SOME PROPERTIES OF THE CALCIUM PUMP OF THE SARCOPLASMIC RETICULUM OF THE SKELETAL MUSCLES OF RABBITS WITH HYPERCHOLESTEREMIA

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Fragments of sarcoplasmic reticulum (SR) isolated from the skeletal muscles of rabbits with marked atherosclerosis have impaired ability to accumulate Ca^{++}. A decrease in the efficiency of the transport process, expressed as a decrease in the Ca/ATP ratio, is accompanied by activation of Ca-ATPase and by a simultaneous increase in the rate of outflow of Ca^{++} from SR. The temperature dependence of Ca-ATPase, plotted between Arrhenius coordinates, normally has an inflection at 20-21°C, but in hypercholesteremia the graph becomes a straight line. Meanwhile the cholesterol/protein ratio in membrane preparations of SR rises sharply in atherosclerosis. The Ca-ATPase of native membranes has an activation energy (E_a) of 15.6 and 28.7 kcal/mole in regions above and below the inflection respectively. The Ca-ATPase of membranes containing an increased amount of cholesterol has an activation energy of 19 kcal/mole over the whole range of temperatures investigated. It is suggested that the cholesterol level in membrane preparations changes not only the physicochemical characteristics of the membranes, but also the enzymic properties of transport ATPase.

KEY WORDS: Ca-ATPase of sarcoplasmic reticulum; cholesterol; fluid characteristics of membranes.

The development of hypercholesteremia is accompanied by a change in protein–lipid interactions in the membranous structures of the cells of such vitally important organs as the brain, heart, and liver [5, 13]. The calcium pump of the sarcoplasmic reticulum (SR) is a convenient model with which to study protein–lipid interaction in the membranous structures of muscle tissue. The properties of the Ca-pump are known to depend on the phospholipid composition of the membranes [11, 14, 18] and, in particular, on the physicochemical state of the phospholipids [9]. Meanwhile the role of cholesterol in the function of the Ca-pump has not been studied, although it is cholesterol which controls the fluid properties of membranes [8, 15].

This paper describes the properties of the Ca-ATPase and Ca-transporting capacity of SR from the skeletal muscles of normal rabbits and rabbits with severe alimentary hypercholesteremia.

EXPERIMENTAL METHOD

Male adult rabbits weighing 2.5-3 kg were kept on a diet including cholesterol (1 g/kg) for 6-7 months. The development of atherosclerosis in the animals was monitored by determining the blood cholesterol concentration periodically by the Éngel'gardt–Smirnova method. The animals were killed, the internal organs examined morphologically, and the degree of atherosclerotic damage to the aorta estimated (this part of the investigation was undertaken by E. D. Klimenko).

Fragments of SR were isolated from the white muscles of the hind limbs (100 g) by differential centrifugation [6]. The Ca-stimulating capacity of the reticulum and Ca-ATPase activity in the transport process were measured by means of a pH-meter [6]. The temperature dependence of ATPase was measured as described previously [1], using a preparation of SR partially purified by alkaline treatment with EDTA [18]. In these experiments the increase in inorganic phosphorus (P_i) was measured [16] and the apparent activation energy calculated by the usual method [4]. The protein concentration was determined by the biuret reaction

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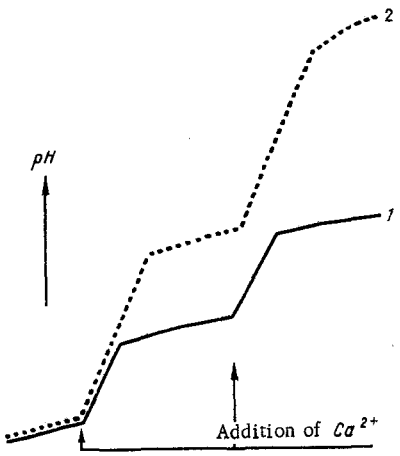

