STRUCTURAL CHANGES IN MEMBRANE LIPIDS OF SKELETAL MUSCLE SARCOPLASMIC RETICULUM IN HYPERCHOLESTEROLEMIA

A. A. Timofeev, O. A. Azizova, and G. V. Chernysheva

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In hypercholesterolemia (HChE) the rate of accumulation of Ca^{2+} by membranes of the sarcoplasmic reticulum (SR) is reduced [6], and this is accompanied by a parallel increase in concentrations of both cholesterol and lipid peroxidation (LPO) products in the membranes [7]. It is natural to suggest that elevation of the cholesterol level and intensification of LPO in the membranes may be the cause of the reduced efficiency of operation of the SR Ca-pump, more especially because both these factors have a similar action *in vitro* [3, 11].

Since the effect of LPO and of a high cholesterol concentration is manifested mainly through changes in the physicochemical properties of lipids, possible structural changes in the lipid phase of the SR membranes during HChE were studied in the investigation described below.

EXPERIMENTAL METHOD

Fourteen male Chinchilla rabbits weighing 2.5-3 kg, divided into two groups (seven animals in each group) were used. Cholesterol (analytically pure, from Reakhim, USSR), in a

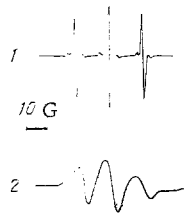

Fig. 1

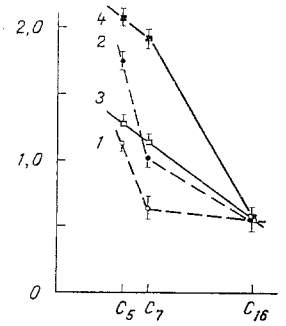

Fig. 2

Fig. 1. Changes in EPR spectrum of tempon in SR preparation in absence (1) and presence (2) of $NiCl_2$. Spectrum recorded in presence of $NiCl_2$ with 30-40-fold increase in sensitivity of the instrument.

Fig. 2. Changes in spin mobility of lipid probes depending on depth of their immersion in lipid phase of SR membranes during HChE. Abscissa, probes arranged in order of their immersion in lipid phase of SR membranes. Origin corresponds approximately to membrane surface (location of carboxyl group of probe). Scale of location of probes along abscissa corresponds to that of completely stretched carbon chain of stearic acid; ordinate) ratio $\tau_{membrane}/\tau_{glycerin}$. 1) Control; 2) control in presence of $NiCl_2$; 3) cholesterol; 4) cholesterol in presence of $NiCl_2$.

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TABLE 1. Changes in Parameters h and τ of EPR Spectra of Tempamine, Tempon, and PCA, Immobilized by SR Membranes, during HChE and in the Presence of $NiCl_2$ (M ± m)

Parameter	Experimental conditions	Tempamine	Tempon	PCA
h, relative units	Control	0,0861±0,003	0,136±0,015	0,132±0,01
	HChE (1 month)	0,0521±0,009*	0,123±0,015*	0,109±0,01*
τ, $\cdot 10^{-9}$ sec	Control	1,24±0,059	1,313±0,09	1,606±0,68
	HChE (1 month)	0,739±0,77*	0,104±0,05*	0,915±0,69

*$P < 0.05$ compared with control.

dose of 1 g/kg body weight, was added to the standard daily diet of the experimental animals. All the rabbits took part in the experiments for 30 days. SR preparations were isolated by the method in [5]. The state of HChE was identified by the serum cholesterol level [9]; the cholesterol concentration in SR membranes was determined by the same method, and protein by Lowry's method. In all the experiments the membranes were kept in buffer: 1 M sucrose, 25 mM imidazole-HCl, pH 7.0 (37°C). The following lipid spin probes were used: tempamine, tempon, 1-oxyl-2,2,5,5-tetramethylpyrroline-4-carboxylic acid (PCA), 5-doxyl stearate (C_5) and 16-doxyl stearate (C_{16}) (from "Syva," USA), and spin-labeled stearic acid with an imidazoline nitroxyl fragment in position 7 of the carbon chain (C_7) [2]. Specimens of SR were treated with spin probes by introduction of 0.001 ml of probe with an original concentration of 10^{-1} M for tempamine, tempon, and TCA, and 10^{-2} M for C_5, C_7, and C_{16} in ethanol (96%) to the bottom of the tube, and then removing the ethanol by evaporation. Next, 0.1 ml of the specimen of SR was added in a concentration of 15 mg protein/ml, and mixed. A solution of $NiCl_2$, made up in the same buffer as the SR preparation, in a concentration of 0.5 M in the sample, was used in the experiments. After addition of the necessary quantity of $NiCl_2$ to the test sample, an equivalent quantity of buffer was added to a parallel sample. Electron paramagnetic resonance (EPR) spectra were recorded on an E-4 spectrometer (Varian, USA) at 37°C. The spin correlation time (τ) for tempamine, tempon, PCA, and C_{16} was calculated by the equation for fast spinning probes, that for C_5 and C_7 for slowly spinning probes [4]. The order-parameter (S) was determined by the method in [1] with a correction for the difference in polarities of the environment of the nitroxyl fragment of the probe in the membrane and in the crystal. The parameter of hydrophobicity (h) was determined by the method in [4] relative to water and to heptane (chemically pure grade, from Reakhim).

