PHARMACOLOGY AND TOXICOLOGY

Effect of Antibodies against AMPA Glutamate Receptors on Brain Neurons in Primary Cultures of the Cerebellum and Hippocampus

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Rabbit antibodies against $GluR_1$ subunit of AMPA glutamate receptors in a concentration of 1 μg/ml significantly increased intracellular Ca^{2+} concentration and decreased mitochondrial potential in hippocampal neurons, *i.e.* produced changes typical of the influence of glutamate in toxic concentrations. In cerebellar neurons rabbit antibodies potentiated glutamate-induced increase in intracellular Ca^{2+} concentration and significantly decreased the mitochondrial potential (compared to the level observed after application of glutamate alone). The exposure of cultured cerebellar neurons to antibodies in a concentration of 0.1 μg/ml for 24 h was followed by a 50% decrease in ATP concentration and development of neuronal necrosis. Our results attest to an important role of autoimmune damage to neurons during hyperstimulation of glutamate receptors.

Key Words: *neurons; glutamate receptors; effect of antibodies*

Antibodies (AB) against autoantigens were found in patients with various neurological diseases, including multiple sclerosis, Guillain—Barre syndrome, multifocal motor neuropathy, Lambert—Eaton myasthenic syndrome, and Rasmussen encephalitis [8,14,15]. Structural intracellular and transmembrane proteins, neurotransmitter receptors, intracellular enzymes, and glycolipids can serve as targets for antigens during autoimmune diseases [8, 9,15]. Histological studies of the brain in patients with autoimmune diseases revealed various disturbances from local disappearance of the target antigen (without appreciable immune response) to mononuclear cell infiltration, deposition of Ig and complement, and tissue destruction by macrophages (multiple sclerosis and Guillain—Barre syndrome) [8,10,14,15].

In patients with Rasmussen encephalitis (epileptic disorder) AB against $GluR_3$ subunit of AMPA glutamate (Glu) receptors cause damage to astrocytes and, to a lesser extent, to neurons. Activation of the complement system is followed by stimulation of the membrane-attacking complex and lysis of nerve cells [15]. Specific immunosorption of anti-$GluR_3$ AB from the blood is used for the therapy of this disease [7]. Autoantibodies against the $GluR_1$ subunit of AMPA Glu receptors play an important role in the pathogenesis of epilepsies [1,2, 4,5,9]. The glutamate cascade of neuronal damage during hyperstimulation of Glu receptors involves the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and energy collapse in neurons [3,4,6,11,13]. The

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molecular mechanisms for neurodestructive activity of AB against $GluR_1$ are poorly understood.

Here we studied the effect of AB against the $GluR_1$ subunit of AMPA Glu receptors on $[Ca^{2+}]_i$, mitochondrial potential, ATP concentration, and cell survival in primary cultures of rat cerebellar and hippocampal neurons.

MATERIALS AND METHODS

The study was performed on a 7-day-old culture of cerebellar neurons and 14-day-old culture of hippocampal neurons from rats. The cell suspension for growth of neuron culture was prepared as described elsewhere [6]. Affinity-purified (in phosphate buffered saline) AB from rabbit serum were used. The rabbit was 4-fold immunized with $GluR_1$ over 63 days.

Cultured cerebellar and hippocampal neurons were loaded with fluorescent probes Fura-2/AM (5 μM, 40 min) and rhodamine 123 (Rh123, 3 μg/ml, 10 min) to measure $[Ca^{2+}]_i$ and mitochondrial potential, respectively. The culture medium was replaced with a buffer solution containing 130 mM NaCl, 5.6 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 20 mM HEPES, and 5.0 mM glucose (pH 7.4). Fluorescence was measured on an Axiovert 200 epifluorescence inverted microscope (Zeiss) equipped with a fluorescence objective (×20) and Lambda 10-2 system with a filter-changing mechanism (Shutter). The excitation wavelengths were 340, 380 (Fura-2), and 488 nm (Rh123). The emission wavelength was 505-530 nm. Emission was recorded using a CCD camera (Roper Science). Image processing was performed with Meta Fluor 6.1 software (Universal Imaging Corp.). $[Ca^{2+}]_i$ was estimated by the fluorescence ratio at 340 and 380 nm. Rh123 fluorescence normalized to the resting fluorescence reflected changes in the mitochondrial potential.

