Effects of Various Hypothermal Ischemia Levels on Myocardial Functional Element ATPase Activity

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Intricate surgical interventions on the heart involve prolonged disengagement of the circulatory system. The success of surgery depends to a great extent on the efficacy of measures aimed at the prevention of ischemic damage to the myocardium. Recently it was demonstrated that myocardial hypothermia was the basic component in the complex of intraoperative protective measures improving the heart's resistance to ischemia [1, 2, 4, 6, 10]. Still, the optimal level of myocardial cooling involving the least injury to the myocardial functional element (MFE) structures is yet to be found [8, 11, 12, 14]. For this reason a morphofunctional approach to elucidating the time course of the processes occurring at the level of the MFE under conditions of various temperatures of cooling of the myocardium during ischemia is particularly important. Some authorities [3, 5] claim that histochemical detection of ATPase activity may be regarded as an objective criterion of MFE status, for this enzyme can be found in the myofibrils, mitochondria, sarcoplasmic reticulum membranes, cardiomyocyte nucleus and carcolemma, and endotheliocyte plasma membrane and attasts to the functinal activity of these structures.

The research was aimed at measuring MFE ATPase activity at various levels of hypothermal ischemia and heart reperfusion.

MATERIALS AND METHODS

Experiments were carried out with 21 adult healthy mongrel dogs of both sexes weithing 18.3±1.2 kg. After premedication with promedol and atropine the animals were cooled under endotracheal ether-oxygen narcosis (III_{1-2}) using a Kholod 2F apparatus and sacks with ice and snow laid around the body. When a rectal temperature of 30°C was attained, thoracotomy was carried out in the right fifth intercostal space and the circulation was cut off from the heart by placing tourniquets on the unpaired vein and venae cavae. Heart ischemia lasted 60 min. The brain temprature over this period was maintained at the level of 20-22°C. In the first series of experiments (11 animals) the intramyocardial temperature during ischemia was maintained at the level of 28-30°C, while in the second series (10 animals) it was kept at 10-12°C using additional cooling of the heart with normal saline (6-8°C). Heart work restoration was started by removing the tourniquets from the unpaired vein and venae cavae, artificial ventilation of the lungs, massage of the heart, intraarterial supply of warm donor blood and polyglucin with epinephrine, and warming of the animal. Left ventricular biopsy specimens in both series of experiments were taken: 1) before occlusion at the height of body hypothermia 28-30°C, 2) 60 min after heart ischemia, and 3) during 120 min of the recovery period (warming of the animals to 35°C). Ten dogs in which left ventricular biopsy specimens were taken under ether-oxygen narcosis were the intact controls. To detect ATPase activity at the electron microscopic levels the lead method [15] in a modification [13] was used. The sections were made with an LKB-III ultratome and examined under an EMV-100A electron microscope. Final reaction product granules were counted using a grid in the capillary epitheliocyte cytolemma,

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cardiomyocyte sarcolemma, myofibrils, and nuclei in 30 electronograms obtained at every stage of the experiment in different animals. The enzyme activity was assessed by the number of lead phosphate granules per unit area of the above-named structures [9].

RESULTS

On the whole MFE ATPase is highly active under conditions of ether-oxygen narcosis, although this enzyme is unevenly distributed in the cardiomyocytes. The highest activity is observed in the nucleus, while the sarcolemma ranks second, and the myofibrils third in activity (Table 1). In the nucleus reaction product is detected at sites of chromatin localization, in the nucleolus, as well as in the karyolemma, presenting as unevenly distributed small granules (Fig. 1, *a*). In the myofibrils round lead phosphate granules are detected mainly near Z-disks, which is characteristic of the intact heart [5]. A considerable share of the enzyme activity is localized in the cardiomyocyte sarcolemma and epitheliocyte plasma membrane. For instance, near capillares in an active state, indicated by marked pinocytosis, uniform sedimentation of lead phosphate granules is observed in the endothelium and sarcolemma (Fig. 1, *b*). Such a picture of the emzyme activity distribution reflects balanced transport processes in the said membrane structures of the normal myocardium.

ATPase activity in the epitheliocytes and cardiomyocytes is changed under moderate (28-30°C) hypothermia of the body. The number of lead phosphate granules in the myofibrils and nuclear ATPase activity are lowered, this indicating inhibition of the synthetic processes in cardiomyocytes under the effect of low temperature. The content of final reaction product increases in the epitheliocyte sarcolemma and plasma membrane, ATPase activation in the epitheliocytes being more marked than in the sarcolemma (Table 1). Such a trend in cardiomyocyte ATPase activity distribution reflects myocardial hypoxia caused by hypothermia. The work of the biochemical systems is discoordinated under such conditions, toxic products are produced and accumulated, plastic processes are disturbed, and ATP synthesis in the myocardium is reduced [10, 11]. Energy deficit in turn results in impairment of cardiomyocyte and endotheliocyte membrane permeability, this leading to reduction of the intracellular potassium and sodium gradients. Cardiomyocyte water-electrolyte homeostasis under such conditions is maintained at the expense of overstress of the energy-