Fig. 1

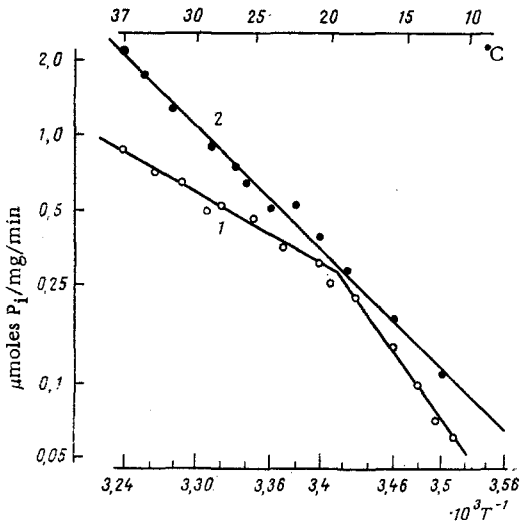

Fig. 2

Fig. 1. Ca^{++} transport by fragments of SR from skeletal muscles of control and experimental rabbits. 1) Control, 2) experiment. Incubation medium: Mg-ATP 2 mM, NaCl 100 mM, imidazole 1.5 mM (pH 7.0), Na oxalate 5 mM, addition of $CaCl_2$ in course of transport 300 nmoles per sample, protein 600 μg, volume of cell 6 ml, temperature 26°C.

Fig. 2. Temperature dependence of EDTA-treated Ca-ATPase of SR from rabbit skeletal muscles. 1) Control, 2) experiment. Incubation medium: KCl 100 mM, EDTA 0.5 mM, $MgCl_2$ 2.5 mM, ATP 2.5 mM, imidazole 20 mM, $CaCl_2$ 0.5 mM, protein 10-20 μg per sample, pH 7.4.

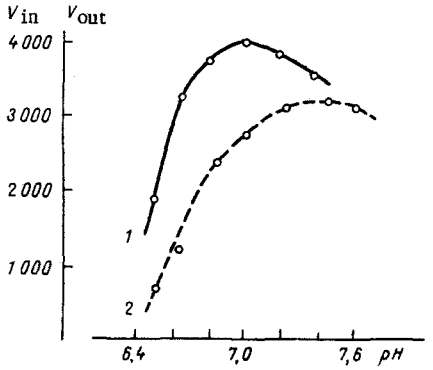

Fig. 3. pH-Dependence of rates (in nmoles P_i/mg/min) of inflow and outflow of Ca^{++} during its transport by SR in hypercholesteremia. 1) Rate of inflow (V_{in}) of Ca^{++}, 2) rate of outflow (V_{out}) of Ca^{++} from reticulum.

or by Lowry's method, and the cholesterol concentration in the membranes by Roschlan's method [17], using the kit of reagents from Boehringer. The composition of the medium and the experimental conditions are illustrated in Figs. 1 and 2.

The Na_2-ATP (from Reanal) was purified by recrystallization [7] and converted into the imidazole form on a column with Dowex 5 × 50 (400 mesh); the concentrations of $CaCl_2$ and $MgCl_2$ were determined by complexonometric titration with Murexide and Erichrome black T respectively [6]. The kinetic analysis of SR function was carried out as described previously [2, 6].

TABLE 1. Correlation between Rates of Inflow (V_{in}) and Outflow (V_{out}) of Ca^{++} during Its Accumulation by Fragments of SR from Control and Experimental Rabbits

Parameters	Control		Experiments	
	33°C	15°C	33°C	15°C
Rate of hydrolysis of ATP, nmoles P_i/mg/min	1175	106	1500	173
Ca/ATP	1,1	0,68	0,63	0,44
V_{in}, nmoles P_i/mg/min	2350	212	3000	346
V_{out}, nmoles P_i/mg/min	1050	140	1950	270

TABLE 2. Cholesterol Content in Membrane Preparations and Activation Energies of Ca-ATPase from SR of Control and Experimental Rabbits

Condition	Cholesterol/protein, mg/mg	Shape of temperature dependence on Arrhenius plot	Activation energy, kcal/mole	
			high temperature	low temperature
Control	0,03	Nonlinear, inflection at 21°C	15,6±1,6	28,7±1,3
Cholesterol diet for 6 months	0,08	The same	16,4*	25,7*
Cholesterol diet for over 6 months	0,15	Linear	19,2±1,9	19,4±1,7

*Measurements made in one series of experiments.

EXPERIMENTAL RESULTS

Keeping rabbits on a high-cholesterol diet for 6-7 months led to a sharp rise in the blood cholesterol level (from 53-117 mg % normally to 350-900 mg %) and to the development of marked lipoidosis in the aorta.

