GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Balance of Neurotransmitter Amino Acids and Integrative Activity of the Brain After Local Ischemic Damage to the Frontal Cortex in Rats: Effects of Glycine and Piracetam

K. S. Raevskii, G. A. Romanova,* V. S. Kudrin, and L. A. Malikova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 4, pp. 370-373, April, 1997
Original article submitted January 22, 1996

Bilateral local compression ischemia in the frontal rat brain cortex modifies the levels of neurotransmitter amino acids at the stage of most pronounced disorders in the brain integrative activity. In this model, neuroprotective properties of the drugs piracetam and glycine are evaluated. Glycine accelerates GABA turnover at the sites of ischemia as well as in the parietal cortex and hippocampus and, similar to piracetam, restores almost completely the conditioned response of passive avoidance impaired by ischemia. Glycine also reduces locomotor activity of both ischemic and sham-operated rats. It is suggested that normalizing effect of glycine on the functional balance of neurotransmitter amino acids immediately after ischemic damage to the cortex promotes restitution of disturbed functions of the central nervous system.

Key Words: *neurotransmitter amino acids; piracetam; glycine; focal ischemia of frontal cortex; conditioned passive avoidance response; locomotor activity*

Recent studies have shown that disturbances of the neurotransmitter function performed by excitatory amino acids such as glutamic and aspartic acid play an important role in the pathogenesis of ischemic injuries to the brain [3,12]. The overproduction of glutamate that occurs under ischemic conditions (as demonstrated by its direct measurements in extracellular spaces of the brain [9]) results in activation of glutamate receptors, excessive rise of glutamate concentration, and intensification of several metabolic processes, including the metabolism of transmitter amino acids and biogenic amines [5,6]. This cascade of neurochemical reactions developing in response to ischemic damage to the brain culminates in neuronal death at the site of ischemia and, consequently, in cerebral infarction. Previously, it was found that local compression of cerebral brain cortex causing a transient ischemia leads to formation of limited necrotic focus (infarction) with perifocal area containing both necrotic and intact neurons [1,4]. It was also shown that bilateral ischemic damage to the frontal cortex significantly impaires both the integrative activity of the brain, which manifestes itself as altered horizontal motor activity in an open field and impaired memory function, as indicated by a shortened latency of the conditioned passive avoidance response (CPAR) [4].

Clinical trials showed that the amino acid glycine produces a neuroprotective (therapeutic) effect in patients with an acute hemispheric ischemic stroke.

Institute of Pharmacology, Russian Academy of Medical Sciences; *Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

0007-4888/97/0004-0319$18.00

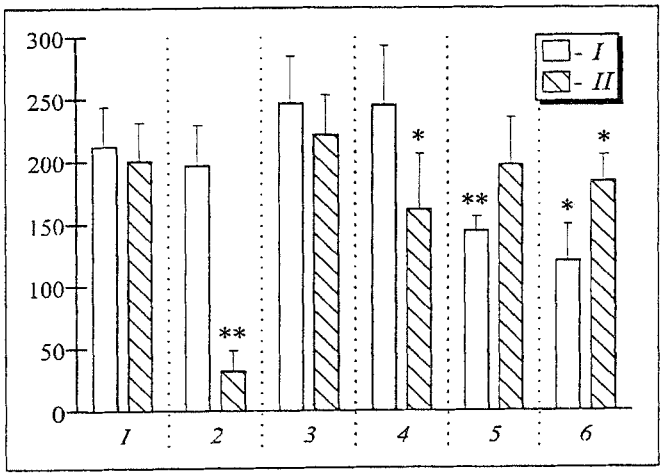

Fig. 1. Horizontal motor activity in the open-field (*I*) and latency of the conditioned passive avoidance response (*II*) in rats on day 9 after bilateral compression ischemia of the frontal cortex. *1*) Sham-operated rats given 0.9% NaCl (10); 2) ischemic rats given 0.9% NaCl (14); *3*) sham-operated rats given piracetam (10); *4*) ischemic rats given piracetam (8); *5*) sham-operated rats given glycine (8); *6*) ischemic rats given glycine (8). Ordinate: number of squares crossed for 300 sec (for *I*) and response latency, sec (for *II*). $p<0.05$: *in comparison with group 2; **in comparison with group 1. The number of rats is given in parentheses.

