MYOCARDIAL ULTRASTRUCTURE OF RATS DIFFERING IN RESISTANCE TO HYPOXIA, AFTER ACUTE AND PERIODIC EXPOSURE TO IT

L. D. Luk'yanova, N. N. Kleimenova, V. N. Arefolov, and A. M. Dudchenko

UDC 616.127-092:612.273.2]-092.9-076

Key words: hypoxia; energy metabolism; cardiomyocytes; resistance.

Differences in the ability of animals to tolerate acute hypoxia [1], which correlates with the resistance of different organs and even cells [2-6, 8], and also with differences in their ability to adapt to hypoxia [4, 8, 10], are based on biochemical mechanisms. Animals with high (HR) and low (LR) resistance differ in the degree of tone of their sympathicoadrenal system, in the acid—base and other properties of the blood, the free-radical activity of their tissues, the state of their energy metabolism, and activity of various enzymes [10]. For example, initially different energy substrates and metabolic pathways of their oxidation are utilized in the myocardium of HR and LR rats, and this accounts for the much faster disturbance of synthesis of high-energy compounds and of the contractile function of the heart in LR compared with HR animals, i.e., their resistance in oxygen-deficiency states [2, 3, 5, 6]. Under conditions of long-term exposure to periodic hypoxia, most biochemical processes undergo transformation, with the breakdown of old mechanisms and their replacement by new, leading to a radical reorganization of the work and energy apparatus and to economy of energy metabolism. This process also takes place differently in HR and LR rats [7-10]. These facts suggest the possibility of ultrastructural differences in the tissue cells of these animals.

The aim of this investigation was to study the submicroscopic features of cardiomyocytes in order to discover differences in the structural organization of the cell responsible for the formation of its individual resistance to hypoxia, and of fundamental importance for maintenance of the functional—metabolic status of the myocardium in hypoxia.

EXPERIMENTAL METHOD

Noninbred male rats weighing 180-200 g, divided beforehand into groups of HR and LR animals on the basis of their "elevation" in a pressure chamber to an altitude of 11,000 m and determination of the survival time (T_s) before the second agonal inspiration, were used in the experiments. T_s of the LR rats was 1-3 min, compared with 10 min or more for the HR rats. Some of the HR and LR animals were adapted to periodic hypoxia (5-6 h daily at 5000 m for 25 days), and their T_s was tested on the 25th day; in the group of LR rats it was increased on average by 3-3.5 times to 8-9 min, whereas in the HR group it was unchanged or even reduced by 15-20%. Thus differences between LR and HR rats with respect to the value of T_s were sharply reduced. For electron-microscopic investigation of the myocardium, HR and LR rats were taken before and after long-term adaptation and control and adapted animals were used immediately after acute "elevation" (to 11,000 m) in the pressure chamber. Pieces of the anterior wall of the left ventricular myocardium (1 × 1 mm) were fixed for 1 h in 1% glutaraldehyde solution in 0.2 M phosphate buffer, and then fixed in 1% OsO_4 in the same buffer (pH 7.4) for 2 h. The tissue was embedded in Araldite and sections were stained with a 2% solution of uranyl acetate and lead citrate by Reynold's method. Sections were examined in the IEM-100B electron microscope.

Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 148-151, August, 1991. Original article submitted January 18, 1991.

