Effect of Dithiothreitol on Cardiomyocyte Ultrastructure in the Prooxidant Zone of Rat Heart in Experimental Cardiac Failure

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Compensatory and degenerative structural changes in rat cardiomyocytes are demonstrated. Lipid peroxidation plays a major role in the degenerative processes in the prooxidant zone. In rats with experimental cardiac failure treated with dithiothreitol, compensatory and repair changes predominate in the prooxidant zone cardiomyocytes. Ultrahistochemical analysis shows that administration of dithiothreitol restores ATPase activity of the sarcoplasmic reticulum.

Key Words: *lipid peroxidation; experimental cardiac failure; dithiothreitol; sulfhydryl groups*

A great role in the pathogenesis of cardiac failure is played by free-radical lipid peroxidation (LPO) yielding high-reactive oxidants exerting a cytotoxic effect on cardiomyocytes [4,8,9]. This is accompanied by impaired antioxidant status of cells [2]. In particular, the glutathione SH/glutathione SS (GSH/GSS) ratio is reduced in cardiac failure [6,12].

It has been recently shown that LPO is enhanced in the prooxidant zone, i.e., on the periphery of the necrotic zone [1] characterized by sharply decreased tissue respiration and the presence of blood vessels communicating with vessels of unaffected myocardium and still carrying blood and oxygen.

In this context, it seems important to examine the effect of dithiothreitol, a donator of SH groups, on the ultrastructure of cardiomyocyte in the prooxidant zone.

Experimental cardiac failure (ECF) was induced by 80 mg/kg isoprenaline sulfate, which causes small necrotic foci in the myocardium [5] and stimulates adrenergic receptors. Stimulation of these receptors induces the release of catecholamines which stimulate LPO.

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MATERIALS AND METHODS

Twenty Wistar rats weighting about 250 g (specific pathogen-free rats, a generous gift of the Germany Division of Charles Rives Laboratories, USA) were maintained in a barrier-type nursery (M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences). The rats were divided into two groups: group 1 rats were controls and rats of group 2 were intraperitoneally injected with 0.5 mg/kg dithiothreitol (Serva) for 5 days.

A specimen for electron microscopy was obtained from the left ventricle, fixed in 0.25% glutaraldehyde (Serva) in 0.1 M phosphate buffered saline (pH 7.4) for 10 h, postfixed in 0.1% OsO_4 (Serva) in 0.1 M phosphate buffered saline (pH 7.3), dehydrated in alcohols, and embedded in Epon-Araldite (Serva). The prooxidant zone was identified on semithin sections. Semithin and ultrathin sections were prepared using an Ultracut E microtome (Reichert Jung) and contrasted with lead citrate [14].

For histochemical study fresh cryostate sections were fixed for 1 h in 2% formaldehyde, kept in incubation mixtures for 45 min at 37°C, and postfixed with OsO_4 with ferricyanide in 0.1 M phosphate

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Fig. 1. Ultrastructure of myocardium in the prooxidant zone in rats with experimental cardiac failure. ×25,000.

a) sarcoplasmic membrane (*SPM*) of two cardiomyocytes, multiple disruptions and loosenings. Damage to the outer and inner mitochondrial membranes (*MT*), the absence of glycogen;

b) multiple disruptions of the nuclear membrane (*NM*), loosening of the sarcoplasmic reticulum (*SPR*) membranes, destruction of myofibrils (*MF*), edema of sarcoplasma, and the absence of glycogen.

TABLE 1. Effect of Dithiothreitol on Cardiomyocyte Degeneration in the Prooxidant Zone in ECF

Model	Cardiomyocytes	
	number	% of degraded cardiomyocytes
ECF	90±10	41.9±1.44
ECF+SH-donator	95±11	13.72±1.86*

Note. *$p<0.001$ compared with ECF group.

buffer. After dehydration, the sections were embedded in Epon-Araldite. The ultrathin sections were examined under a JEM-120EX electron microscope (Jeol).

Cardiomyocytes were counted at ×10,000 The data were processed statistically using the Student's *t* test for independent variables.

RESULTS

Morphological study revealed numerous small necrotic foci in the myocardium of animals with ECF, which is consistent with previous data [3,16].

Ultrastructural study revealed two opposite processes in cardiomyocytes of the prooxidant zone: compensatory-reparative and destructive.

The destructive process manifested itself as the following morphological changes: sarcolemmal disruption, abnormal ultrastructure of myofibrils, thinning of actin filaments, convolution and discontinuation of Z disks, edema of tubules of the sarcoplasmic reticulum (SPR) and stroma, swelling of mitochondria, appearance of a dense matrix between cristae and their degradation, condensation of nuclear chromatin and its aggregation with the nuclear membrane, glycogen depletion, and accumulation of degradation products in the form of lipoprotein complexes (Fig. 1, *a*, *b*).

