ROLE OF LIPID PEROXIDATION IN HYPERCHOLESTEROLEMIA-INDUCED STRUCTURAL CHANGES IN MUSCLE SARCOPLASMIC RETICULUM Ca-ATPase

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A state of protracted hypercholesterolemia (HCh) in animals induces cholesterol accumulation in cell membranes and also raises the level of lipid peroxidation in them [3-5]. As a result, operation of ionic pumps and, in particular, of the Ca-pump of the sarcoplasmic reticulum (SR) of skeletal muscles, is disturbed [8, 11, 12]. The decrease in efficiency of functioning of Ca-ATPase of SR in this case is probably linked with changes in physicochemical properties of the membrane. It was shown previously that in HCh the mobility of Ca-ATPase of skeletal muscle SR is reduced and its structure changed, as a result of accumulation of cholesterol in SR membranes [10, 11]. However, the possible role of lipid peroxidation (LPO) in modification of the structure of this enzyme in HCh has not been investigated.

The aim of this investigation was to study changes in structure of SR membranes in HCh and the action of α-tocopherol under these conditions.

EXPERIMENTAL METHOD

Experiments were carried out on 24 male chinchilla rabbits weighing 2.5-3 kg. The animals were divided into four groups with six rabbits in each group: 1) control, 2) the standard diet was supplemented with cholesterol in a dose of 1 g/kg daily, 3) together with cholesterol a 10% solution of α-tocopherol acetate in oil was added in dose of 30 mg/kg, 4) the animals were given α-tocopherol acetate together with their ordinary diet. All the rabbits were used in the experiments for 30 days. Preparations of SR were obtained from skeletal muscles by the method in [7] and the protein concentration was determined by Lowry's method. As spin label, 2,2,6,6-tetramethylpiperidine-1-oxyl-4-maleimide, which reacts with protein thiol groups, was used. Ca-ATPase from SR was incubated with the spin label for 12-14 h with constant mixing in medium containing 1 M sucrose, 25 mM imidazole-HCl, pH 7.0, at 5°C, with a ratio of concentration (in moles) of label to protein of 5:1 (the protein concentration in the sample was 5 mg/ml, which is roughly the same as a $5 \cdot 10^{-5}$ M concentration of Ca-ATPase molecules). EPR spectra were recorded on an EPR-spectrometer (Varian, USA). The rotary correlation time (τ) of the spin label was estimated by the method in [6].

EXPERIMENTAL RESULTS

The rate of binding of the spin label with thiol groups of Ca-ATPase of SR in HCh was lower than in the control (Fig. 1). This change may be due to a decrease in the number of SH-groups accessible to the label or to a change in the surface structure and a decrease in mobility of the enzyme in the membrane, or as a result of their clinical modification. Parallel with this, accessibility of spin-labeled thiol groups of Ca-ATPase for sodium ascorbate and potassium ferricyanide was observed (Figs. 2 and 3a), in agreement with previous data [11]. Changes in accessibility of spin-labeled thiol groups of Ca-ATPase for paramagnetic ions indicate that in HCh there is a change in structure of the enzyme molecule in the region of the spin level. This evidently also explains the decrease in the rate of bind-

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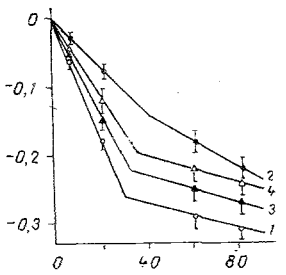

Fig. 1

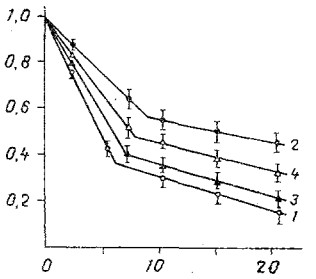

Fig. 2

Fig. 1. Kinetics of binding of maleimide spin label with thiol groups of SR Ca-ATPase. Temperature 22°C, pH 7.0 ratio of concentrations of label to protein 2:1. Abscissa, time (in min); ordinate, logarithm of amplitude of low-field component of EPR spectrum of spin label not bound with protein, normalized for amplitude in initial time period. 1) Control, 2) cholesterol, 3) cholesterol + α-tocopherol, 4) α-tocopherol.

