Function of Ca-Pump in Sarcoplasmic Reticulum of Rat Myocardium during Adaptation to Electromagnetic Field

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We studied the effect of adaptation to electromagnetic field on functional state of Ca-pump in sarcoplasmic reticulum, intensity of lipid peroxidation, and energy metabolism in rat myocardium. Membrane viscosity was assessed in crude preparation of myocardial sarcoplasmic reticulum. Adaptation to electromagnetic field did not change the rate of endogenous respiration, including ouabain-sensitive respiration, but potentiated coupling between catalytic activity and Ca-transporting capacity of Ca-pump. It is hypothesized that modification of structural and functional properties of the membrane due to inhibition of free radical processes increases functional activity of Ca-pump in sarcoplasmic reticulum.

Key Words: *electromagnetic field; energy metabolism; Ca-pump; sarcoplasmic reticulum; lipid peroxidation*

Adverse effects of electromagnetic field (EMF) on human health are generally accepted. EMF induces ion migration in biological tissues, changes in their concentration in various cell compartments and intracellular space, polarization of biomolecules, and breakage of hydrogen bonds. Adaptation to EMF promotes compensatory ionic processes in muscles [6,7]. However, the mechanisms underlying the effect of EMF on pathologic changes in biological tissues are unknown. Our aim was to study the effect of adaptation to EMF on functional state of Ca-pump in sarcoplasmic reticulum (SR) playing an important role in contractile activity of the myocardium.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino rats weighing 200-250 g. Adaptation to EMF was performed as follows: the rats were placed in front of a PC monitor at the distance of 4-6 cm for 6-8 hours daily, 6 days per week for 3 weeks. The parameters of EMF were 72 V/m at 2 kHz and 9 V/m at 2-400 kHz; magnetic field 1960 nT at 5-2000 Hz and 196 nT at 2-400 kHz; electrostatic potential 2.53 kW. The control rats were subjected to the same manipulations, but the monitor was turned off. The relative microviscosity of the membranes in crude microsomal fraction was assessed by lateral diffusion of hydrophobic probe pyrene. Microviscosity of membrane lipid bilayer and the area of lipid-protein contacts were assessed at excitation wavelength of 334 and 286 nm, respectively [3]. Incubation of SR-containing suspension with pyrene (3 μmol/ml suspension) was carried out at 25°C for 1 min under constant stirring. Fluorescence intensity of pyrene dimers and monomers was measured in Aminco Bowman Series 2 spectrofluorimeter (Thermo Spectronic) [1]. The intensity of LPO in the myocardium was assessed by the content of malonic dialdehyde (MDA) [9]. Myocardial energy metabolism was assessed by the rate of O_2 utilization by tissue homogenates. Homogenization medium contained (in mM): 140 NaCl, 6.2 KCl, 1.5 $MgCl_2$, 12 Na_2HPO_4, 6% sucrose, pH 7.5. The rate of O_2 utilization was measured using type 5221 oxymeter supplied with type 5972 Teflon-coated electrode. Respiration rate was measured at 37°C in a 1-ml cell in sucrose-free isolation

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medium. Ouabain-sensitive respiration was calculated by the difference in O_2 utilization in the presence and absence of 0.1 mM ouabain. To isolate crude microsomal fraction, the myocardium was homogenized in a medium containing 0.3 M sucrose, 3 mM sodium azide, 30 mM Tris HCl, pH 8.3. Cell fragments, myofibrils, and mitochondria were precipitated by centrifugation (10,000*g* for 20 min) [8]. The supernatant was centrifuged at 35,000*g* for 60 min. Activity of Ca-pump of SR was assessed in re-suspended precipitate using cation-selective electrodes. Amplified and digitized signals were fed into PC and processed with original software. The rate of ATP hydrolysis was evaluated by H^+ concentration, transport of Ca^{2+} ions across the membrane was evaluated by a decrease in Ca^{2+} activity in the incubation medium. Incubation medium contained (in mM): 5 Tris HCl buffer, 100 KCl, 3 $MgCl_2$, 3 ATP, and 40 µl microsomal preparation at pH 7.4 and 25°C. Volume of measuring cell was 4 ml. The reaction was initiated by adding 40 µl 3 M $CaCl_2$ [8].

The results were analyzed statistically using Student's *t* test.

RESULTS

Adaptation to EMF significantly increased the rate of ATP hydrolysis by Ca-pump in myocardial SR. The rate of Ca entry from the incubation medium also increased by several times, which resulted in an increase in Ca^{2+}/ATP ratio (Table 1). Therefore, adaptation to EMF increased coupling between catalytic activity and transporting capacity of Ca-pump.

Since Ca-ATPase is an energy-dependent enzyme, we also assessed the level of energy metabolism in the myocardium during adaptation. Our experiments revealed no changes in myocardial metabolism during adaptation to EMF. The rate of oxygen consumption by the myocardium was the same in control and experimental rats. Similarly, there were no changes in ouabain-sensitive respiration controlled by Na,K-ATPase, another energy-depending enzyme (Table 1). Stability of endogenous ouabain-sensitive respiration and enhancement of catalytic activity of Ca-pump attest to energy redistribution in favor of Ca-pump and inhibition of other energy-depending processes in rat myocardium during adaptation to EMF.

Functional state of Ca-pump depends on activity of free radical processes [2,5]. Therefore, we measured MDA content to study the mechanisms of activation of Ca-pump in myocardial SR. Under normal conditions, free radical processes are an essential element of metabolism. In particular, reactive oxygen species induce physicochemical modification of biological membranes. In addition, they serve as the second messengers in regulation of cell metabolism [4]. We found that myocardial content of MDA significantly decreased in adapted rats (Table 1). This moderation of free radical processes could trigger a number of adverse events in the cell: modulation of activity of membrane-bound enzymes, disturbances in the regulation of metabolic processes, and increase in membrane fluidity. The latter was observed in our study: microsomal membranes from adapted rats were characterized by increased fluidity of the lipid bilayer and decreased fluidity of lipid-protein contacts (Table 1).

Therefore, adaptation to EMF was accompanied by pronounced activation of Ca-pump in myocardial SR. This increase in enzyme activity can inhibit other

TABLE 1. Effect of Adaptation to Electromagnetic Field on Rat Myocardium (*M*±*m*, *n*=9-10)

Index	Control	Experiment	
		abs.	Δ, % of control
MDA, µmol/g tissue (*n*=15)	1.18±0.18	0.89±0.09	-25.0*
Microviscosity, rel. units			
lipid bilayer	0.0842±0.0024	0.0971±0.0021	15.3**
lipid-protein contacts	0.0940±0.0028	0.0792±0.0070	-19.7*
Respiration, µmol O_2/g tissue/h			
endogenous	53.7±4.8	55.4±6.2	
ouabain-sensitive	19.6±4.8	20.1±2.4	
Activity of Ca-pump per 1 mg protein/h			
ATP-hydrolysis capacity, µmol P_i	17.58±2.00	24.59±2.56	39.9**
Ca-accumulation capacity, µmol Ca^{2+}	42.87±5.66	310.00±29.78	809.9*
Ca/ATP	2.44±0.33	12.61±1.41	416.7*

Note. *p<0.01, **p<0.05 compared to the control. P_i: inorganic phosphate.

energy-dependent processes in the cells, which was confirmed by stability of endogenous respiration in the myocardium under conditions of enhanced catalytic activity of Ca-pump. Presumably, activation of Ca-pump in our experiments was induced by changes in physicochemical properties of SR membrane caused by inhibition of free radical processes during EMF.

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