EFFECT OF CHOLESTEROL ON MEMBRANE Na,K-DEPENDENT ADENOSINE TRIPHOSPHATASE ACTIVITY

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By feeding rats on a high-cholesterol diet or by treating rat brain membranes with cholesterol oxidase, preparations of membrane Na,K-ATPase differing in their cholesterol content were obtained. The cholesterol/protein ratio varied from 0.034 to 0.251 (normally 0.152). An increase in this ratio was shown to straighten Arrhenius' plots for Na,K-ATPase versus temperature, whereas a decrease increases their curvature. A change in the temperature dependence on enzyme activity takes place simultaneously with the corresponding change in water repellency in experiments with the spin-label analog of androstane. It is concluded that the cholesterol level in the brain membranes controls Na,K-ATPase activity.

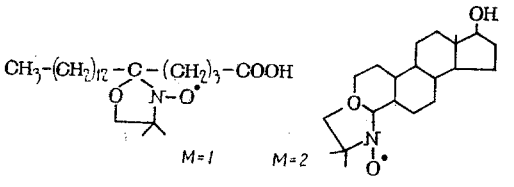
KEY WORDS: brain Na,K-ATPase; cholesterol; fluid characteristics of membranes

Na,K-dependent adenosine triphosphatase (Na,K-ATPase) is an enzyme of the plasma membranes whose activity determines the function of the Na pump, which is responsible for creating the gradient of Na^+ and K^+ ions in cells. One of the distinguishing features of Na,K-ATPase, which reflects its location in the membrane, is the curvature of the Arrhenius' plot versus temperature. The inflection of the temperature curve at 20 ± 2°C corresponds to the region of structural changes in lipids and reflects control of the activity of the Na pump by the cell membranes [1]. Proof of the presence of this control is given by the dependence of the position of the inflection on the Arrhenius' plot during reactivation of Na,K-ATPase on the physicochemical properties of the phospholipids used for reactivation [2]. Further evidence is given by the fact that Mg^{2+} ions, which have a structurizing influence on membrane lipids, straighten the Arrhenius' plots for ATPase so that it becomes linear for the spectral parameters of spin probes in the same membranes [3]. A powerful factor controlling the fluid properties of membranes is known to be the cholesterol level in them [4].

The object of this investigation was to study correlation between the cholesterol content in the brain membranes and the character of the temperature dependence of the Na,K-ATPase of these membranes.

EXPERIMENTAL METHOD

Female albino rats weighing 150-200 g, kept for 15-20 days under normal conditions or for the same period on a high-cholesterol diet (15 mg cholesterol per rat daily) were used in the experiments. Na,K-ATPase was isolated from the gray matter of rat brain [5]. Protein and inorganic phosphate (P_i) were determined by the usual method [1, 3], the cholesterol content in the membranes by the "Boehringer" test kit [6], and phospholipids by Folch's method [7] or by thin-layer chromatography [8]. ATP from "Reanal" (Hungary), recrystallized and converted into the imidazole salt, NaCl and KCl from "Soyuzreaktiv" (USSR), recrystallized twice with EDTA; $MgCl_2$ of spectral purity; and the "Boehringer" kit for determination of cholesterol and cholesterol oxidase were used. The labels used in the electron paramagnetic resonance (EPR) experiments were:



and were generously provided by É. K. Ruuge (Moscow, USSR) and J. Inesi (Pennsylvania, USA), and the EPR spectra were recorded on a "Varian" spectroscope.

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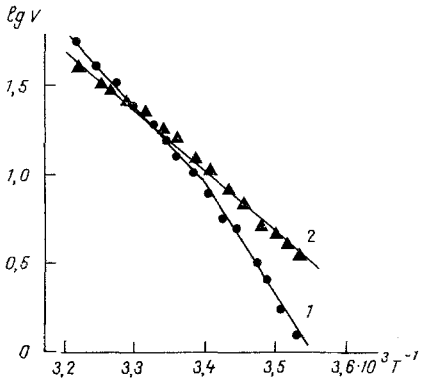

Fig. 1. Brain Na,K-ATPase activity of normal rats (1) and rats on high-cholesterol diet (2) as a function of temperature. Conditions of incubation: 3 mM ATP, 1 mM ATP, 1 mM $MgCl_2$, 130 mM NaCl, 20 mM KCl, 30 mM imidazole, pH 7.4. Abscissa, reciprocals of absolute temperature (T); ordinate, logarithm of maximal reaction velocity.

TABLE 1. Comparison of Cholesterol Content in Membrane Na,K-ATPase Preparations and Apparent Activation Energies of This Enzyme Determined within the Range 10-30°C

Preparation of Na,K-ATPase	Ratio		Experimental conditions	Activation energy, kcal/mole	
	cholesterol/protein	cholesterol/phospholipids		above 20°C	below 20°C
Normal	0,152	0,205	$MgCl_2$ 1 mM, ATP 3 mM	19,5±1,5	34,2±2,1
			$MgCl_2$ 5 mM, ATP 1 mM	17,4±2,0	17,5±0,9
Experimental hypercholesteremia	0,231	0,250	$MgCl_2$ 1 mM, ATP 3 mM	19,6±1,4	20,0±1,9
After treatment of preparation with cholesterol oxidase	0,034	—	$MgCl_2$ 5 mM, ATP 1 mM	7,6±1,2	17,4±0,8

EXPERIMENTAL RESULTS

As was shown previously [3], the appearance of the Arrhenius' plots for Na,K-ATPase depends on the $MgCl_2$/ATP ratio in the incubation medium. In the presence of 3 mM ATP and 1 mM $MgCl_2$ the curve had an inflection at 20°C. The temperature dependence curve of Na,K-ATPase isolated from the brain of rats kept on a high-cholesterol diet was without this inflection (Fig. 1). The sharp change in the appearance of the Arrhenius' curve suggested certain radical changes in the structure of the membranes as a result of cholesterol loading.