ATP concentration in supernatant aliquot was measured by the luminescent method using luciferin-luciferase (Calbiochem) in 0.1 M Tris-acetate buffer (pH 7.75). The measurements were performed after extraction with 2% trichloroacetic acid and 2 mM ethylenediaminetetraacetic acid, neutralization of extracts with 3 M KOH/1.5 M Tris, and centrifugation. ATP concentration was standardized by protein content (measured after Lowry). The data were expressed in percents of the control level. ATP concentration under control conditions was taken as 100%.

Death of cultured neurons was studied by counting neurons stained with fluorescent dye ethidium bromide (EtBr, 10 mg/ml) at excitation and emission wavelengths of 550 and 610 nm, respectively.

RESULTS

AB in a concentration of 1 μg/ml increased $[Ca^{2+}]_i$, but decreased the mitochondrial potential in hippocampal neurons (Fig. 1, *a*, *b*).

$[Ca^{2+}]_i$ and mitochondrial potential returned to the basal level after washout (*i.e.* anti-$GluR_1$ AB modulated $[Ca^{2+}]_i$ and energy metabolism only when they were present in the cell medium). We failed to find evidence that antibodies against the $GluR_1$ subunit affect cultured neurons of the cerebellum or hippocampus. Hence, we compared the results

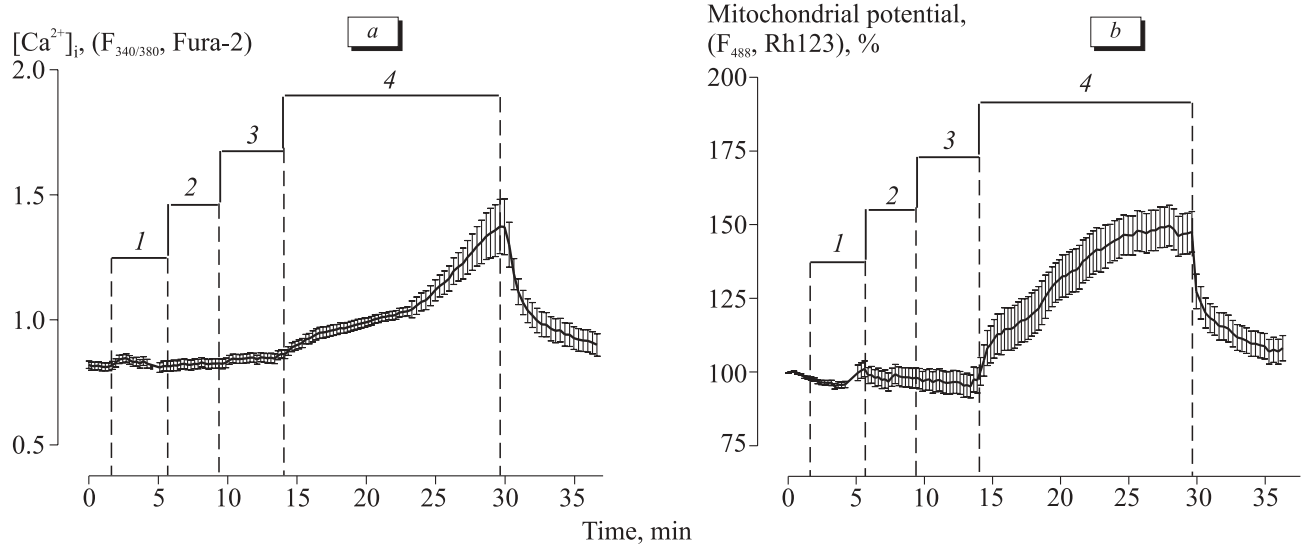

Fig. 1. Effects of AB against the $GluR_1$ subunit of AMPA Glu receptors on $[Ca^{2+}]_i$ and mitochondrial potential in rat hippocampal neurons. *a:* effect of AB in concentrations of 0.01 (*1*), 0.02 (*2*), 0.1 (*3*), and 1.0 μg/ml (*4*) on $[Ca^{2+}]_i$ (n=14). *b*: effect of AB in various concentrations on the mitochondrial potential (n=14). *n*, number of studied neurons.

