Ultrastructure of Rabbit Myocardium in the Late Period of Immobilization Stress

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Ultrastructure of working myocardium was studied in rabbits subjected to 70-day immobilization in narrow cages excluding active movements. The structure of working cardiomyocytes, nervous apparatus, and microvascular bed in auricles were studied. Particular attention was focused on intercalated disks in muscle fibers, where separation of *fasciae adherents* was observed, while desmosomes in the lateral area of these contacts were preserved. The role of these findings in triggering of discoordinated contractions of rabbit auricular cardiomyocytes is discussed.

Key Words: *myocardial ultrastructure; hypokinesia*

Experimental hypokinesia negatively affects the functional state of basic organospecific animal systems, including the cardiovascular and endocrine ones [9, 11]. Particular attention was given to the action of the heart, which determines the general state of living organism [2,10]. However, few studies directly analyzed the structure and, particularly, ultrastructure of this organ during hypokinesia, which is a risk factor of many cardiovascular diseases in men [1,7,12]. In many studies, the development of electrical instability of the heart is explained by disturbances in myocardial architectonics and interaction of cardiomyocytes effected via specialized cellular contacts [4,8, 13,14]. Since ultrastructure of rabbit myocardium during immobilization stress (IS) is little studied, our aim was to clarify this point.

MATERIALS AND METHODS

The specimens were taken from the auricles of intact Chinchilla rabbits (n=5) and rabbits (n=5) subjected to 70-day IS. For IS modeling the rabbits were kept in tight metal cages made according to body size and form and practically excluding animal movements [9]. The rabbits were sacrificed by injection of 3% procaine into the posterior marginal vein. The preparations for electron microscopy were prepared as described previously [5,6]. Tissue specimens were fixed in aldehyde fixative and postfixed in OsO_4. The specimens were embedded in epoxy resins and examined under a JEM-100CX electron microscope operated at 80 kV.

RESULTS

Cardiomyocytes isolated from rabbits subjected to IS contained numerous vacuoles surrounded by small atrial granules (AG). No pronounced alterations of myofibrils were observed in these cells; mitochondria were condensed and varied in size, large AG clusters were located between them (Fig. 1, *a*). Some myofibrils were contracted, especially in the subsarcolemmal parts of the cells. The sarcolemma formed scalloped processes into the connective tissue matrix. The cytoplasm contained coated vesicles, enlarged T-tubules, and glycogen granules (Fig. 1, *b*). AG clusters with various degree of osmiophilia were seen in the subsarcolemmal cytoplasm. Sometimes these granules were located extracellularly in the connective tissue matrix, which attested to exocytosis of these organel-

