PHARMACOLOGY AND TOXICOLOGY

Effects of Trimethazidine on Brain Metabolism in Acute Ischemia Combined with Hypoxia

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Trimethazidine in a dose of 25 mg/kg prevents disturbances of energy metabolism and activation of lipid peroxidation in rat brain after acute ischemia and hypoxia.

Key Words: *trimethazidine; brain; ischemia with hypoxia; metabolism*

Acute circulatory disorders are an important medical and social problem. A growing arsenal of drugs protecting the brain from ischemia and hypoxia includes glutamate antagonists and glutamate receptor modulators (Riluzole, Lubeluzole), calcium antagonists (nimodipine) [9], antihypoxic (cytochrome C, Amtisol) [2,5] and antioxidant (tyrilazole mesylate, superoxide dismutase) [6] compounds. A new derivative of piperazine, trimethazidine (TM; Preductal, Servier or Vastarel, Biopharm) is of particular interest. This potent cytoprotective drug acts at the cellular level and is beneficially used for the treatment of ischemic heart disease [12].

Our objective was to study the protective effects of TM under conditions of acute brain ischemia complicated with hypoxia. This combination occurs when acute disorders of cerebral circulation are accompanied by respiratory deficiency of central origin. [11].

MATERIALS AND METHODS

Experiments were carried out on adult male rats weighing 180-200 g. Three groups were formed (8 rats in each): 1) sham-operated (control); 2) exposed to acute ischemia and hypoxia; 3) treated with intraperitoneal TM (Vastarel, Biopharm, 25 mg/kg) 30 min prior to ischemia-hypoxia. Ischemia was caused by occlusion of both common carotid arteries under ether anesthesia. The animals were then elevated to an altitude of 8000 m in a pressure chamber for 90 min. The brain was fixed by immersing the head in liquid nitrogen. The metabolic state of cerebral hemispheres was assessed by measuring the content of glycogen, glucose, pyruvate, lactate [7], creatine phosphate [1], ATP, ADP, AMP, and inorganic phosphate, and by determining the energy potential of the adenine nucleotide system [4]. The intensity of lipid peroxidation (LPO) was assessed by the content of lipid peroxides and malonic dialdehyde. The functioning of the antioxidant system was assessed by the content of reduced glutathione and catalase and superoxide dismutase (SOD) activities as described previously [8]. The data were analyzed statistically using Student's t test.

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RESULTS

Occlusion of the carotid arteries in combination with hypoxia caused dramatic changes in brain metabolism (Table 1). Cerebral content of glycogen decreased by 53% with glucose increasing by 44%. Considerable rise in lactate content occurred simultaneously with the decrease in pyruvate content. As a result, the lactate/pyruvate ratio increased more than six-fold, which was indicative of intensified anaerobic glycolysis. It is known that occlusion of the carotid arteries in rats considerably reduced cerebral circulation, and dis-

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TABLE 1. Effects of Trimethazidine on Energy Metabolism in the Brain after Acute Ischemia and Hypoxia (*M±m*, *n*=8)

Index	Control	Ischemia+hypoxia	TM+ischemia+hypoxia
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**
Adenine nucleotides, μmol/g	4.16±0.08	5.59±0.35*	4.29±0.16**
Energy potential	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**

Note. Here and in Table 2 $p<0.05$: *in comparison with control animals; **in comparison with animals exposed to ischemia and hypoxia.

TABLE 2. Effects of Trimethazidine on Lipid Peroxidation in the Brain after Exposure to Acute Ischemia and Hypoxia (*M±m*, *n*=8)

Index	Control	Ischemia+Hypoxia	TM+Ischemia+Hypoxia
MDA, μmol/g	13.8±0.25	34.9±3.92*	15.0±1.68**
Peroxides, units	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, unit/mg protein	2.89±0.20	2.14±0.15*	2.52±0.21
Catalase, unit/mg protein	0.297±0.041	0.182±0.020*	0.201±0.023

turbed oxygenation in the brain. Under these conditions pO_2 in the center of ischemic area is as low as 4-8 mm Hg [11], which is the cause of oxidative phosphorylation impairment. Despite activated compensatory biochemical mechanisms (glycolysis and other alternative metabolic pathways), cerebral ischemia is accompanied by energy deficit. Our data showed that creatine phosphate and ATP contents decreased by 66 and 32%, respectively, with a simultaneous increase in the concentration of ADP, AMP, and inorganic phosphate. Changes in the adenine nucleotide pool were accompanied by a decrease in the energy potential of the ATP+ADP+AMP system that regulates the rate of energy production and utilization in the cell. Thus, acute ischemia in combination with hypoxia is characterized by disturbed energy metabolism and metabolic acidosis in rat brain.

Postischemic and posthypoxic metabolic products are toxic for cell membranes and stimulate LPO processes. The ischemia-hypoxia-induced energy deficiency is associated with LPO activation. Increased intracellular concentration of ADP and AMP and a high level of reduced pyridine nucleotides due to enhanced anaerobic glycolysis stimulate the formation of chelated Fe^{2+} complexes which initiate LPO [3]. Neuronal membranes are enriched with unsaturated lipids, which in combination with low concentration of antioxidant enzymes and generation of free radicals promotes oxidation of membrane structures. As seen from Table 2, acute brain ischemia, aggravated by hypoxia, was accompanied by accumulation of LPO products: the contents of lipid peroxides and malone dialdehyde increased by 47 and 150%, respectively. At the same time, the level of reduced glutathione in brain hemispheres decreased by 21% and SOD and catalase activities decreases by 21 and 39%, respectively.

Pretreatment with TM prevented metabolic changes after combined exposure to ischemia and hypoxia. Being administered prior to ischemic episode, TM reduced the decrease in pyruvate content and prevented lactate accumulation and increase in the lactate-pyruvate ratio, thus inhibiting metabolic acidosis in the brain after ischemia-hypoxia. TM preserved the content of glucose and glycogen in the brain at a high level. The contents of creatine phosphate and ATP did not differ from the control, the concentrations of ADP and AMP were lower than in untreated rats and the energy potential of the adenine nucleotide system was restored (Table 1). The drug prevented accumulation of LPO products and inhibition of antioxidant en-

zymes in tissues. The content of reduced glutathione in the brain of TM-treated animals did not differ from the control (Table 2).

Thus, pretreatment with TM prevents severe metabolic disturbances in the brain induced by acute ischemia and hypoxia. The drug prevents the development of energy deficit and metabolic acidosis, inhibits LPO processes, and prevents suppression of the antioxidant system.

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