Antiamnestic Effects of Antibodies to Glutamate in Experimental Alzheimer's Disease

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Experiments on rats showed that neurodegenerative brain damage caused by administration of neurotoxic fragment of β-amyloid protein $A\beta_{25-35}$ in a dose of 2 μg into Meynert giant cell nucleus leads to long-term memory impairment in rats. Intranasal administration of antibodies to glutamate in a dose of 300 μg/kg 1 h after damage restores learning capacity of the experimental animals in the conditioned passive avoidance paradigm.

Key Words: *antibodies to glutamate; amyloid peptide $A\beta_{25-35}$; Meynert giant cell nucleus; cognitive impairments*

Alzheimer's disease (AD) is a severe chronic brain disease affecting people of the elderly and senile age and leading to progressive dementia. Memory impairments are among the most common symptoms of AD. Recently, the main mechanisms of cognitive disorders, particularly memory disorders, in AD were clarified in chronic neurodegenerative disorders of the brain. Disorders in neurotransmitter brain functions are an important element of the pathogenesis of neurodegenerative disorders. Moreover, disturbances in the glutamatergic system leading to neuron death play the key role [10].

Glutamate (Glu) is the main excitatory neurotransmitter in CNS. It is involved in various processes in the brain, including cognitive functions [7]. Under conditions of a neurodegenerative pathological process, excessive Glu activation produces a neurotoxic effect on cortical and subcortical neurons, which results in cognitive impairments, memory loss, and progressive dementia. Antibodies to Glu (Glu-Ab) produced in response to long-term excessive Glu release in CNS may appear to be one a mechanism regulating Glu content. The existence of a tight relationship and mutual regulation between CNS and immune system is now beyond doubts. The immune system is known to respond by antibody production to all disturbances in CNS. The possibility of Glu-Ab production was previously demonstrated [1,4,5]. We recently demonstrated the production of Glu-Ab in AD [2,3] and revealed peculiarities of Glu-Ab production in patients with various forms of AD. Low level of autoantibodies to Glu in the serum was observed in patients with early disease onset, whereas in patients with late AD onset the serum level of Glu-Ab was high. A correlation between serum level of autoantibodies to Glu and impairment of cognitive functions in AD patients was established. One may assume that Glu-Ab are involved in the development of cognitive disturbances, particularly in memory disorders, but their role in these processes remains unclear.

Administration of a neurotoxic fragment of β-amyloid protein $A\beta_{25-35}$ into Meynert basal giant cell nuclei is an experimental model most close by the mechanisms of AD development and most adequately reproducing the symptoms of memory disorders in this pathology [6]. The development of disseminated degenerative changes in neurons in the frontal cortex and hippocampus manifesting in certain forms of cognitive disorders was experimentally proven [6,9]. The neurotoxic action of $A\beta_{25-35}$ is partially mediated via activation of the excitotoxic cascade induced by Glu [8]. This experimental model of AD provides the possibility of assessing the effects of Glu-Ab in cognitive

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deficit developing in this chronic neurodegenerative pathology.

The objective of this study was to investigate the effects of Glu-Ab on memory disorders, particularly on learning capacity of experimental animals, in rats after $A\beta_{25-35}$ administration into Meynert giant cell nuclei. The effects of intranasal administration of Glu-Ab were studied, since this method of passive immunization provides rapid delivery of antibodies to the brain [11].

MATERIALS AND METHODS

Experiments were performed on 74 male Wistar rats weighing 280-330 g and grown in a vivarium of Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences. The animals were kept under standard vivarium conditions with free access to food and water at 12-h light cycle. The experiments were carried out in accordance with principles of Directive of European Union Council 86/609/EEC on animal use in experiments.

The rats were divided into 5 groups: intact, sham-operated; $A\beta_{25-35}$+H_2O (rats with bilateral administration of neurotoxic fragment $A\beta_{25-35}$ and intranasal administration of 10 μl of distilled water 1 h after surgery); $A\beta_{25-35}$+Glu-Ab (intranasal administration of Glu-Ab water solution in a dose of 300 μg/kg according to the same scheme); $A\beta_{25-35}$+γ-globulin (animals receiving water solution of rabbit γ-globulin from intact animals in the same dose and by the same scheme).

Glu-Ab were obtained from rabbits routinely immunized with Glu-BSA conjugate synthesized by the modified method using glutaraldehyde. The titer of Glu-Ab measured by ELISA was 1:1000. γ-Globulin fractions from the serum of immunized and intact rabbits were extracted using by ammonium sulfate precipitation, lyophilized, and stored at 4°C.