Moreover, as shown by measurements of the transmitter amino acids in patients with severe stroke, glutamate and aspartate predominate in the cerebrospinal fluid immediately after the stroke onset, while the inhibitory neurotransmitter glycine appears 9-10 h later, which probably reflects activation of protective inhibitory mechanisms in the brain.

Using a rat model of bilateral local compression ischemia of the frontal cortex, we attempted to evaluate the role of transmitter amino acids in the mechanisms by which integrative activity of the brain is impaired after ischemic injury and to check up the hypothesis that the nootropic drug piracetam and the inhibitory transmitter amino acid glycine act as neuroprotectors.

MATERIALS AND METHODS

A total of 58 random-bred male rats weighing 200-250 g were used. Preoperatively, their motor and exploratory activities were evaluated in an open-field test using a Rodeo-1 automatic apparatus, after which CPAR was elaborated, and the latency of the movement from the light to the dark compartment of the box was determined.

Symmetrical bilateral compression ischemia in the frontal brain cortex was produced as previously [1,4]. On day 9 after the operation, motor activity and the ability to exhibit the learned response were evaluated. Sham-operated rats in which bilateral trepanation of the skull was performed without damaging the cerebral cortex served as controls. Piracetam or glycine were administered orally in a daily dose of 500 mg/kg 9 days, the first dose being given 2 h after surgery.

All rats were divided into six groups: sham-operated and ischemic rats given physiological saline (groups 1 and 2, respectively), sham-operated and ischemic rats given piracetam (groups 3 and 4), and sham operated and ischemic rats given glycine (groups 5 and 6). On postoperative day 9, when functional changes in brain integrative activity were most pronounced (as indicated by repeated tests for motor activity and CPAR), rats were decapitated, their brains were removed, and the following structures were isolated [7]: frontal cortex (at the sites of ischemia), parietal cortex, striatum, and hippocampus. The levels of neurotransmitter amino acids (aspartate, glutamate, glycine, taurine, and γ-aminobutyric acid, GABA) were then measured in these structures by high performance liquid chromatography with electrochemical detection as described [11]. The data were statistically analyzed using Student's *t* test.

RESULTS

Ischemic rats exhibited slightly reduced motor activity and abnormal CPARs on day 9 after surgery (Fig. 1, *1* and *2*). Thus, horizontal activity in the open-field test decreased by 7% ($p>0.05$) and the CPAR latency by 84% ($p<0.05$), indicating that the memory trace was markedly impaired.

Ischemic rats treated for 9 days with the nootropic drug piracetam (group 4) or the transmitter amino acid glycine (group 6) showed CPAR latencies close to those in the respective control groups (73% and 93% of the control values, respectively) (Fig. 1, *4* and *6*). It should be noted that in ischemic rats glycine caused a significant reduction in locomotor activity (by 39%), while piracetam slightly increased it (by 24%, $p>0.05$), compared with the control. Glycine also reduced motor activity in sham-operated rats (by 32%, $p<0.05$) without altering the CPAR latency.

The contents of transmitter amino acids in brain structures (ischemic areas of the frontal cortex, parietal cortex, striatum, and hippocampus) on day 9 after surgery are given in Table 1. In group 2 (ischemic rats given NaCl) in comparison with group 1 (sham-operated controls given NaCl), the glutamate, taurine, and GABA contents were not significantly altered in the ischemic area, while the aspartate and taurine contents were significantly elevated in the parietal cortex (by 40% and 50%, respectively). Glutamate and taurine were significantly lowered in the hippocampus (by 21% and 41%, respectively), and only

TABLE 1. Levels of Neurotransmitter Amino Acids in Brain Structures of Rats on Day 9 After Local Ischemia of the Frontal Cortex (*M±m*)

