ISCHEMIC DAMAGE TO THE SARCOPLASMIC RETICULUM OF SKELETAL MUSCLES: THE ROLE OF LIPID PEROXIDATION*

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The development of ischemia was shown to be accompanied by inhibition of the Ca^{2+} enzyme transport system (ETS) (a decrease in the Ca^{2+}/ATP ratio and in activity of Ca^{2+}-dependent ATPase), which correlates with accumulation of the primary and secondary molecular lipid peroxidation products (POL) in vivo and in the membranes of the sarcoplasmic reticulum (SR) of the skeletal muscles. Administration of antioxidants (2,6-di-tert-butyl-4-methylphenol, α-tocopherol) prevents activation of POL in the ischemic muscle and partially protects the Ca^{2+} ETS against injury. Restoration of the blood flow after prolonged ischemia leads to further inhibition of the Ca^{2+} ETS while the concentration of POL products remains unchanged.
KEY WORDS: ischemia; sarcoplasmic reticulum; peroxidation of lipids; muscle.

An important factor in muscle pathology is a disturbance of the function of the sarcoplasmic reticulum (SR) of the muscle fiber [2, 20], which links the processes of excitation and contraction through regulation of the concentration of Ca^{2+} cations in the sarcoplasm by means of an enzyme transport system (ETS) [19]. The function of this system is dependent on the presence of a calcium pump (Ca^{2+}-dependent ATPase) and low passive permeability of the SR membranes to Ca^{2+} ions [18, 21]. Molecular oxygen [7] and its active forms generated by single-electron carriers [8, 16], are natural modifiers of the structural and functional characteristics of the SR membranes. In ischemic tissues the level of the natural inhibitors of free-radical oxidation is lowered and the concentration of lipid peroxidation products (POL) rises; the intensity of these processes, moreover, correlates with the functional activity and structural damage of the organ [1, 3].

The object of this investigation was to study the connection between various parameters of the Ca^{2+} ETS and POL in the SR membranes in experimental ischemia of the skeletal muscles.

EXPERIMENTAL METHOD

Experiments were carried out on 96 male August rats weighing 150-170 g. Ischemia of the hind limbs was induced by application of a tourniquet to the upper third of the thigh to arrest the blood flow completely for 1, 3, and 6 h. Tissue for investigation was removed immediately after the end of the period of ischemia or 4 h after removal of the tourniquet. In experiments to study survival, the animals remained under observation for 1.5 months after removal of the tourniquet. Ionol (2,6-di-tert-butyl-4-methylphenol) was injected intraperitoneally in a dose of 120 mg/kg 4 h before application of the tourniquet; α-tocopherol was given by intraperitoneal injection in a dose of 50 mg/kg daily for 3 days; the value of pO_2 in the limb muscles was investigated in vivo by a polarographic method using a pair of Cu/Hg and Cd electrodes with a type M-95 microammeter. The electrodes were calibrated in physiological saline at atmospheric pressure (pO_2 = 155 mm Hg) and in a solution

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TABLE 1. Indices of Ca^{2+} ETS and POL in SR of Rat Skeletal Muscles during Ischemia (M ± m)

Series of investigations	Activity of Ca^{2+}-ATPase, μmoles P_{inorg}/min/mg protein	Ca^{2+}/ATP	Hydroperoxides, nmoles/mg lipids	Diene conjugation products	pO_2 in muscle, mm Hg	Survival rate of rats, %
Control	4,50±0,16	0,7±0,15	3,6±0,5	2,19±0,2	50,0±7.7	—
Ischemia:						
1 h	4.53±0.33	0,46±0,05	8,9±0,8†	2.88±0,63	4,2±0,7‡	100
3 h	4.28±0,34	0.35±0,07	11,4±1.5‡	3.16±0,26†	2.3±0,2‡	100
6 h	2,77±0.12†	0.25±0,09*	11,0±0,7‡	3,26±0,14‡	2,2±0.2‡	0
Ionol	3.97±0,25	0,48±0,15	5,9±0,1**	1.9±0,21††	—	
α-Tocopherol	4.29±0,45	0,44±0,06	4,2±0,7‡‡	2.34±0,31	—	

Note: Here and in Table 2, diene conjugation products measured in optical density units of solution of lipids (1 mg/ml) at wavelength of 232 nm.

*P < 0.05.

†P < 0.01.