To test this hypothesis experiments were carried out with spin-labeled compounds reflecting the state of different parts of the membrane. Label M-1 was introduced into relatively fluid regions of the membranes, and oriented transversely in the bimolecular lipid layer. The character of the temperature dependence of the correlation time for this label showed no change after cholesterol loading. The M-2 label, androstane in nature and resembling cholesterol in structure, not only fitted better into the Na,K-ATPase preparations from the experimental rats, but also gave a different type of temperature dependence (Fig. 2). The inflection on the Arrhenius' plot was smoothed, in harmony with the known effect of cholesterol on the physicochemical characteristics of artificial lipid membranes [4]. These experiments suggested that in alimentary hypercholesteremia cholesterol is built into the brain membranes and causes a change in their properties and in the character of operation of the ionic pumps.

The cholesterol/protein and cholesterol/phospholipids ratios in brain membranes from normal and experimental rats were compared (Table 1). The results confirm that with the model used both ratios increased. Differences in the activation energy above and below the region of inflection, which under normal conditions amounted to 15 kcal/mole, disappears, reflecting the removal of structural conversions of the lipids with an increase in the cholesterol level in the membranes.

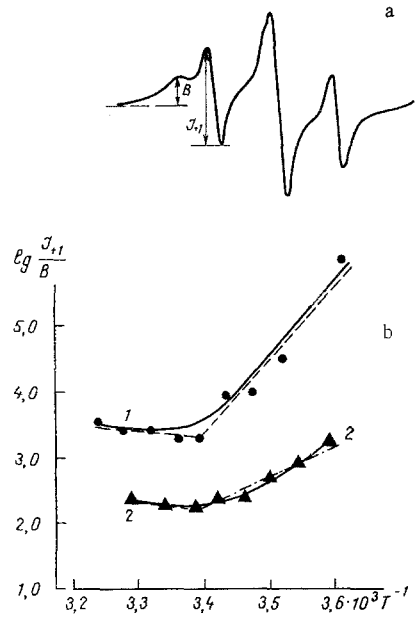

Fig. 2

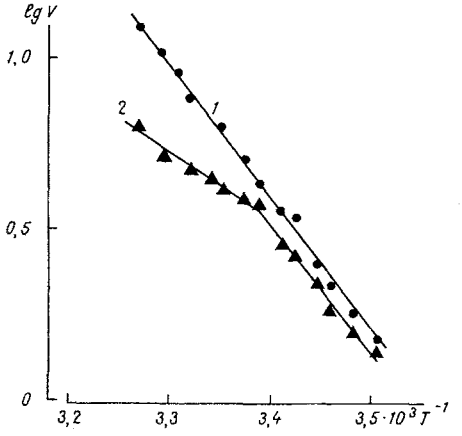

Fig. 3

Fig. 2. Appearance of spectrum of M-2 label at room temperature (a) and spectral parameter I_{+1}/B as a function of temperature in normal conditions and after high cholesterol diet (b). 1) Normal; 2) after high-cholesterol diet.

Fig. 3. Activity of Na,K-ATPase as a function of temperature under normal conditions (1) and after treatment of preparation for 8 h with cholesterol oxidase (2). Conditions of treatment: ratio between protein of Na,K-ATPase and protein of cholesterol oxidase 100 : 1. Conditions of determination of activity: 1 mM ATP, 5 mM $MgCl_2$, 130 mM NaCl, 20 mM KCl, 30 mM imidazole, pH 7.4. Remainder of legend as in Fig. 1.

In other experiments cholesterol oxidase was used to reduce the cholesterol contents in the membrane preparations in vitro. After treatment of the Na,K-ATPase with cholesterol oxidase for 8 h 50-80% of the cholesterol in the membranes was oxidized (Table 1). The graph of temperature dependence of this enzyme in this case showed an inflection even in the presence of a high $MgCl_2$ concentration (Fig. 3). A cholesterol deficiency in the membranes, it must be noted, led to a decrease in the activation energy in the temperature region above the inflection. Mg^{2+}, on the other hand, controls the temperature dependence of the enzyme in the low-temperature region.

The experiments showed that, just as in artificial membranes, the cholesterol level controls the fluid properties of the lipids and enzyme activity of the proteins in native membrane structures. Changes in the cholesterol level in the membranes thus must characterize the degree of their nativeness. Another possible index reflecting the state of the membrane structures may be the character of the temperature dependence of Na, K-ATPase. Since the model of alimentary hypercholesteremia as used in these experiments is a first approximation to a model of atherosclerosis, the principles revealed could be of practical importance.

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