GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Effect of Trimetazidine on Cerebral Metabolism during Acute Ischemia Complicated by Hypoxia

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In experiments on rats, trimetazidine (25 mg/kg) prevented disturbances in energy metabolism and LPO activation in the brain under conditions of acute ischemia aggravated by hypoxia.

Key Words: *trimetazidine; brain; ischemia; hypoxia; metabolism*

Acute disturbances of cerebral circulation are an important medical and social problem. Among drugs protecting the brain against ischemia and hypoxia special place is occupied by glutamate antagonists and various modulators of glutamate receptors (ryluzol, lubeluzol), calcium antagonists (nimodipine) [10], antihypoxants (cytochrome C and amtizol) [2,5], and antioxidants (tirilazad mezylate, SOD) [6]. Of particular interest is trimetazidine (TM), a piperazine derivative (Preductal, Servier; Vastarel, Biopharma). This efficient cytoprotective preparation acting at the cellular level is successfully used in the treatment of cardiac ischemia [8,13]. The protective effect of TM against cerebral hypoxia and ischemia was not studied.

Our aim was to study the protective effects of TM during acute cerebral ischemia complicated by hypoxia. This combination often develops in patients with acute disturbances of cerebral circulation accompanied by respiratory insufficiency of central origin [11].

MATERIALS AND METHODS

The study was carried out on mature male rats weighing 180-200 g. The animals were divided into three groups (8 rats in each): group 1 comprised sham-operated control rats, group 2 consisted of rats with acute ischemia complicated by hypoxia, and group 3 rats were intraperitoneally injected with TM (Vastarel, Biopharma) in a dose of 25 mg/kg 30 min before hypoxia and ischemia modeling. Cerebral ischemia was produced under ether anesthesia by occlusion of both carotid arteries, then the rats were "elevated" to an altitude of 8000 m for 90 min. The rats were killed by decapitation. The brain was frozen in liquid nitrogen. The metabolic status of cerebral hemispheres was assessed by the content of glycogen, glucose, pyruvate, lactate [7], creatine phosphate [1], ATP, ADP, AMP, inorganic phosphate, and energetic charge of the adenine nucleotide system [4]. The intensity of LPO was assessed by the content of lipid hydroperoxides and malonic dialdehyde (MDA), while the functions of antioxidant systems were evaluated by the content of reduced glutathione and activity of catalase and superoxide dismutase (SOD). The results were statistically analyzed using Student's t test.

RESULTS

Hypoxia and occlusion of the carotid arteries profoundly changed cerebral metabolism (Table 1). The content of glycogen in the cerebral hemispheres decreased by 53%, glucose content increased by 44%. The content of lactate increased profoundly against the background decreased pyruvate content. The lactate/pyruvate ratio increased more than 6-fold, which in-

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dicates drastic acceleration of anaerobic glycolysis. Ligation of the carotid arteries in rats drastically reduces blood flow in the cerebral hemispheres and disturbs oxygen supply to the brain. Under these conditions the value of Po_2 in the ischemia focus drops to 4-8 mm Hg [12], which causes disturbances of oxidative phosphorylation. Despite activation of compensatory biochemical mechanisms (activation of glycolysis and other alternative metabolic pathways), cerebral ischemia is accompanied by progressive energy deficiency. In our experiments the content of cerebral creatine phosphate and ATP decreased by 66 and 32%, respectively, while the content of ADP, AMP, and inorganic phosphate increased. These changes in adenine nucleotide pool were accompanied by a decrease in the energy charge of the ATP+ADP+AMP system controlling the rate of energy production and utilization in the cell. Thus, acute cerebral ischemia combined with hypoxia in rats was characterized by disturbances in energy metabolism and the development of metabolic acidosis.

Abnormal metabolites in the brain subjected to ischemia and hypoxia are memranotoxic and stimulate LPO. There are close relationships between energy deficiency and activation of LPO. Specifically, accumulation of ADP, AMP, and reduced pyridine nucleotides caused by activation of the anaerobic glycolysis, promotes the formation of chelated bivalent iron complexes initiating LPO [3]. Neuronal membranes are enriched with unsaturated lipids, while low activity of antioxidant enzymes and free radicals formed in neurochemical reactions provide conditions for oxidation of membrane structures. Acute cerebral ischemia complicated by hypoxia was accompanied by accumulation LPO products in cerebral tissue: the content of lipid hydroperoxides increased by 47% and MDA by 150% (Table 1). Simultaneously, the content of reduced glutathione in cerebral hemispheres decreased by 21%, while activity of SOD and catalase dropped by 21 and 39%, respectively.

