MORPHOLOGY AND PATHOMORPHOLOGY

Chondriome Ultrastructure of Rat Cardiomyocytes After Apparent Death and in the Postresuscitation Period

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Twelve-minute blockade of blood circulation caused different changes in cardiomyocyte organelles, particularly in the mitochondria. The initial cardiomyocyte structure was restored within 3.5 h of the postresuscitation period. Ultrastructural changes in cardiomyocytes were observed again 1 month after resuscitation. They disappeared after 5 months.

Key Words: *cardiomyocytes, ultrastructure, mitochondria, apparent death, reanimation*

Investigation of basic processes of myocardial adaptive reactions is important for the understanding of some pathological processes. Study of changes occurring in the body during the postresuscitation period (PRP) is important because numerous clinical and experimental data show that various structural and functional disturbances persist for a long time (weeks and months) after terminal state [9].

There is evidence that apparent death is accompanied by insufficient blood circulation resulting from myocardial contractility disturbances and leading to irreversible changes in tissues and organs [2]. Therefore, it is important to study the reparation processes occurring in the myocardium during PRP as well as to determine the reversibility of pathological changes and the periods of ultrastructural normalization. The information on myocardial changes during the early PRP is scarce [4, 5]. The purpose of the present study was to examine cardiomyocyte ultrastructure immediately after apparent death and in the late postreanimation period.

MATERIALS AND METHODS

Outbred male albino rats (body weight 180-200 g) were used. Apparent death lasting for 12 min was achieved by complete ligation [7] of the heart vascular bundle with a special hook without thorax opening. The animals were resuscitated by indirect heart massage, artificial lung ventilation with air, and intratracheal injection of 0.1 mg/kg norepinephrine.

Left and right ventricle myocardium was collected for examination on the 12th min of apparent death, 3.5 h after it and after 1 and 5 months of PRP.

The tissue was fixed in 4% glutaraldehyde, postfixed in 1% OsO_4 and embedded in Epon. Ultrathin sections were counterstained by uranyl acetate and lead citrate and examined in a JEM-100B electron microscope.

RESULTS

The reaction of cardiomyocytes was the same in the right and left ventricles and remained unchanged throughout the entire observation period. Since the mitochondria (MC) are the key system reacting to functional changes in the myocardium [11-13], their ultrastructure was examined with special attention.

In normal cardiomyocytes MC have densely packed parallel cristae and are arranged in rows along myofibrils, in the perinuclear and subsarcolemmal zones (Fig.1, *a*). In cardiomyocytes MC are joined into a common system via special intermitochondrial contacts (IMC) [1] (Fig.1, *b*).

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The blockade of blood circulation caused destructive changes in the myocardium. Morphological heterogeneity of cardiomyocytes as well as local and mosaic structural changes, manifested as different degrees of cell destruction were observed. In some cells sarcoplasmic edema, swelling of the sarcoplasmic reticulum, and microlysis and thinning of myofibrils occurred. Chromatin condensation developed in the nucleus, the nucleus borders became uneven, and large secondary lysosomes appeared in the perinuclear zone. Chondriome was significantly changed: MC showed acute swelling, matrix transparency, chaotic orientation of the cristae, and a decrease in their number. In some cells MC preserved orthodox conformation but their cristae lost parallel orientation and became curved and fragmented. In some cardiomyocytes both normal and swollen MC were seen (Fig. 2). IMC were present in the cells with orthodox MC but were absent from cardiomyocytes with swollen MC.

Mitochondrial swelling is a typical reaction occurring in various pathological states. For example, it was observed in chronic cardioischemia [11] and alcoholic cardiomyopathy [12]. Similar ultrastructural changes in cardiomyocytes were found after sudden death in man [14].

The differences in cardiomyocyte changes probably reflect structural and functional heterogeneity of the normal myocardium that can be manifested in varying content of redox enzymes, glycogen and ribosomes in cardiomyocytes [10].

Similar heterogeneity of the cardiomyocyte reaction has been observed in myocardial infarction, adaptation to hypoxia [13], and alcoholic cardiomyopathy [12]. Both intact and pathologically changed cardiomyocytes were observed after sudden death [14].

