The similar modification of the catalytic properties of MAO discovered in various extremal states (hyperoxia, hypoxia, exposure to cold) was thus due to two factors: extrusion of the enzyme into the cytoplasm as a result of disturbance of membrane structures and a change in the molecular properties of MAO, as shown by an increase in $K_m$ and changes in the substrate specificity of the enzyme.

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# PYRENE PROBE STUDY OF MEMBRANE LIPIDS OF THE SARCOPLASMIC RETICULUM IN ISOPROTERENOL MYOCARDITIS


L. V. Lyzlova, V. R. Persianova, and A. E. Antipenko



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KEY WORDS: isoproterenol myocarditis; viscosity of lipids; sarcoplasmic reticulum.


The cardiac rhythm is largely determined by operation of the Ca pump of the myocardial sarcoplasmic reticulum (SR). In turn, in various forms of pathology of the heart, disturbance of the working of this pump and its regulation [1] leads to incompleteness of diastole and, consequently, of systole also. Membrane-bound enzyme function also is known to depend largely on the state of the surrounding lipids, which determines the flowability and microviscosity of the membrane [6].

Accordingly, in the investigation described below, the state of the lipid layer of SR membrane and activity of the Ca,Mg-ATPase of SR of the heart muscle were studied in isoproterenol myocarditis.

## EXPERIMENTAL METHOD

Myocarditis was induced in rabbits weighing 1.5-2.0 kg by subcutaneous injection of a 1% aqueous solution of isoproterenol (1 mg/kg body weight). The animals were decapitated 24 h after injection of the drug. Ischemic tissue from the left ventricle was investigated and the same regions of the myocardium of healthy animals served as the control. The microsomal fraction was isolated by the method in [7]. Excimerization of pyrene in SR was


Department of Biochemistry, A. A. Zhdanov Leningrad University.


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TABLE 1. Ca,Mg-ATPase Activity and MDA Concentration in SR of Heart with Isoproterenol Myocarditis

| Regions of myocardium | Ca,Mg-ATPase activity, μmoles $P_i$/mg protein/min | MDA concentration, nanomoles/mg protein |
|---|---|---|
| Control | 0,62±0,08 (10) | 1,1±0,06 (6) |
| Affected | 0,36±0,05 (9) | 2,6±0,22 (7) |

Legend. Number of animals shown in parentheses.

observed in 20 mM Tris-HCl buffer, pH 6.8, 0.6 M KCl, 0.25 M sucrose. The protein concentration in the probe was 0.5 mg/ml. Twice recrystallized pyrene was dissolved in alcohol immediately before the experiment and 2.5 μmoles in 10 μl of alcohol was gradually introduced (final pyrene concentration in the sample 10-15 μM). Fluorescence was measured on an MPF-4 spectrofluorometer (Hitachi, Japan). The spectra were corrected automatically. Activity of Ca,Mg-ATPase of SR was measured as accumulation of inorganic phosphate in medium of the following composition: 20 mM Tris-HCl (pH 7.2), 5 mM $MgCl_2$, 5 mM ATP, 100 mM KCl, 5 mM $NaN_3$, 2.5 mM Na oxalate, 0.15 mM $CaCl_2$, 0.1 mg protein in 1 ml (30°C). The intensity of lipid peroxidation (LPO) in the SR preparations was judged from malonic dialdehyde (MDA) formation [8].

## EXPERIMENTAL RESULTS

To study viscosity of the lipids in SR membranes, the fluorescent hydrophobic probe pyrene was used; pyrene inserts itself into the region of the fatty acid residues of the membrane phospholipids and diffuses in the membrane to form excimers. The degree of excimerization of pyrene is directly proportional to the rate of diffusion and inversely proportional to the microviscosity of the lipid layer of the membrane [10]. The microviscosity of membranes falls as the temperature rises, and this leads to an increase in the degree of excimerization of pyrene. Changes taking place under certain conditions in the lipid layer of the membrane can thereby be demonstrated in the form of differences of temperature-dependence on microviscosity of the membrane lipids. In the present investigation excimerization of pyrene in SR was studied between the temperature range from 15 to 45°C.

