ELECTRON-MICROSCOPIC STUDY OF PERMEABILITY OF THE SARCOLEMMA OF RAT CARDIOMYOCYTES IN ACUTE MYOCARDIAL ANOXIA

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The permeability of the sarcolemma of cardiomyocytes of the perfused rat heart during acute anoxia was studied. The radioactive complex ^{99m}Tc-Sn-pyrophosphate, the localization of which in the cell was determined by the direct method of electron-microscopic autoradiography with gold chloride, was used as the tracer. A residue of metallic gold began to be observed in the cell after 5 min of anoxia. After 15 min of anoxia, reoxygenation for 15 min completely prevented penetration of ^{99m}Tc-Sn-pyrophosphate within the cardiomyocytes. Further anoxia led to irreversible changes in the sarcolemma. Accumulation of the radioactive complex by the cells correlated directly with the decrease in their creatine phosphate and ATP content.

KEY WORDS: myocardial anoxia; permeability of the sarcolemma; creatine phosphate.

A wealth of factual material resulting from the study of the ultrastructural changes in the cardiomyocytes in hypoxia (and in its extreme variant, anoxia) of the myocardium of different genesis has now accumulated in the literature. Hypoxia leads initially to relaxation of the myofibrils, disappearance of glycogen granules, widening of the Z bands, and the appearance of additional N bands within the I disk [10, 14]. By the 20th min of anoxia or sooner, depending on the experimental conditions, the changes already described are supplemented by swelling of the mitochondria with fragmentation of the cristae, dilatation of the lumen of the T tubules and tubules of the sarcoplasmic reticulum, margination of the nuclear chromatin, accumulation of lipid drops, focal myolysis, and widening of the space of the intercalated disks [1, 5, 6, 8, 10]. Many of these changes arising later are evidently connected with overloading of the cells with calcium [9]. As the work of Jennings [11] showed, the appearance of electron-dense inclusions in the mitochondria containing lipids and calcium phosphates, and also fragmentation of the sarcolemma, observed 60 min after the beginning of anoxia, point to definitely irreversible changes in the cardiomyocytes. Evidence is increasingly being obtained that defects in the sarcolemma are among the earliest changes during the transition from reversible to irreversible injuries [11].

However, it is not yet known what ultrastructural changes are directly connected with the decrease in contractility of the heart during hypoxia, for the earliest of them are found after contractile weakness of the

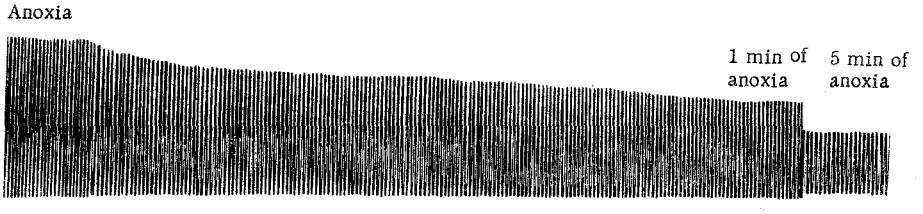

Fig. 1. Pressure within left ventricle of rat during anoxia. Maximal systolic pressure begins to fall immediately after creation of anoxia and after 5 min is only 40% of its initial value.