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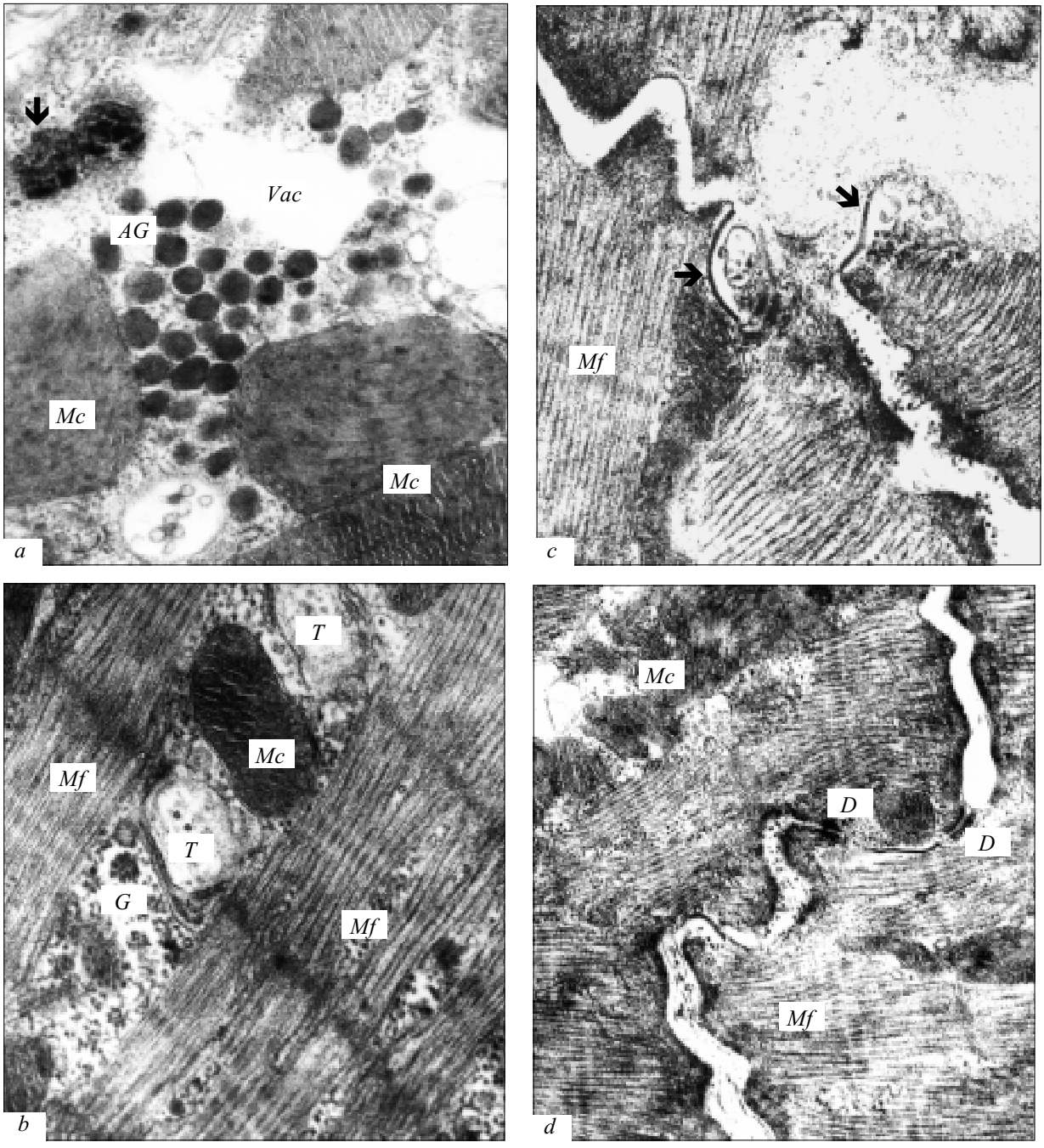

Fig. 1. Changes in working auricular cardiomyocyte in rabbits subjected to 70-day immobilization stress. *a*) working myocyte from the left auricle of experimental rabbit. Arrow shows lysosome, ×33,100; *b*) dilated T-tubules (*T*) and glycogen granules (*G*) located between myofibrils (*Mf*) in a working myocyte ×40,000; *c*) moderate separation of *fasciae adherents* in intercalated disk. The arrows show nexuses, ×49,200; *d*) intact desmosomes (*D*) in the lateral part of the intercalated disk separated along *fasciae adherents,* ×28,300. *Mc*: mitochondria, *AG*: atrial granules, and *Vac*: vacuole.

les during chronic hypokinesia. The most pronounced changes were observed near the intercalated disks. In some myocytes these changes were minor, while in others they were pronounced (Fig. 1, *c*, *d*; Fig. 2, *a*, *b*). In some myofibrils, separation of intercalated disks between the adjacent cells along *fasciae adherents* was observed (Fig. 1, *c*). However, the nexuses of these cells and the basal membrane surrounding the myofibril remained intact. Relatively intact desmosomes were observed in the lateral part of intercalated disks (Fig. 1, *d*). Membrane structures and glycogen granules were seen in the clefts between neighboring myocytes forming end-to-end contacts (Fig. 2, *a*). In these clefts some nexuses in the lateral parts of inter-