The rate of diffusion of pyrene and, correspondingly, the viscosity of the lipid layer are characterized by the ratio $I_E/I_M$, where $I_M$ denotes the intensity of fluorescence of pyrene monomers at a wavelength of 395 nm and $I_E$ denotes the intensity of fluorescence of pyrene excimers at a wavelength of 485 nm. Microviscosity was determined by exciting fluorescence of pyrene with light at wavelengths of 280 and 330 nm. With a wavelength of 280 nm, excitation of the tryptophan residues of proteins incorporated into the SR membranes, mainly of Ca,Mg-ATPase, takes place. The wavelength of fluorescence of tryptophanyls coincides with the wavelength of excitation of pyrene (330 nm), as a result of which energy is transferred from the tryptophan residues to pyrene located in the lipid bilayer in the immediate vicinity of the protein globules (not more than 0.3 nm away), by induction-resonance energy transfer (IRET).

The fluorescence spectrum of SR proteins of intact heart muscle [1], the fluorescence spectrum of pyrene in SR after excitation by IRET (excitation wavelength 280 nm), and the fluorescence spectrum of pyrene in these same SR preparations with excitation wavelength of 330 nm are given in Fig. 1. The efficiency of IRET in this case was about 67-70%, which is a little less than in SR preparations from rabbit skeletal muscles [3]. Excimerization of pyrene was observed both in the region of lipids in immediate contact with proteins ("bound" lipids) and in the region of "free" lipids. Typical curves showing $I_E/I_M$ as a function of temperature at excitation wavelengths of 280 and 330 nm, obtained in preparations of SR from intact and damaged heart muscle (control) are shown in Fig. 2. At both excitation wavelengths an increase in the degree of excimerization of pyrene and, consequently, a reduction in microviscosity of the SR membranes in the region of 15-35°C, followed by an increase in microviscosity in the region of 35-42°C, were observed in the control. In the case of insertion of pyrene into SR obtained from the myocarditic heart an increase in microviscosity took place at lower temperatures and more rapidly than in preparations of healthy muscle. The same picture was observed in the region both of "bound" and of (especially) "free" SR lipids.

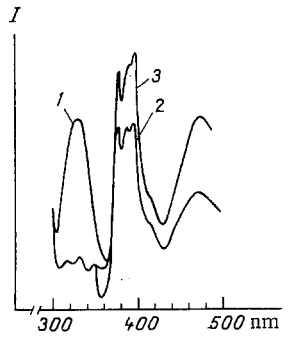


Fig. 1

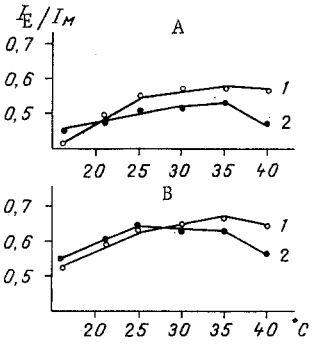


Fig. 2

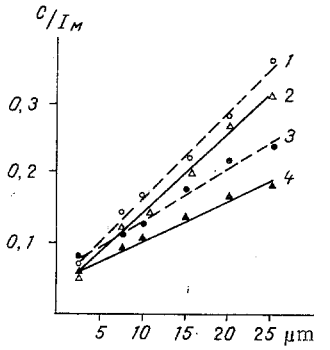


Fig. 3

Fig. 1. Fluorescence spectra: 1) protein chromophores in SR membrane; 2) pyrene in SR membrane, excitation wavelength 280 nm; 3) pyrene in SR membrane, excitation wavelength 330 nm.

Fig. 2. Dependence of degree of excimerization of pyrene in SR membranes on temperature. $I_E$) Intensity of fluorescence of pyrene excimers at $\lambda$ = 485 nm; $I_M$) intensity of fluorescence of pyrene monomers at $\lambda$ = 395 nm. A) Excitation wavelength 280 nm, B) 330 nm. 1) Normal heart, 2) myocarditis.

Fig. 3. Dependence of $C/I_M$ on C in SR membranes at 37°C. 1) "Bound" lipids (normal heart); 2) "bound" lipids (myocarditis); 3) "free" lipids (normal heart); 4) "free" lipids (myocarditis).

In SR preparations from the heart in isoproterenol myocarditis the level of LPO products was raised (Table 1). Under the influence of these products quenching of pyrene fluorescence is known to be observed [4]. As a result of this, comparing viscosities of SR membranes in the normal heart and in isoproterenol myocarditis by the pyrene excimerization method may give distorted results. To rule out this possibility we used a different method of determining viscosity of the lipids, namely, that based on dependence of $C/I_M$ on C, where C stands for the pyrene concentration. In this case the pyrene quenching effect can be disregarded [4]. Typical curves showing dependence of $C/I_M$ on C in the region of "free" and "bound" lipids in SR preparations in myocarditis and in the control at 37°C are shown in Fig. 3. The angle of slope of the straight line characterizes the diffusion constant of pyrene: In the region of "free" lipids diffusion of pyrene in SR of the affected heart was about 14% less than in the control. In the region of "bound" lipids diffusion of pyrene into SR obtained from the "myocarditic" heart was about 10% less than into SR from healthy muscle. It can accordingly be concluded that in isoproterenol myocarditis the viscosity of the cardiomyocyte SR membrane is higher than that in the intact heart in the region of both "free" and "bound" lipids.