It is believed that heterogenous cardiomyocyte reactivity is based on cyclic renewal of intracellular structures. Cell vulnerability of damaging factors is different at different stages of he cycle [10].

Reparative changes in the cardiomyocyte ultrastructure were observed as soon as after 3.5 h of PRP. The nucleus borders became even, chromatin condensation was less pronounced, and sarcoplasmic swelling decreased in comparison with cardiomyocytes examined immediately after apparent death. In addition, considerable amount of small MC was observed in the perinuclear zone, while swollen MC disappeared. The majority of MC had the orthodox conformation and were connected by IMC. Nevertheless, the orientation of cristae changed significantly: they lost parallel arrangement and became sinuous, curved and fragmented with anastomoses between fragments. Some cristae were seen as a net on the section (Fig. 3).

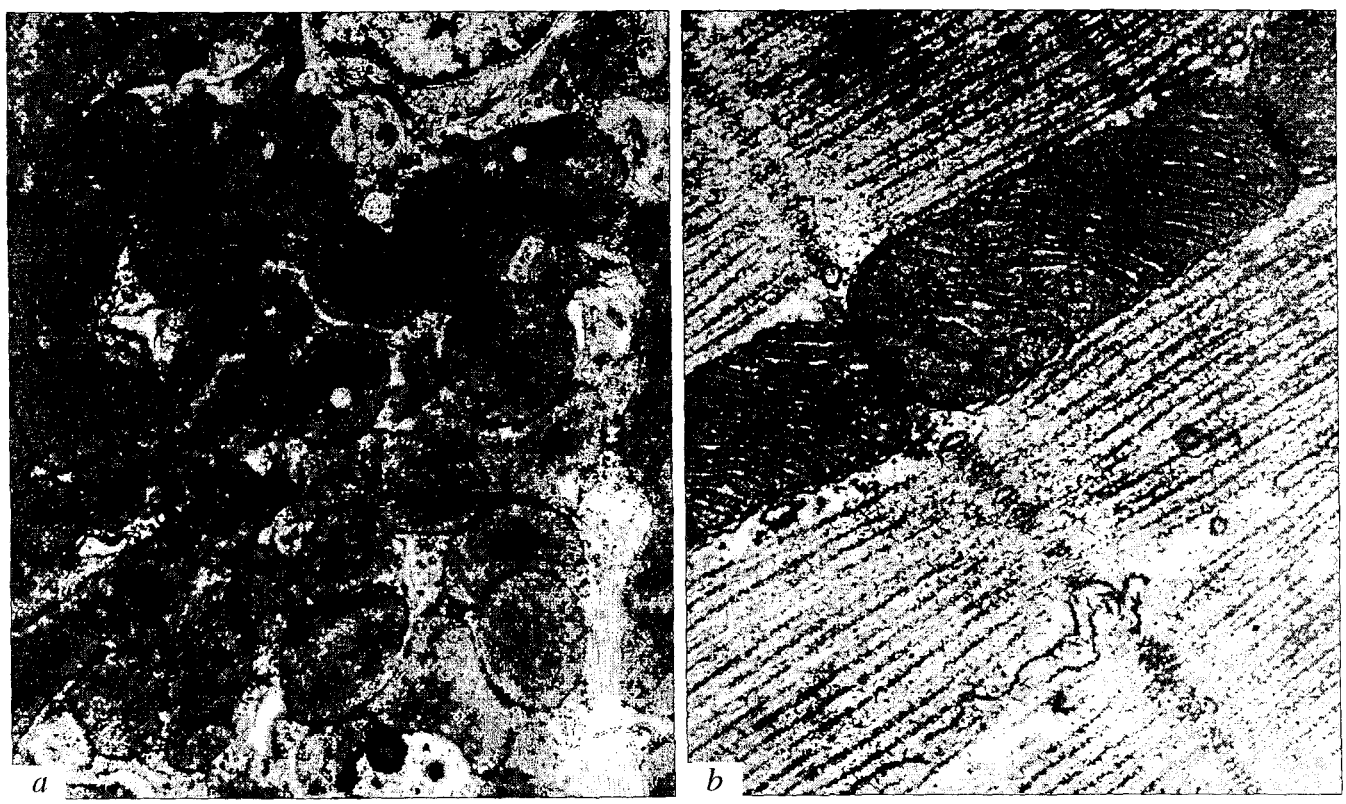

Fig. 1. Perinuclear mitochondrial zone (*a*), ×16,000) and intermitochondrial contacts (*b*), ×50,000) in control cardiomyocytes.