Fig. 2. Kinetics of quenching of low-field component of EPR spectrum of spin label bound with SR Ca-ATPase and with sodium ascorbate. Temperature 4°C, sodium ascorbate concentration 20 times higher than protein concentration. Abscissa, time (in min); ordinate, amplitude of low-field component of EPR spectrum, normalized for amplitude in initial time period. Remainder of legend as to Fig. 1.

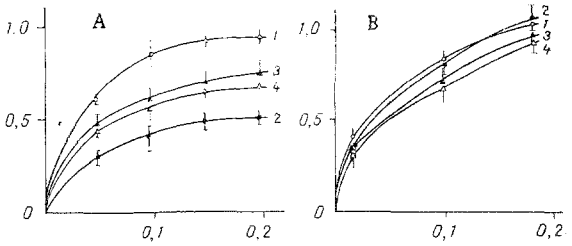

Fig. 3. Changes in width of low-field component of EPR spectrum of spin labels bound with SR Ca-ATPase under the influence of paramagnetic ions: potassium ferricyanide (A) and nickel chloride (B). Temperature 22°C. Abscissa, concentration of ion (in M); ordinate, half-width of low-field component of EPR spectrum at half-height (in G). Remainder of legend as to Fig. 1.

ing of label with the enzyme, probably due to a sharp decrease in accessibility of the label itself for protein SH-groups.

A study of the parameter of spin labels covalently bound with SR Ca-ATPase gives an estimate of the mobility of the spin-label fragment of the enzyme. The mobility of this fragment of Ca-ATPase was reduced in HCh (Table 1), probably as a result of the altered physicochemical properties of the SR membranes under the influence of cholesterol accumulation and intensification of LPO in SR [9].

On the one hand, an increase in hydrophobicity of the environment of spin-labeled thiol groups of the enzyme and restriction of protein mobility in the membrane [11] may be due to an increase in microviscosity of the lipid bilayer of SR and an increase in hydrophobicity of the carbohydrate region of the SR membrane, which is observed in experiments *in vitro* during induction of LPO and with an increase in cholesterol concentration in the membranes [13, 2]. On the other hand, since the antioxidative activity falls in SR membranes in HCh and the level of LPO products rises [11], the direct action of peroxide radicals of the membrane phospholipids on the Ca-ATPase molecule is possible, followed by a change in the native

TABLE 1. Effect of α-Tocopherol *in Vivo* on Mobility of Spin-Labeled Fragments of Ca-ATPase in SR Membranes in HCh (M ± m)

Experimental conditions	$\tau, \cdot 10^{-7}$ sec
Control	3,1±0,2
Cholesterol	9,5±0,8*
Cholesterol + α-tocopherol	4,1±0,3**
α-Tocopherol	4,2±0,1

Legend. *P < 0.01 compared with control, **P < 0.01 compared with animals of group 2.

structure of the enzyme as a result of irreversible cross-linkages either with phospholipids or with minor proteins of SR, or between Ca-ATPase molecules themselves. It can thus be tentatively suggested that activation of LPO and an increase in cholesterol concentration in SR membranes in HCh are complementary to each other in their modifying action on the Ca-pump of SR.

Since the maleimide spin label binds with the SH-groups of the enzyme close to the active center [14] and the change in surface structure of the Ca-ATPase molecule takes place exactly in the region of the spin label, it can be postulated that this determines the decline in efficiency of operation of the SR Ca-pump in HCh. Restriction of mobility of Ca-ATPase evidently potentiates this effect still more.

