GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Induction of Autoantibodies to Glutamate in Patients with Alzheimer's Disease

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Autoantibodies to glutamate were found in blood plasma from patients with Alzheimer's disease. The content of autoantibodies to glutamate in blood plasma from patients with moderate and severe dementia was 2-fold higher compared to patients with mild dementia.

Key Words: *Alzheimer's disease; dementia; autoantibodies; glutamate*

Glutamate plays an important role in the pathogenesis of Alzheimer's disease (AD) [2,3,4]. Glutamate produces an excitotoxic effect and causes death of cortical and subcortical neurons due to prolonged calcium influx, which finally leads to the development of progressive dementia, the main symptom of AD [5]. Production of autoantibodies to glutamate in response to long-lasting chronic release of considerable amounts of this compound can be a mechanism of regulation of glutamate concentration in CNS.

Here we studied the production of autoantibodies to glutamate in AD patients and patients with different severity of cognitive disorders.

MATERIALS AND METHODS

We examined 40 patients with AD (women, 75-90 years) and 22 mentally healthy women of similar age. The diagnosis of AD was made from the results of psychiatric, neurological, and psychological examination and computer tomography of the brain according to criteria of ADRDA and ICD, 10th Revision (WHO, 1994). The severity of cognitive disorders was scored using the MMSE test. The blood was taken once.

The content of autoantibodies to glutamate in blood plasma was measured by enzyme-linked immunosorbent assay (ELISA) in 96-well polystyrene plates sensitized with the test antigen (glutamate-BSA conjugate). The conjugate was synthesized by a modified method using bifunctional reagent glutaraldehyde [6]. The test antigen (100 μl, final concentration of 0.3 μg/well) was put in wells of a Dynatech plate. Incubation was performed at 4°C for 18 h. The plates were washed 4-5 times with physiological saline containing 0.05% Tween-20. Plasma samples (100 μl) were diluted in 0.05 M phosphate buffered saline (pH 7.4) containing 0.05% Tween-20 (dilution 1:50). After 1-h incubation at 37°C, the plates were washed and treated with secondary antibodies to human IgG labeled with horseradish peroxidase (dilution 1:2000). The plates were washed after 1-h incubation. The substrate mixture of 10 ml 0.2 M $Na_2HPO_4 \times 2H_2O$, 10 ml 0.1 M citric acid, 8 mg *o*-phenylenediamine (Sigma), and 8 μl 33% H_2O_2 was added to wells (100 μl). Incubation was performed in darkness at

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TABLE 1. Content and Frequency of Detection of Auto-antibodies to Glutamate in AD Patients

Group	Number of patients	Frequency of detection of autoantibodies to glutamate, %	Content of autoantibodies to glutamate in blood plasma, arb. units
Control	22	12.5	0.95±0.05
Whole	40	47.6	1.40±0.09*
With mild dementia	10	0	0.95±0.02
With moderate and severe dementia	30	83.3	1.80±0.06*+x

Note. $p<0.001$: *compared to the control group; +compared to patients with mild dementia; xcompared to the whole group.

room temperature for 1 h. The reaction was stopped by addition of 6 N H_2SO_4. The content of antibodies in each well was estimated by measuring optical density of the plasma on a Mini-reader device (Dynatech) at 495 nm and expressed in arbitrary units of activity (ratio of optical density of the plasma from each patient to the mean optical density of plasma samples from healthy donors). When this ratio exceeded 1.0, we concluded that blood plasma contains antibodies.

The results were analyzed by Student's *t* test (PRIMER software).

RESULTS

The content of autoantibodies to glutamate in blood plasma from AD patients was higher than in mentally healthy donors (Table 1). Autoantibodies to glutamate were detected in 47.6% AD patients and 12.5% healthy donors. The patients with AD were divided into 2 groups depending on the severity of cognitive disorders (MMSE test): mild (MMSE score 22.0±1.5) and moderate-to-severe dementia (MMSE score 9.0±0.5). The MMSE score for healthy patients was 27.4±2.5. The content of autoantibodies to glutamate in plasma samples from patients with moderate and severe dementia increased and was much higher compared to patients with mild dementia (Table 1). The content of autoantibodies to glutamate in patients with moderate and severe dementia exceeded that in the whole group of AD patients and patients with mild dementia. The content of autoantibodies to glutamate in these patients corresponded to the age norm.

Our results show that patients with AD are characterized by increased production of autoantibodies to glutamate. It should be emphasized that the frequency of detection and content of autoantibodies to glutamate in blood plasma increased with increasing the severity of cognitive disorders in patients. In patients with mild dementia not requiring help, free autoantibodies to glutamate were not found in plasma samples, while in patients with moderate and severe dementia that could not live without assistance, the content of autoantibodies to glutamate reached 83.3%. The content of autoantibodies to glutamate in blood plasma from these patients 2-fold surpassed that in patients with mild dementia. It seems most likely that autoantibodies to glutamate in patients with mild dementia are utilized for inactivation of glutamate molecules and are in a bound state; therefore, free antibodies are not detected at the early stage of the disease. The development of severe dementia in patients is accompanied by death of most neurons and decrease in glutamate release. Blood plasma in these patients contains considerable amounts of free autoantibodies to glutamate. Autoantibodies to glutamate probably inactivate excess glutamate, maintain optimal level of glutamate in CNS, and prevent glutamate excitotoxicity. Published data show that autoantibodies to glutamate modulate the behavior of experimental animals with low behavioral activity and high anxiety, which manifested in a decrease in anxiety and memory improvement [1]. Further studies are required to evaluate the role of autoantibodies to glutamate in the pathogenesis of AD.

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