Fig. 2. Heterogeneity of mitochondria in different cardiomyocytes after 12 min apparent death. Here and in figs. 3-5: ×16,000.

Similar changes of MC cristae were observed in various cardiopathological states such as alcoholic cardiomyopathy [12], chronic cardioischemia [11], and 6 h of PRP in dogs [5].

Although biochemical data point to the restoration of respiration rate and phosphorylation to the control levels within 1-1.5 h of PRP [15] complete recovery of MC structure was not achieved after 3.5 h of PRP.

Reparation of the MC system observed after 3.5 h of PRP is in good agreement with physiological data pointing to the normalization of central hemodynamics in dogs during 2-4-h PRP [2].

Nevertheless, the recovery was not permanent. One month after PRP, the left- and right- ventricle cardiomyocytes were morphologically heterogenous, which was observed immediately after apparent death. Some cells retained the ultrastructure typical of normal cardiomyocytes. Their MC had the orthodox conformation and contained densely packed cristae. Some small MC were located not only in perinuclear zone, but also intrafibrillary and in the perivascular zone, which points to reparative processes in cardiomyocytes. Some cells contained MC with chaotically oriented cristae that formed a network similar to that observed after 3.5 h of PRP (Fig. 4, *a*).

The ultrastructure of other cells reflects pathological processes in the myocardium. Nuclear chromatin condensation, sarcoplasmic swelling, thinning and separation of myofibrils, and microlysis of microfilaments followed by destruction of some sarcomeres were observed in these cells. Ultrastructurally, two types of swollen MC were defined: (1) MC with transparent matrix, destruction of cristae and a decrease in their number and (2) MC with preserved matrix with cristae located at a long distance from each other. IMC could be observed only in the cells with normal ultrastructure, they were absent in cardiomyocytes with swollen MC (Fig. 4, *b*).

Previously, MC swelling was shown in rat hypothalamic neurons at the late stages (6 weeks) of PRP [16].

It is believed that MC swelling is associated with the disturbances in the internal membrane permeability and opening of nonspecific calcium-dependent channels. It was shown that activation of lipid peroxidation (LP) in PRP leads to disturbances of cell membrane permeability and excessive calcium influx into cardiomyocytes [3, 9]. The swelling of cardiomyocyte MC may reflect the intercellular processes which are induced by hypoxia during apparent death and then by subsequent recirculation and reoxygenation. This can

Fig. 3. Ultrastructure of perinuclear mitochondria 3.5 h after resuscitation.