Group	Amino acid levels, μmol/g tissue					Glutamate/ GABA
	aspartate	glutamate	glycine	taurine	GABA	
Frontal cortex (ischemic area)						
Sham operation+0.9% NaCl (10)	2.4±0.1	7.04±0.4	0.8±0.01	7.0±0.8	1.5±0.1	5.0±0.2
Ischemia+0.9% NaCl (14)	2.6±0.3	6.5±0.6	1.2±0.2	6.0±0.8	1.4±0.2	5.0±0.3
Sham operation+glycine (8)	2.8±0.3	7.2±0.8	1.3±0.2	6.1±0.7	1.3±0.1	5.8±0.4
Ischemia+glycine (8)	2.3±0.2	6.0±0.6	1.3±0.2	5.4±0.5	0.8±0.1*	7.7±1.0
Parietal cortex						
Sham operation+0.9% NaCl (10)	2.5±0.3	7.0±0.9	0.7±0.2	5.9±0.5	1.5±0.3	5.4±0.7
Ischemia+0.9% NaCl (14)	3.5±0.5**	8.6±0.6	1.1±0.1	8.9±0.7**	2.1±0.2	4.1±0.1
Sham operation+glycine (8)	3.7±0.2	9.4±0.4	1.2±0.1	9.3±0.5	1.8±0.1	5.4±0.3
Ischemia+glycine (8)	3.6±0.3	9.0±0.7	1.6±0.3*	8.5±0.7	1.7±0.1	5.3±0.2
Striatum						
Sham operation+0.9% NaCl (10)	1.6±0.2	4.84±0.6	0.7±0.1	8.0±0.4	1.9±0.1	2.8±0.1
Ischemia+0.9% NaCl (14)	1.6±0.1	4.6±0.2	0.8±0.1	6.8±0.4**	2.1±0.2	2.3±0.2
Sham operation+glycine (8)	2.4±0.2	6.2±0.4	0.9±0.1	8.9±0.7	2.5±0.3	2.6±0.2
Ischemia+glycine (8)	1.9±0.2*	6.7±0.4*	1.3±0.2*	9.7±0.7*	3.0±0.2*	2.3±0.2
Hippocampus						
Sham operation+0.9% NaCl (10)	1.6±0.2	5.6±0.5	0.8±0.1	9.3±1.4	1.8±0.3	3.3±0.2
Ischemia+0.9% NaCl (14)	1.4±0.1	4.4±0.2**	0.6±0.1	5.5±0.4**	1.6±0.02	3.0±0.3
Sham operation+glycine (8)	1.3±0.1	4.4±0.4	0.9±0.1	6.3±0.8	1.4±0.1	3.3±0.1
Ischemia+glycine (8)	1.6±0.1	5.5±0.2*	0.6±0.05	4.9±0.3	1.4±0.1	4.0±0.3

Note. $p<0.05$: *in comparison with the "ischemia+0.9% NaCl" group; **in comparison with the "sham operation+0.9% NaCl" group. The number of rats is given in parentheses.

taurine was significantly lowered in the striatum (by 15%). In group 6 (glycine-treated ischemic rats) in comparison with group 2 (ischemic rats given NaCl), GABA was significantly lowered in the frontal cortex (by 43%), glycine was significantly elevated in the parietal cortex (by 45%), and all five amino acids were significantly elevated in the striatum (aspartate by 30%, glutamate by 46%, glycine by 63%, taurine by 43%, and GABA by 50%). Of special note is the considerable increase in the glycine-treated ischemic rats of GABA turnover rates (as assessed by the glutamate/GABA ratio) in the frontal cortex (7.7 *vs.* 5.0 in the NaCl-treated ischemic rats), parietal cortex (5.3 *vs.* 4.1), and hippocampus (4.0 *vs.* 3.0) (Table 1).

The degree of functional changes in the integrative activity of the brain caused by its ischemic damage reflects to a large extent the imbalance between excitatory and inhibitory events [10]. Glycine increased GABA turnover rates at the sites of ischemia (frontal cortex), parietal cortex, and hippocampus, which are brain structures involved in preserving memory traces [2,8,13].

Accelerated turnover of GABA probably indicates enhancement of inhibitory function of this neurotransmitter. Another important result from the therapeutic use of glycine during the recovery period after ischemic damage was elevation of all major amino acids in the striatum. These findings support the concept that the beneficial effect of the inhibitory transmitter glycine on the CPAR elaborated prior to ischemic damage to the frontal cortex is due primarily to normalization of the relation between excitatory and inhibitory processes in the cortex and hippocampus. This concept is consistent with the reduced locomotor activity shown by glycine-treated rats in the open field test on day 9 after surgery.

