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STRUCTURAL AND FUNCTIONAL CHANGES IN ERYTHROCYTE MEMBRANES IN EXPERIMENTAL ATHEROSCLEROSIS

T. I. Torkhovskaya, L. G. Artemova, B. G. Khodzhakuliev, T. S. Rudenko, V. A. Polesskii, and O. A. Azizova

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Changes in the functional state of cell membranes in the development of atherosclerosis have attracted the attention of many research workers. According to one hypothesis [5, 12], one of the early stages in the development of atherosclerosis, namely proliferation of the smooth muscle cells of the aorta, may be due to a decrease in membrane activity and in the activity of membrane-bound enzymes as the result of an increase in the content of nonesterified cholesterol (Ch) in the membrane.

The object of this investigation was to study changes in Na,K-ATPase activity and in the structural characteristics of the membranes of erythrocytes which, like smooth muscle cells of the aorta, are mesenchymal in origin [1], and also activity of the enzyme in homogenates of the aorta of animals with alimentary atherosclerosis.

EXPERIMENTAL METHOD

Experiments were carried out on 28 chinchilla rabbits. Experimental atherosclerosis was induced in 13 rabbits by feeding them daily for 4 months with Ch in a dose of 0.2 g/kg body weight. In 8 rabbits total involvement of the thoracic aorta was found at autopsy, and in 5 rabbits there were single lipid plaques; 15 rabbits served as the control. Blood was collected in tubes containing heparin (1000 units to 5 ml blood). Erythrocyte membranes were isolated by centrifugation at 28,000g after hemolysis of the erythrocytes in 10 mM histidine solution [2]. The protein content was determined in the suspension of membranes by the method in [10], and after extraction with a mixture of chloroform and methanol (2:1), the Ch content was determined on a Technikon A II automatic analyzer and the phospholipid content by Svannborg's method [14]. Activity of Na,K-ATPase was determined by the concentration of inorganic phosphate [13] after incubation in a medium containing 150 mM NaCl, 20 mM KCl, 30 mM $MgCl_2$, and 30 mM ATP [7]. The reaction was stopped by the addition of 1 ml acetate buffer, pH 4.7.

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TABLE 1. Na,K-ATPase Activity and Cholesterol Content in Erythrocyte Membranes and Aortic Homogenates (M ± m)

Degree of involvement of a vessel in animals	Plasma cholesterol concentration, mg%	Molar ratio Ch/PL in membrane	Na,K-ATPase activity, μmoles P_{inorg}/mg protein/h · 10^2		Cholesterol content in aortas, mg/100 g wet weight of tissue
			in erythrocyte membranes	in aortic homogenate	
Control	34±8	0,80±0,05	167±6,4	194±26	316±42
Experimental atherosclerosis					
solitary lipid plaques in aorta	441±28*	0,92±0,04 †	49±2,3*	120±18 †	528±39*
total involvement of aorta	840±56*	1,20±0,10 †	44±1,2*	114±18 †	897±76*

*P < 0.01.

†P < 0.05.

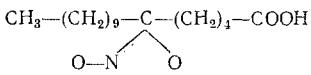
TABLE 2. Structural Parameters of Erythrocyte Membranes According to EPR Data (M ± m)

Degree of involvement of a vessel in animals	Parameter of orderliness S at 37°C, relative units	Difference between extrema of spectrum $2T_{11}$ at 37°C, G	Constants of HFI, G	
			10°C	37°C
Control	0,641±0,007	51,8±0,2	15,29±0,10	14,56±0,10
Experimental atherosclerosis				
Solitary lipid plaques in aorta	0,658±0,007	52,2±0,2 †	15,5±0,1	14,70±0,10
Total involvement of aorta	0,668±0,007*	53,5±0,2 †	15,67±0,1*	14,75±0,1

*P < 0.02.

†P < 0.05.