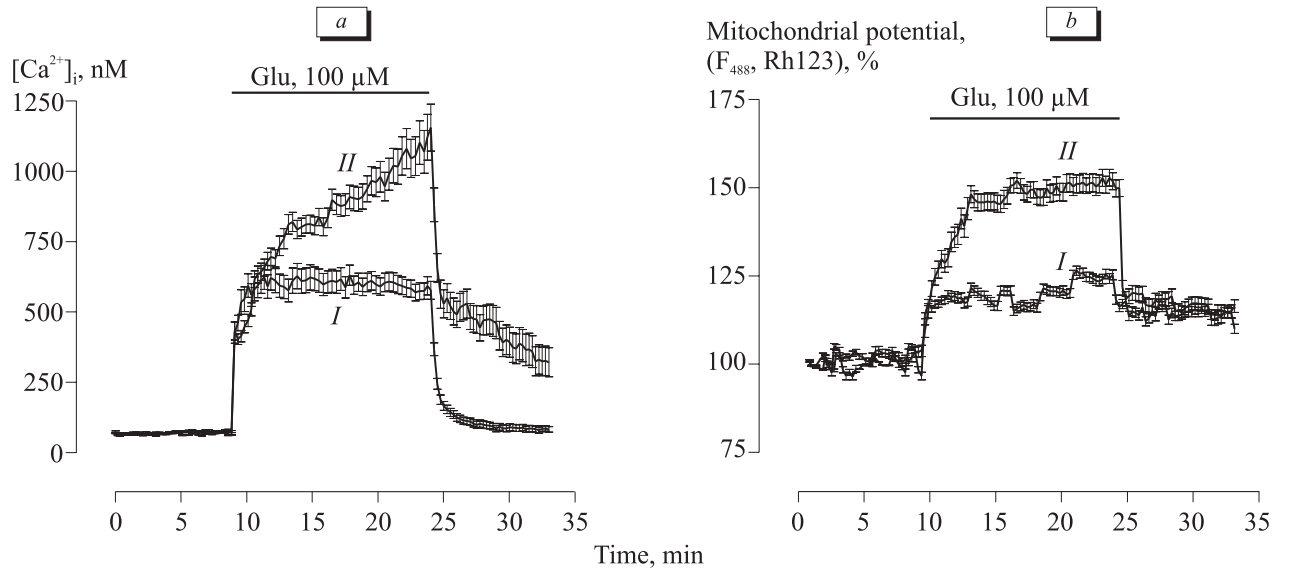

Fig. 2. Potentiation of the effect of toxic concentration of Glu on cerebellar neurons by AB against the $GluR_1$ subunit of AMPA Glu receptors. a: dynamics of $[Ca^{2+}]_i$ after treatment with 100 μM Glu alone (*I*) or in combination with 1 μg/ml anti-$GluR_1$ AB (*II*). *b*: mitochondrial potential after treatment with 100 μM Glu alone (*I*) or in combination with 1 μg/ml anti-$GluR_1$ AB (*II*). Each series was performed on 31-35 neurons.