0007-4888/91/0008-1103$12.50

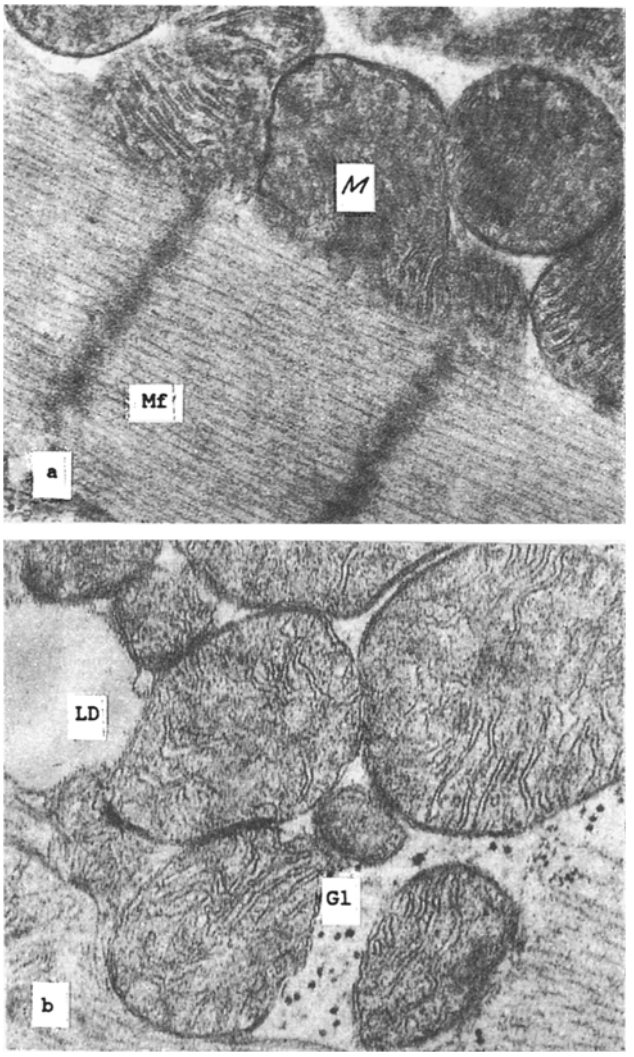

Fig. 1. Ultrastructure of cardiomyocytes of LR and HR rats under normoxic conditions: a) reduced number of granules and lipid droplets in cytoplasm of cardiomyocytes of LR rats. M) Mitochondria; Mf) myofibrils. 35,000×; b) Glycogen granules (Gl) and lipid droplets (LD) in cytoplasm of cardiomyocytes of HR rats. 60,000×.

EXPERIMENTAL RESULTS

Electron-microscopic investigation of the myocardium of intact (control) HR and LR rats showed no difference in the structure of their nuclei, nucleoli, mitochondria, endoplasmic reticulum (EPR), and sarcolemma of the cardiomyocytes. Their shape, structure, density, and arrangement in the cytoplasm did not differ from those described in the literature, and were the same in the two groups of animals. However, the number of polysomes and the number of glycogen and lipid granules in the cardiomyocytes of the HR rats was significantly greater than in the myocardial cells of the LR rats (Fig. 1a, b). These data indicate an initially different intensity of the working of the protein-synthesizing system, and also of glycogenolysis (or glycolysis) and lipolysis in the myocardium of the HR and LR rats. This confirms and supplements data obtained by the writers previously concerning differences in activity of the two principal pathways of oxidation of the energy substrates of the respiratory chain [2, 3, 5, 6]. In light of these findings, we can evidently regard the relatively high glycogen and lipid concentrations in the myocardium of the HR rats compared with LR as a reflection of the lower intensity of oxidation of carbohydrates and fatty acids under normoxic conditions.