The structural characteristics of the membranes were investigated by the electron paramagnetic resonance (EPR) of spin probes method [6]. The nitroxyl derivative of palmitic acid:



was used as the probe. The concentration of the probe was 10^{-4}M. EPR spectra were recorded on the E-4 radiospectrometer (Varian) with temperature attachment, at a microwave power of 10 mW, amplitude of modulation of 1 and 4 G, and a magnetic field scanning time of 100 G/8 min. The temperature of the specimen was monitored by means of a thermocouple with an accuracy of 0.5°C. Parameters of the EPR spectra (Fig. 1) – the distance between the outer extrema ($2T_{11}$), characterizing the fluidity of the lipids, the parameter of orderliness (S), and the constant of hyperfine interaction (HFI), depending on the hydrophobicity of the microenvironment of the probe, were calculated by equations in [3].

EXPERIMENTAL RESULTS

Results of determination of the Ch level and of enzyme activity in erythrocyte membranes and aortic homogenates of rabbits with experimental atherosclerosis, compared with control animals, and also the Ch concentration in plasma, are given in Table 1. They show that the increase in Ch concentration in the plasma from 34 to 441 mg% in rabbits with solitary lipid plaques in the aorta is accompanied by a small, but statistically significant (P < 0.05) increase in the molar ratio of cholesterol/phospholipids (Ch/PL) from 0.81 to 0.92; activity of Na, K-ATPase fell under these circumstances from 1.67 to 0.49 μmole P_{inorg}/mg protein/h (P < 0.01).

In rabbits with total involvement of the aorta, the molar ratio Ch/PL increased even more – to 1.2. However, activity of Na,K-ATPase was reduced by the same degree as in animals with solitary lipid plaques in the aorta.

It can be tentatively suggested that in rabbits with mild lesions of the aorta even a small change in the Ch/PL ratio in the membranes would lead to a disturbance of the structural characteristics of the membranes necessary for normal functioning of Na,K-ATPase, an enzyme sensitive to the fluidity of the membrane lipids [4]. Evidence of such disturbances is given by changes in the temperature dependence of the parameter $2T_{11}$,

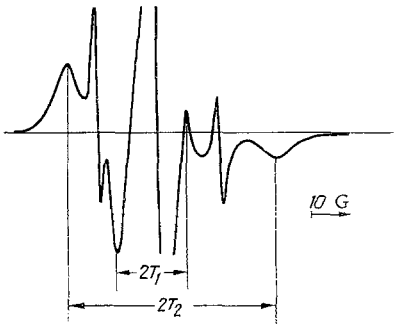


Fig. 1

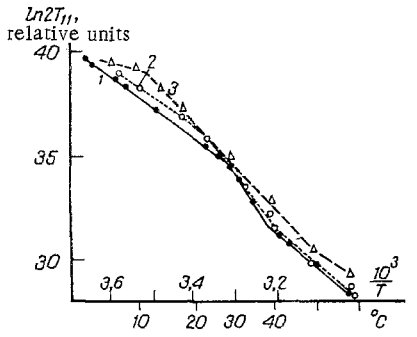


Fig. 2

Fig. 1. EPR spin probe spectrum in erythrocytes at 37°C.

Fig. 2. Temperature dependence of parameter of EPR spin probe spectrum in erythrocytes ($2T_{II}$; between Arrhenius coordinates). 1) control; 2, 3) experimental atherosclerosis: 2) single lipid plaques in aorta, 3) total involvement of aorta.

characterizing fluidity of lipids, between Arrhenius coordinates (Fig. 2). For erythrocyte membranes of healthy rabbits, sudden inflections of the curve were found at 29 and 37°C, indicating the existence of phase changes in the lipid regions of the membrane. Similar inflections have been observed at different temperatures for membranes of mitochondria and microvessels of the rat and sheep liver, plasma membranes of the rat liver, and membranes of microorganisms [9]. For erythrocyte membranes of rabbits, with a small increase in the ratio Ch/PL (0.92) and with mild changes in the aorta, the inflections at the above-mentioned temperatures disappeared, as was observed previously under the influence of cholesterol in membranes of microorganisms and in phospholipid liposomes [8, 11]. However, inflections appeared at 24 and 40°C, i.e., the temperature range of the phase changes was widened. This was manifested to an even greater degree in rabbits with marked atheromatosis and a high Ch/PL ratio in the membrane [1, 2]: Phase changes were observed only at 12 and 48°C. In the erythrocyte membranes of these animals, changes appeared in other parameters of the EPR spectrum also: an increase in the parameter S and in the content of HFI (Table 2).

