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QUANTITATIVE ENZYME-HISTOCHEMICAL CHANGES IN RED AND WHITE SKELETAL MUSCLE FIBERS IN THE LIMBS DURING TEMPORARY ISCHEMIA AND POSTISCHEMIC RECIRCULATION

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The dynamics of changes in metabolism of ischemized skeletal muscles of the limbs has so far been examined morphologically mainly at the descriptive level [5, 7, 9]. Quantitative enzyme histochemical studies of skeletal muscle tissue during ischemia have been few in number and have not allowed for muscle heterogeneity [4] and, in particular, changes in red and white muscle fibers (RMF and WMF, respectively) have been studied only in chronic ischemia of the limbs [2].

The dynamics of metabolic processes in skeletal muscle was studied in the present investigation, taking into account heterogeneity of the muscle, during temporary acute occlusion of the main limb arteries and early postischemic recirculation.

EXPERIMENTAL METHOD

Acute occlusion of the hind-limb arteries was created by the method in [1] in experiments on 69 mongrel dogs of both sexes, weighing 13-18 kg. The duration of ischemia of the limbs was 3, 6, 9, and 12 h, and revascularization for 2 h was carried out after each period of ischemia. Enzyme-histochemical investigation of the soleus muscle was undertaken on frozen sections 10 μ thick. Activity of the following enzymes was determined: succinate dehydrogenase (SDH) — the Krebs' cycle, lactate dehydrogenase (LDH) — anaerobic glycolysis, glutamate dehydrogenase (GDH) and β-hydroxybutyrate dehydrogenase (HBDH) — protein and lipid catabolism, and NAD- and NADP-diaphorases. Activity of oxidoreductases was demonstrated with nitro-BT by the usual methods [11]. ATPase activity was determined by the calcium method [10]. To differentiate between muscle fibers when determining enzyme activiy, the classification given in [10, 15] was used. Changes in enzyme activity were assessed quantitatively with an MIF-7 integrating photometric microscope, based on the logarithmic screen method [3]. The object was measured under oil immersion in monochromatic light at a wavelength of 546 nm (magnification 600). From one transverse section 11 RMF and 11 WMF were investigated: Each fiber was subjected to photometry separately in three regions, followed by calculation of arithmetic mean values and standard error.

EXPERIMENTAL RESULTS

It is generally accepted that the main source of energy of WMF (fast contracting) is anaerobic glycolysis, whereas that of RMF (slowly contracting) is metabolic processes of the Krebs' cycle and processes associated with protein and lipid metabolism [8, 12, 14, 15]. The results of the present cytophotometric investigations confirmed this enzyme-histochemical het-

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erogeneity of striated muscle fibers. For instance, SDH, NAD, and NADP activity was highest in RMF, GDH and LDH activity was lower, and HBDH activity was lowest of all. In WMF the relative levels of activity of these enzymes was different: activity of LDH was highest (24% higher than in RMF), activity of SDH, NAD, and NADH was lower (53, 47, and 57% lower, respectively, than in RMF), and GDH and HBDH activity was lowest (57 and 45% lower, respectively, than in RMF). After ischemia for 3 h activity of enzymes of the Krebs' cycle (SDH), of NAD- and NADP-diaphorases, and of enzymes of protein and lipid catabolism (GDH and HBDH) was clearly lower than in the control in both types of muscle fibers: by 21, 23, 22, 31, and 30%, respectively, in RMF and by 26, 25, 26, 17, and 30%, respectively in WMF (Figs. 1, 2, and 3). With simultaneous blocking of oxidative phosphorylation and a rapid fall in the ATP reserves (by 33% in RMF and by 16% in WMF) it was possible to restore levels of high-energy compounds by activation of glycogenolysis. LDH activity, for instance, increased in the two types of muscle fibers by 21 and 15%, respectively. These enzyme-histochemical changes were mild and reversible. This was shown by the increase in activity of oxidative enzymes and the decrease in activity of enzymes of glycolysis to normal during subsequent postischemic recirculation for 2 h.

After 6 h of ischemia activity of enzymes of aerobic oxidation continued to decline in all muscle fibers: SDH activity in RMF fell by 60%, NADP- and NAD- diaphorases by 49% and 45%, respectively, GDH by 60%, and HBDH by 47%; in WMF they fell by 56, 29, 38, 59, and 45%, respectively. Meanwhile activity of LDH, an enzyme responsible for anaerobic glycolysis, also fell (by 5% in RMF, by 15% in WMF), by contrast with the rise in its activity after ischemia for 3 h. Possible collapse of compensatory reactions of the metabolic systems of ischemic muscles is suggested most clearly by the postischemic recirculation period, during which no recovery of the activity of these enzymes was observed. Moreover, a considerable decrease in their activity was found compared with the parameters after 6 h of ischemia. In RMF, for instance, activity of SDH fell by 74%, of NADP-diaphorase by 69%, of NAD-diaphorase by 70%, GDH by 72%, HBDH by 62%, LDH by 38%, and ATPase by 85%; the corresponding falls in WMF were by 73, 67, 62, 64, 58, 32, and 84%, respectively.

