Characteristics of Albumin Molecule Binding Centers in Patients with Anxious Depression. Study by the Fluorescence Quenching Method

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The state of binding centers in albumin molecule in patients with anxious depression was studied by the method of quenching of fluorescence of molecular probe (dimethylaminonaphthaleic acid carboxyphenylimide) with nitrate ions. Serum samples from 24 donors without somatic and mental diseases and 26 patients were analyzed. In the absence of the quenching agent, specific fluorescence of the probe (standardized by albumin concentration) was lower in patients with depression. The fluorescence quenching constant and the percentage of fluorescence available for quenching were also lower in serum samples from patients. These data indicate that the parameters of binding centers in albumin molecule in patients with anxious depression are significantly modified in comparison with normal subjects. The detected changes can play a role in the pathogenesis of depressive disorders.

Key Words: *fluorescence; quenching; albumin; anxious depression*

Depression is one of the most prevalent disorders in psychiatric and common somatic practice. According to modern forecasts, by 2020 depression is expected to rank second among the causes of disability after cardiovascular diseases. WHO study showed that depression and anxiety are the most frequent concomitant disorders in primary medical practice. Anxiety is detected in 42-100% patients with depression [9-11].

Depressive disorders are paralleled by significant disturbances in the cardiovascular, gastrointestinal, autonomic nervous, immune, and endocrine systems, and by other abnormalities [6,7]. However, the molecular processes underlying these changes are poorly understood. Dysfunctions of transporting proteins in depression are virtually not studied, for example, changes in albumin, the main transporting protein of human liquid media, remain little studied.

Fluorescence quenching is an effective method for the study of conformation changes in proteins. The gist of this phenomenon is the decrease in substance fluorescence (ligand, probe) during its reaction with another substance (quenching agent), for example, in diffusion collision or during the formation of a nonfluorescent complex with this agent. The conditions of interactions between the fluorophore and quencher can be evaluated by the degree of quenching. If the fluorophore is bound to a protein, the dynamic and spatial characteristics of protein molecule binding domain can modulate quenching efficiency [3,5].

We compared albumin binding centers in patients with anxious depression and healthy indivi-

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duals by the method of fluorescence quenching for a probe bound to serum albumin.

MATERIALS AND METHODS

Blood sera from 26 patients hospitalized at Moscow Institute of Psychiatry and 24 donors without somatic and mental diseases (reference group) were analyzed.

The inclusion criteria were signs of depressive disorders (section F 3. IDC-10), predominance of anxiety affect in the structure of depression, and age 16-50 years. Patients with depressive disorders with concomitant hallucinatory delirious disorders, chronic alcoholism, narcomania, mental disorders associated with organic disorders of the CNS were not included in the study. Patients received no antidepressants for at least 2 weeks before the study. All patients gave informed consent to participation in the study.

According to IDC-10 criteria, the patients' status was diagnosed as F.32.1 (moderate depressive episode) in 6 patients and F.33.1 (recurrent depressive disorder, episode of moderate severity) in 20.

Blood for the analysis was collected after overnight fasting, the serum was separated by centrifugation after 45-min clotting at ambient temperature.

Fluorescent probe dimethylaminonaphthalic acid N-carboxyphenylimide (K-35; CAPIDAN) synthesized by B. M. Krasovitskii (Institute of Monocrystals, Kharkov, Ukraine) served as the ligand for albumin center testing. Being added to the serum, the probe selectively fluoresces from albumin [1,4],

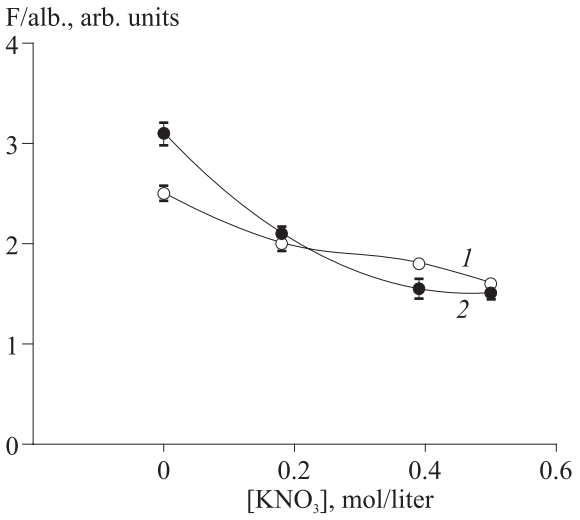

Fig. 1. Averaged curves of K-35 fluorescence quenching with nitrate in the sera from patients with depression (*1*) and donors (*2*). Ordinate: K-35 fluorescence intensity from serum, standardized by serum albumin concentration.

due to which there is no need in isolation of this protein and albumin centers can be therefore studied in a maximally native status.

The fluorescence of albumin-bound probe was quenched using mixtures containing KNO_3 and KCl (Sigma) in different proportions: 0.5 ml mixture was added to 1.5 ml serum diluted 20-fold with PBS (pH 7.4; Sigma). Nitrate ion is the agent quenching the fluorescence. Its final concentration in the samples varied from 0 to 0.5 mol/liter. KCl was added for maintenance of ionic strength of the samples (0.635 mol/liter).