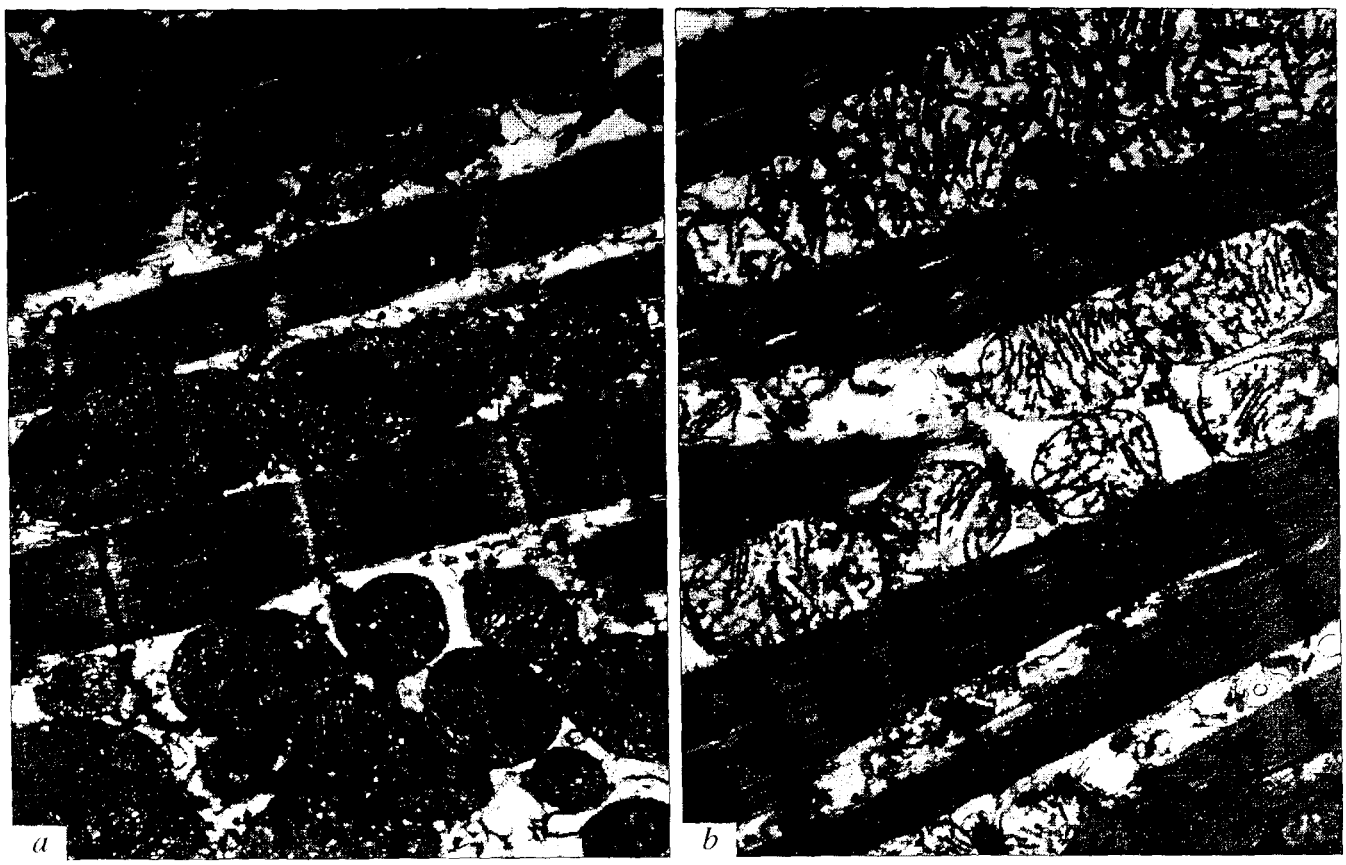

Fig. 4. Heterogeneity of normal (*a*) and swollen (*b*)mitochondria in different cardiomyocytes 1 month after resuscitation.

be regarded as morphological reflection of so called "postresuscitation disease" [9].

It was shown that myocardial ultrastructure normalizes within 2 weeks of PRP [4, 5]. The discrepancy between these data and our data can be explained by the species-specific differences and by prolongation of apparent death. The effectiveness of reanimation depends on the time of apparent death, being 87, 65, 40 and 20% at 5, 10, 15, and 20 min after death respectively [7]. The degree of postreanimation ultrastructural damage to rat hypothalamic neurons was shown to depend on the time of apparent death [16]. It can be suggested that the degree and duration of postresuscitation myocardial pathological changes depend on the time of apparent death.

After 5 months of PRP, the ultrastructure of cardiomyocytes normalized. Mitochondrial matrix of medium electron density was packed with cristae. In some cells MC had partially fragmented cristae. Perinuclear and perivascular MC clusters consisted of small MC. In all cell subpopulations, MC were connected by IMC (Fig. 5, *a*).

Although in the majority of animals cardiomyocyte chondriomes normalized after 5 months of PRP some of them showed heterogeneity which manifested itself at different stages of reparation of the cardiomyocyte mitochondrial system. Some cells had normal ultrastructure and contained normal mitochondria. Other cells showed heterogeneity in MC structure: together with normal cristae these MC contained large vacuoles with membrane-bound myelin-like structures (Fig. 5, *b*). These changes are probably associated with individual reaction to apparent death and with reparative capacity of the body. There is evidence that the degree of stress-induced destructive changes in rat myocardium is individual and depends on animal's emotional state and its position in hierarchy [6]. It was shown that the lethality in rats after a 12-min circulation blockade is 53% [8]. In the other study [3] dealing with immunological aspects of postreanimation myocardium lesion the rats with moderate or pronounced changes in the immune system were selected. These data point to the individual reaction of a body to terminal state and subsequent PRP.

