Structural Abnormalities and Changes in Na,K-ATPase Activity in Erythrocyte Membranes in Patients with Neurotic Disorders

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We studied structural and metabolic characteristics of erythrocyte membranes in 19 patients with neurotic disorders (neurasthenia and dysadaptation). Microviscosity of the lipid phase in erythrocyte membranes increased in 13 patients, and Na,K-ATPase activity decreased in 6 patients. Various changes in test parameters are probably associated with different adaptive capacities of the organism under stress conditions.

Key Words: *neurotic disorders; membrane; erythrocyte; fluorescent probes; Na,K-ATPase activity*

Psychic dysadaptation providing the basis for the development of neurotic disorders manifests itself at various biological levels, including the subcellular (impairment of bioenergetic, molecular, and biological processes), cellular (structural changes in membranes and organelles), systemic (changes in the equilibrium constants for physiological systems), organism (changes in individual behavioral parameters and emotional reactivity), and supraorganism levels (changes in intraspecies relationships) [4]. Neurotic disorders are accompanied by visceral dysfunction, which is considered by some authorities as the main sign of neurosis [1,3,11,12]. However, the mechanisms underlying the development of somatic disturbances in patients with neuroses are poorly understood. Moreover, the interpretation of these processes is contradictory. Here we studied somatic response of macroorganisms to psychogenic factors. We evaluated structural and metabolic characteristics of membranes in erythrocytes that act as a "cellular dosimeter" for the effects of facultative and obligate exogenous and endogenous factors.

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MATERIALS AND METHODS

We examined 19 patients (17 women and 2 men, 19-48 years) with neuroses. The diagnosis of dysadaptation (F43.2, ICD-10) and neurasthenia (F48.0, ICD-10) were made in 12 and 7 patients, respectively. These patients were not alcohol and drug abusers and had no somatic and neurological diseases. The patients were examined before the start of psycho- and pharmacotherapy. The control group included 36 healthy donors of the same age.

The blood was taken from the cubital vein after 10-12-h starvation and stabilized with 50 U/ml heparin. Membrane preparations were obtained by hypoosmotic hemolysis of erythrocytes [13]. Protein content in the membrane suspension was estimated by the biuret method. Pyrene, N-phenyl-2-naphthylamine (PNA), and 1-anilinonaphthalene-8-sulfonate (ANS, Sigma) were used as fluorescent probes. Spectral characteristics of the interaction between membranes and fluorophores were estimated on a MPF-4 spectrofluorometer (Hitachi) using a rectangular quartz cuvette (optical path 1 cm) at 25°C. Pyrene fluorescence was measured in a medium containing 145 mM NaCl and 10 mM Tris (pH 7.4). Pyrene (ethanol solution) was added to the cuvette with erythrocyte ghosts (protein

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concentration 0.3 mg/ml) to a final concentration of 10 μM and incubated for 10 min under constant mixing. To estimate the degree of pyrene excimerization in the region of annular and total lipids, we calculated the ratio between the intensity of fluorescence I_{470}/I_{370} at excitation wavelengths (λ_E) of 285 and 340 nm, respectively [6]. The I_{370}/I_{390} ratio was calculated at λ_E=340 nm to evaluate polarity of the microenvironment in pyrene molecules [5]. The index for energy migration from tryptophan residues in proteins to pyrene was calculated by the formula described elsewhere [5].

Fluorescence polarization of PNA was assayed to estimate microviscosity of the medium surrounding this probe in the membrane [6]. Standard film polarizing filters served as the polarizer and analyzer. The solution of PNA in methanol was added to the suspension of erythrocyte membranes (protein concentration 0.3 mg/ml) to a final probe concentration of 10 μM. Excitation was performed with linearly polarized light (wavelength 350 nm). Maximum fluorescence of polarized light was recorded at λ_E=440 nm.

Binding of ANS was assayed in 2 experiments with titration. Titration I was performed with the protein in various concentrations (0.3-0.6 mg/ml) at a constant concentration of the fluorophore (5 μM). Titration II was performed with the fluorophore in various concentrations (5-20 μM) at a constant concentration of the protein (0.3 mg/ml). Fluorescence of ANS was induced at λ_E=360 nm. The intensity of fluorescence was measured at λ_E=490-510 nm. The binding constant and the number of ANS binding sites on the membrane were estimated graphically in double inverse coordinates [6].

Na,K-ATPase activity in erythrocyte ghosts was estimated by an increase in the content of inorganic phosphorus (P_i) in an incubation medium containing 125 mM NaCl, 25 mM KCl, 3 mM $MgCl_2$, 0.5 mM EDTA, 2 mM ATP, and 50 mM Tris-HCl (pH 7.4) [8]. Incubation was performed at 37°C for 1 h. The reaction was stopped by the addition of 20% trichloroacetic acid. Na,K-ATPase activity was calculated as the difference between ATPase activities under these experimental conditions and in a medium of the same composition containing 125 mM KCl (without NaCl).

