MODIFICATION OF FUNCTIONAL PROPERTIES OF SARCOPLASMIC RETICULUM Ca-ATPase IN HYPERCHOLESTEROLEMIA

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KEY WORDS: Ca-ATPase; sarcoplasmic reticulum; skeletal muscles; hypercholesterolemia; cholesterol.

It was shown previously [5, 7] that the development of hypercholesterolemia (HChE) in rabbits is accompanied by disturbance of the functioning of the Ca-pump in the membranes of the sarcoplasmic reticulum (SR) of skeletal muscles. Under these circumstances some accumulation of cholesterol (ChS) was found in SR membranes, with an increase in the concentration of lipid peroxidation (LPO) products, and changes in the fatty acid composition of the membranes [1, 6]. The importance of the changes in lipid composition of SR membranes during disturbance of the Ca-transporting function of the Ca-pump is not yet clear, for the development of AChE is accompanied by changes in protein composition of the membranes also: products of oligomerization of proteins appear and the concentration of free SH-groups falls [1, 4].

In the investigation described below methods of delipidation and reconstruction were used to study the role of changes in the lipid composition of SR membranes observed in HChE, in a modification of the catalytic function of Ca-ATPase.

EXPERIMENTAL METHOD

Male Chinchilla rabbits weighing 2.5-3 kg were used. Experimental HChE was induced by keeping the rabbits on a diet with the addition of ChS in a dose of 1 g/kg body weight daily (one meal a day) for 3 months. The development of HChE in the animals was monitored by periodic determination of the blood ChS level. ChS in the blood serum was determined on a "Trace III System" automatic analyzer, with a standard kit of reagents from Beckman (West Germany).

Fragments of SR from white muscles were taken from a rabbit by the method described previously [3]. The SR preparations were delipidized by gel-filtration on a column (1.1 × 25 cm) filled with Ultragel AcA 34 [3]. The solubilized preparation of SR (10 mg protein) in a volume of 2.5 ml in column equilibrating medium was applied in samples of 2.5 ml to the column and eluted with the same medium at 4°C. The volume of the fractions collected was 1 ml. To determine the degree of delipidization and the ability of ATPase to be reactivated by ovolecithin (OL) the fractions were analyzed for their protein content and activity of the enzyme in them was determined in the absence of phospholipid and after addition of OL in cholate.

Proteoliposomes containing Ca-ATPase were prepared from the delipidized SR preparation and OL by chromatography on an anion-exchange resin [3].

ATPase activity was measured pH-metrically [2, 3]. The temperature dependence of Ca-ATPase activity over the temperature range from 0 to 40°C was studied as described previously [3].

EXPERIMENTAL RESULTS

Previous investigations of the functional properties of Ca-ATPase from SR showed that HChE leads to a decrease in the rate of ATP hydrolysis in the process of Ca^{++} transport. To

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TABLE 1. Changes in Ca-ATPase Activity in SR Membranes during HChE (M ± m)

SR preparation	ATPase activity, μmoles P_i/min/mg protein
Control	20,00±1,00
HChE	16,70±2,00

TABLE 2. Specific Activity of Undelipidized and Delipidized SR Membranes and Preparations of Proteoliposomes from OL

Preparations	ATPase activity, μmole P_i/min/mg protein	
	control	HChE
SR membranes	20,0	16,7
The same after delipidization	0	0
Proteoliposomes	23,0	16,7

Legend. Ca-ATPase activity was measured in medium containing 100 mM NaCl, 4 mM $MgCl_2$, 5 mM sodium oxalate, 25 μM $CaCl_2$, 2 mM ATP, and 2.5 mM imidazole, pH 7.2 (37°C). The incubation mixture contained 3 μg/ml of alamethicin.

study changes in true catalytic activity of Ca-ATPase, the rate of ATP hydrolysis was measured in SR membranes from control and hypercholesterolemic animals in the presence of the antibiotic alamethicin. On the one hand, alamethicin increases the permeability of SR membranes for Ca^{++}, and thereby abolishes control of ATPase activity by the Ca gradient; on the other hand, it creates channels of permeability for ATP in the membrane, making it possible for ATPase molecules, turned with their active center facing the inner space of the SR vesicles, to function [3, 8].