After ischemia for 9 h the decrease in activity of the above-mentioned enzymes was even greater: in RMF, activity of SDH fell by 80%, of NADP-diaphorase by 62%, NAD-diaphorase by 65%, GDH by 69%, HBDH by 74%, and LDH by 67%; in WMF the falls were 76, 57, 63, 69, 76, and 58%, respectively.

In the period of postischemic recirculation the decrease in activity of these enzymes continued to progress, and after ischemia for 12 h followed by revascularization for 2 h the decrease was greater still.

When the time course of activity of glycolytic enzymes, on the one hand, and of enzymes of aerobic oxidation, on the other hand, is compared, the irregular character of the changes

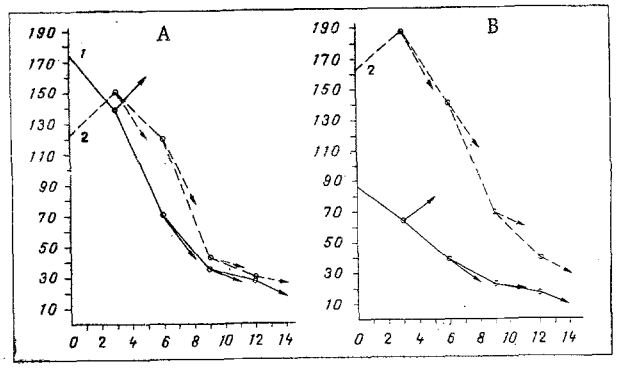

Fig. 1

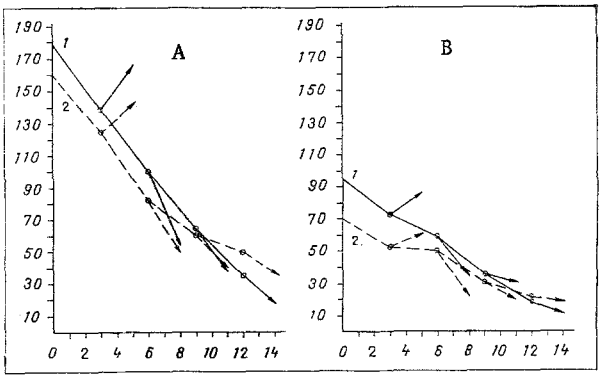

Fig. 2

Fig. 1. Time course of SDH (1) and LDH (2) activity in RMF (A) and WMF (B) of dog soleus muscle during temporary acute ischemia and early postischemic recirculation. Here and in Figs. 2 and 3: abscissa, mean optical density; ordinate, time (in h). Arrow indicates recirculation.

Fig. 2. Time course of changes in NAD- (1) and NADP-diaphorase (2) activity in RMF (A) and WMF (B) of dog soleus muscle during temporary acute ischemia and early postischemic recirculation.

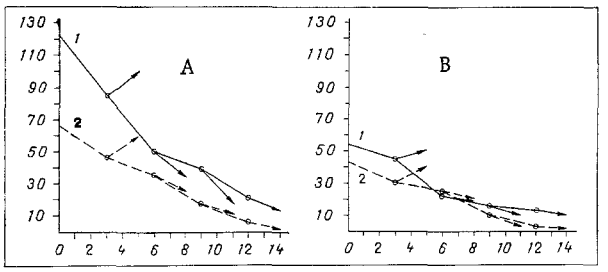

Fig. 3. Time course of GDH (1) and HBDH (2) activity in RMF (A) and WMF (B) of dog soleus muscle during temporary acute ischemia and early postischemic recirculation.

will be noted. Under anaerobic conditions the most drastic decline was observed in activity of oxidative enzymes, whereas activity of enzymes of anaerobic glycolysis was considerably increased in the early stages of ischemia and fell slowly during the later stages of prolonged acute ischemia (6, 9, and 12 h). This fact confirms the unequal resistance of the different enzyme systems to the action of ischemia [6].

It will also be noted that at all stages of acute ischemia and subsequent postischemic recirculation metabolism in RMF was more severely disturbed than in WMF. Similar heterogeneity of the stages and types of injuries to striated muscle fibers also has been noted by other investigators [5, 7, 9].

The results of the present investigation confirm data in the literature [5, 13] indicating that the metabolic changes after ischemia of the limb for 3 h are reversible. Irreversibility of metabolic changes in acute ischemia more than 6 h in duration indicates possible damage to compensatory and adaptive mechanisms. This general rule agrees to some degree with results of qualitative enzyme histochemistry of rat skeletal muscles during ischemia [9]. At the same time, it will be noted that metabolic disturbances at this critical period were observed principally in RMF, whereas WMF, which are more resistant to hypoxia, were affected less severely.

It can be concluded from these results that operative restoration of the blood flow in the limbs must be carried out in the early period after acute occlusion of the main arteries. Revascularization after 6 h of ischemia is followed by marked depression of metabolism in the muscle tissue.

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