Effects of Afobazole on the Content of Neurotransmitter Amino Acids in the Striatum in Global Transient Ischemia

V. S. Baykova, I. A. Kadnikov, M. V. Voronin, T. S. Ganshina, A. V. Gnezdilova, A. A. Gorbunov, P. C. Mirzoyan, and S. B. Seredenin

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Anxiolytic agent afobazole (10 mg/kg intraperitoneally) 24 h after ischemia restores impaired balance of excitatory and inhibitory amino acids in the striatum of mongrel rats, normalizes their content to control levels, and activates endogenous taurine-dependent system of neuroprotection.

Key Words: *afobazole; amino acids; global ischemia; striatum*

Hypoxic and ischemic brain lesions are associated with dramatic increase in glutamate release and hyperactivation of glutamate receptors during the first 3-6 h, which results in the development of neuronal excitotoxicity. Inhibitory neurotransmitter amino acids GABA, glycine, and taurine counteract with excitatory neurotransmitters and compensate excitotoxic neuronal damage providing endogenous neuroprotection. Therefore, information concerning the balance of excitatory and inhibitory amino acids in brain structures during ischemia is important characteristic of pathological process intensity. It was previously established that afobazole (5-ethoxy-2-[2-(morpholino)-ethylthio] benzimidazole hydrochloride) apart from its anxiolytic effects possesses neuroprotective properties [2]. Published reports mainly contains information about the effects of neuroprotective agents on neurotransmitter amino acid content during the first hours of ischemia [14].

The objective of this study was to investigate delayed effects of afobazole on the levels of neurotransmitter amino acids in brain structures of mongrel rats under conditions of global ischemia modeled by occlusion of the common carotid artery with simultaneous BP lowering.

V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** veronica_xc@mail.ru. V. S. Baykova

MATERIALS AND METHODS

Afobazole was synthetized at V. V. Zakusov Institute of Pharmacology, Russian Academy of Medical Sciences.

The experiments were carried out on albino mongrel male rats weighing 260-300 g, 8-10 animals in each experimental group. Twelve hours before the experiments, the animals were deprived of food but allowed free access to water. Experiments were carried out in the fall during the first half of the day.

Global transient ischemia in anesthetized (chloral hydrate 325 mg/kg intraperitoneally) rats with natural breathing was induced by 10 min occlusion of both common carotid arteries with simultaneous BP lowering down to 40 mm Hg by bleeding with subsequent reinfusion.

Bleeding was performed using polyethylene cannula inserted into the femoral vein. Blood in a volume of 4-6 ml was taken with a syringe and reinfused after ischemia [12]. Afobazole was administered intraperitoneally 10 mg/kg 30 min after the reperfusion.

The rats were decapitated 24 h after reperfusion, the brains were taken out, and brain stem and cerebellum were separated. Hypothalamus, frontal cortex, striatum, hippocampus, and adjacent nuclei were separated at a temperature of melting ice (0-4°C) and immediately frozen. The material was stored in cryotubes at -70°C until chromatographic investigation.

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Separated rat brain structures were crushed in glass–glass homogenizer No. 7725 (Pyrex) in 0.1 M $HClO_4$ containing 1 nM homoserine as an internal standard. The samples were centrifuged at 6000*g* for 10 min. The supernatant (20 μl) of homogenized structures was derivatized for 21 min in the presence of orthophthalic aldehyde 10 μl and 40 μl derivate was used for separation at analytical column BDS C18 μm 250×4.6 mm (Agilent Technologies). Amino acids were separated using 2 mM phosphate buffer with 3% acetonitrile (pH 5.6) as a mobile phase. Identification of amino acids was carried out on chromatographic station LC 1100 (Agilent Technologies) using glass-carbon electrode (potential+0.85 V *vs*. Ag/AgCl reference electrode) and electrochemical detector DE-104. Analog signal entered an analog-digital converter (Ampersand) and was processed on a computer using Multichrom 1.5 software (Ampersand).

Significance of obtained results was assessed using one-way ANOVA (post-hoc: Fisher LSD-test).

RESULTS

The study included measurements of the levels of neurotransmitter amino acids in the brain of rats from the following groups: intact, sham-operated, ischemic, and ischemic with subsequent afobazole administration 10 mg/kg intraperitoneally 40 min after ischemia. Five brain structures were investigated: hypothalamus, frontal cortex, striatum, hippocampus, and adjacent nuclei. Among chosen structures the most prominent changes were documented in the striatum.