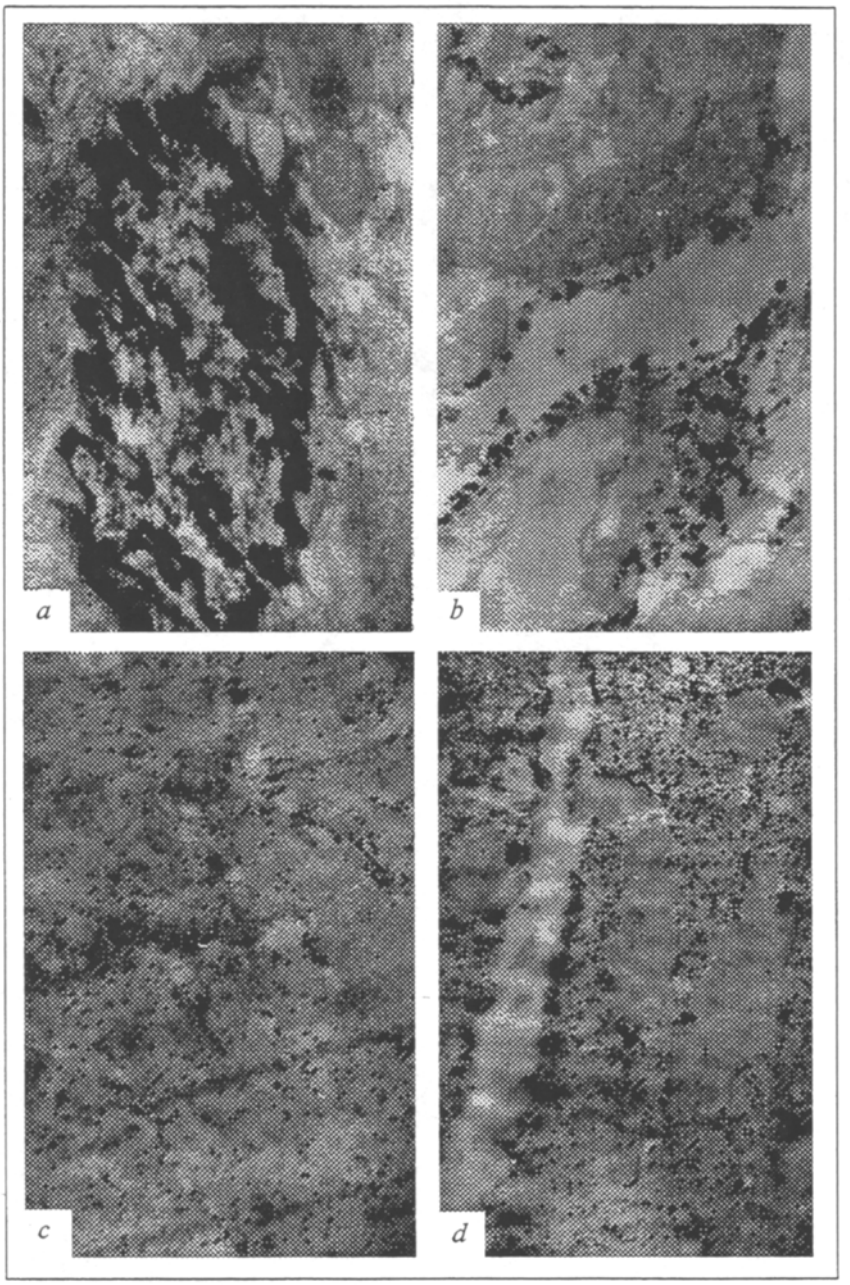

Fig. 1. ATPase activity in MFE structures in normothermia and under various conditions of hypothermal ischemia of the myocardium. *a*) ether–oxygen narcosis: high activity of nuclear ATPase (×18,000; *b*) ether–oxygen anesthesia: uniform precipitation of lead phosphate granules in endotheliocyte plasma membrane and sarcolemma (×18,000); *c*) 28–30°C hypothermal ischemia of myocardium diffuse distribution of lead phosphate granules in myofibrils (×18,000); *d*) 10–12°C hypothermal ischemia of myocardium: low ATPase activity in myofibrils (×20,000).

TABLE 1. ATPase Activity in Myocardial Functional Element Structures in Normothermia and for Cooling of Animals to 26–30°C, Various Levels of Hypothermal Myocardial Ischemia, and Reperfusion ($M \pm m$, $n = 30$)

MFE structure	Period of investigation					
	intact (ether-oxygen anesthesia)	28-30°C hypothermia	hypothermal ischemia		reperfusion after ischemia	
			28-30°C	10-12°C	28-30°C	10-12°C
Cardiomyocyte						
nucleus	35.0±3.6	25.1±2.3[a]	21.6±1.9[a]	23.0±3.6[a]	8.0±1.3[a]	25.0±2.9[a,d]
myofibrils	14.5±2.4	14.1±0.95	14.7±2.0	7.9±1.8[a,b,c]	9.0±0.9[a,b]	11.0±1.6[b]
sarcolemma	23.8±2.1	25.0±0.3	28.0±2.3	22.9±1.7[c]	20.7±2.2	26.8±2.5[d]
Endotheliocyte						
plasma membrane	21.8±2.4	29.8±2.5[a]	30.6±2.4[a]	36.0±3.5[a,b]	18.0±1.5[b]	40.6±4.5[a,b,d]

Note. a) reliable differences from intact animals; b) from 28–30°C hypothermia; c) between levels of hypothermal myocardial ischemia; d) between hypothermal myocardial ischemia levels during reperfusion.

dependent processes, this being expressed in a more intensive functioning of the endotheliocyte plasma membrane and sarcolemma ATPase. The mosaic increase of the enzyme activity in these structures seems to be due to irregular changes in the physical characteristics of the membrane components under the effect of hypothermia [7].