In some cells in the degenerative stages we observed compensatory morphological changes. Chromatin in these cells was decondensed, and in some of them nucleolar organizer regions were seen. There were focal aggregates of small (newly formed) mitochondria around the nuclei; some of these mitochondria were moderately hypertrophied, which was probably related to enhanced energy metabolism in some cardiomyocytes induced by necrobiotic processes in the prenecrotic zone.

Ultrahistochemical study revealed a considerable drop of ATPase activity in the SPR and its slight decrease in myofibrils. Degraded cardiomyocytes constituted 41.9±1.44%.

In animals with ECF treated with dithiothreitol, cardiomyocytes at the stage of postnecrotic restoration were predominant (Fig. 2, *a*, *b*). No defects in the plasma and intercellular membranes, swelling of SPR channels, or myofibril damage were seen. The structures involved in protein synthesis and processing (nucleus, granular endoplasmic reticulum, and Golgi apparatus) were well developed. Lysosomes and peroxisomes with distinct membranes were seen around the Golgi apparatus, whereas in animals with ECF no lysosomes or lysosomes with loose membrane were observed. There were no autophagic vacuoles containing membrane fragments.

The development of tissue necrosis is accompanied by accumulation of the degradation products. After injection of the SH-donator to rats with ECF, no lipoprotein complexes were found. The inner and outer mitochondrial membranes were intact, and no dense granules were seen in the matrix.

Ultrahistochemistry showed that ATPase activity in the SPR channels returned to the control. The number of degraded cardiomyocytes after injection of dithiothreitol was 13.72±1.86% *vs.* 41.9±1.44% before the treatment ($p<0.001$).

It has been established that LPO plays a major role in the development of degenerative processes in the prooxidant zone. A decrease in the GSH/GSS ratio stimulates LPO [1,6,12]. Oxidation of membrane phospholipids is the main cytotoxic effect of LPO [13]. It has been recently demonstrated that the inner mitochondrial membrane participating in oxidative phosphorylation is very susceptible to LPO [15]. Disturbances of oxidative phosphorylation lead to the formation of intercrista inclusions [9]. The observed restoration of mitochondrial ultrastructure and of the integrity of plasma and intracellular membranes in animals treated with dithiothreitol is due to inhibition of LPO in the prooxidant zone.

Restoration of ATPase activity in the SPR channels to the control level provided by the SH-donator is presumably related to reduction of thiols in Ca-ATPase protein of SPR, which agrees with published data [7]. The observed reduction in the edema of SPR and stroma results from restoration of membrane integrity and reduction of the thiol groups of Ca-ATPase protein in SPR.

Thus, ultrastructural and ultrahistochemical studies and quantitative evaluation (Table 1) of cardiomyocytes in the prooxidant zone in dithiothreitol-treated and nontreated rats with ECF showed that augmentation of the antioxidant system capacity by dithiothreitol promotes compensatory-repair processes in cardiomyocytes of the prooxidant zone, which probably influences the size of necrotic zone.