However, the equal degree of lowering of Na,K-ATPase activity in the erythrocyte membranes of the two groups of animals (Table 1) is evidence that the most important change in the structure of the membranes, affecting enzyme activity, is widening of the temperature ranges of the phase changes in membrane lipids. Such changes are observed even when the Ch/PL ratio is increased only a little (to 0.92).

The parallel fall in Na,K-ATPase activity in the aortic homogenates of these same animals (Table 1) suggests that similar chemical and structural changes may take place in aortic cell membranes also. Under these circumstances, according to the hypothesis of Papahadjopoulos [12], the change in the fluidity of the membrane and in Na,K-ATPase activity may be one cause of proliferation of the smooth muscle cells of the aorta and of the development of the atherosclerotic process.

The structural and enzymic changes discovered in the erythrocyte membranes of rabbits with experimental atherosclerosis, together with the parallel changes in enzyme activity of the aortic homogenate, thus provide indirect evidence in support of the "membrane" hypothesis of the pathogenesis of atherosclerosis.

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CHANGES IN SURFACTANT SYSTEM OF THE LUNGS DURING AND AFTER STARVATION

V. V. Shishkanov and I. A. Serebrovskaya

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KEY WORDS: Surfactant system; starvation; vitamin A.

Data in the literature [4, 6, 11] are evidence that starvation can cause a decrease in lung surfactant activity. However, they do not give the final answer to the question of the functional integrity of the surfactant system during starvation or of the possibility of its restoration.

The object of this investigation was to study the state of the lung surfactant system by a combination of physical and morphological methods in rats after starvation, to determine the possibility of its restoration, and to attempt to accelerate this process by means of vitamin A, which has the property of stimulating maturation of the surfactant system [9].

EXPERIMENTAL METHOD

Experiments were carried out on 76 noninbred male albino rats weighing 190-230 g. Of these animals 51 were completely deprived of food for 4-5 days, but allowed free access to water. At that stage 21 rats (series I) were killed by cutting the throat. Nine animals (series II) were killed in the same way two weeks later, and another 10 rats (series III) four weeks after resumption of normal feeding. The remaining 11 starved animals (series IV) were given additional vitamin A (0.02 ml of a 3.44% oily solution of retinol acetate, 100,000 i.u./ml) by mouth daily; 25 intact rats served as the control (series V).

The lung index was calculated:

$$LI = \frac{\text{weight of lungs}}{\text{weight of rat}} \times 100$$

To characterize the surfactant system the following were investigated; 1) the surface tension (ST) of lung washings and extracts on surface scales [2]; 2) the coefficient of stability (CS) of bubbles expressed from pieces of the lungs [1, 3]; 3) luminescence in UV light of cryostat sections of the lungs stained with rhodamine 6G [1, 7]; 4) the number of birefringent structures in frozen unstained sections of fixed lungs [8, 10].

Alveolar washings were obtained by repeated injection of 7-8 ml physiological saline from the syringe into the trachea, followed by its aspiration until enough had been collected to fill the cuvette of the surface scales (100 ml). An extract was prepared from the washed, homogenized lungs (1 g tissue to 100 ml physiological saline). Minimal and maximal values of ST were determined and the stability index (SI) calculated by the formula:

$$SI = 2\,\frac{ST_{max} - ST_{min}}{ST_{max} \times ST_{min}}$$

The state of ST of the extract is an over-all index of surfactant activity of the lung tissues; ST of the washings, CS of the bubbles, and luminescence microscopy mainly characterize the state of the alveolar lining. By po-

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