GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effect of Antibodies to Glutamate on the Content of Neurotransmitter Amino Acids in Brain Structures of Rats with Ischemic Damage to the Prefrontal Cortex

G. A. Romanova, Yu. N. Kvashennikova, F. M. Shakova, and T. V. Davydova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 1, pp. 17-20, January, 2012
Original article submitted March 24, 2011

Experiments on the model of bilateral photothrombosis in the prefrontal cortex showed that antibodies to glutamate administered intranasally 1 h after ischemic damage to the brain cortex led to a decrease in glutamate content in the hippocampus and prefrontal cortex and had no effect on aspartate concentration in these structures of the brain.

Key Words: *antibodies to glutamate; glutamate; photothrombosis; prefrontal cortex of the brain*

Impairment of glutamatergic neurotransmission in the brain is one of the key mechanisms of ischemic and traumatic injury to the brain. Glutamate is the major excitatory neurotransmitter in CNS and plays a role in various processes in the brain, including cognitive functions. Glutamate hyperactivation under conditions of ischemic brain injury produces a neurotoxic effect leading to death of cortical and subcortical neurons due to long-term calcium influx [7]. Previous studies demonstrated increased production of autoantibodies to glutamate (Glu-AB) in rats with bilateral ischemic injury to the prefrontal cortex by day 8 after surgery [3]. Moreover, intranasal administration of Glu-AB to rats 1 h after bilateral photochemical thrombosis of blood vessels in the prefrontal cortex promoted retention of conditioned passive avoidance response learned before injury [3]. The protective effect of Glu-AB in ischemic injury to the brain can be determined by their capacity to decrease glutamate overproduction.

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** romanovaga@mail.ru. G. A. Romanova

Here we studied the effect of Glu-AB on glutamate content in brain structures (prefrontal cortex, hippocampus, and hypothalamus) of rats with ischemic injury to the prefrontal cortex. These structures are related to the realization of cognitive functions.

MATERIALS AND METHODS

Experiments were performed on 30 male outbred rats weighing 200-220 g and obtained from the nursery of the Institute of General Pathology and Pathophysiology. The animals were maintained in a vivarium at the 12:12 h light-dark cycle and had free access to food and water.

Our study was conducted in accordance to the Directives of European Community Council (86/609/EEC) on the protection of animals used for experimental and other scientific purposes.

The animals were divided into 5 groups: group 1 (n=6) consisted of intact rats; group 2 (n=6) included sham-operated rats; group 3 (n=6) comprised animals with bilateral ischemic infarction of the prefrontal cortex and intranasal administration of 7 μl physio-

0007-4888/12/1531-0013

logical saline 1 h after surgery; group 4 rats (n=6) intranasally received aqueous solution of Glu-AB in a dose of 250 μg/kg (according to the same scheme); and group 5 rats (n=6) received aqueous solution of rabbit γ-globulin from intact animals (the same dose, similar scheme of treatment).

Glu-AB were obtained from rabbits immunized with glutamate-BSA conjugate by the standard scheme. The conjugate was synthesized by a modified method using bifunctional reagent glutaraldehyde [6]. The titer of Glu-AB was 1:1000 (determined by ELISA). γ-Globulin fractions were isolated from the serum of immunized and intact rabbits (over-precipitation with ammonium sulfate), purified from BSA (affinity chromatography), lyophilized, and stored at 4°C.

Bilateral focal ischemic infarction of the prefrontal cortex (areas Frl and Frl2) [5] was induced by the method of photochemical thrombosis [7]. The animals were intraperitoneally anesthetized with 300 mg/kg chloral hydrate. A photosensitizing dye Bengal rose (40 mg/kg; Sigma) was injected intravenously. The rat was fixed in a stereotaxis. The periosteum was separated after a longitudinal skin incision. Special device for irradiation was composed of a cold light source (halogen lamp, 250 W) and fiber-optic light guide (inner diameter 3 mm). The fiber-optic light guide was placed at a distance of 1 mm from the cranial surface (2 mm rostral to bregma, 2 mm lateral to the sagittal suture). The cranial surface was bilaterally irradiated with cold light for 15 min. Sham-operated rats were subjected to the same manipulations except for administration of Bengal rose.