As Table 1 shows, measurement of the rate of ATP hydrolysis at 37°C in the presence of alamethicin revealed some decrease in specific Ca-ATPase activity (10-30% depending on the preparation) in HChE.

More important changes in the functional properties of ATPase were found during a study of the temperature dependence of ATPase activity. In agreement with data obtained previously [1, 5] it was found that if ATPase activity was measured in the absence of alamethicin, straightening out of the graph of temperature dependence of ATPase activity between Arrhenius' coordinates was observed in HChE (Fig. 1). If, however, the measurements were made in the presence of alamethicin, the graph of temperature dependence of ATPase activity between Arrhenius' coordinates both for the control preparations and for preparations isolated from animals with HChE had a kink. This fact was unexpected, on the grounds that alamethicin has no modifying effect on the temperature dependence of catalytic activity of the Ca-ATPase of SR [3]. Straightening of the graph of temperature dependence of Ca-ATPase between Arrhenius' coordinates can evidently be explained on the grounds that permeability of SR membranes for Ca^{++} is increased in HChE so considerably in the region of high temperatures that it has an effect on the character of temperature dependence of Ca-ATPase. In other words, straightening of the graph of temperature dependence of Ca-ATPase in HChE can be explained by additional activation of the enzyme in the temperature region 20-40°C because of the sharp rise in permeability of SR membranes for Ca^{++} under these conditions. This may explain the apparent decrease in the effectiveness of the transport process (the value of Ca/ATP) observed in HChE [5, 6]. Alamethicin abolishes the barrier of permeability of SR membranes for Ca^{++} and, consequently, also abolishes the effect of the change in membrane permeability on the character of temperature dependence of Ca-ATPase, and for that reason straightening of the graph between Arrhenius' coordinates is not observed in the presence of alamethicin.

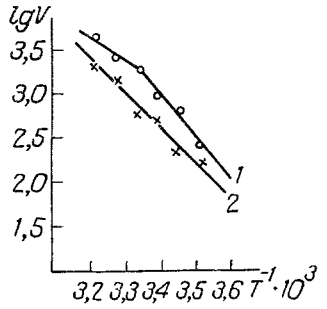

Fig. 1

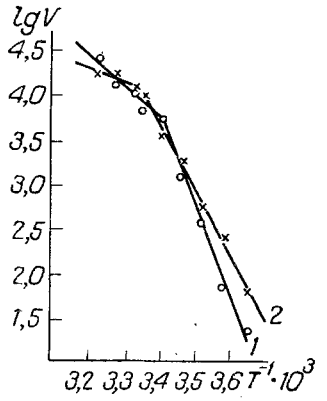

Fig. 2

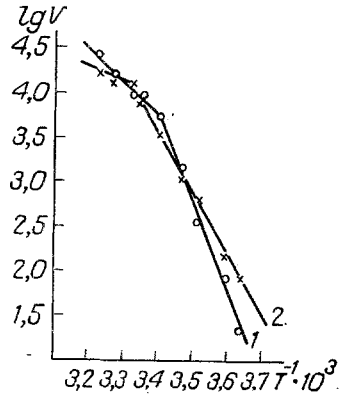

Fig. 3

Fig. 1. Temperature dependence of Ca-ATPase activity of SR under normal conditions (1) and in HChE (2).

Fig. 2. Temperature dependence of Ca-ATPase activity of SR membranes: 1) control, 2) HChE. Incubation mixture contained 3 μg/ml of alamethicin.

Fig. 3. Temperature dependence of Ca-ATPase activity of preparation of SR membranes, reconstituted with OL (1), and preparation of SR membranes isolated from rabbits with HChE and reconstituted with OL (2). Incubation mixture contained 3 μg/ml of alamethicin.

The study of temperature dependence of Ca-ATPase activity in the presence of alamethicin in SR preparations isolated from control animals and from animals with HChE revealed modification of the polypeptide responsible for catalytic function in HChE.