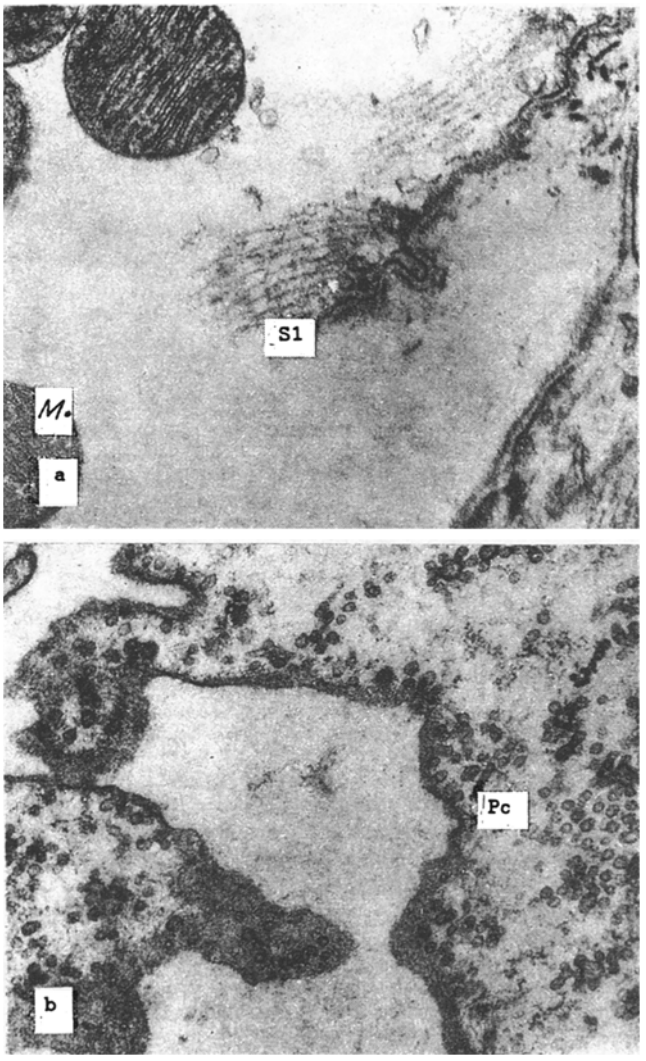

Fig. 2. Cardiomyocyte ultrastructure of LR and HR rats following single exposure to hypoxia ("elevation" to 11,000 m): a) rupture of sarcolemma (Sl) and outflow of mitochondria (M) into intercellular space. 23,000×; b) increase in number of pinocytotic vesicles (Pc) in cytoplasm of endothelial cells of capillaries. 60,000×.

The hypoxic load ("elevation" in the pressure chamber to an altitude of 11,000 m) of both LR and HR rats did not lead to changes in structure of the mitochondria and myofibrils in the cardiomyocytes. Meanwhile, the number of vesicles in the nucleus was increased (to two or three), hypertrophy of the EPR was observed, especially of the T-system, and the number of polysomes and elements of the rough endoplasmic reticulum was increased. After a single acute "elevation" to an altitude, rupture of the sarcolemma and release of the mitochondria into the intercellular space were frequently observed (Fig. 2a). The endothelial cells of the capillaries were swollen, leading to narrowing of the capillary lumen, and indicating disturbance of osmoregulation, and the number of microvilli was considerably increased. There was a sharp increase in the number of mitochondria, ribosomes, and pinocytotic vesicles in the cytoplasm of the endothelial cells (Fig. 2b), evidence of activation of exchange between the blood and cardiomyocytes and intensification of synthetic processes. These changes were expressed more strongly in the myocardium of LR than of HR rats. Meanwhile, glycogen granules and lipid droplets almost completely disappeared in the cardiomyocytes of the LR rats, and their numbers were reduced in the cells of HR rats (Fig. 2). Thus a single exposure to hypoxic stress did not affect the structure of the nuclei and mitochondria, evidence of the high resistance of the genetic and energy-producing apparatus to it. Meanwhile, the marked activation of the protein-synthesizing system is evidence that even a single exposure to high altitude hypoxia can leave a "trace" in the cell memory, and this

