CHANGES IN STRUCTURE AND ACTIVITY OF ATPase IN ERYTHROCYTE MEMBRANES IN ANGIOGRAPHICALLY CONFIRMED CORONARY DISEASE

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One of the hypotheses on atherosclerosis of the coronary arteries is the "membrane" hypothesis of Jackson and Gotto [6], put forward some years ago on the basis of contemporary views on the structure of biological membranes and, in particular, the condensing effect of cholesterol on the phospholipid bilayer [5]. They postulate similar influences also in smooth-muscle cell membranes of the vessel wall and regard these changes as the first step in biochemical processes which result in cellular proliferation [6]. Experimental confirmation of this hypothesis is difficult at present because of the impossibility of isolating membranes from smooth muscle cells in a pure form. Meanwhile the view has been put forward in the literature that changes in membranes of different cells may take place in the same direction. For instance, changes in the fatty-acid composition of dietary fats cause corresponding changes in the membranes of many cells in the body, and this affects the degree of orderliness of arrangement of the hydrocarbon chains of the fatty acids [8]. Feeding animals with cholesterol causes an increase in its concentration in membranes of different types, and a decrease in activity of the lipid-dependent membrane enzyme Na,K-ATPase has been demonstrated both in plasma membranes of the brain [1] and in erythrocyte membranes, with a simultaneous decrease in fluidity of the membranes as revealed by EPR studies of spin probes [4]. The writers previously demonstrated correlation between the depth of changes in Na,K-ATPase in erythrocyte membranes and in homogenates of aortas with experimental atherosclerosis [4]. This indicates the possibility of parallel structural changes in the membranes of erythrocytes and smooth-muscle cells as a result of the predominance of cells of this type in the aorta [10] and the membrane localization of Na,K-ATPase.

It was decided to study whether the fluidity of erythrocyte membranes changes under more natural conditions, namely during the development of atherosclerosis of the coronary arteries in man without any sudden changes in the plasma cholesterol level characteristic of experimental atherosclerosis [9].

For this purpose structural characteristics and Na,K-ATPase activity of erythrocyte membranes were investigated in patients with ischemic heart disease (IHD) and with angiographically confirmed coronary arterial involvement.

EXPERIMENTAL METHOD

Erythorcytes from 26 healthy men and 60 patients with IHD were used. The presence and absence of arterial lesions were determined by selective coronary arteriography. Erythrocytes were isolated and Na,K-ATPase activity and the cholesterol and phospholipid levels in the membrane were determined as described previously [4]. A suspension of erythrocytes (100 µl) were poured into tubes containing the spin probe — the nitroxyl derivative of palmitic acid with an oxazolidine ring in the 6th position of the fatty acid chain (the spin probe was generously provided by R. I. Zhdanov). The solvent for the probe, ethyl alcohol, was evaporated beforehand. The final concentration of probe in the erythrocytes was 10^{-5} M. EPR spectra were recorded on an E-4 radiospectrometer (Varian, USA) with thermostatically controlled flat cuvette. Conditions for recording the EPR spectra were: microwave power 10 mW, amplitude of modulation 1-2 G, magnetic field development 100 G/8 min. To estimate the structural characteristics of regions where the spin probe was located quantitatively, the

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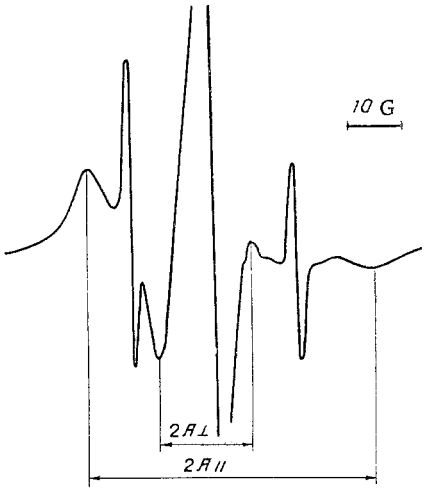

Fig. 1. EPR spectrum of spin probe in healthy human erythrocyte membrane (temperature 37°C).

parameter of orderliness S was used. This parameter reflects the degree of orientation of the fatty-acid chains of phospholipids in the bilayer membrane of erythrocytes and varies from 0 to 1 during transition from isotropic rotation to complete orientation. S was calculated by the equation [2]:

