AGE DIFFERENCES IN LIPID FLOWABILITY IN THE SARCOPLASMIC RETICULUM OF THE RAT HEART

V. K. Kol'tover, N. V. Gerasimovich,
A. P. Kirilyuk, A. A. Milyutin,
and S. A. Romanov

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The view has often been expressed in the literature that disturbances of myocardial function observed during aging and in some cardiovascular diseases (arterial hypertension, ischemia, and so on) are connected with changes in the membrane systems regulating calcium transport [6, 9]. Membranes of the sarcoplasmic reticulum (SR) of the cardiomyocytes are among the most important components of these systems [6, 8, 9]. Meanwhile it is known that the efficiency of function of SR, like other membranes, depends essentially on the physical state of their lipids, and in particular, on the degree of orderliness and mobility (flowability) of the lipids of the bilayer [1, 4-6, 8]. The need has thus arisen for a comparative study of the flowability of membrane lipids depending on the age of the animal and its physiological state. The most informative tools for such investigations at the present time are fluorescent and spin probes [1, 2, 4, 5].

This paper gives the results of a study of lipid flowability in membranes of the myocardial SR of adult and old rats using fluorescent and spin probes.

EXPERIMENTAL METHOD

Membranes of SR were isolated from the left ventricles of the hearts of adult (aged 12 months) and old (24 months) male Wistar rats by the method in [11], using medium containing 20 mM Tris-HCl (pH 7.4), 0.25 M sucrose, and 1 mM dithiothreitol, after which they were frozen in the same medium and kept at −196°C. Activity of the membrane preparations was estimated from their ability to accumulate calcium ions in medium containing 50 mM imidazole (pH 7.0), 100 mM KCl, and 0.1 mM EGTA, using the isotope ^{45}Ca (2 μCi/ml) and a millipore filter technique [9, 11]. In experiments to study inhibition of Ca^{++} transport by rotenone and oligomycin (in the presence of oxalate or succinate as precipitating anion) it was shown that contamination of the SR preparations with mitochondria did not exceed 5%. The fluorescent probe DPHT (1,6-diphenyl-1,3,5-hexatriene (from Sigma, USA) was introduced into the membranes from a solution of the probe in tetrahydrofurane (1 mM). For this purpose 2 ml of a suspension containing 125 μg of membrane protein was incubated with 5 μl of the solution of DPHT for 30 min at room temperature, and thereafter for 10 h at 4°C. Steady-state fluorescence of DPHT was measured at 22°C on an SLM spectrofluorometer (USA), radiation with wavelength λ = 430 nm being recorded, with excitation by light with λ = 360 nm. Anisotropy of fluorescence r_s was calculated with corrections for scattering of light and natural polarization of the monochromator. The parameter of orderliness S_f of the DPHT probe was calculated from the value of r_s, taking r_0 = 0.395 [1, 12]. Curves of quenching of fluorescence were recorded on a PRA pulse fluorometer (Canada) on single photon counting mode at the same wavelength of excitation and emission, and analyzed by "Minc-11" microcomputer (USA). The spin probe 5NS (5-nitroxide stearate, from Syva, USA) was introduced into lipid bilayers of SR, from which all dithiothreitol had previously been washed out, as in [7]. For this purpose a solution of 5NS in ethanol (1 mM) was evaporated under a current of argon and the film of radical thus formed was incubated with the membrane suspension for 15 min at room temperature. The membranes were washed to

Institute of Chemical Physics, Academy of Sciences of the USSR, Chernogolovka, Moscow Region. Institute of Radiobiology, Academy of Sciences of the Belorussian SSR, Minsk.
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TABLE 1. Values of Parameters of Fluorescent Probe DPHT (mean values + standard error of the mean) and of Parameter of Orderliness of Spin Probe 5NS (mean values + standard deviation) in SR of Cardiomyocyte Membranes of Rats of Different Ages

Age, months	DPHT					5NS
	τ_1, nsec	τ_2, nsec	fluorescence with τ_2, %	r_s	S_f	S_e
12	2,30±0,14 (7)	9,33±0,12 (7)	87,6±0,5 (7)	0,284±0,015 (4)	0,82±0,03 (4)	0,686±0,005 (4)
24	1,52±0,16* (7)	8,31±0,22* (7)	81,0±2,6* (7)	0,236±0,009* (4)	0,72±0,02* (4)	0,675±0,005** (4)

Legend. Temperature 22°C. Number of experiments given in parentheses. Age differences significant: *p = 0.01, **p = 0.05.

remove excess of the probe and concentrated by centrifugation to obtain a satisfactory signal/noise ratio. EPR spectra were recorded on an E-104 spectrometer (USA), the temperature being controlled with an accuracy of ±0.2°C by means of a thermocouple, located in the sample. The parameter of orderliness of the probe SE was measured from the EPR spectra, with a correction for polarity of its environment in the membrane [2, 5]. The molar ratio of probe/lipid did not exceed 1:300 for DPHT and 1:100 for 5NS.