Fragments of SR isolated from the skeletal muscles of the experimental animals contained a large quantity of cholesterol (up to 150 μg/mg protein compared with the normal 30. It was also found that these preparations possessed higher Ca-ATPase activity than normally and they accumulated calcium for a longer time (Fig. 1). The calculated values of the efficiency of action of the Ca-pump (Ca/ATP) thus differed significantly: normally 1.9, experimentally 0.9 (pH 7.0 at 26°C). Sharp differences in the efficiency of Ca^{++} accumulation also were observed at both the higher (33°C) and the lower (15°C) temperature. To explain this phenomenon the rates of inflow and outflow of Ca^{++} were calculated in the course of its transport [2]. The increase in the rate of ATP hydrolysis was found to be accompanied by an increase in the rate of both the inflow and outflow of Ca^{++}, but the latter process was more marked, for the efficiency of Ca^{++} accumulation fell (Table 1).

To assess the properties of Ca-ATPase in native and cholesterol-loaded membranes, the temperature dependence of the enzyme, treated with EDTA in an alkaline medium, was determined. After treatment in this way the vesicles of SR become permeable to Ca^{++}; no Ca^{++} gradient is created during hydrolysis of ATP and, for that reason, the ATPase activity changes with time [3, 6]. The temperature dependence of this treated Ca-ATPase, when plotted between Arrhenius coordinates, under normal conditions has an inflection at 21°C (Fig. 2), which can be explained by structural modifications in the membrane lipids [3, 10, 12]. Meanwhile the Ca-ATPase from ST of the experimental animals was a linear function of temperature. The change from nonlinear to linear temperature dependence was observed when the cholesterol/protein ratio increased from the normal 0.03 to 0.08-0.15 mg/mg (Table 2). Activation energies characterizing Ca-ATPase under normal conditions are 15.6 kcal/mole in the region above the inflection and 28.7 kcal/mole below 21°C. When the cholesterol/protein ratio rose to 0.15 mg/mg the Arrhenius plot became linear and the activation energy was averaged to 19 kcal/mole.

Conversion of the nonlinear temperature dependence of the enzyme to linear was accompanied by activation of ATPase (compared with the control) in temperature regions above and below the previous position of the inflection (Table 1, Fig. 2). This activation was accompanied by an increase in the rate of inflow of Ca^{++} into the reticulum in the course of its active transport. However, the rate of outflow of Ca^{++} increased by a greater degree at the same time, so that the efficiency of action of the Ca-pump was reduced at all temperatures.

The pH-dependence of the Ca^{++} inflow and outflow during transport was expressed by typical curves with an optimum at 6.8-7.0 and 7.3-7.5 respectively (Fig. 3). The profile of the curves was unchanged by an increase in the cholesterol content in the membranes, and the optimal values also remained the same. Differences

between the pH-dependencies of Ca^{++} inflow and outflow have been reported in the literature [2, 3, 6]. Comparison of the rate of outflow of Ca^{++} during its transport by SR fragments of the control and experimental animals (Table 1) indicates that the conformation change responsible for the outflow of Ca^{++} takes place more readily in membranes loaded with cholesterol.

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CHANGES IN TISSUE ENERGY METABOLISM IN ANIMALS EXPOSED TO CONTINUOUS AND INTERRUPTED LOW-FREQUENCY VIBRATION

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The action of continuous low-frequency vibration on rats for 1 month caused no changes in the total adenine nucleotides of the brain but led to a marked decrease in the ATP content and total adenine nucleotides in the limb muscles. After exposure to vibration for 3 months considerable exhaustion of the total adenine nucleotides of both muscles and brain was found. In the case of interrupted exposure to vibration the state of the adenine–nucleotide system depended on the duration of the pauses between periods of continuous exposure to vibration. During vibration with the shortest pauses (4 min) between successive periods of 30 min of vibration no changes were observed in the energy metabolism of the muscles and brain. Vibration with pauses of 8 and 15 min was found to be unfavorable for the adenine nucleotides of the muscles and vibration with a pause of 8 min for the brain.

KEY WORDS: adenine nucleotides; brain; muscles; continuous and interrupted low-frequency vibration.

Exposure to vibration causes various functional, structural, and metabolic disturbances in man and animals [4, 7, 9]. The effect of low-frequency vibrations on energy metabolism, however, has been inadequately studied.

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