Platelet Content of Serotonin-Modulated Protein SMP-69 in Patients with Schizophrenia

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Enzyme immunoassay showed that serotonin-modulated protein SMP-69 from the brain of albino rats displayed no tissue specificity and was present in platelets. Platelet content of SMP-69 in patients with schizophrenia surpassed that in healthy humans. Our findings indicate that this method can be used in forensic psychiatry and clinical practice to control the state of patients in therapy.

Key Words: *platelets; serotonin-modulated protein SMP-69; schizophrenia; enzyme immunoassay*

In the 1950s, it has been proposed that serotonin play a role in the pathogenesis of schizophrenia [14]. Recent studies suggest that the pathogenesis of schizophrenia is associated with impaired serotonin metabolism in the brain [14]. Postmortem examination of the frontal cortex from patients with schizophrenia showed that the number of binding sites (B_{max}) for ^{3}H-spiperone, a selective 5-HT_2 receptor ligand, decreased, while K_d remained unchanged [5]. These data indirectly indicate the elevation of serotonin content in the frontal cortex in patients with schizophrenia.

Noninvasive methods for estimating serotonin metabolism in brain cells of patients with schizophrenia are important for clinical practice. Parameters of serotonin metabolism in platelets, including the number of specific receptors and activity of enzymes involved in serotonin synthesis and degradation are similar to those in brain cells [9,10]. Therefore, platelets are a good model for analyzing serotonin metabolism in nerve cells in various brain diseases. The density (B_{max}) for 5-HT_2 receptors in platelets from patients with depression and suicidal behavior increases [4, 7,8]. At the same time, B_{max} of ^{3}H-imipramine binding sites in platelets from patients with panic psychoses decreases, but K_d remains unchanged [15].

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Serotonin changes the activity of the genetic apparatus in nerve cells by modulating synthesis of some proteins [13], which mediate its effects in various cells [6]. Experiments with intracerebral administration of antibodies against serotonin-modulated protein SMP-69 (molecular weight 69 kDa, pI 6.0) to rats demonstrated that this agent is involved in the regulation of exploratory behavior [2] and consolidation of memory traces [3]. Here we measured the content of SMP-69 in platelets from patients with psychotic schizophrenia.

MATERIALS AND METHODS

Total proteins of the liver, testes, brain, pancreas, and platelets from 4 rats were extracted in a homogenizer in 0.05 M phosphate buffer (pH 7.2) containing 0.3 M NaCl, 5 mM ethylenediaminetetraacetate (EDTA), and 0.1% Triton X-100.

The blood (2 ml) from the ulnar vein of patients with psychotic schizophrenia (n=23) and healthy subjects (n=15) was collected into tubes with 300 μl 5% EDTA preventing coagulation and centrifuged at 1500 rpm for 5 min. The plasma was collected, platelets were precipitated by centrifugation at 6000 rpm for 10 min, and proteins were extracted by the above described method. The content of SMP-69 in protein extracts was measured by enzyme-linked immunosorbent assay (ELISA) on polystyrene plates (4 wells for

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each probe) [1]. Tissue protein extracts (250 µl in 0.1 M Tris-HCl buffer, pH 8.6) were incubated for 20 h at room temperature, the wells were washed 4 times with 0.15 M NaCl containing 0.05% Tween-20, and rabbit immunoglobulins to SMP-69 diluted 1:30 with 0.01 M phosphate buffer (pH 7.2) containing 0.15 M NaCl, 0.05% Tween-20, and 1 mg/ml BSA were added. The wells were washed after 24 h and incubated for 3 h with horseradish peroxidase-conjugated goat antirabbit immunoglobulins (Cardiology Center, Moscow) diluted 1:2000 with the same buffer. After washout, *o*-phenylenediamine (horseradish peroxidase substrate) in 0.05 M citrate-phosphate buffer (pH 4.5) containing 0.4 µl/ml 30% H_2O_2 was added. The reaction was stopped 15 min later by adding 50 µl 3 M NaOH, and optical density was measured at 492 and 405 nm on a Multiscan photometer. The results were analyzed by Student's *t* test.

RESULTS

The content of SMP-69 in the pancreas, testis, and liver cells was the same. At the same time, SMP-69 content in platelets surpassed that in the brain (Table 1). Thus, SMP-69 had no tissue specificity and was found in various tissues, including platelets.

The content of serotonin in platelets insignificantly varies between adult men and women [12] and, therefore, the measurements were performed without considering this parameter. Platelet content of SMP-69 in patients with psychotic schizophrenia estimated by absorbance at 432 nm surpassed that in healthy subjects (0.446±0.004 and 0.336±0.002, respectively, $p<0.001$).

High content of SMP-69 in platelets from patients with psychotic schizophrenia indicates activation of brain serotoninergic system. Our findings are consistent with the data on serotonin content in postmortem preparations of the brain from patients with panic psychoses [15].

Changes in the content of SMP-69 in platelets from patients with psychotic schizophrenia and low data spread in patients and healthy humans indicate that ELISA with immunoglobulins to SMP-69 can be used for the laboratory diagnostics, when clinical observations are not sufficient for the diagnosis of this disease. This method holds much promise for controlling efficiency of therapy and dynamics of schizophrenia. Furthermore, ELISA can be used in forensic psychiatry to confirm the diagnosis of schizophrenia, if the symptoms are suspected to be aggravated, or if patients with schizophrenia feign mental health.

TABLE 1. Content of SMP-69 in Various Tissues of Albino Rats ($M\pm m$, $n=4$)

Tissue	Absorbance at 405 nm
Brain	2.064±0.045
Pancreas	2.027±0.06
Testes	1.976±0.032
Platelets	2.352±0.006*
Liver	2.154±0.049

Note. *$p<0.05$ compared with the brain.

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