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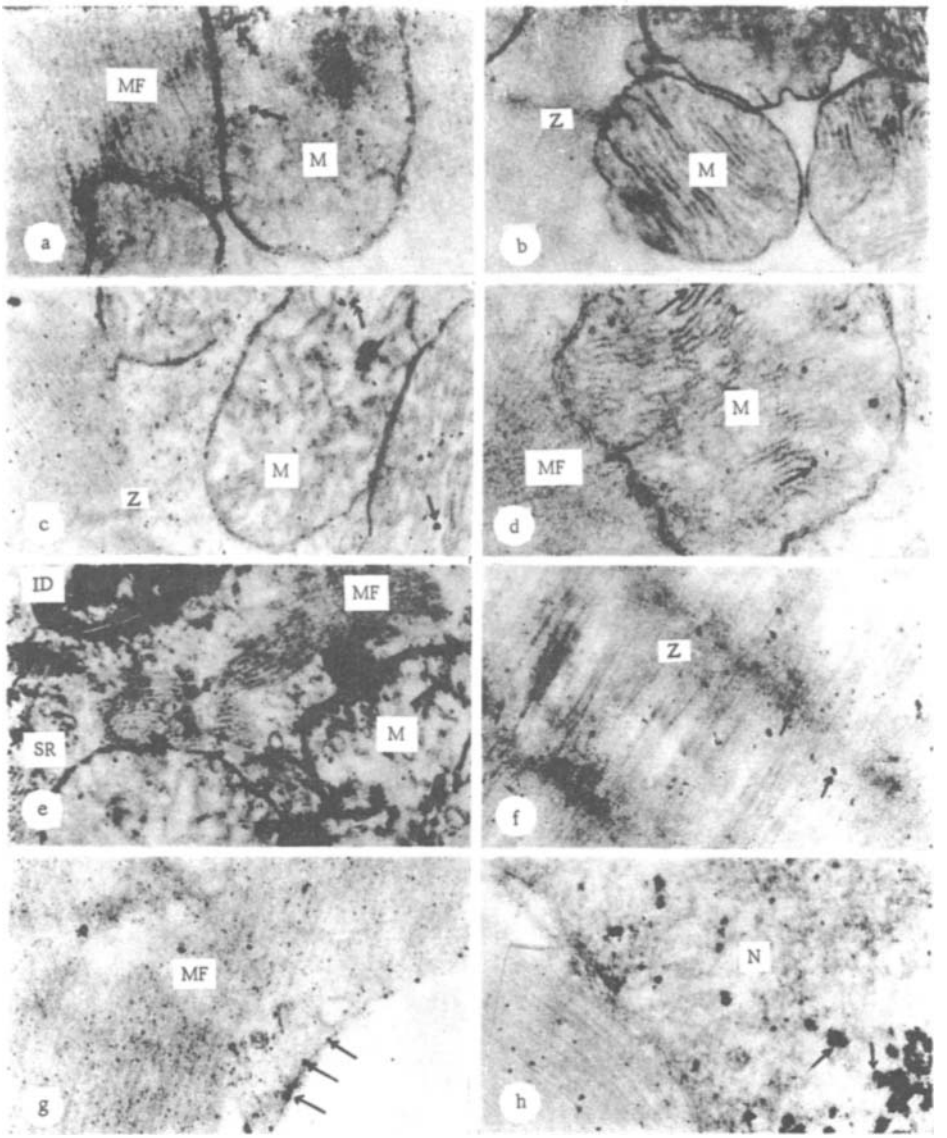

Fig. 2. Ultrastructure of myocardium in anoxia. Heart tissue embedded in Epon–Araldite mixture, unstained ultrathin sections exposed for 5 min in 0.000001% gold chloride solution, 30,000×. a) 5 min of anoxia; fine deposit of metallic gold scattered among myofilaments (MF), larger tracks visible on membranes of mitochondria (M) and inside their matrix (largest tracks indicated by arrows); b) 10 min of anoxia and 15 min of reoxygenation: tracks in cell absent; Z) Z band; c) 20 min of anoxia: large tracks visible in mitochondria and in contact with other cell membranes; d) 20 min of anoxia and 15 min of reoxygenation: fine deposit of metallic gold visible among myofilaments, larger tracks located in mitochondria; e) 1 h of anoxia: large tracks in close proximity to membranes of sarcoplasmic reticulum (SR), intercalated disk (ID), and mitochondria, and with myofilaments; f) 1 h of anoxia: deposition of large granules in region of I disk of sarcomeres; g) 1 h of anoxia: large tracks of membrane of sarcolemma and among myofilaments; h) 1 h of anoxia: largest deposits of metallic gold in nucleus (N) of cardiomyocyte.

myocardium has developed and they cannot be its cause. In particular, in hypoxia of the isolated perfused rabbit heart without peripheral resistance no ultrastructural changes whatsoever could be found in the course of 15 min, whereas the contractile power of the organ fell sharply [10].