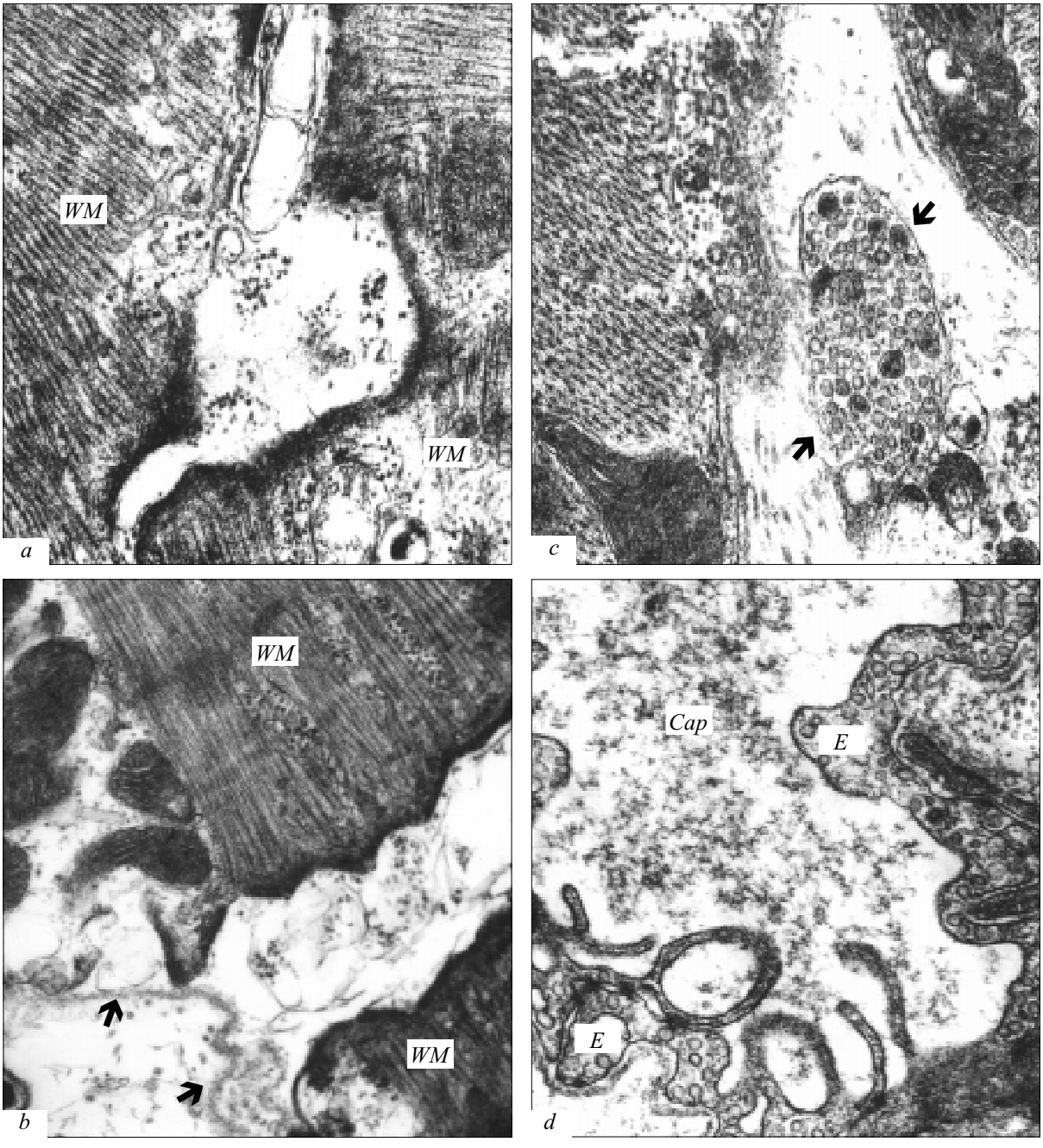

Fig. 2. Changes in interstitium, nerve fibers, and capillaries in working myocardium from rabbits subjected to 70-day immobilization stress. *a*) membrane structures and glycogen in the cleft between separated working myocytes (*WM*), ×53,300; *b*) intact basal membrane (arrows) around myofibril consisting of CM separated along the intercalated disk, ×27,000; *c*) nerve terminal (arrows) near auricular WM. Preserved synaptic vesicles, ×43,300; *d*) endotheliocyte (*E*) processes of various shape protruded into capillary lumen (*Cap*), ×32,900.

calated disks were located near the plasmalemma of only one myocyte. In other myofibrils, the clefts between cells forming the end-to-end contacts were more pronounced, but the integrity of basal membrane was not disturbed (Fig. 2, *b*). These alterations attest to disturbance of contractile function in some myofibrils of auricular myocardium in rabbits subjected to long-term hypokinesia, although some individual myocytes in these myofibrils preserved their contractile capacity.

Ultrastructural analysis showed that some efferent nerve terminals located near myofibrils and capillaries contained a large number of small agranular vesicles (Fig. 2, *c*), while other terminals were empty. Moreover, the terminals contained intact mitochondria and few vacuoles. In capillaries of working myocardium luminal membranes of endotheliocytes formed long or ring-shaped processes (Fig. 2, *d*). At the same time, the contact structures of these endotheliocytes remained