‡P < 0.001 compared with control.

**P < 0.05.

††P < 0.01 between experiments with and without ionol, after ischemia for 3 h.

‡‡P < 0.01 between experiments with and without α-tocopherol, after ischemia for 3 h.

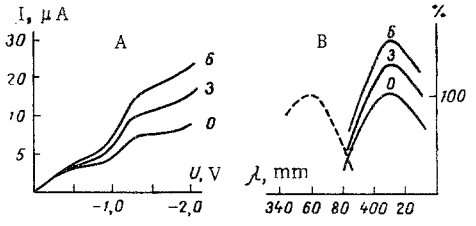

Fig. 1

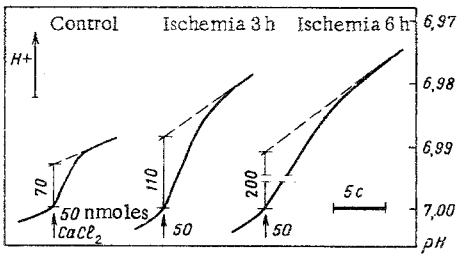

Fig. 2

Fig. 1. Accumulation of POL products in lipids of SR of ischemic muscles. A) Polarographic determination of lipids (2 mg/ml; methanol+benzene, 1 : 2; 0.25 M LiCl) isolated from muscle SR membranes of control animals (0) and after ischemia for 3 and 6 h (3 and 6). LP-7 Polarograph; B) emission (continuous line) and excitation (broken line) fluorescence spectra of same lipids (0.1 mg/ml; heptane+methanol, 1 : 5); Hitachi MPF-2A spectrofluometer.

Fig. 2. Changes in pH of incubation medium (5 mM Tris-HCl; 100 mM NaCl; 5 mM Na oxalate; 2 mM $MgCl_2$; 2 mM ATP; 0.5 mg SR protein; 37°C) during Ca^{2+}-dependent hydrolysis of ATP by SR membranes from control and ischemic muscles.

from which oxygen was removed by a current of nitrogen (pO_2 = 0 mm Hg) [4, 12]. The fraction of SR membranes was isolated from the muscles by differential centrifugation [22] and the parameters of the Ca^{2+} ETS were recorded in vitro with a pH-meter during acidification of the incubation medium in the course of Ca^{2+}-, Mg^{2+}-dependent hydrolysis of ATP [9]. The protein concentration of SR was determined by the biuret reaction [17]. Lipids were isolated [15] and their peroxidation products were analyzed (hydroperoxides of lipids polarographically [5]; diene conjugation products UV-spectrophotometrically [13]; secondary POL products of the Schiff base type fluorometrically [14]).

EXPERIMENTAL RESULTS AND DISCUSSION

The results in Table 1 and Fig. 1 show that as a result of interruption of the circulation in the limbs the Ca^{2+} ETS was inhibited and primary and secondary molecular POL products accumulated in vivo. The intensity of the two effects depended on the duration of ischemia. Whereas a concentration of POL products rose steadily during 1, 3, and 6 h of ischemia, the changes in the parameters of ETS differed qualitatively after different periods of ischemia: After 1 and 3 h of ischemia there was a tendency for Ca^{2+} transport to be inhibited while activity of Ca^{2+}-dependent ATPase was unchanged, whereas after ischemia for 6 h a further decrease in the Ca^{2+}-transporting ability of the SR fragments was accompanied by inhibition of Ca^{2+}-dependent ATPase

TABLE 2. Indices of Ca^{2+} ETS and POL in SR of Rat Skeletal Muscles during Ischemia for 6 h before and after Restoration of Blood Flow (M ± m)

Series of investigations	Activity of Ca^{2+}-ATPase, μmoles Pinorg/min/mg protein	Ca^{2+}/ATP*	Diene conjugation products
Control	4.88±0.23	0,34±0,03	1,87±0,38
Ischemia for 6 h	2,79±0,24	0	3,48±0,14
Ditto + restoration of blood flow for 4 h	1,86±0,09†	0	3,45±0,61

*The sharper changes are probably due to the fact that the experiments were carried out in spring.

†$P < 0.01$ between experiments before and after restoration of blood flow.