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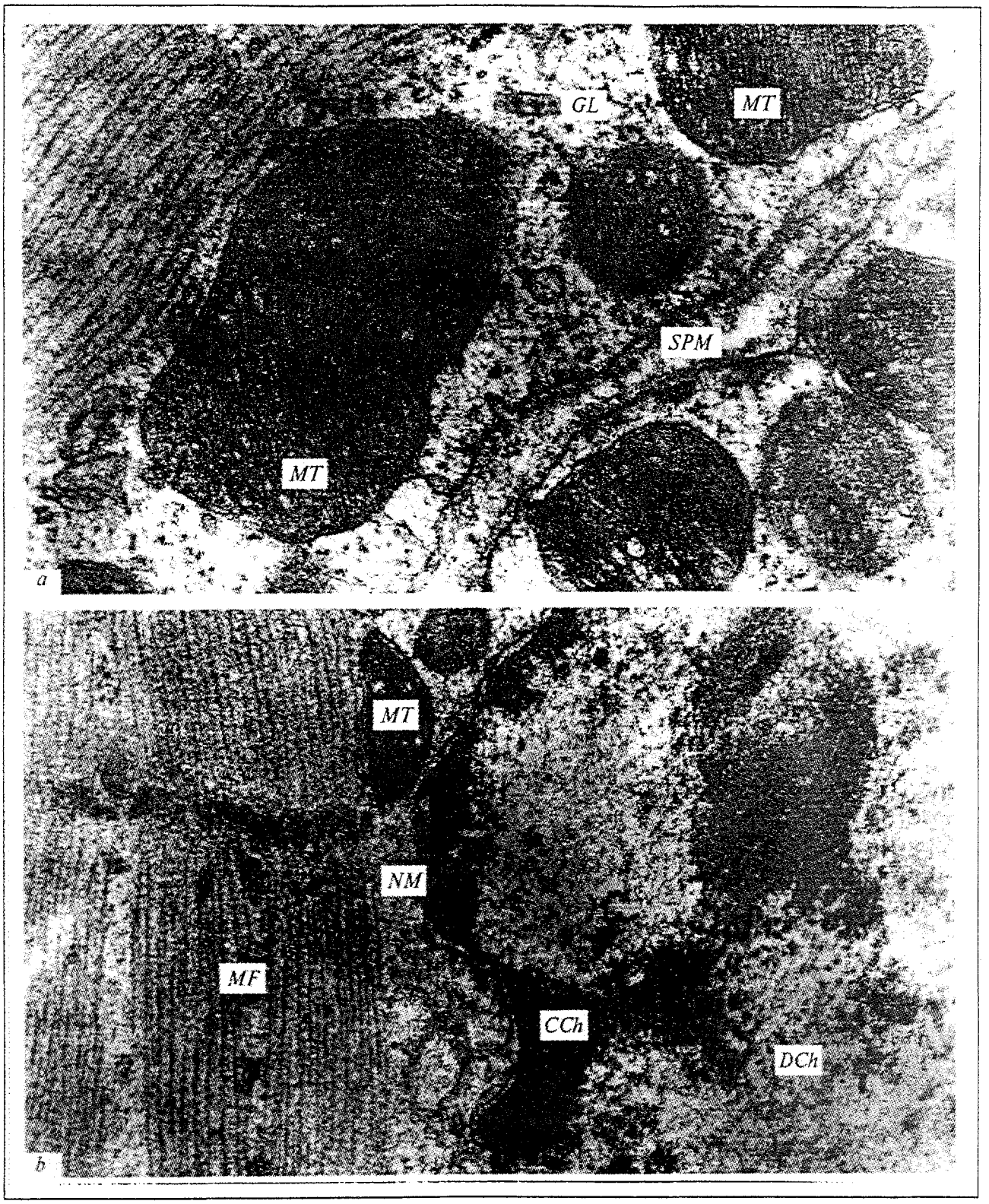

Fig. 2. Ultrastructure of myocardium in the prooxidant zone in dithiothreitol-treated rats with experimental cardiac failure. ×25,000.

a) sarcoplasmic membrane (*SPM*) of two neighboring cardiomyocytes. The integrity of SPM and the transport of substances through SPM are seen. Mitochondria (*MT*) have regular inner and outer membranes, glycogen granules (*GL*) are seen;

b) intact nuclear membrane (*NM*), condensed (*CCh*) and decondensed (*DCh*) chromatin.
MF: myofibrils.

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Effect of Electrical Cutaneous Stimulation on the Level of Succinate Dehydrogenase Activation and Changes in Glutamatergic Synaptic Transmission in Response to Sensory Stimulation

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It is shown that the rise of succinate dehydrogenase activity in the hippocampus depends on the number of sensory stimuli presented before decapitation, which correlates with changes in the efficiency of glutamatergic synaptic transmission in hippocampal sections from the same animal. Electrocutaneous stimulation potentiates the activation of succinate dehydrogenase induced by sensory stimulation probably due to enhanced glutamate release.

Key Words: *succinate dehydrogenase; population spike of the hippocampus; frequency-dependent facilitation; frequency-dependent depression*

The major afferent pathways of the hippocampus are glutamatergic [3,10]. Hence, long-term rearrangements caused by different types of sensory stimulation are probably mediated through modulations of glutamate metabolism [4,8,9,12,14].

Glutamate is taken up from the synaptic gap by nervous endings or glial cells [7,11]. It has been shown that part of glutamate released during excitation replenishes the pool of the transmitter in the synaptic gap, while the other part becomes involved into energy metabolism in astrocytes at the level of α-ketoglutarate and succinate [5,6,13]. Thus, in any case glutamate should be expected to influence the level of succinate dehydrogenase (SDH) activity.

A correlation was previously found between SDH activity and the parameters of synaptic transmission in the hippocampus (population spike amplitude, PSA, and the direction of plastic processes in rhythmic stimulation) [2].

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