Addition of the antioxidant α-tocopherol to the cholesterol-enriched diet caused an increase in the rate of binding of the spin label with SR Ca-ATPase, an increase in accessibility of the thiol groups of the enzyme for sodium ascorbate and potassium ferricyanide, and also an increase in mobility of Ca-ATPase (Figs. 1, 2, and 3a; Table 1). Spin-labeled SH groups of the enzyme were equally accessible for nickel ions (Fig. 3b), probably because of the short radius of the Ni^{++} ion compared with ions of ascorbate and ferricyanide.

The change observed in structure of Ca-ATPase of SR in HCh thus was largely prevented by addition of α-tocopherol to the animals' diet. According to data in the literature [1], administration of the antioxidants to an animal causes enrichment of the cell membranes with readily oxidized phospholipids with unsaturated fatty acid residues. This, in turn, must lead to a decrease in microviscosity of the lipid bilayer of the membranes and, consequently, to an increase in mobility of the membrane-bound enzymes. This change in the lipid composition of the membranes may compensate sufficiently effectively the structural effect of cholesterol on the membrane. Meanwhile addition of α-tocopherol to the diet increased the antioxidant activity of the SR membranes sharply [9] and, as a result, lowered the LPO level; this must ultimately preserve the original lability of the membranes and must also reduce the harmful action of LPO products on the Ca-ATPase molecule.

It can thus be postulated that not only the increase in mobility of Ca-ATPase, but also preservation of the native structure of the enzyme molecule to a sufficient degree, ultimately determine the increased efficiency of operation of the SR Ca-pump under the influence of α-tocopherol *in vivo* against the background of HCh. Since the disturbances of structure and function of the Ca-pump of SR membranes of the skeletal muscles observed in HCh can be prevented by the addition of α-tocopherol to the animals' diet, this suggests that the decrease in efficiency of operation of the Ca-pump in HCh [11] is due mainly to activation of LPO in SR membranes.

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INDIVIDUAL RESPONSIVENESS MANIFESTED AS CHANGES IN MITOCHONDRIAL CYTOCHROME CONCENTRATION IN CIRRHOSIS

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There is much information in the literature on changes in the content and stoichiometry of the mitochondrial cytochromes, which are responsible for the terminal stage of oxidative metabolism, coupled with energy accumulation, but the nature and role of these changes remain unexplained.

The aim of this investigation was to study the content of cytochromes in mitochondria (Mc) of hepatocytes during progressive cirrhosis of the liver in rabbits. With the more accurate determination of their concentrations, it was possible not only to track the dynamics of changes in the mean parameters, but also to analyze individual changes in the content of mitochondrial cytochromes in the animals.

EXPERIMENTAL METHOD

Three chronic experiments were conducted on mature male chinchilla rabbits. The model of cirrhosis was devised and built by V. N. Tugarinova [7]. Cirrhosis was induced by four cycles of combined treatment with the hepatotoxic agent CCl_4 and hepatogenic antigen [2]. Repeated biopsy operations for the morphological and metabolic investigations were performed simultaneously three times on the control and experimental rabbits: after 2-3 weeks, at the end of the 2nd and 4th cycles, and 2 months after the end of the injurious procedures. In all three years the experiments were done between February and June. The cytochrome concentrations were determined in biopsy material from 22 experimental and 22 control rabbits. Pieces of tissue were cooled in ice-cold 0.9% KCl solution and homogenized in medium of the following composition: sucrose 0.2 M, KCl 20 mM, KH_2PO_4 2 mM, $MgCl_2$ 0.5 mM, Tris-buffer 30 mM, versene 1 mM, pH 7.4, temperature 0°C. MC were isolated by differential centrifugation at −2°C: at 700g for 5 min and at 12,000g for 10 min. The residue of MC was suspended in the same medium. For spectrophotometry, 0.1 mM of 2,4-dinitrophenol and 2 mM amytal were added to the medium. To convert cytochromes a, b, c_1, and c into the reduced state 5 mM succinic acid (pH 7.4) and 3 mM KCN were added to one of the cuvettes; cytochrome b_5 in both cuvettes was reduced by the addition of 0.5 mM NADH. Difference spectra of the cytochromes were recorded on a multifunction differential spectrophotometer [3], constructed at the

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