EXPERIMENTAL RESULTS

After the rabbits had been kept on a diet with added cholesterol for 1 month the cholesterol level in the blood serum was increased tenfold and in SR membranes it was doubled, in agreement with previous observations [7]. It was observed previously that activation of LPO and accumulation of cholesterol in SR membranes in HChE lead to changes in the structural organization of Ca-ATPase [8], as a result of changes in the physicochemical properties of the lipid matrix of the SR membranes.

The structural organization of the lipid part of SR membranes in HChE was studied with the aid of spin probes (tempamine, tempon, PCA, C_5, C_7, C_{16}). The first three probes are known to pass through the SR membrane [10-13], so that the internal space of SR vesicles and also the inside of the SR membrane can be investigated. However, the EPR spectrum of each of these probes in the SR preparation is the resultant of superposition of two spectra: of the free and immobilized probe; the EPR spectrum of the former, moreover, completely screens the spectrum of the latter (Fig. 1). To record the spectrum of tempamine, tempon, and PCA, inserted into SR vesicles, it was necessary to remove the strong signal of the free probe. To do this, $NiCl_2$ was used in a concentration of 0.5 M, at which it broadened the EPR spectrum of the free probe in solution completely. Since under these conditions virtually no $NiCl_2$ passed through the SR membrane [10-13], the spectra of tempamine, tempon, and PCA gave information on the state of the inside of the SR vesicles (Fig. 1).

The results of a study of changes in the parameter of hydrophobicity and the spin correlation time of tempamine, tempon, and PCA in SR preparations in HChE are given in Table 1. The value of h for tempamine is evidence that this probe, located inside the SR vesicles, is mainly in the free state. In HChE the hydrophobicity of the environment of the probe was depressed, but spin mobility increased. A similar effect was observed for tempon and PCA, although these probes must evidently interact to a greater degree with the SR membrane, as is

TABLE 2. Change in Parameters h, S, and τ of Probes C_5, C_7, and C_{16} in SR Membranes during HChE and in the Presence and Absence of $NiCl_2$ (M ± m)

Parameter	Experimental conditions	C_5	C_7	C_{16}
h, relative units	Control	0,687±0,010	0,774±0,008	0,861±0,016
		0,633±0,011*	0,692±0,009*	0,930±0,009*
	HChE	0,855±0,008*	0,879±0,008*	0,932±0,009*
		0,713±0,007 †	0,754±0,007 †	0,970±0,005 †
S	Control	0,513±0,016	0,511±0,004	—
		0,608±0,008*	0,548±0,008*	—
	HChE	0,607±0,006*	0,527±0,006*	—
		0,651±0,009 †	0,586±0,005 †	—
τ, $\cdot 10^{-9}$ sec	Control	4,85±0,27	4,10±0,15	0,879±0,027
		7,46±0,67*	6,49±0,71*	0,880±0,270
	HChE	5,51±0,30*	7,32±0,41*	0,891±0,019
		8,86±0,67 †	12,36±1,23 †	0,881±0,018
	Glycerin (100%)	4,273	6,401	1,570

*$P < 0.05$ compared with control without $NiCl_2$.
†The same in the presence of $NiCl_2$.