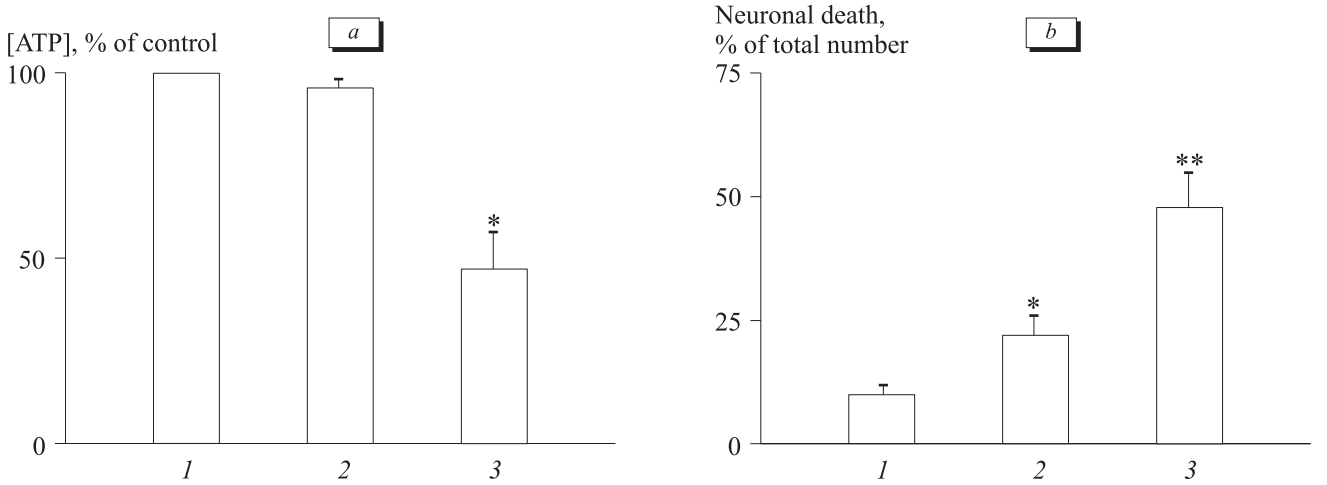

Fig. 3. Decrease in ATP concentration and necrosis of cerebellar neurons after treatment with AB against the $GluR_1$ subunit of AMPA Glu receptors. *a*: effect of AB (0.1 μg/ml) on ATP concentration. ATP concentration under control conditions (6.4±0.4 nmol/mg protein) is taken as 100%. Control (*1*); AB treatment for 1 h (*2*); AB treatment for 24 h (*3*). Each series was performed on 5 cultures. *p<0.01 compared to the control. *b*: neuronal death after 24-h treatment with AB. Control (*1*); 0.1 μg/ml AB (*2*); 1 μg/ml AB. Each series was performed on 5-6 cultures. *p<0.01 and **p<0.001 compared to the control.

of our study with published data on the influence of AB against the $GluR_3$ subunit of Glu receptors [15]. Our results are consistent with published data that AB against the terminal peptide region in the $GluR_3$ subunit of Glu receptors cause neuronal death by activating the receptor ion channel [12]. The mechanisms of brain injury with excess autoantibodies against the $GluR_3$ subunit were studied in patients with Rasmussen encephalitis. Greater severity of damage to astrocytes compared to neurons in hippocampal cultures was probably related to low expression of proteins in glial cells protecting them during activation of the complement system [15]. It should be emphasized that death of hippocampal astrocytes and neurons was observed under the influence of AB against the $GluR_3$ subunit in a concentration of 100 μg/ml [15]. In our study the concentration of AB against the $GluR_1$ subunit was lower by 100 and 1000 times.

Addition of anti-$GluR_1$ AB in a concentration of 1 μg/ml to cultured granular cells of the cerebellum had no effect on $[Ca^{2+}]_i$ in resting neurons, but addition of 100 μM Glu increased $[Ca^{2+}]_i$ by 2. 5 times (Fig. 2, *a*). $[Ca^{2+}]_i$ decreased after the re-

moval of AB and Glu from the medium. Under these conditions $[Ca^{2+}]_i$ returned to the basal level more slowly compared to treatment of with Glu alone.

Previous studies on young cerebellar neurons showed that administration of 100 μM Glu is followed by easily reversible depolarization of mitochondria [6]. In our experiments anti-$GluR_1$ AB significantly increased mitochondrial depolarization observed under the influence of Glu (Fig. 2, *b*). Similar changes in the mitochondrial potential were found after administration of Glu in combination with mitochondrial inhibitors and nitric oxide donor nitrosocysteine [6,3,13].

Treatment of cultured cerebellar neurons with AB (0.1 μg/ml) for 24 h was followed by a 50% decrease in intracellular ATP concentration and increase in the number of EtBr-positive neurons (necrotic death). These changes were most pronounced under the influence of AB in a concentration of 1 μg/ml (48±7%, Fig. 3, *a*, *b*). Necrotic damage promotes activation of autoimmune processes and is related to catalytic activity of AB [8].

Our results suggest that high level of autoantibodies against the $GluR_1$ subunit of AMPA Glu receptors in patients with epilepsy contributes to $[Ca^{2+}]_I$ increase, deenergization of mitochondria, and necrotic damage. These changes are probably associated with hyperstimulation of Glu receptors. Activation of autoimmune processes in patients with hyperstimulation of Glu receptors is followed by an increase in the concentration of AB against the $GluR_1$ subunit of AMPA Glu receptors in the blood and brain, which contributes to neuronal damage.

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