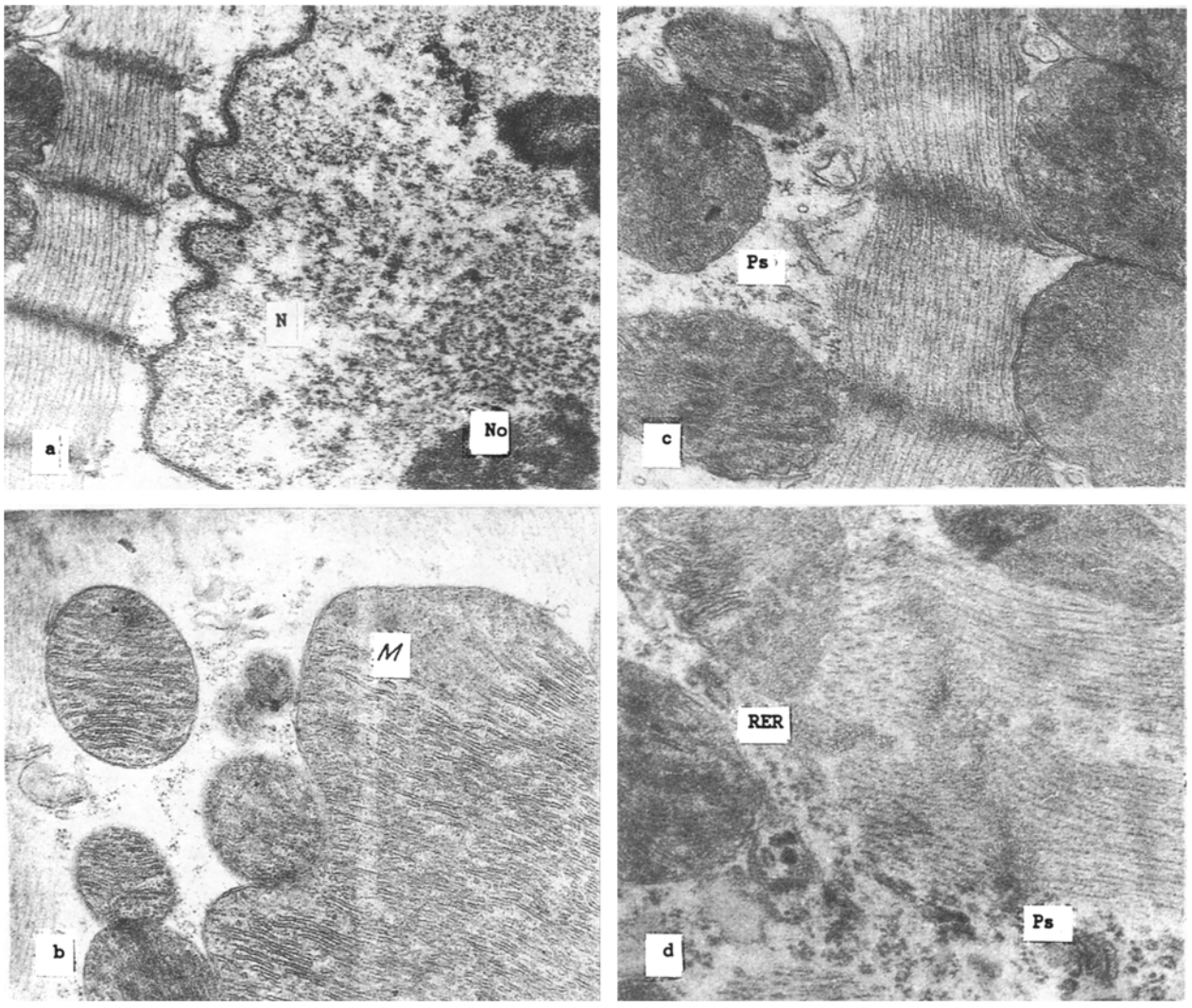

Fig. 3. Cardiomyocyte ultrastructure of NR and HR rats during long-term adaptation to hypoxia: a) increase in number of nucleoli (No) in nuclei (N). 20,000×; b) hypertrophy of mitochondria (M). 30,000×; c) increase in number of polysomes (Ps) and glycogen granules in cytoplasm of LR rats. 40,000×; d) increase in number of elements of rough endoplasmic reticulum (RER) and of polysomes (Ps) in cytoplasm of cardiomyocytes of HR rats. 50,000×.

evidently constitutes the basis for activation of the enzyme systems connected with the formation of adaptive features. Reduction of the number of glycogen granules and lipid droplets, or their complete disappearance, points to activation of oxidation of carbohydrates and fatty acids under these conditions. Consequently, their accumulation is a positive feature, for it constitutes the energy reserve of the cell, to be used in a stress situation.

On the whole, the results of this series of experiments indicate that the experimental model of acute hypoxia (elevation to an altitude of 11,000 m) is an extraordinary stimulus, leading to destructive changes in the plasma membrane, to activation of exchange processes between the blood and cardiomyocytes, to intensification of synthetic processes, and also to exhaustion of energy substrates. It must be pointed out that all these changes in the cardiomyocytes were reversible and they disappeared completely after only 25 days, including changes in the endothelial cells and sarcolemma.