The results were analyzed by Student's *t* test and nonparametric tests.

RESULTS

Spectral characteristics of the interaction of fluorescent probes pyrene, PNA, and ANS with erythrocyte membranes in patients with dysadaptation and neurasthenia allowed complex structural analysis of these membranes in the region of polar heads and hydrophobic tails.

Microviscosity of the lipid phase in the region of lipid-protein contacts (λ_E=285 nm) and lipid bilayer of erythrocyte membranes (λ_E=340 nm) was estimated by excimerization of the nonpolar probe pyrene that diffused into the hydrophobic compartment of membranes. Pyrene fluorescence spectra were recorded during its interaction with erythrocyte membranes in patients with neurotic disorders. Pyrene excimerization (I_{470}/I_{370}) at λ_E=340 nm decreased by 32% compared to the control, which indicates that microviscosity of the lipid bilayer increased. However, the analysis of differences in the arrangement of annular lipids and protein-free lipid phase revealed no structural changes in the region of protein-lipid contacts in erythrocyte membranes from patients with neuroses (Table 1).

TABLE 1. Studies of Erythrocyte Membranes in Patients with Neurotic Disorders with Fluorescent Probe Pyrene (*X*±*m*)

Parameter	Healthy donors	Patients with neurotic disorders
Fluorescence, arb. units		
I_{470}/I_{370} (λ_E=285 nm)	0.412±0.015	0.382±0.028
I_{470}/I_{370} (λ_E=340 nm)	0.650±0.019	0.439±0.050*
I_{370}/I_{390} (λ_E=340 nm)	0.907±0.019	0.959±0.012**
Energy migration from tryptophan to pyrene, %	62.35±0.79	64.52±1.49

Note. *p<0.001, **p<0.05 compared with healthy donors.

TABLE 2. Studies of Erythrocyte Membranes in Patients with Neurotic Disorders Using Fluorescent Probes PNA and ANS (*X*±*m*)

Parameter	Healthy donors	Patients with neurotic disorders
Fluorescence polarization of PNA, arb. units	0.044±0.004	0.036±0.004
Binding constant for ANS, μM^{-1}	0.061±0.004	0.080±0.012
Binding constant for ANS, μmol×mg/ml	1.40±0.21	1.89±0.49

Microviscosity of the lipid phase in erythrocyte membranes can increase due to lengthening of the acyl chain in lipids, high concentration of bivalent inorganic cations, and decreased content of unsaturated fatty acids in the bilayer [2,6]. It cannot be excluded that decreased fluidity of the lipid phase in erythrocyte membranes is a result of intensification of lipid peroxidation (LPO) in patients with neurotic disorders [9]. These patients were characterized by a marked increase in I_{370}/I_{390} at λ_E=340 nm. Our results indicate that polarity of the microenvironment surrounding the probe in the integral lipid phase of erythrocyte membranes increases due to an increase in the content of polar H_2O molecules during intensification of LPO.

It should be emphasized that despite changes in fluorescence of pyrene in erythrocyte membranes, test parameters in 6 of 19 patients with neurotic disorders (32%) did not differ from the control. Therefore, structural characteristics of membranes in these patients should be analyzed individually. Changes in spectral characteristics of the interaction between erythrocyte membranes and ANS were found in only patients. This probe binds to the surface of the membranes because of the presence of negatively charged sulfonyl groups. However, in patients with neurotic disorders the average binding constant and number of ANS binding sites on the erythrocyte membranes did not differ from the control (Table 2). We revealed no changes in spectra reflecting the interaction between erythrocyte membranes and fluorophore PNA. PNA molecules are localized in hydrophobic regions of the membrane that include carbonyl groups and glycerol residues of phospholipids (Table 2).

Structural modification of the lipid phase in erythrocyte membranes induces conformational changes of proteins [7]. The activity and properties of transport ATPases in plasma membranes are determined by structural characteristics of the lipid matrix [10]. We measured activity of membrane-bound ion-transporting Na,K-ATPase. The mean enzyme activity in patients did not differ from the control (0.037± 0.002 and 0.038± 0.001 μmol P_i/h×mg protein, respectively, p>0.05). However, in 6 of 19 patients (32%) Na,K-ATPase activity was lower than in healthy donors (0.026±0.001 μmol P_i/h×mg protein). In other 6 patients (32%) enzyme activity increased to 0.055± 0.001 μmol P_i/h×mg protein. In 7 patients (36%) activity of this ion-transporting enzyme was similar to that in healthy donors (0.037±0.001 μmol P_i/h×mg protein).

Structural and metabolic characteristics of erythrocyte membranes in patients with neurotic disorders may be considered from the viewpoint of normal and pathological states of the organism. Various changes in test parameters are probably associated with differences in the strain of regulatory mechanisms, functional state of individual systems and organisms, and adaptive capacities under stress conditions.

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