In agreement with data in the literature [3], the graph of temperature dependence of Ca-ATPase activity in the present experiments, measured in the presence of alamethicin, had a kink at 20°C. The most characteristic change in the graph of temperature dependence of Ca-ATPase activity for all preparations of SR membranes studied from animals with HChE was that the kink was shifted toward higher temperatures (Fig. 2). No tendency was observed for a change in the apparent activation energies in HChE. The changes in the character of the graph of temperature dependence of activity in HChE clearly shows that HChE causes modification of the catalytic properties of Ca-ATPase. Previous investigations showed [1, 6] that HChE leads to transverse cross-linkage of the membrane proteins of SR, to a change in the accessibility of SH groups for thiol reagents, and to a change in the fatty acid composition of the membrane lipids.

To study the cause of the change in character of temperature dependence of Ca-ATPase in HChE, the SR membranes were delipidized on a column with Ultragel AcA 34 in the presence of cholate, after which Ca-ATPase activity was reactivated by reconstitution of proteoliposomes from OL. Proteoliposomes were reconstituted avoiding the stage of isolation of purified Ca-ATPase, i.e., by using whole protein material of SR membranes. This approach was used in order to exclude any possible artefacts during isolation of the enzyme from the membranes of animals with HChE. Since cross-linkage of membrane proteins probably takes place in HChE, during isolation of ATPase from SR membranes by extraction with cholate, separation of the modified and unmodified enzyme could take place. Functional characteristics of proteoliposomes reconstituted from SR membranes of control animals and of animals with HChE are given in Table 2.

In agreement with previous data [3], delipidization of control preparations of SR membranes led to total loss of ATPase activity. The delipidized preparations of SR membranes from animals with HChE likewise did not possess catalytic activity. This is indirect evidence that covalent bonding of lipids with Ca-ATPase does not take place in HChE, despite considerable cross-linkage of protein. The ATPase activity of delipidized control SR membranes and SR membranes from animals with HChE was completely restored on reconstitution of proteoliposomes from OL. The difference of ATPase activity under normal conditions and in HChE, observed on SR membranes, also was preserved with the reconstituted preparations. Consequently, the small decrease in ATPase activity at 37°C observed in HChE cannot be explained by a change in the phospholipid composition of the SR membranes.

Arrhenius plots of temperature dependence of ATPase activity of SR membranes in HChE and of proteoliposomes reconstituted from these membranes are illustrated in Fig. 3. It will be clear from Fig. 3 that replacement of the native lipid environment of Ca-ATPase by OL did not restore the normal character of the graph of temperatre dependence of activity, i.e., did not shift the kink into the low-temperature region (20°C), as is characteristic of proteoliposomes and SR membranes of control animals [3].

The results thus show that modification of the temperature dependence of Ca-ATPase activity in HChE is connected with changes in the polypeptide which performs the catalytic function and is not the result of a change in the phospholipid environment of the enzyme.

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MONOCLONAL ANTIBODY STUDY OF CYTOCHROME P-450 ISOFORMS IN THE LIVER OF RATS TREATED WITH PHENOBARBITAL, 3-METHYLCHOLANTHRENE, AND AROCLOR 1254

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Cytochrome P-450, the terminal stage in the enzyme system oxidizing various exonobiotics (including drugs, carcinogens, and so on), is found in the body as a family of isoforms. As a result of introduction of various inducing agents into the body, different isoforms of cytochrome P-450 are synthesized. The distribution of these isoforms in the tissues of the body varies [12], and the isoforms themselves differ from each other in different tissues [10]. The distribution of these isoforms among the cells of an organ also is not uniform [2].

This paper describes an attempt to discover, with the aid of three types of monoclonal antibodies, the distribution of certain isoforms of cytochrome P-450 in the liver of rats treated with various inducers.

EXPERIMENTAL METHOD

BaLB/c mice were immunized with cytochrome P-450 isolated from the liver of male Wistar rats. The rats were treated beforehand with phenobarbital in a dose of 70 mg/kg body weight or 3-methylcholanthrene in a dose of 40 mg/kg body weight. Cytochrome P-450 was isolated by the method in [7]; the procedure stopped at the first stage of purification (i.e., at precipitation with ammonium sulfate). Spleen cells of immune mice were fused with myeloma cells of strain P3-X-63 Ag 8.653 by the standard method [3]. The resulting clones were analyzed for the presence of staining on sections of rat liver by the immunofluorescence method. Paraffin

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