intact. Similar alterations in myocardial capillaries were observed in the heart with disturbed innervation [3,10]. This probably implies neurotrophic nature of observed changes in the myocardium. This hypothesis is corroborated by the fact that the early period of hypokinesia is characterized by enhanced release of catecholamines from adrenal medulla [11].

REFERENCES

1. L. A. Bokeriya, E. Z. Golukhova, E. R. Pavlovich, and I. P. Polyakova, *Vestn. Ross. Akad. Med. Nauk*, No. 5, 3-9 (1996).
2. E. A. Kovalenko and N. N. Turovskii, *Hypokinesia* [in Russian], Moscow (1980).
3. E. R. Pavlovich, *Morphological Analysis of Basic Subdivisions of the Conducting System in Intact and Vagotomized Heart*, Abstract of Cand. Biol. Sci. Dissertation, Moscow (1977).
4. E. R. Pavlovich, in: *Biological Membranes* [in Russian], Saransk (1984), pp. 103-106.
5. E. R. Pavlovich, in: *Actual Problems of Medical Morphology* [in Russian], Issue 2, Pt. 3, Izhevsk (1993), pp. 452-456,.
6. E. R. Pavlovich, *Tsitologiya*, **40**, Nos. 2-3, 119-126 (1998).
7. E. R. Pavlovich, *Ibid.*, **40**, No. 11, 934-942 (1998).
8. P. P. Rumyantsev, *Cardiomyocytes in the Processes of Reproduction, Differentiation, and Regeneration* [in Russian], Leningrad (1982).
9. V. V. Tyavokin, *Hypokinesia and Cardiovascular Pathology* [in Russian], Saransk (1975).
10. I. A. Chervova and E. R. Pavlovich, *Byull. Eksp. Biol. Med.*, **84**, No. 9, 378-381 (1978).
11. V. N. Shvalev, R. A. Kargina-Terent'eva, K. L. Mar'yan, and E. R. Pavlovich, *Arkh. Anat.*, **89**, No. 8, 97-102 (1985).
12. K. Kawamura and T. N. James, *J. Moll. Cell. Cardiol.,* **3**, No. 1, 31-60 (1971).
13. N. J. Severs, *Int. J. Cardiol.*, **26**, No. 2, 137-173 (1990).
14. J. R. Sommer, *J. Mol. Cell. Cardiol.*, **27**, No. 1, 19-35 (1995).