CHANGES IN CARDIOMYOCYTE DEHYDROGENASE ACTIVITY IN ACUTE EXPERIMENTAL OCCLUSION OF THE LIMB ARTERIES

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An urgent problem in modern vascular surgery is reduction of the postoperative mortality among patients with acute occlusion of the limb arteries [3, 7, 8]. The high levels of mortality (15-35%) are due, according to many research workers, to disturbances of homeostasis developing after operative resotration of the blood flow to the limbs. The so-called postischemic syndrome, or "reperfusion syndrome," which develops in 20-50% of patients with acute occlusion of the aortic-iliac zone [8, 10, 12] is conventionally divided into three forms: cardiovascular, pulmonary, and hepatorenal [3, 8, 10]. The pathogenesis of the cardiovascular disorders under these conditions has been inadequately studied, and this prevents their adequate clinical correction [1, 5]. A reliable functional-morphological test of the state of this cell when exposed to various factors is demonstration of the activity of key enzymes, especially dehydrogenases [2]. However, no information could be found in the literature on quantitative estimation of their activity in cardiomyocytes in acute ischemia of the limbs and early postischemic recirculation.

The aim of this investigation was to study the effect of temporary ischemia of the limbs, restoration of the blood flow in them, and pharmacologic correction of postischemic disorders on the state of activity of certain dehydrogenases in cardiomyocytes.

METHODS

Experiments were carried out on 60 mongrel dogs of both sexes weighing 13-18 kg, in which acute arterial occlusion of the hind limbs was produced by the method in [4]. Trimeperidine was used for premedication. The duration of ischemia was 3, 6, 9, and 12 h. Recirculation for 2 h was carried out after each period of ischemia. To prevent the development of postischemic disorders pharmacologic correction was used and was aimed at making good the deficiency of the circulating blood volume, restoring the functional state and activity of the principal neurohumoral adaptive systems, normalizing the hemostasis and the blood rheology, abolishing disturbances in the microcirculatory system, and preventing activation of lipid peroxidation. The combination of therapeutic agents used included rheopolyglucin, heparin, trental, gordox, vitamin E, and droperidol. The animals were killed by intravenous injection of hexobarbital. Four pieces were excised from each heart from the walls of the left and right ventricles, ventricular septum, and left posterior papillary muscle. Activity of the following dehydrogenases was determined on cryostat sections 10 μ thick with the aid of nitroblue tetrazolium [11]: succinate dehydrogenase (SDH), an indicator of metabolic processes in the Krebs cycle, lactate dehydrogenase (LDH), characterizing anaerobic glycolysis, and NAD-diaphorase (NADD), reflecting to some degree the state of the energy supply. Quantitative estimation of dehydrogenase activity was carried out on a "Microvideomat" television image analyzer (Opton, West Germany), controlled by a Wang-720C computer (USA), working on a psecial program of photometric analysis of histologic sections. The parameter for estimation was the average optical density, proportional to enzyme activity in the tissues. The significance of differences was determined by Student's test.

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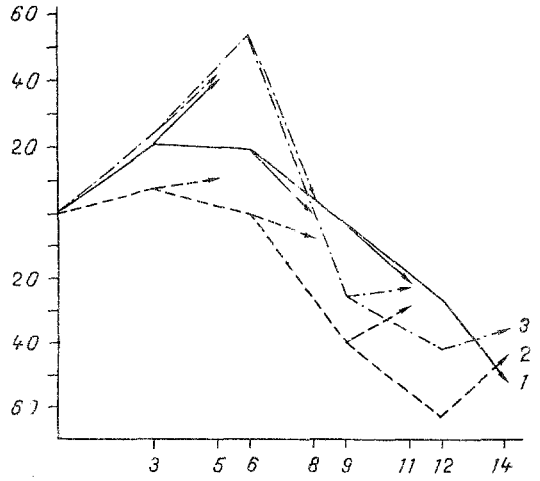

Fig. 1

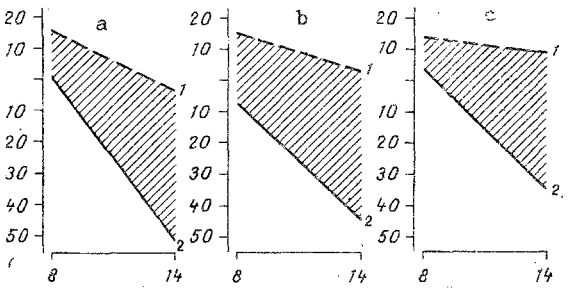

Fig. 2

Fig. 1. Time course of changes in SDH (1), LDH (2), and NADD activity (3) in cardiomyocytes in acute experimental occlusion of the limb arteries (in % of control of the limb arteries (in % of control). Arrows indicate postischemic period. Here and in Fig. 2: abscissa, duration of limb ischemia (in h); ordinate, dehydrogenase activity.