Bilateral administration of $A\beta_{25-35}$ into Meynert basal giant cell nuclei (AP -1.4; OL 2.7; H 8.7) in a volume of 2 μl and dose of 2 μg for each side was carried out using a standard stereotaxic device for rats via a cannula connected to a Hamilton syringe [9]. The rats were anesthetized with chloral hydrate (300 mg/kg intraperitoneally). Sham-operated animals received with the same volume of physiological saline.

Passive avoidance (PA) conditioning was performed routinely on day 14 after $A\beta_{25-35}$ administration into Meynert nuclei [9]. At the time of training, the latency of transition into the dark compartment (L_1; manifestation of the hole reflex) was determined. Immediately after rat transition into the dark compartment, the door between the compartments was closed and electrocutaneous stimulus was delivered through the wire floor (0.8 mA, 3 sec), after which the rat was taken away and placed into the home cage. PA was tested after 24-h. To this end, the rat was placed into the light compartment with opened door and the time of transition into the dark compartment was measured (L_2). The test duration was 3 min. The memory about the electric shock was reflected in the difference between the latencies of transition into the dark compartment during PA conditioning and after 24 h (ΔL, sec).

Statistical processing of the results was performed using one-way non-paramentric Kurskal–Wallis ANOVA (H test) with subsequent post-hoc analysis using Mann–Whitney *U* test. The level of statistical significance for testing the null hypothesis was set at 0.05.

RESULTS

Significant intergroup differences were revealed in L_2 values: $H(4, n=74)=20.46253$, $p=0.0004$, and in ΔL values: $H(4, n=74)=23.56948$, $p=0.0001$. On day 14 after bilateral administration of $A\beta_{25-35}$ into the brain, substantial memory impairment was observed in rats, what manifested in a decrease in L_2 and ΔL values (Table 1) compared to control animals according to *U* test ($p<0.05$). After PA conditioning, the electric shock was remembered by 70% of rats in the control group *vs.* 25% in the experimental group, which attests to an amnestic effect of $A\beta_{25-35}$. In rats receiving Glu-Ab 1 h after $A\beta_{25-35}$ administration, the learning capacity during PA conditioning on day 14 did not differ from that in control animals and 77% rats remembered electric shock. Intranasal administration of Glu-Ab more than 4-fold reduced mnestic deficit induced by $A\beta_{25-35}$ administration into Meynert nuclei. Control intranasal administration of γ-globulin 1 h after $A\beta_{25-35}$ administration did not affect learning. These animals remembered electric shock in 28% of cases. L_2 and ΔL values in rats intranasally receiving γ-globulin did not differ from that in animals with experimental AD.

Thus, we confirmed the previously described impairment of PA performance after bilateral administration of the neurotoxic fragment $A\beta_{25-35}$ into Meynert giant cell nuclei [9]. We also showed that the amnestic effect of administration of $A\beta_{25-35}$ into Meynert nuclei can be prevented by intranasal administration of Glu-Ab 1 h after neurotoxic treatment. Intranasal administration of γ-globulin 1 h after $A\beta_{25-35}$ injection did not correct memory impairments. These findings can be regarded as an important experimental evidence of the protective effects of Glu-Ab during the development of cognitive disorders in neurodegenerative brain damage, *e.g.* in AD. This protective effects is probably determined by their capacity to reduce the effect of Glu hyperproduction and prevent neuronal death. Recently, the protective effects of systemic administration of

TABLE 1. Effects of Intranasal Administration of Glu-Ab and γ-Globulin on Memory Impairments Induced by $A\beta_{25-35}$ Administration into Meynert Basal Nuclei (*M*±*m*)

Group	Time of transition to dark compartment, sec		
	L_1	L_2	ΔL, sec
Intact rats	6.4±1.7	138.3±13.3*	131.9±12.9*
Sham-operated rats	4.4±1.5	148.8±19.3*	144.4±20.4*
$A\beta_{25-35}$	10.8±2.8	52.5±17.1	41.7±17.1
$A\beta_{25-35}$+Glu-Ab	5.2±1.9	145.8±18.9*	140.6±19.2*
$A\beta_{25-35}$+γ-globulin	16.5±6.2	67.6±35.7	51.1±35.4

Note. $p<0.05$ compared to $A\beta_{25-35}$ administration.

Glu-Ab were demonstrated on experimental models of epileptic and neurogenic pain syndrome characterized by enhanced Glu production in brain structures [4,5]. Experiments aimed at evaluation of the effects of intranasal administration of Glu-Ab on PA training in intact rats demonstrated the absence of Glu-Ab effects on mnestic functions. Thus, Glu-Ab administration to intact rat did not change ΔL in comparison with control animals (147.8±26.2 and 142.4±30.7 sec, respectively). Thus, Glu-Ab produce protective effects in mnestic impairments under conditions of intensified Glu production induced by neurodegenerative brain damage.

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