indicated by the values of h and τ (Table 1), and also by the partition coefficient for methyl oleate/water, which is 1.7 for tempon and 1.0 for PCA [11]. Incidentally, for all three water-soluble probes used [10-12] the mobility of the molecules inside SR vesicles was much less than in solution. For instance, for tempamine in solution τ was $0.14 \cdot 10^{-10}$ sec, for tempon $0.11 \cdot 10^{-10}$ sec, and for PCA $0.17 \cdot 10^{-10}$ sec, under the same conditions of recording of the spectra. What is the possible mechanism of such a great reduction in mobility of the probes inside the SR vesicles? The probe was evidently uniformly distributed on both sides of the SR membrane, because the SR preparation was preincubated with the probe (10 min, 20°C) with $NiCl_2$ before each measurement. There are likewise no grounds for considering that active transport of the probe takes place inside vesicles. The composition of the medium inside the vesicles was identical with that outside, as was ensured when the preparation was made ready for the experiments [5]. In that case it might be supposed that the great reduction of mobility of the probe inside SR vesicles is due to the presence of two populations of probe molecules: adsorbed on the inner surface of the membrane, and diffusing freely in the internal state of the vesicles. Probably the first probe population makes the main contribution to the increase in τ, considering that the unadsorbed probe inside the vesicles has the same mobility as the free probe in solution.

The fatty acid probes C_5, C_7, and C_{16} can be used to study the hydrophobic zone of SR membranes; moreover, the carboxyl group of the probe molecule can act as a distinctive "anchor," located evidently in the region of the polar heads of the phospholipids. The state of HChE in an animal induced an increase in hydrophobicity and order of the microenvironment of these three probes, sharply reducing their mobility (Table 2). An increase in the concentrations of LPO products and cholesterol, observed previously in SR membranes during HChE [5], evidently leads to denser packing of lipid molecules into a bilayer, and this phenomenon may extend to the region of the polar heads of the phospholipids (especially of the inner monolayer of the vesicular membrane), and thus reduce the degree of interaction of tempamine, tempon, and PCA with the membrane. As a result the fraction of the probes present in the free state inside the vesicles was increased, and this led to an increase in spin mobility and to a decrease in hydrophobicity of their microenvironment during HChE (Table 2).

The use of $NiCl_2$ in a sufficiently high concentration could affect the structure of SR membranes. To evaluate the contribution of $NiCl_2$, spectra of C_5, C_7, and C_{16} were recorded both in the presence and in the absence of $NiCl_2$ (Table 2). $NiCl_2$ was shown to affect the parameters of the EPR spectra of the probes. During HChE and in the control, for instance, $NiCl_2$ reduced hydrophobicity and increased the order of the environment surrounding the C_5 and C_7 probes, and reduced their spin mobility. The contribution of $NiCl_2$ to the value of the parameter h during HChE was 17.6% for C_5 and 7.3% in the control; for C_7 probes during HChE it was 14.2 and 10.5% respectively. The same effect was observed for the parameters S and τ. For C_7, for instance, the contribution of $NiCl_2$ to the value of the parameter S during HChE was 8.3%, and 3.5% in the control; the deeper the nitroxyl fragment of the probe was inserted into the membrane, moreover, the weaker the action of $NiCl_2$. The contribution of $NiCl_2$ to the parameters of the EPR spectra of tempamine, tempon, and PCA must evidently be very small.

Treatment of a preparation of SR with $NiCl_2$ thus led to a change in structure of the membrane, and this effect was more marked in HChE. This result may evidently be further proof of a change in the physicochemical properties of the lipid part of the SR membranes in HChE. To compare the spin mobility of the different probes and to express the spin mobility of the probes as a function of depth of SR membranes, the physical properties of each probe must be taken into consideration separately. To do this, values of τ in 100% glycerin were determined at 30°C (Table 2). From the EPR spectra of the probes in glycerin it was possible to calculate the value of τ by the same equations as in SR membranes. It was found that the ratio $\tau_{membrane}/\tau_{glycerin}$, characterizing mobility of the probes in the membrane, can be represented as a function of depth of insertion into the membrane, provided that the probes are arranged along the abscissa in the order of depth of their insertion into the lipid bilayer (Fig. 2). In HChE, the reduction in spin mobility of the probes was found to be inversely proportional to the depth of insertion of the nitroxyl group of the probe; the differences disappeared, however, toward the center of the membrane.

Thus the microviscosity, hydrophobicity, and orderliness of the lipid bilayer of SR membranes are increased on the whole in HChE. Since a disturbance of function of the calcium pump of SR in HChE is accompanied by cholesterol accumulation and by intensification of LPO in SR membranes [7], it can be tentatively suggested that the changes observed in the lipid phase of the membranes in HChE are the result of the processes mentioned above.

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