As has already been pointed out, long-term adaptation to periodic hypoxia led to a three-fourfold increase in T_s of the LR rats but did not change, or even reduce, the value of this parameter in HR rats. Marked hypertrophy of the nuclei, and increase in the number of nucleoli (Fig. 3a) and of elements of the rough endoplasmic reticulum (RER), an increase in the number of ribosomes (Fig. 3c, d) and glycogen granules and lipid droplets, and also hypertrophy and hyperplasia of the mitochondria were observed, the latter sometimes attaining the length of 5-7 sarcomeres (Fig. 3b). The changes were

more marked in the myocardium of the LR rats, in which the content of glycogen and lipids and activity of RNA synthesis were initially higher than in the myocardium of the HR rats. Thus, as a result of long-term adaptation of rats to periodic hypoxia, significant morphological changes appeared in the subcellular structures, responsible for the basic metabolic processes in the cell, and the intensity of synthetic and transport processes also was increased. It can be postulated, for example, that significant changes took place in the oxidative phosphorylation system. Consequently, the increased working efficiency of the respiratory chain which we noted previously in adapted animals against the background of a lowered intensity of respiration, i.e., economy of oxidative processes, and also activation of a number of enzymes of energy metabolism under these conditions [7-10], correlate with structural changes in the mitochondria.

The increase in the number of glycogen granules and lipid droplets observed during long-term adaptation to hypoxia in the cardiomyocytes of LR rats, accompanied by comparatively small changes in their number in the myocardium of HR animals, largely canceled out any initial differences in the values of these parameters in the two groups of rats. They indicated an increase in the intensity of glycogenolysis and lipolysis, especially in the myocardium of the LR animals. In other words, under the influence of adaptation a change took place in the type of metabolism as a whole. This observation is in full agreement with data obtained previously on the transformation of metabolic flows, which are the suppliers of energy substrates, and on the reduction in the role of carbohydrates in the myocardial energy metabolism of LR animals as a result of adaptation and a consequent decrease in the importance of the role of NAD-dependent substrates [7-10].

"Elevation" of the adapted rats to an altitude of 11,000 m caused changes in the test structures similar qualitatively to those in unadapted animals, but quantitatively, they were expressed less strongly in the former. In particular, for instance, there was a distinct decrease in the content of glycogen and lipids in the cardiomyocytes.

The results thus indicate higher activity of the protein-synthesizing system and a lower intensity of oxidation of carbohydrates and lipids in the myocardium of unadapted HR rats compared with those in LR rats, and relative equalization of these metabolic parameters in the myocardium of the two groups of animals during adaptation. At the same time, it must be pointed out that activation of protein-synthesizing processes and RNA synthesis took place not only as a result of long-term adaptation to hypoxia, but also after a single exposure to a very strong hypothermic stimulus. The ability of the myocardium to utilize intracellular reserves of glycogen and lipids as energy-yielding substrates under the conditions of acute hypoxia and an increase in the reserves of glycogen and lipids in the cardiomyocytes under the influence of adaptation to hypoxia may also be postulated.

LITERATURE CITED

1. V. A. Berezovskii, Individual Sensitivity and Hypoxia [in Russian], Kiev (1972).
2. A. A. Korneev and L. D. Luk'yanova, Patol. Fiziol., No. 3, 53 (1987).
3. A. A. Korneev, O. A. Popova, S. V. Zamula, and L. D. Luk'yanova, Byull. Éksp. Biol. Med., No. 7, 60 (1990).
4. L. D. Luk'yanova and A. V. Korobkov, Physiological and Clinical Problems of Adaptation and Hypoxia, Hypodynamia, and Hypothermia [in Russian], Vol. 2, Moscow (1981), pp. 73-76.
5. L. D. Luk'yanova, A. A. Korneev, and O. A. Popova, Scientific Methods and Techniques for Protection of Ischemic Tissues [in Russian], Ustinov (1986), pp. 14-16.
6. L. D. Luk'yanova, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1989), pp. 11-44.
7. V. E. Romanova and G. N. Chernobaeva, Physiology and Bioenergetics of Hypoxia [in Russian], Minsk (1990), pp. 85-86.
8. G. N. Chernobaeva and L. D. Luk'yanova, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1989), pp. 160-164.
9. G. N. Chernobaeva, V. E. Romanova, and L. D. Luk'yanova, Physiology and Bioenergetics of Hypoxia [in Russian], Minsk (1990), pp. 20-21.