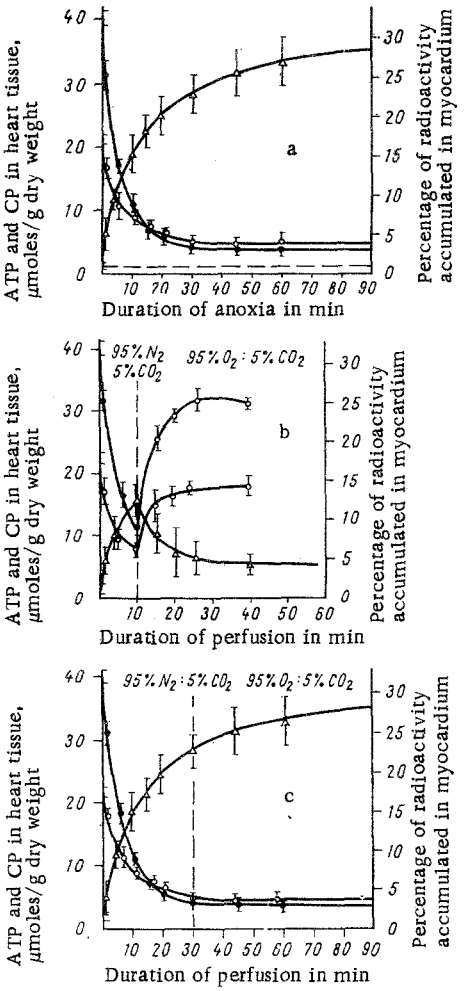

Fig. 3. Dynamics of concentrations of ATP (empty circles), CP (filled circles), and radioactivity (triangles) in the myocardium during anoxia and reoxygenation. Abscissa: a) duration of anoxia (in min); b and c) duration of perfusion (in min). Ordinate: left) ATP and CP concentrations in heart tissue (in μmoles/g dry weight); right) percentage of radioactivity accumulated in myocardium. a) Changes in ATP and CP concentrations and radioactivity during anoxia without reoxygenation; b, c) changes in ATP and CP concentrations and radioactivity after 10 and 30 min respectively of anoxia (time of ending of anoxia indicated by vertical broken line) followed by reoxygenation.

The sarcolemma in cardiomyocytes is known to be the location of many important processes regulating contraction itself and ensuring normal functioning of the cell. It was shown comparatively recently that the slow calcium current triggering contraction and regulating its force, is energy-dependent [2], and that the enzyme creatine phosphokinase (CPK) [7], necessary to maintain the local ATP pool, is located on the sarcolemma. The question accordingly arises: Is damage to the sarcolemma one of the main components in the genesis of myocardial weakness and the development of subsequent ultrastructural changes in the cardiomyocytes during hypoxia? To examine this problem the investigation described below was undertaken.

EXPERIMENTAL METHODS

Experiments were carried out on the isolated Wistar rat heart perfused by Langendorff's method [12]. The heart was perfused at 37°C with Krebs–Henseleit bicarbonate buffer. To create anoxia, the buffer was saturated with a gas mixture of 95% N_2 and 5% CO_2. For control perfusion the buffer was saturated with a mixture of 95% O_2 and 5% CO_2, with the addition of 11 mM glucose. The electron-microscopic marker was ^{99m}Tc-Sn-pyrophosphate, used in clinical practice for the diagnosis of acute myocardial infarction. ^{99m}Tc is a short-lived γ-ray source which, after only a few hours, is converted into a weak β-emitting isotope. In the appropriate dose, the latter has been found to be suitable for autoradiography. Accordingly, 100 μCi of an eluate was extracted from a ^{99m}Tc generator (Hoechst, West Germany) and was used, but only after a period of 2 days, to prepare a ^{99m}Tc-Sn-pyrophosphate complex suitable for electron-microscopic autoradiography. The autoradiographic investigations were carried out by the direct method with gold chloride [13]. Accumulation of the radioactive complex, prepared from the fresh technetium eluate, by the heart muscle cells was measured by means of a 1185 γ-counter (from Nuclear Chicago, USA). The concentrations of ATP and creatine phosphate (CP) were determined by methods adopted in the Laboratory of Metabolism of the Myocardium, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR [3]. The contractility of the heart was recorded on a Mingograph (Elema-Schonander, Sweden).