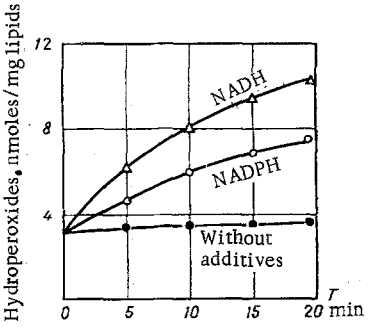

Fig. 3. Accumulation of lipid hydroperoxides during incubation of SR fragments in the presence of NADH (0.5 mM), NADPH (0.5 mM), and without additives. Conditions of incubation: 100 mM NaCl; 10 μM $FeSO_4$; 50 mM Tris-HCl; pH 7.4; 37°C.

also (Fig. 2). These results provide an explanation of the sharp decrease in the ATP concentration and increase in the inorganic phosphate concentration in ischemic muscles [6, 10]. The decrease in the level of "coupling" of Ca^{2+} transport with ATP hydrolysis observed during 3 h of ischemia, i.e., the decrease in efficiency of the calcium pump, although activity of Ca^{2+}-dependent ATPase remained high, must inevitably lead to rapid exhaustion of the pool of endogenous ATP, whose synthesis under anoxic conditions cannot compensate for these unproductive losses of energy.

The experiments in vitro showed that accumulation of products of free-radical lipid oxidation in the SR membranes was accompanied in the initial stages (up to 10 nmoles hydroperoxide/mg lipids) by inhibition of Ca^{2+} transport by the SR fragments (as a result of an increase in the permeability of the SR membranes for Ca^{2+} ions), whereas later (over 20 nmoles hydroperoxides/mg lipids) it was also accompanied by inhibition of Ca^{2+}-dependent ATPase [7]. The accumulation of POL products observed in SR membranes in vivo during ischemia correlates with the degree of injury to ETS ($r = -0.73$; $P < 0.01$) less strongly than during induction of POL in SR membranes in vitro by a nonenzymic system of Fe^{2+} + ascorbate ($r = -0.99$; $P < 0.01$). It must be remembered, however, that the concentration of lipid peroxides recorded in vivo represents only their steady-state concentration, reflecting both the accumulation of hydroperoxides and their breakdown catalyzed by reduced ions of metals of variable valency and very likely to occur under conditions of ischemia, i.e., when the redox potential of the muscle tissue is negative [11].

The contribution of accumulation of POL products to the damage caused by ETS in the SR membranes can be estimated by the use of antioxidants inhibiting POL in vivo. Experiments showed (Table 1) that the products of free-radical oxidation of lipids accumulating in vivo were evidently only part of the mechanism of damage to ETS taking place during 3 h of ischemia.

The possible mechanisms of the more intensive generation of lipid peroxides during ischemia are an important problem. In principle, a decrease in pO_2 in the ischemic muscle can only reduce the rate of POL. However, even at a comparatively low pO_2 level, generators of active forms of oxygen, with high affinity for O_2 [8], are able to catalyze the POL reactions provided that sources of reducing equivalents are present. It is pertinent to recall that the content of reduced pyridine nucleotides in skeletal muscles is relatively high during ischemia of short duration [6]. Accordingly the possibility of NADH- and NADPH-dependent POL taking place in SR membranes was investigated and the results showed that SR membranes of the skeletal muscles contain enzyme systems capable of acting as POL generators and activated by reduced pyridine nucleotides (Fig. 3).

Restoration of the blood flow after prolonged ischemia of organs does not cause the concentration of POL products to fall to their initial level [1]. A similar effect was observed in the SR membranes of skeletal muscles after ischemia for 6 h and restoration of the blood flow for 4 h (Table 2). Meanwhile removal of the tourniquet frequently leads to a further decrease in enzyme activity [6]. In this respect the ETS of the SR membranes was no exception, for during 4 h of the postischemic period the Ca-transporting capacity remained completely blocked and further inhibition of Ca^{2+}-dependent ATPase developed. Correlation between the time of the fall in Ca^{2+}-dependent ATPase activity and the time of death of the animals with an ischemic limb, as well as the further decrease in this index after removal of the tourniquet after 6 h of ischemia suggests that this criterion can be used to assess the degree of reversibility of ischemic damage in skeletal muscles.

It can be concluded that damage to the Ca^{2+} ETS in the membranes of SR, due partly to accumulation of POL products, can lead to an increase in the Ca^{2+} concentration in the sarcoplasm and can thus be a cause of hypertonia of the skeletal muscles in ischemia.

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