Significant increase in glutamate level was detected in the striatum of ischemic mice in comparison to intact animals. Afobazole (10 mg/kg) reduced glutamate level in comparison with ischemic animals: this parameter approached the values detected in intact rats (Table 1, Fig. 1).

Aspartate content in ischemic rats decreased compared to the control group. Afobazole improved aspartate level in comparison to ischemic rats, but this parameter did not significantly differ from that in intact and sham-operated animals.

GABA level was significantly decreased in the striatum of ischemic rats. After afobazole administration, GABA level improved and reached the control values recorded in intact animals.

Rats under the conditions of global ischemia were characterized by insignificant increase in taurine level (Table 1, Fig. 1). In ischemic animals, increase in taurine level appeared to be significant after afobazole administration compared to that in intact and sham-operated animals.

Glycine level significantly increased in ischemic animals in comparison with sham-operated rats after afobazole administration, but this parameter did not significantly differ from that in intact animals (Table 1).

The striatum is known to be among the structures most sensitive to ischemic lesions [9], where changes induced by global ischemia with subsequent reperfusion can be found over a protracted period after the experiment [5]. Our study established significant increase in glutamate level 24 h after global ischemia with subsequent reperfusion, which indicates potential damage to neurons due to excitotoxicity. After administration of 10 mg/kg afobazole to rats 40 min after global ischemia, glutamate level in the striatum significantly decreased, which should be regarded as a sign of nervous system recovery after ischemic lesion.

Radioligand assay of receptor targets carried out previously showed afobazole affinity for σ_1-receptors, most probably with agonistic activity [3]. It is known that σ_1-receptor agonists administered during perfusion significantly lowered concentration of extracellular glutamate, which correlates with reduction of nervous tissue damage area [4,11]. Thus, the obtained data are in line with the results of studies demonstrating the possibility of realization of the neuroprotective action of σ_1-receptor agonists via reduction of glutamate concentration.

Reduction of GABA level in the striatum in the group of ischemic animals 24 h after the start of the experiment, as is shown in a number of studies on various experimental models, may indicate exhaustion of GABA-ergic mechanisms and decrease in neuroprotective potential of neurons [15].

TABLE 1. Amino Acid Levels in Brain Structures (*M*±*SEM*; nmol/mg tissue)

Group	Aspartate	Glutamate	Glycine	Taurine	GABA
Intact	8.87±0.80	49.14±7.57	21.78±3.90	100.90±29.56	53.04±6.15
Sham-operated	9.43±1.11	50.91±3.00	16.04±2.47	93.82±24.10	42.55±4.47
Ischemia	6.15±0.54*	81.48±14.92^{+*}	25.14±2.78	150.34±44.44	35.70±3.33*
Ischemia+afobazole	9.06±0.53	68.05±10.19	26.91±4.37^{+}	214.57±37.77^{+*}	47.24±7.09

Note. $p<0.05$ in comparison *with intact animals (ANOVA; Fisher LSD-test), $^{+}$with sham-operated animals (ANOVA; Fisher LSD-test).