EXPERIMENTAL RESULTS

After 5 min of anoxia the contractility of the heart was reduced by 60% (Fig. 1). No changes could be found in the ultrastructure of the cardiomyocytes compared with the control material. However, electron-microscopic autoradiographic investigations carried out at that time showed penetration of the ^{99m}Tc-Sn-pyrophosphate complex inside the cells. Small tracks could be seen above all membranes of the cardiomyocytes, including myofibrils, by the 5th minute of anoxia. Larger tracks were located above mitochondrial membranes (Fig. 2a). After 10 min of anoxia, followed by 15 min of reoxygenation, tracks above the cardiomyocytes were absent (Fig. 2b). After 20 min of anoxia, large tracks were visible above all cell membranes (Fig. 2c), and 15 min of reoxygenation after this period of anoxia did not prevent penetration of the radioactive complex into the cardiomyocytes: There was only a decrease in the number of large tracks above the various cell organelles (Fig. 2d). After 1 h of anoxia large tracks were still found in large numbers above the mitochondrial membranes and tubules of the sarcoplasmic reticulum (Fig. 2e), above the myofibrils with some predominance of accumulation in the zone of the I disks (Fig. 2f), and above the sarcolemma (Fig. 2g); particularly large deposits of metallic gold appeared in the nuclei of the cardiomyocytes (Fig. 2h). Reoxygenation after 1 h of anoxia had no effect on the character of distribution of the radioactive tracks. In the control material, no deposition of metallic gold was observed.

The results of the electron-microscopic study of the permeability of the sarcolemma correlated completely with the results of the concurrent biochemical tests. After only 5 min of anoxia there was a sharp fall in the CP level accompanied by a less marked fall in the ATP concentration and a simultaneous accumulation of radioactivity in the myocardium (Fig. 3a). Continuation of the anoxia led to a further decrease in the concentration of high-energy compounds in the myocardium and accumulation of radioactivity. If the duration of anoxia did not exceed 10–15 min, reoxygenation led to restoration of the ATP and CP levels in the heart tissue by 80–92%, whereas the quantity of accumulated radioactivity fell almost to its initial level, namely 3–4% (Fig. 3b). Reoxygenation after 20–30 min of anoxia caused no significant change in the concentrations of ATP and CP in the myocardium or in the accumulation of radioactivity (Fig. 3c).

The results indicate disturbance of permeability of the sarcolemma of the cardiomyocytes in the early stages of myocardial anoxia. Since disturbances of permeability responsible for the appearance of the ^{99m}Tc-Sn-pyrophosphate complex inside the cardiomyocytes can be completely abolished by reoxygenation after anoxia for 10–15 min, this period can be regarded as the upper limit for the existence of reversible changes in the sarcolemma under the conditions of the 15-min reoxygenation used in these experiments. These disturbances provide a morphological explanation of the onset of myocardial weakness during anoxia against the background of an apparently unchanged cardiomyocyte ultrastructure. The appearance of radioactive tracks simultaneously above all organelles of the cardiomyocytes evidently indicates that during hypoxia permeability not only of the sarcolemma is changed, but also of membranes of other organelles and, in particular, of the mitochondria, where the largest deposits of metallic gold accumulate. Changes in the permeability of the sarcolemma and other membranes correlate directly with the sharp fall in the CP and ATP concentrations in the cells. The fall in CP, a carrier of energy to all the energy-dependent systems of the cardiomyocytes [4], is most probably the primary cause of the acute weakness of the myocardium in hypoxia. Immediately

after the fall in CP a decrease in the ATP concentration and disturbance of the permeability of the sarcolemma and other organs are observed; these are the morphological reflections of the inadequacy of the energy supply to these membranes.

Permeability of the sarcolemma for the ^{99m}Tc-Sn-pyrophosphate complex in the early, reversible stages of hypoxia suggest that, in principle, it is possible for other substances, whose administration could lead to improvement in the state of the heart, to be introduced into the cardiomyocytes under these conditions also.

The results show that the radioactive complex ^{99m}Tc-Sn-pyrophosphate can be used to study permeability of the membranes of cardiomyocytes, especially in the early stages of hypoxic damage.

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ULTRASTRUCTURE OF THE MYOCARDIUM IN YAKS LIVING AT ALTITUDES OF OVER 3000 m ABOVE SEA LEVEL

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The ultrastructure of the myocardium was studied in yaks living constantly at an altitude of over 3000 m above sea level. The pattern of Z lines in semithin and ultrathin sections was sharply defined and produced distinct cross-striation of the myofibrils. Numerous lipid granules were seen in the cytoplasm of the cardiomyocytes. An increase in the number of intercalated disks was found in many zones of the myocardium. The results are discussed from the point of view of increased activity of yak heart muscle and its hyperfunction at high altitudes in the mountains.

KEY WORDS: high mountains; yaks; myocardium; intercalated disks; lipids.

Yaks are typical representatives of high-mountain animals. They have great powers of endurance, can work satisfactorily all the year round, and can be found in the open air whatever the weather. The study of the structure of the myocardium of these animals from the standpoint of adaptation is therefore particularly

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