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, \(\alpha\)-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; \(\alpha\)-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\textsuperscript{2+} ETS and POL in SR of Rat Skeletal Muscles during Ischemia (M ± m)

| Series of investigations | Activity of \( \text{Ca}^{2+}\)-ATPase, \( \mu \text{moles Pinorg/min/mg protein} \) | \( \text{Ca}^{2+}/\text{ATP} \) | Hydroperoxides, \( \mu \text{moles/mg lipids} \) | Diene conjugation products | \( \text{pO}_2 \) in muscle, \( \text{mm Hg} \) | 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\textsuperscript{†}    | 2.88±0.53                   | 4.2±0.7\textsuperscript{†} | 100                    |
| 3 h                   | 4.26±0.34                       | 0.35±0.07      | 11.4±1.5\textsuperscript{†}   | 3.16±0.26\textsuperscript{†} | 2.3±0.2\textsuperscript{†} | 100                    |
| 6 h                   | 2.77±0.12\textsuperscript{†}    | 0.25±0.09\textsuperscript{†} | 11.0±0.7\textsuperscript{†}   | 3.26±0.14\textsuperscript{†} | 2.2±0.2\textsuperscript{†} | 0                      |
| lonol                 | 3.97±0.25                       | 0.48±0.15      | 5.9±0.1\textsuperscript{†}    | 1.9±0.21\textsuperscript{†}  | —                      |                        |
| \( \alpha \)-Tocopherol | 4.29±0.45                      | 0.44±0.05      | 4.2±0.7 \textsuperscript{+\#} | 2.84±0.51                   | —                      |                        |

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 lonol, after ischemia for 3 h.

‡‡ \( P < 0.01 \) between experiments with and without \( \alpha \)-tocopherol, after ischemia for 3 h.

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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 spectrofluorometer.

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

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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\textsuperscript{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\textsuperscript{2+} transport to be inhibited while activity of Ca\textsuperscript{2+}-dependent ATPase was unchanged, whereas after ischemia for 6 h a further decrease in the Ca\textsuperscript{2+}-transporting ability of the SR fragments was accompanied by inhibition of Ca\textsuperscript{2+}-dependent ATPase.