The resultant relationships between the probe fluorescence intensity and the quenching agent concentration were analyzed using Lehrer's model [8]. The reduction of the probe fluorescence intensity after addition of the quenching agent was described by two parameters: biomolecular quenching constant (K_Q) and percentage of quenched fluorescence (f):

$$f = \frac{F_0 - F_\infty}{F_0};$$

$$\frac{F_0}{F_0 - F} = \frac{1}{f} + \frac{1}{f \times K_Q \times [KNO_3]},$$

where F_0 and F are probe fluorescence intensities without quenching agent and in the presence of its infinity concentration, respectively. The fragment of the curve reflecting the relationship between $F_0/(F_0-F)$ and $1/[KNO_3)$, cut on the vertical axis, is equal to $1/f$, the curve slope being equal to $1/(f \times K_Q)$.

Serum albumin concentrations were evaluated by the fluorescent method [10].

The samples fluorescence intensities were recorded on a Hitachi F-4000 spectrofluorometer in a standard 1×1-cm cuvette at excitation wavelength λ=425 nm and emission wavelength λ=525 nm. In order to compare the intensities of fluorescence measured on different days, the resultant values were standardized by the fluorescent signal of the reference sample (fluorescent simulator of serum composition GSO 6296-91 [1]).

The results were processed using MS Excel XP and Statistica 6.0 software. The significance of differences between the groups was evaluated using Mann—Whitney nonparametric test.

RESULTS

In the absence of the quenching agent, the fluorescent signal of the probe from albumin was higher in healthy donors than in patients (p=0.03; Fig. 1). These differences can be explained by changed

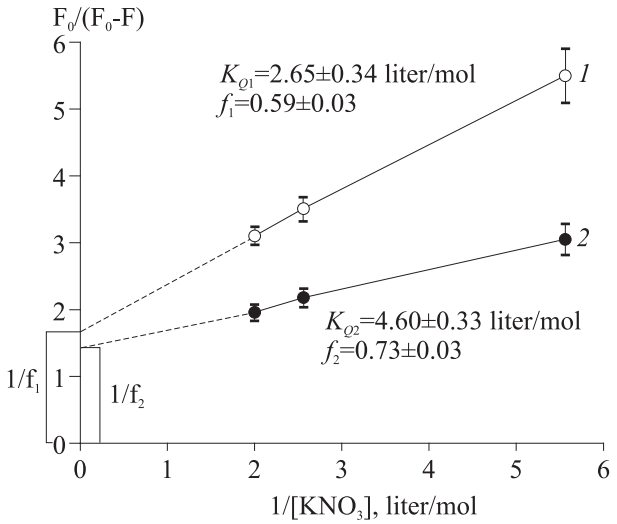

Fig. 2. Averaged Lehrer's curves for calculating the parameters of nitrate quenching of fluorescence of albumin-bound K-35 probe in sera. *1*) sera from patients with depression; *2*) donor sera. Ordinate: F_0 and F: probe fluorescence intensity from albumin without and with nitrate; f_1 and f_2 probe fluorescence intensity available for quenching; K_Q: quenching constant.

characteristics of albumin centers, which led to reduction of binding and/or quantum yield of fluorescence of albumin-bound K-35 molecules. In the presence of the quenching agent, K-35 fluorescence intensity decreased, but this effect was more pronounced in normal subjects, and therefore probe fluorescence intensity from albumin in donor sera was lower than in serum samples from patients ($p<0.001$).

The constant of K-35 fluorescence quenching by nitrate was lower in patients with depression and the percentage of fluorescent available for quenching was lower in them than in donors ($p<0.01$; Fig. 2).

The results are in line with the data indicating that specific fluorescence of K-35 from albumin under conditions of physiological ionic strength is reduced in patients with anxious depression [12]. We detected similar shifts at ionic strength of 0.635 mol/liter (Fig. 1).

Hence, interactions between K-35 probe and albumin binding centers changed significantly in patients with anxious depression in comparison with normal subjects. Presumably, the chemical structure or conformation of binding centers in albumin molecule was modified in them.

Albumin transport of ligands (endogenous and exogenous) is determined by the properties of binding centers in albumin molecule. Irrespective of the molecular mechanism of the detected disorders, changes in conditions of albumin binding of natural metabolites (fatty acids, bilirubin, *etc.*) and xenobiotics (including drugs) can lead to redistribution of concentrations of low-molecular-weight substances in the body and hence, to disorders in the metabolic processes and homeostasis in general.

This can be significant for the formation of depressive disorder, its course, and dynamics of therapy, and can be essential for the course of anxious depression complications. Registration of changes in albumin molecule in depression can serve as a method for evaluating the status of the organism, which will help to develop recommendations for predicting treatment efficiency and thus reduce treatment duration, which will improve the patients' quality of life.

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