The concentration of excitatory (aspartate and glutamate) and inhibitory neurotransmitter amino acids (GABA, glycine, and taurine) in brain structures (prefrontal cortex, hippocampus, and hypothalamus) on day 8 after surgery was measured by the standard method of HPLC with electrochemical detection [4]. Since native amino acids are weak chromophores (not absorb UV light) and do not exhibit electrochemical activity, their detection requires chemical pre-modification (derivatization). To this end, *o*-phthalic aldehyde (OPA) fluorescing upon binding to amino acid was used.

GABA, aspartate, glutamate, taurine, and glycine (0.1 μmol/ml in 0.1 N $HClO_4$) were used as a standard mixture for calibration. Incubation was performed at room temperature for 15 min. The solution (20 μl) was applied on an Agilent Hypersil ODS column (5 μM, 4.6×250). The separation products were detected on an Agilent 1100 fluorescence detector at the excitation and emission wavelengths of 230 and 392 nm, respectively. The mobile phase consisted of 0.05 M phosphate buffer (pH 5.6), 0.025 mM EDTA, and 5% acetonitrile. The mobile phase flow rate was 1.5 ml/min.

The mobile phase was filtered through a cellulose filter (pore diameter 0.02 μ) using a vacuum pump. This phase was thoroughly degassed under vacuum conditions before each chromatographic analysis.

The solution of aspartate, glutamate, glycine, taurine, and GABA in a concentration of 0.5 mmol/liter was used as a standard to measure the content of amino acids in rat brain structures.

The data were subjected to nonparametric analysis of variance (Kruskall-Wallis test) and intragroup comparison (Mann-Whitney U test) using Statistica 6.0 software.

RESULTS

Differences between the groups were found in the concentration of excitatory neurotransmitter glutamate in the prefrontal cortex [H (4,N=30)=9.33333, p<0.0533]. On day 8 after surgery, the content of glutamate in the prefrontal cortex of rats with ischemic injury to the cortex intranasally receiving Glu-AB was much lower than in animals of the physiological saline group with photochemical thrombosis of vessels in the prefrontal cortex [Z=2.081666, p=0.037374].

Significant differences between the groups were revealed in the content of the following neurotransmitter amino acids in the hippocampus: glutamate [H (4,N=30)=10.6667, p=0.0306], aspartate [H (4,N=30)=19.28571, p=0.0007], glycine [H (4,N=30)=12.0000, p=0.0174], and GABA [H (4,N=30)=17.33333, p=0.0173]. No differences in the concentration of taurine were found [H (4,N=30)=5.33333, p=0.2548].

Glutamate content in the hippocampus of rats with ischemic injury to the prefrontal cortex increased on day 8 after surgery [Z=-2.72218, p=0.006486 compared to the control] (Table 1). On day 8 of the study, hippocampal glutamate concentration in rats with ischemic injury to the prefrontal cortex intranasally receiving Glu-AB 1 h after surgery did not differ from that in the control (intact animals; Z=-2.40192, p=0.16310). On day 8 of the study, hippocampal glutamate concentration in rats receiving γ-globulin from intact rabbits 1 h after ischemic injury was higher than in intact animals [Z=-2.72218, p=0.006486] and did not differ from that in animals with photochemical thrombosis of vessels in the prefrontal cortex.

Aspartate content in the hippocampus of rats with ischemic injury was shown to increase on day 8 after photothrombosis of the prefrontal cortex [Z=-2.64211, p=0.008239] compared to intact animals. Hippocampal aspartate concentration in rats with ischemic injury to the prefrontal cortex was also elevated after intranasal administration of Glu-AB [Z=-2.64211, p=0.008239] and γ-globulin from intact rabbits [Z=-2.88231, p=0.003948].

Elevated contents of inhibitory neurotransmitter amino acids GABA and glycine were found in the

TABLE 1. Effect of Glu-AB on the Content of Neurotransmitter Amino Acids in Brain Structures of Rats with Ischemic Injury to the Frontal Cortex (mmol/g)