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} + {}^1/_2 (A_{xx} + A_{yy})} \cdot \frac{\frac{A_{xx} + A_{yy} + A_{zz}}{3}}{a'},$$

where the constant parameters for this particular spin probe were taken from [2], and the values of $A_{\parallel}$ and $A_{\perp}$ were determined as shown in Fig. 1. The constant of hyperfine interaction (HFI), designated a', characterizing polarity of the spin probe, was calculated by the equation:

$$a' = \frac{A_{\parallel} + 2A_{\perp}}{3}.$$

EXPERIMENTAL RESULTS

The results of measurement of parameters of the spin probe in the erythrocyte membrane of patients with IHD and normal subjects (at 37°C) are given in Table 1. The higher values of the parameters $2A_{\parallel}$ and S in the erythrocytes of patients with coronary arterial disease indicate the greater orderliness of the fatty-acid chains of phospholipids in these patients compared with healthy subjects. The increase in the parameter a' in these same patients is evidence that the environment of the nitroxyl fragments of the fatty-acid spin probe, located in erythrocyte membranes, becomes more polar than that for healthy subjects. This may perhaps be due to the greater accessibility of the radical for water molecules and for hydrogen bond formation in the radical fragments.

Characteristically in patients with IHD and without arterial lesions, as shown by coronary arteriography, values of the parameters S and a' were similar to those in healthy subjects. The EPR spectrum of this probe thus revealed structural changes in erythrocyte membranes of patients with coronary arterial disease by contrast with erythrocyte membranes of patients without arterial disease and of healthy subjects.

One cause of the structural changes described above in the erythrocyte membranes of patients with IHD is evidently an increase in the molar cholesterol/phospholipid (CH/PL) ratio observed in all the patients investigated (Table 2). As Table 2 shows, this value rose from 0.87 in normal subjects to 1.15 in patients with IHD. A similar increase in the molar CH/PL ratio was observed previously in the erythrocyte membranes of parients with IHD [3]. One result of structural changes in erythrocyte membranes of patients with IHD was drastic inhibition of Na,K-ATPase, an enzyme whose activity depends on the state of the lipid environment [7].

It must be pointed out, however, that unlike changes in the structural characteristics (Table 1), both Na,K-ATPase activity and the CH/PL ratio showed changes even in patients with no evidence of coronary arterial damage. The reason for this is not yet clear. It may perhaps be because the erythrocyte membranes in these patients were in fact damaged, although in microregions that differed from the sites of the probe used. Perhaps despite the absence of visible lesions in the aorta, initial molecular changes capable of leading to lesions of the vessels were already present in the membranes of the aortic cells, just as in erythrocyte membranes of these patients.

TABLE 1. Parameters of EPR Spectrum of Spin Probe in Erythrocyte Membranes

Group of subjects tested	Distance between extrema, $(2A_{\parallel})$, G	Constant of hyperfine interaction (a'), G	Parameter of orderliness (S), relative units
Healthy individuals	51,16±0,32	14,52±0,15	0,611±0,007
Patient with IMD:			
without vascular involvement	51,00±0,22	14,60±0,02	0,603±0,007
with vascular involvement	53,00±0,47	14,87±0,10	0,642±0,009

TABLE 2. Molar Cholesterol/Phospholipid (CH/PL) Ratio and Na,K-ATPase Activity of Erythrocyte Membranes

Group of subjects tested	CH/PL, mole/mole	Na,K-ATPase activity, μmoles P_i/mg protein/h × 10^2
Healthy individuals	0,87±0,01	148,93±4,35
Patients with IMD:		
without vascular involvement	0,92±0,05	116,00±4,60
with vascular involvement	1,15±0,02	45,07±2,39

The results are evidence of a profound change in structure of the erythrocyte membranes in IHD, leading to inhibition of the membrane-bound enzyme Na,K-ATPase. The presence of such changes indicates a possible connection between damage to the arteries, beginning probably with injury to smooth-muscle cell membranes [6], and the state of the erythrocyte membranes.

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EFFECT OF TESTOSTERONE ON RATE OF TOTAL PROTEIN SYNTHESIS IN THE FETAL RABBIT REPRODUCTIVE TRACT *IN VITRO*

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In the modern view androgens determine the development of the male reproductive system in the critical period of embryonic development [4]. Whereas the molecular basis for the direct effect of androgens on tissues of target organs has been studied quite well [3], the mechanism of their action on growth and differentiation of the reproductive tract of mammalian fetuses has not hitherto been investigated.

The most active androgen is testosterone, secreted by Leydig's cells in the testis. Its stimulating effect on the synthesis of various proteins, including receptor proteins, in target organs has been demonstrated in adult mammals [3, 7]. Nevertheless, the study of the action of testosterone on biosynthesis in fetuses is of great interest for elucidation of the role of hormones in early ontogeny.

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