Fig. 2. Changes in SDH (a), LDH (b), and NADD activity (c) in cardiomyocytes following pharmacologic correction of postischemic disorders (1), compared with corresponding parameters in untreated animals (2).

RESULTS

Activity of the dehydrogenases studied in the control group was taken as 100%. Under the influence of ischemia of the limbs of varied duration, significant changes were found to take place in cardiomyocyte metabolism, the time course of which is illustrated in Fig. 1. A significant ($P < 0.05$) increase in SDH, NADD, and LDH activity by 21, 23, and 8% respectively was observed 3 h after creation of occlusion. After 6 h of ischemia of the limbs the greatest increase was observed in NADD activity in the cardiomyocytes (by 53%) and ADH activity remained at about the same level as at the preceding period of the experiment. Against this background, a decrease in LDH activity almost to the control level will be noted. After 9 h of the experiment dehydrogenase activity in the cardiomyocytes was reduced. The decrease in SDH activity amounted to only 3% compared with the control ($P > 0.05$), whereas LDH activity was inhibited by 39% and NADD activity by 25% ($P < 0.05$). After ischemia of the limbs for 12 h, conditions for cardiomyocyte metabolism are evidently even more unfavorable, for SDH activity was 26%, NADD activity 41%, and LDH activity 62% below the control levels ($P < 0.05$). The results are evidence of definite general rules governing the time course of changes in SDH, NADD, and LDH activity depending on the duration of limb ischemia. A characteristic feature was an initial increase in activity of the dehydrogenases studied in the early stages of acute occlusion of the limb arteries, as reflected in intensification of aerobic and anaerobic oxidation and function of the energy-transport systems in the cardiomyocytes. Later, however, under the influence of ischemia of the limbs, the adaptive powers of the enzyme systems of the cardiomyocytes were evidently exhausted. An important fact is the comparatively rapid fall in the level of anaerobic glycolysis as a source of energy, confirmed by a progressive decline in LDH activity in the cardiomyocytes. Restoration of the blood flow in the ischemic limbs in the course of 2 h led to distinctive changes in carbiomyocyte metabolism, which depended mainly on the duration of the preceding ischemic period (Fig. 1). It will be recalled that during recirculation the role of intoxication, due to the entry of toxic products from the zone of ischemia into the general blood flow, which was particularly marked in the late stages of the experiment [5], becomes more important. Recirculation after ischemia of the limbs for 3 h leads to an increase in dehydrogenase activity in the cardiomyocytes: SDH by 40%, NADD by 41%, and LDH by 11% compared with the control ($P < 0.05$). The postischemic period after occlusion of the limb arteries for 6 h was characterized by a fall of SDH and NADD activity almost to the control level ($P > 0.05$) and of the LDH level by 7% ($P < 0.05$). Restoration of the blood flow in the limbs at the later stages of ischemia (9 and 12 h) was not followed by normalization of

dehydrogenase activity. Characteristically there was a small increase in LDH and NADD activity after recirculation compared with the period of ischemia, but their level was considerably below the control. It must be pointed out that in the postischemic period, after occlusion of the limb arteries for 9 and 12 h, a progressive weakening of oxidative processes in the Krebs cycle was observed in the postischemic period, and was confirmed by a fall of SDH activity by 20 and 51% respectively compared with the control level ($P < 0.05$).

The effect of pharmacologic correction of the postischemic disorders on the functional-morphological state of the cardiomyocytes is shown in Fig. 2. The results are evidence of a considerable rise in the level of metabolism in the cardiomyocytes in response to therapeutic measures compared with that in the postischemic period without correction. The increase in dehydrogenase activity in the cardiomyocytes was seen most demonstratively after therapeutic correction of limb ischemia lasting 12 h. SDH activity rose by 98%, NADD by 68%, and LDH by 85% compared with their activity in the cardiomyocytes during recirculation after limb ischemia for 12 h without correction.

The experimental results, obtained by television histophotometry, thus indicate definite general principles in the time course of SDH, NADD, and LDH activity in the cardiomyocytes under the influence of acute arterial occlusion of the limbs. These data must be taken into account during combined functional and morphological evaluation of the state of myocardial contractility in the presence of ischemia of the limbs and in the postischemic period. The increase in dehydrogenase activity in the arly stages of ischemia of the limbs (3 and 6 h) and during subsequent recirculation can evidently be interpreted as a manifestation of adaptive processes in the cardiomyocytes. The considerable decline of dehydrogenase activity in the later stages (9 and 12 h) indicates the existence of metabolic disturbances in the cardiomyocytes, which must be taken into account during the treatment of patients with acute occlusion of the limb arteries. The data showing an improvement of the metabolic situation in the cardiomyocytes under the influence of pharmacologic correction, which confirms the justification for the use of this combination of therapeutic measures, may be of definite interest in this connection.

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