Group	Aspartate	Glutamate	Glycine	Taurine	GABA
Frontal cortex					
Intact	0.0048±0.0009	3.1938±0.5131	0.1922±0.0255	22.5580±2.4889	0.1322±0.0190
Sham-operated	0.0053±0.0009	3.2117±0.5208	0.1913±0.0227	21.9015±1.7791	0.1337±0.0214
Ischemic injury	0.0068±0.0003	4.0813±0.1841*	0.2198±0.0112	26.8868±0.6487	0.1648±0.0129
Ischemic injury+Glu-AB	0.0060±0.0004	3.3450±0.17413+	0.2041±0.0153	27.4670±1.2530	0.1525±0.0085
Ischemic injury+ γ-globulin	0.0066±0.0002	4.0380±0.1310	0.2228±0.0126	27.0793±0.7389	0.1738±0.0137
Hippocampus					
Intact	0.0157±0.0019	15.0290±1.5296	0.4695±0.0615	87.5185±5.9914	0.2500±0.2459
Sham-operated	0.0160±0.0019	14.6315±1.2592	0.4728±0.0570	88.2060±5.9856	0.2538±0.2508
Ischemic injury	0.0273±0.0028*	23.4300±1.2600*	0.7160±0.0374*	107.1953±8.7861	0.3690±0.0102*
Ischemic injury+Glu-AB	0.0240±0.0014*	17.8553±2.3608	0.6858±0.0405*	113.8033±5.7345	0.3513±0.0260*
Ischemic injury+ γ-globulin	0.0288±0.0015*	23.2538±1.1302*	0.7100±0.0308*	111.7233±5.1905	0.3693±0.080*
Hypothalamus					
Intact	0.0098±0.0016	7.5582±0.9683	0.2326±0.0527	74.3760±21.9141	0.1028±0.0173
Sham-operated	0.0102±0.0017	7.5000±0.6261	0.2323±0.0531	64.7236±9.1521	0.1040±0.0176
Ischemic injury	0.0112±0.0009	7.7090±0.7865	0.2655±0.0243	48.8738±5.1641	0.1047±0.0087
Ischemic injury+Glu-AB	0.0117±0.0013	6.6513±1.3049	0.2550±0.0275	52.9035±2.4339	0.1125±0.0153
Ischemic injury+ γ-globulin	0.0233±0.0113	8.6043±0.3610	0.2676±0.0163	48.2195±4.9131	0.1032±0.0085

Note. $p<0.05$: *in comparison with intact controls; +in comparison with ischemic injury.

hippocampus of rats with ischemic injury to the prefrontal cortex, as well as in animals with ischemia and intranasal administration of Glu-AB or γ-globulin from intact rabbits (Table 1). Our results are consistent with published data that the amount of inhibitory amino acids in brain structures increases during focal ischemia of the cerebral cortex [2].

We found no differences between the groups in the content of excitatory (aspartate and glutamate) and inhibitory neurotransmitter amino acids (GABA, glycine, and taurine) in the hypothalamus.

We conclude that Glu-AB have a normalizing effect on the content of excitatory neurotransmitter amino acid glutamate in animals with focal ischemia of the prefrontal cortex. Previous studies showed that cerebral ischemia is accompanied by an increase in the concentration of excitatory amino acids aspartate and glutamate [2]. Intranasal administration of Glu-AB 1 h after surgery was followed a decrease in glutamate content in the prefrontal cortex and hippocampus, but had no effect on aspartate content in these structures on day 8 after photothrombosis, which attests to a specific effect of Glu-AB on enhanced production of glutamate. Our results explain the protective effect of Glu-AB during acute neurodegenerative damage to cognitive functions of the brain [3].

REFERENCES

1. E. I. Gusev and V. I. Skvortsova, *Cerebral Ischemia* [in Russian], Moscow (2001).
2. G. A. Romanova, *Dysregulation Pathology*, Ed. G. N. Kryzhanovskii [in Russian], Moscow (2002), pp. 605-615.
3. G. A. Romanova, F. M. Shakova, V. Yu. Gorbatov, *et al*., *Byull. Eksp. Biol. Med*., **149**, No. 3, 261-264 (2010).
4. W. R. Pearson, *Genomics*, **11**, No. 3, 635-650 (1991).
5. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates. 3rd Ed.*, San Diego (1997).
6. P. Seguela, M. Geffard, R. Buijs, and M. Le Moal, *Proc. Natl. Acad. Sci. USA*, **81**, No. 12, 3888-3892 (1984).
7. B. D. Watson, W. D. Dietrich, R. Busto, *et al*., *Ann. Neurol*., **17**, No. 5, 497-504 (1985).