EXPERIMENTAL RESULTS

Measurements of ATP-dependent Ca^{++} transport showed that at 37°C the SR preparations from animals of different ages had different values of maximal velocity (40.8 ± 3.0 and 22.2 ± 0.5 nmoles $^{45}Ca^{++}$/min/mg protein, repectively, for adult and old rats), whereas values of the Michaelis constant were equal (0.59 ± 0.02 and 0.63 ± 0.01 μM). The results of the probe experiments are given in Table 1. Fluorescence of DPHT in SR membranes is characterized by two values of duration of the excited state: a relatively short τ_1 and a longer τ_2; the latter, moreover, characterizes more than 80% of the fluorescence. Fluorescence of DPHT in liposomal membranes of synthetic phospholipids has one single value of τ in the 7-9 nsec interval [1, 12]. It can be tenatively suggested that most DPHT molecules with time τ_2 are located between the acyl chains of the lipids in the lipid bilayers of the membranes, whereas fluorescence at τ_1 belongs to molecules of the probe located in regions of protein-lipid junctions ("boundary lipid"). Values of τ_1 and τ_2 and of the parameter of anisotropy of fluorescence r_s were smaller for membranes of old animals than membranes of adult rats. A decrease in the value of r_s with simultaneous decrease in the life span of fluorescence indicates an increase in mobility of the probe [1, 12]. The DPHT molecule performs random movements in the membrane (rotations, deviations from the normal to the surface of the bilayer) within the limits of a cone, whose angle is characterized by the parameter of orderliness S_f. The closer the value of S_f to 1, the higher the degree of orderliness, and the lower the mobility of the acyl chains of the lipid molecules, and it thus reflects the flowability of the lipid bilayer [1]. Consequently, a lower value of the parameter of orderliness of DPHT in the membranes of old animals compared with those of adult rats is evidence of an increase in lipid flowability in the membranes of SR with age.

This conclusion is confirmed by the results of the spin probe experiments (Table 1). The probe 5NS, containing a paramagnetic nitroxide fragment attached to the 5th carbon atom of the acyl chain, "probes" the bilayer in the region of the polar heads of the lipids, and performs random rotary movements which, like the mobility of the DPHT probe, are characterized by a parameter of orderliness [2, 5]. It follows from the data given in Table 1 that the value of SE of the spin probe in membranes of old animals is less than that in the membranes of adult rats, in agreement with data obtained by the fluorescent probe method. However, with an increase of temperature the difference between values of the parameter of orderliness of the spin probe in the membranes of old and adult rats decreases, and at 37°C it disappears. At this (physiological) value of the temperature SE = 0.591 ± 0.005 for membranes of SR of adult rats and 0.588 ± 0.006 for membranes of old animals (p = 0.05).

It was shown previously that lipid flowability in membranes of the endoplasmic reticulum of the liver, sarcolemma of the cardiomyocytes, brain synaptosomes, and several other membranes stabilizes (at physiological temperatures) during aging in rats [5, 7, 10]. The

mechanisms of this stabilization are evidently linked with enzymes of synthesis of cholesterol and other structural components of the membranes, and have so far received little study. Nevertheless, it can be postulated that the corresponding enzyme systems function with limited reliability [3]. Evidence in support of this view is given, in particular, by the appearance of age-related structural differences both in membranes of SR (in the present investigation) and also in other membranes [3, 5, 7], when the temperature falls below physiological values. The reduced reliability of the system for stabilizing lipid flowability in the membranes of the old animal may be manifested not only when the temperature falls, but also in the presence of other deviations from the "norm," including in stress situations. Besides the diminished ability of the SR membranes to accumulate calcium and other changes in all the calcium transport systems of the myocardium [9], this fact may play an essential role in the disturbances of cardiac function during aging.

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