Thus, our results showed that 12-min blockade of blood circulation in rats causes morphologically heterogenous changes in cardiomyocytes, in their mitochondrial system. In the course of PRP, rapid (3.5 h) recovery of cardiomyocyte structure is followed by significant ultrastructural which normalize after 5 months of PRP.

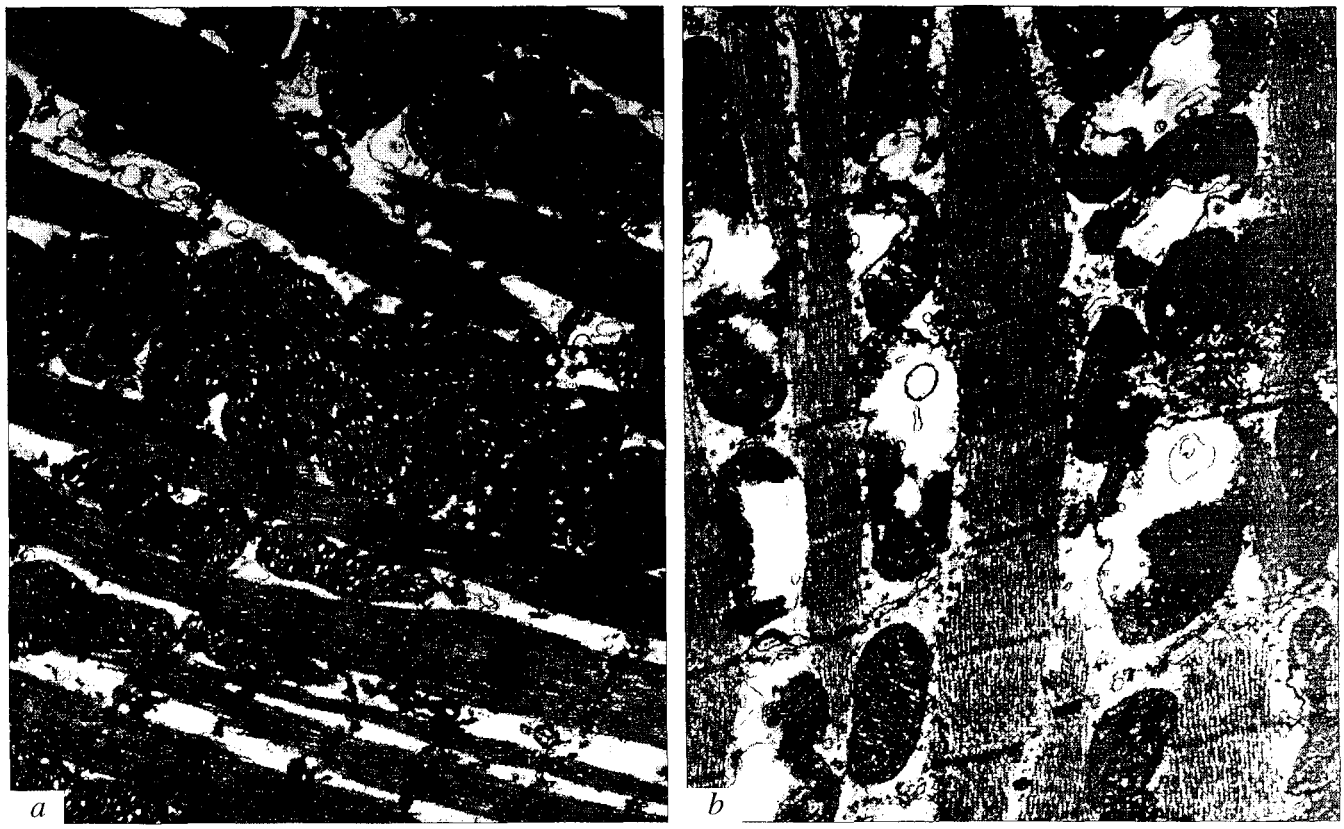

Fig. 5. Ultrastructural recovery (*a*) and local swelling (*b*) of mitochondria 5 months after resuscitation.

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