Sixty-minute myocardial ischemia in the presence of overall moderate hypothermia of the body is characterized by a progressive reduction of ATPase activity in the nucleus. Myofibrillar ATPase activity was the same as in intact animals, but the redistribution of lead phosphate granules was diffuse, as is characteristic of asystole (Fig. 1, *c*). ATPase activity was higher in the capillary endothelium and sarcolemma than in the intact heart, but these increases were nonuniform: endotheliocyte sites with high activity of the enzyme were adjacent to cardiomyocyte sites with very low activity. This discrepancy is indicative of both intra- and inter-cellular water-electrolyte homeostasis shifts under conditions of prolonged myocardial ischemia at 28-30 °C [7].

Myofibrillar ATPase activity was reduced as against the norm during restoration of cardiac activity after cooling of the myocardium as low as 28-30°C, this indicating disorders in energy utilization processes during reperfusion. Plastic processes in the cardiomyocytes were inhibited too because of low nuclear ATPase activity. Endotheliocyte ATPase activity was increased vs. the norm and vs. that during ischemia, whereas the activity of the enzyme in the sarcolemma was unchanged (Table 1). Such an imbalance of NFE energy-dependent processes during reperfusion was associated with heart contractility reduction and development of grave acute heart failure [7].

A reliable reduction of ATPase activity in all the examined MFE structures was observed after 60-min hypothermal (10-12°C) myocardial ischemia as against the similar stage of control time in the first series of experiments. A drastic inhibition of the enzyme activity is indicative of a more effective reduction of the rate of myocardial metabolism under conditions of deep cooling of the myocardium. The ATPase activity drop was particularly marked in the nucleus and myofibrils with diffuse distribution of the reaction product (Fig. 1, *d*). The enzyme activity was decreased below the norm in the capillary endothelium and sarcolemma; interestingly enough, the values here were almost the same indicating a better balanced water-electrolyte exchange between the cells, intercellular medium, and capillaries in comparison with that in 28-30°C hypothermal ischemia of the myocardium.

Recovery of heart function after 10-12°C hypothermal myocardial ischemia was associated with stimulation of the energy-dependent processes in the myocardium, a positive time course of ATPase in the examined MFE structures being evidence of this. Activation of nuclear and myofibrillar ATPase, with its values approaching those in the intact heart, indicates recovery of the plastic processes and myocardial contractile function. A significant increase of the enzyme activity in the capillary endothelium and sarcolemma was conducive to maintenance of the water-salt balance of the myocardium and to a more favorable course of heart work recovery than after 28-30°C hypothermal ischemia. According to the data of similar experiments [7], extracellular edema does not develop and intracellular edema is arrested after 10-12°C hypothermal ischemia, and an earlier and more complete recovery of the heart contractile and pumping functions is observed after infusions of lesser volumes of inotropic agents and other means in comparison with that after 28-30°C hypothermal ischemia. Hence, the results demonstrate that cooling of the myocardium to 10-12°C, signiificantly reducing MFE ATPase activity during ischemia, better prevents ischemic and reperfusion disturbances in the energy-dependent processes than 28-30°C hypothermal ischemia. Recovery of MFE ATPase activity during reperfusion after 10-12°C hypothermal ischemia of the myocardium maintains heart contractile function in the postischemic period at a sufficiently high level.

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Electron Microscopic Study of Erythrocyte Shape after Ultraviolet and Red Coherent Extracorporal Irradiation of the Blood

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At present quantum hemotherapy has gained wide acceptance in clinical practice. The literature available definitely proves the importance and dissimilar action of different bands of the electromagnetic spectrum on the organism and, in particular, on the blood [1, 3-11].

The aim of the present study was to investigate the effect of ultraviolet (UV) and red coherent radiation on the shape of erythrocytes after extracorporal irradiation of the blood.

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MATERIALS AND METHODS

Two experimental series involving extracorporal irradiation of the total blood volume (TBV) were performed on 23 adult male and female dogs weighing 15-18 kg, subjected to quarantine and veterinary examination. A helium-neon laser (HNL, red coherent irradiation, λ=632.8 nm, 1 mW) and a DRB-8 lamp (UV irradiation, λ=254 nm, 8 W) were used for irradiation of the blood. A single irradiation was performed during one hour during the blood flow through a PK 11-05 disposable system for blood transfusion: in the first series the irradiation was performed by a defocused laser beam over 10 cm of the tube; in the second se-