Considering changes in the levels of excitatory and inhibitory amino acids together with those in brain integrative activity induced by local compression ischemia of the frontal cortex, we have concluded that pharmacological agents capable of normalizing the balance of neurotransmitter amino acids immediately after ischemic damage to the cortex can promote restoration of impaired functions of the central nervous system. Piracetam and glycine are pharmacological agents effective in this respect.

REFERENCES

1. I. V. Barskov and I. V. Viktorov, *Pat. Fiziol.*, No. 2, 57-59 (1994).

2. R. I. Kruglikov, *Neurochemical Mechanisms of Learning and Memory* [in Russian], Moscow (1981).
3. K. S. Raevskii, *Pat. Fiziol.*, No. 1, 3-9 (1990).
4. G. A. Romanova, I. V. Barskov, A. N. Sovetov, and I. V. Viktorov, *Byull. Eksp. Biol. Med.*, **118**, No. 12, 568-571 (1994).
5. S. P. Butcher, R. Bullock, D. I. Graham, *et al.*, *Stroke*, **21**, No. 12, 1727-1733 (1990).
6. M. J. Globus, R. Busto, W. D. Dietrich, *et al.*, *J. Neurochem.*, **51**, 1455-1464 (1988).
7. J. Glowinski and L. L. Iversen, *J. Neurochem.*, **13**, 655 (1966).
8. B. Kolb, *Brain Res. Rev.*, No. 8, 65-98 (1984).
9. H. Manev, M. Favaron, S. Vicini, *et al.*, *J. Pharmacol. Exp. Ther.*, **252**, 419-427 (1990).
10. Y.-T. Mordecai, M. J. Globus, R. Busto, E. Martinez, *et al.*, *J. Neurochem.*, **57**, No. 2, 470-478 (1991).
11. S. J. Pearson, C. Czudek, K. Merar, and G. P. Reynolds, *J. Neural. Transm.*, **86**, 151-157 (1991).
12. W. Pulsinelli, *Lancet*, **339**, 533-536 (1992).
13. R. J. Sutherland, B. Kolb, and I. Q. Whishaw, *Neurosci. Lett.*, **31**, 271-276 (1982).

Use of Phosphatidylcholine Liposomes for Correction of Mitochondrial Phospholipid Composition in the Medulla Oblongata and Frontal Lobes in Hemorrhagic Shock

G. F. Leskova and V. I. Udovichenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 4, pp. 374-377, April, 1997
Original article submitted January 29, 1996

Phosphatidylcholine liposomes normalize phosphatidylcholine and lysophospholipid levels in mitochondrial membranes of the medulla oblongata of cats with hemorrhagic shock. In the frontal lobes of brain hemispheres of liposome-treated cats, phospholipid levels in the mitochondria and their membranes are close to the norm, with the exception of phosphatidylserine and lysophosphatidylserine: their contents in the inner mitochondrial membranes remain below the control. It is concluded that phosphatidylcholine liposomes exert protect brain mitochondrial membranes in hemorrhagic shock.

Key Words: *phospholipids; mitochondria; brain; hemorrhagic shock; liposomes*

Hemorrhagic shock involving long-lasting pronounced hypotension is accompanied by impaired cerebral circulation and brain hypoperfusion and hypoxia. Of particular significance in such situations is the damage sustained by mitochondrial membranes that are the principal site of energy generation. Impaired metabolism of membrane phospholipids is associated with altered energy generation in the mitochondria [1,13]. It was shown [4,5] that phosphatidylcholine (PCh) liposomes, which inhibit lipid peroxidation [2], exert an appreciable membrane-protecting effect in hepatocytes of cats subjected to hemorrhagic shock. The purpose of the present study was to explore the possibility of using PCh liposomes for correction of the phospholipid composition of mitochondrial membranes in two different parts of the brain in hemorrhagic shock.

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

MATERIALS AND METHODS

The study was performed on 17 cats (body weight 3.0±0.5 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally). Hemorrhagic shock was produced by the method of Wiggers—Fine [17]. In order to prevent blood coagulation in catheters the cats were

0007-4888/97/0004-0322$18.00