TM injected prior to ischemic episode and "ascent" to high altitude prevented the development of metabolic disturbances in the brain. TM prevented the decrease in the content of pyruvate, accumulation of lactate, and the rise of lactate/pyruvate ratio, which attests to its capacity to inhibit the development of metabolic acidosis in cerebral tissue provoked by ischemia combined with hypoxia. Injection of TM preserved high level of cerebral glucose and glycogen. The content of creatine phosphate and ATP in the brain did not significantly differ from the control, while the content of ADP and AMP decreased, and the energetic charge of adenyle nucleotide system returned to normal (Table 1). Injection of TM before occlusion of the carotid arteries and hypoxia prevented accumulation of LPO products and inhibition of the

TABLE 1. Effect of TM on Energy Metabolism and LPO in the Brain during Acute Ischemia Complicated with Hypoxia (*M±m*)

Index	Control	Ischemia+hypoxia	
		without TM	with TM
Glycogen, mg/g	1.14±0.13	0.54±0.07*	0.67±0.07
Glucose μmol/g	2.16±0.29	3.11±0.31*	2.89±0.37
Pyruvate, μmol/g	0.24±0.01	0.14±0.01*	0.28±0.01+
Lactate, μmol/g	1.58±0.18	5.93±0.35*	4.47±0.34+
Lactate/pyruvate	6.67±0.38	42.42±2.51*	19.66±1.15+
Creatine phosphate, μmol/g	3.61±0.65	1.22±0.30*	4.00±0.66+
ATP, μmol/g	2.82±0.07	1.93±0.10*	2.67±0.92+
ADP, μmol/g	0.82±0.02	2.15±0.17*	1.02±0.13+
AMP, μmol/g	0.53±0.02	1.24±0.24*	0.61±0.06+
Total adenine nucleotide, μmol/g	4.16±0.08	5.59±0.35*	4.29±0.16+
Energy charge	0.775±0.004	0.555±0.028*	0.743±0.020+
Inorganic phosphate, μmol/g	7.41±0.92	10.27±0.53*	8.23±0.53+
MDA, μmol/g	13.8±0.25	34.9±3.92*	15.0±1.68+
Hydroperoxides, U	0.032±0.002	0.047±0.003*	0.050±0.003
Reduced glutathione, μmol/g	41.8±0.13	37.1±1.10*	40.1±0.69+
SOD, U/mg protein	2.89±0.20	2.14±0.15*	2.52±0.21
Catalase, U/mg protein	0.297±0.041	0.182±0.020*	0.201±0.023

Note. $p<0.05$ *compared to control and **compared to TM-untreated animals.

antioxidant system in cerebral tissue, while the content of reduced glutathione did not differ from the control (Table 1).

Therefore, injection of TM prevented the development of severe disturbances of cerebral metabolism induced by acute ischemia complicated by hypoxia. TM prevented the development of energy deficiency and metabolic acidosis, activation of LPO, and inhibition of the antioxidant system.

REFERENCES

1. A. M. Alekseeva, *Biokhimiya*, **16**, No. 2, 97-103 (1951).
2. S. F. Barsukov, E. I. Dedov, G. I. Antonov, *et al.*, *Antihypoxants and Actoprotectors: Results and Perspectives* [in Russian], Issue 2, St. Petersburg (1994).
3. M. V. Bilenko, *Ischemic and Reperfusion Damage to Organs (Molecular Mechanisms, Prevention, and Treatment)* [in Russian], Moscow (1989).
4. I. V. Zarubina and B. I. Krivoruchko, *Ukr. Biokhim. Zh.*, **54**, No. 4, 437-439 (1982).
5. V. A. Zinchenko, in: *Cytochrome C and Its Clinical Application* [in Russian], Leningrad (1990), pp. 39-46.
6. J. E. Cottrell, *Anest. Reanimatol.* No. 2, 81-85 (1996).
7. *Methods of Biochemical Studies*, Ed. M. I. Prokhorova [in Russian], Leningrad (1982).
8. *Myocardial Cytoprotection: From Stenocardia to Thrombolysis*, Ed. M. E. Bertran [in Russian], Moscow (1995).
9. A. V. Smirnov, I. V. Zarubina, and B. I. Krivoruchko, *Eksp. Klin. Farmakol.*, **59**, No. 5, 56-58 (1996).
10. Z. A. Suslina and L. A. Geraskina, *Klin. Farmakol. Ter.*, **5**, No. 4, 80-82 (1996).
11. K. S. Ternovoi, Yu. P. Butylin, and Yu. I. Bobylev, *Urgent States: Pathophysiology, Clinics, and Treatment* [in Russian], Kiev (1984).
12. E. S. Flamm, M. L. Seligman, and H. B. Demopoulos, in: *Anesthesia and Neurosurgery*, Eds. J. E. Cottrell and H. Turndorf, St. Louis (1980), pp. 248-266.
13. C. Harpey, P. Clauser, C. Labrid, *et al.*, *Cardiovasc. Drug Rev.*, **6**, No. 4, 292-312 (1989).