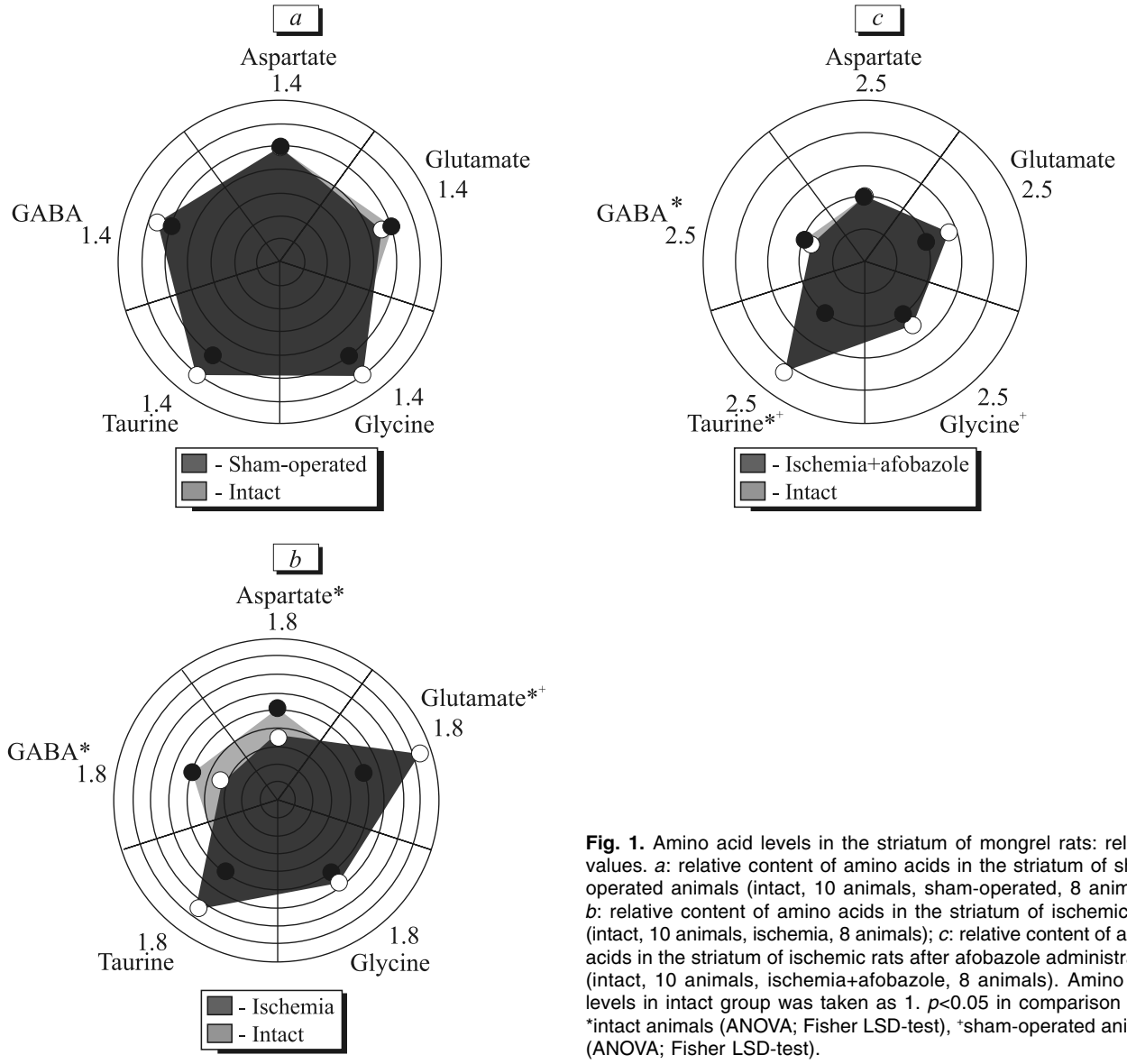

Fig. 1. Amino acid levels in the striatum of mongrel rats: relative values. *a*: relative content of amino acids in the striatum of sham-operated animals (intact, 10 animals, sham-operated, 8 animals); *b*: relative content of amino acids in the striatum of ischemic rats (intact, 10 animals, ischemia, 8 animals); *c*: relative content of amino acids in the striatum of ischemic rats after afobazole administration (intact, 10 animals, ischemia+afobazole, 8 animals). Amino acid levels in intact group was taken as 1. $p<0.05$ in comparison with: *intact animals (ANOVA; Fisher LSD-test), +sham-operated animals (ANOVA; Fisher LSD-test).

Afobazole administration resulted in restitution of striatal GABA content, which approached the control values; this should promote recovery of neuroprotective systems related to inhibitory influences [8].

It is currently established that apart from GABA system, taurine is also involved in implementation of endogenous neuroprotective mechanisms. Special attention is paid to this amino acid in discussion of the issue of neuron damage during ischemia [10]. Thus, taurine was established to play a crucial role in tissues requiring intensive oxygen supply. Taurine possesses antioxidant properties, on the one hand, and inhibits the development of ischemia-induced glutamate-mediated toxicity and prevents subsequent metabolic disturbances on the other [6,7]. We revealed significant increase in striatal taurine level in ischemic animals, which can be regarded as an element of compensatory mechanism development. This statement is supported by the results of Finnish researchers demonstrating increased taurine levels in brain structures in ischemia [10]. Afobazole administration to ischemic animals resulted in more substantial increase in taurine level in the striatum. Agonistic interaction between afobazole and σ_1-receptors, possibly underlies this phenomenon that may indirectly lead to protein kinase C activation with subsequent activation of taurine synthesis [13]. Thus, the increase in taurine level alongside with GABA can be regarded as components of recovery therapy of brain ischemia. Our results are in line with previously obtained data on neuroprotestive effects of afobazole on models of ischemic and hemorrhagic stroke [1,2].

Our findings led us to a conclusion that afobazole administered in a dose of 10 mg/kg to the animals 40

min after modeling of global ischemia restored the impaired balance of excitatory and inhibitory amino acids in the striatum, normalized their content to control values, and activates endogenous taurine-dependent neuroprotection system within 24 h.

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