial matrix and membranes.

The protective effect of myophedrine was exhibited most clearly in the state of the microvessels of the ischemic and distant zones of the heart was estimated quantitatively (Fig. 4). A reduction of hydration of the cells and an increase in the osmiophilia of their membranes were evidently due to the stabilizing effect of myophedrine on the cell membranes. This stabilization may be the result of depression of peroxidation processes in the membrane due to the antioxidant activity of this drug [4].

The results of this investigation thus suggest that myophedrine premedication in ATCI has a twofold protective action on myocardial ultrastructure: membrane-stabilizing and metabolic. The membrane-stabilizing effect is manifested in the ischemic zone and leads to reduced hydration of the cardiomyocytes and a reduced degree of hypercontraction of the myofibrils, reduced swelling of the endotheliocytes of the capillaries and a decrease in the number of erythrocytes in them. The metabolic effect was manifested chiefly in the zone remote from the injured area, as an increase in size of the nuclei, in the glycogen content, in the number of polysomes and ribosomes, and also in the density of the mitochondrial matrix of the cardiomyocytes.

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