The observed increase in viscosity of SR membranes in isoproterenol myocarditis was accompanied by a fall in activity of SR Ca,Mg-ATPase, against the background of an increase in the concentrations of LPO products (Table 1).

It can be tentatively suggested that the raised LPO level is one cause of the changes in viscosity of the lipid components of SR membranes and of a corresponding decline in activity of membrane-bound Ca,Mg-ATPase in isoproterenol myocarditis. We know, for instance, that auto-oxidation of catecholamines (to such products as adrenochrome, for instance) leads to the generation of highly toxic free radicals, and causes ischemia of the heart muscle [9]. As a result of LPO the ionic permeability of the cardiac membranes is disturbed and this leads, in particular, to "overfilling" of the cytosol with Ca ions. This last state of affairs is, as we know, a critical factor in the development of profound changes in the cardiomyocyte [5] and it may be largely responsible for the disturbance of working of the Ca-pump of SR, and also of its regulation by means of cAMP-dependent phosphorylation of the target proteins in SR, as the writers showed previously in circulatory hypoxia and myocardial infarction [1, 2]. An excess of $Ca^{++}$ in the sarcoplasm may lead to activation of phosphorylase $A_2$ and to a corresponding rise in the level of arachidonic acid, which in turn is metabolized with the formation of free radicals [8]. This positive feedback may be an essential factor in the development of injury to the cardiomyocyte membranes induced by catecholamines.

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# MECHANISMS OF DISTURBANCE OF HORMONAL REGULATION OF ADENYLATE CYCLASE IN IN VITRO IRRADIATED LIVER PLASMA MEMBRANES OF RATS IN EARLY ONTOGENY

T. E. Ushakova, L. V. Slozhenikina, L. A. Fialkovskaya, and A. M. Kuzin*

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The adenylate cyclase (AC) system of plasma membranes participates in the regulation of many intracellular metabolic processes which are disturbed by the action of ionizing radiation. This explains the increased interest in the state of AC after irradiation. The main data on radiation modification of AC have been obtained on mature tissues [6, 10, 11]. The study of AC in the developing organism is particularly important because its reaction product cAMP also participates in the regulation of cellular proliferation and differentiation, which are damaged by irradiation. The writers previously described the effect of $\gamma$-irradiation of isolated plasma membranes of the fetal rat liver in doses of 1-1000 Gy on basal and hormone-stimulated (by isoproterenol, via $\beta$-adrenoreceptors) AC activity. Isoproterenol-stimulated AC activity was found to be most vulnerable to irradiation in the region of high doses [2, 3].

The aim of the present investigation was to study the possible causes of radiation disturbance of hormonal regulation of AC.

## EXPERIMENTAL METHOD

Experiments were carried out on 20-day Wistar rat fetuses. Plasma membranes [2] or the membrane fraction [3] were isolated from the liver. The membrane fraction was used in experiments with binding of [$^3$H]-dihydroalprenolol ([$^3$H]-DHA). The conditions of irradiation of the isolated membranes and determination of AC activity were described previously [3]. Irradiation was given in a dose of 300 or 500 Gy. The latter dose reduced almost by half the degree of stimulation of AC by isoproterenol [3]. Binding of [$^3$H]-DIIA was carried out by the method in [7] in siliconized tubes at 30°C for 10 min in 0.5 ml of medium of the following composition: 25 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10-60 nM [$^3$H]-DHA (1.924 TBq/millimole). The reaction was started by addition of about 200 $\mu$g membrane protein and was stopped by addition of 5 ml of ice-cold 25 mM Tris-HCl buffer, containing 5 mM $MgCl_2$. Filtration was carried out in vacuo through GF/C glass fiber filters. The filters were washed with the above buffer solution in a total volume of 15 ml, dried, and counted in ZhS-8 scintillation fluid, using an SL-30 scintillation counter (Intertechnique, France). Specific binding was assessed from the dif-

*Corresponding Member, Academy of Sciences of the USSR.

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