cholesterolemia and a tendency toward atherosclerosis, as has frequently been demonstrated previously [1, 3, 4, 9]. Elevation of the Cp/Tf ratio and, correspondingly, of the blood serum AOA, however, is evidence of resistance to cholesterol accumulation and to the development of atherosclerosis.

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EFFECT OF CHOLESTEROL ON COOPERATIVENESS OF Ca-ATPase OF THE SARCOPLASMIC RETICULUM OF RABBIT SKELETAL MUSCLES

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Ca-ATPase of the sarcoplasmic reticulum (SR) is an oligomeric allosteric enzyme consisting of several functional units or protomers, Ca-ATPase function is not governed by the traditional Michaelis—Menten kinetics; in particular, an abnormal curve of enzyme activity versus substrate concentration is observed with additional activation at high ATP concentrations [3-5]. According to one hypothesis, the nature of the allosteric effect of ATP can be explained by the presence of a special allosteric center [14, 15]. More recently, however, data have been published to show that cooperative interactions existing between the protomers of Ca-ATPase may be modified in the course of function so that the hydrolytic center of one protomer becomes the allosteric center of the other [1]. It is quite possible that hydrolytic and transport functions of this enzyme are controlled by a change in cooperative interactions between the protomers of Ca-ATPase. It is accordingly interesting to study factors influencing the cooperativeness of interaction between Ca-ATPase protomers and, in particular, modifiers of the phase state of the phospholipid environment of Ca-ATPase [2].

The aim of this investigation was to study the effect of cholesterol, one regulator of the phase state of membrane lipids, on cooperativeness of interaction between Ca-ATPase protomers, using ATP and UTP as substrate.

EXPERIMENTAL METHOD

Experiments were carried out on 12 male chinci-la rabbits weighing 2.5-3 kg. To increase the cholesterol concentration in SR membranes nine experimental animals were kept on a diet to which cholesterol was added in a dose of 1 g/kg body weight for 1, 3, and 6 months [8, 11]. The SR fraction was isolated from white muscles of the hind limbs of the rabbits

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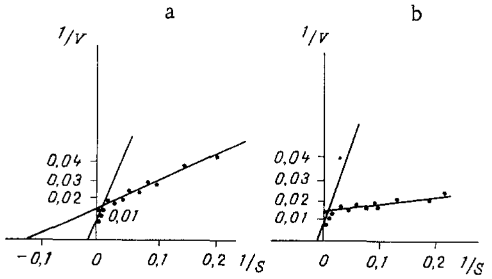

Fig. 1

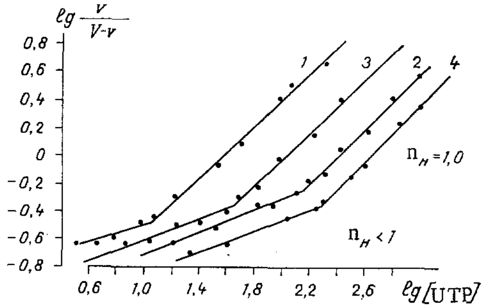

Fig. 2

Fig. 1. Activity of Ca-ATPase during hydrolysis of ATP (a) and UTP (b) as a function of substrate concentration (in Lineweaver—Burk coordinates). Abscissa, 1/S, where S denotes substrate concentration (in μM); ordinate, 1/v, where v denotes rate of hydrolysis (in μmoles P_i/2 mg protein/h). Here and in Fig. 2 all values studied were calculated from data for 5-7 experiments.

Fig. 2. Effect of high cholesterol diet on cooperativeness of Ca-ATPase relative to UTP (in Hill's coordinates). Abscissa, log of UTP concentration (in μM); V) maximal reaction velocity. 1) Normal, 2, 3, 4) high cholesterol diet for 1, 3, and 6 months respectively.

TABLE 1. Values of n_H for ATP and UTP (M ± m)

Substrate	Region of concentrations	n_H
ATP	100 μM	0,40±0,06
	100 μM	1,00±0,09
	1 mM	3,20±0,46
UTP	10 μM	0,43±0,05
	10 μM	1,00±0,04

Legend. Here and in Table 2 all values were calculated from data for 5-7 experiments.

TABLE 2. Maximal Values of n_H for ATP and UTP under Normal Conditions and in Hypercholesterolemia (M ± m)

Substrate	Control	Duration of hypercholesterolemia, months		
		1	3	6
ATP	3,20±0,46	1,50±0,17	2,00±0,11	1,60±0,21
UTP	1,00±0,04	1,10±0,07	1,10±0,05	1,00±0,06

by the method in [6]. The membrane preparation of Ca-ATPase was purified by treatment with EDTA [9]. To calculate the kinetic constants (37°C, pH 7.7) an express method was used [9]. The protein concentration was determined by Lowry's method. Preparations of ATP and UTP for use in the work were recrystallized beforehand.

EXPERIMENTAL RESULTS

Besides ATP, the Ca-pump of SR membranes is known to be able to utilize UTP, ITP, GTP, and CTP as substrates [13]. However, the kinetics of hydrolysis has not been adequately studied for all nucleotides, and for that reason only UTP was used in addition to ATP as substrate.

A graph showing Ca-ATPase activity in the ATPase (a) and UTPase (b) reaction of function of substrate concentration, between Lineweaver—Burk coordinates, is shown in Fig. 1. The two groups of points on the graph suggest that activity of the enzyme is characterized by the presence of two different K_m values, i.e., an allosteric effect is observed for both substrates. This conclusion is in full agreement with data in the literature on Na, K-ATPase, according to which all substrates responsible for ion transport exert an allosteric action on this enzyme [12].

It could be concluded from the construction of graphs of ATP and UTP hydrolysis as functions of substrate concentration in Hill's coordinates and calculation of coefficients of cooperativeness that with an increase in substrate concentration from micromolar to millimolar values there was an increase in the values of Hill's ratios (n_H). Under these circumstances n_H for ATP, initially (at low substrate concentrations) having values below 1 (negative cooperativeness), increased to 3.2 (positive cooperativeness), whereas n_H for UTP, which also was initially below 1, increased to 1 (Table 1). These values of cooperativeness of Ca-ATPase correlate with values for the efficiency of ionic transport (the efficiency of ionic transport expresses the ratio between the velocity of Ca^{++} accumulation and the velocity of substrate hydrolysis), which, as was observed in preliminary experiments, were significantly higher when ATP was used as substrate than when UTP was used. We also know that with an increase in the ATP concentration the efficiency of work of the Ca-pump increases [7], and this also correlates with the increase in cooperativeness of Ca-ATPase for ATP under these conditions (Table 1). It can be postulated that an increase in cooperativeness of interaction between the protomers of Ca-ATPase leads to an increase in efficiency of Ca^{++} transport.

An increase in the cholesterol concentration in SR membranes leads to a significant decrease in cooperativeness of the enzyme for ATP over the whole region of ATP concentrations studied. Maximal values of n_H did not exceed 2 (Table 2). An increase in cholesterol concentration in SR membranes did not lead to a change in the values of n_H in the region of both high and low UTP concentrations, although it induced a marked increase in the UTP concentration at which the values of n_H reached 1.0 (Fig. 2). An increase in the cholesterol concentration in SR membranes is known to lead to an increase in viscosity of the lipid bilayer, as a result of which the probability of cooperative interactions between the protomers of Ca-ATPase is evidently reduced, and this causes a decrease in the efficiency of work of the Ca-pump [8, 11].

It can thus be considered that interaction between protomers of the Ca-pump in the membrane is controlled by the physicochemical state of their lipid environment.

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