ГИПОКСИЯ И АНТИГИПОКСАНТЫ, В ФОКУСЕ ЧЕРЕПНО-МОЗГОВАЯ ТРАВМА

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© ***П.Д. Шабанов, И.В. Зарубина***

ФГБВОУ ВО «Военно-медицинская академия им. С.М. Кирова» Минобороны России, Санкт-Петербург

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В экспериментах на крысах была показана индивидуальная чувствительность животных к острой гипоксии в ранний период после механической черепно-мозговой травмы. Антигипоксант этомерзол (25 мг/кг, 3 дня внутрибрюшинно), введенный после травмы, уменьшал поведенческие расстройства у крыс с разным уровнем устойчивости к гипоксии, сохраняя структуру индивидуального поведения и предупреждая метаболические нарушения в головном мозге. Монотерапия последствий черепно-мозговой травмы антидепрессантом пиразидолом (1 мг/кг) вызывала анксиолитический эффект у крыс с высокой устойчивостью к гипоксии и активирующий эффект у низкочувствительных к гипоксии крыс. Лечение бемитилом (25 мг/кг), антигипоксантом бензимидазольной структуры, оказывало церебропротекторное действие и нормализовало индивидуальные поведенческие паттерны, параметры энергетического обмена и состояние антиоксидантных систем головного мозга у крыс с высокой и низкой чувствительностью к гипоксии. Наиболее значимо защитный эффект бемитила проявлялся у крыс с высокой устойчивостью к гипоксии. Комбинированное применение бемитила и пиразидола вызывало аддитивный эффект в обеих подгруппах крыс. Эта комбинация нормализовывала поведенческие реакции и предотвращала развитие метаболических расстройств в головном мозге.

◆ **Ключевые слова:** устойчивость к гипоксии; антигипоксанты; черепно-мозговая травма; этомерзол; бемитил; пиразидол; поведение; метаболизм.

HYPOXIA AND ANTIHYPOXANTS, FOCUS ON BRAIN INJURY

© *P.D. Shabanov, I.V. Zarubina*

S.M. Kirov Military Medical Academy, Saint Petersburg, Russia

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Experiments on rats showed that the individual resistance of the body to acute hypoxia is of decisive importance in the early recovery period after mechanical craniocerebral trauma. Antihypoxant ethomersol administration (25 mg/kg, 3 days, intraperitoneally) following trauma decreased behavioral impairments in rats with different levels of resistance to acute hypoxia, restored the structure of individual behavior, and prevented metabolic disturbances in the brain. Monotherapy of consequences of craniocerebral trauma with antidepressant pyrazidol (1 mg/kg) produced an anxiolytic effect in animals highly resistant to hypoxia and activating effect on low resistant animals. Treatment with bemithyl, an antihypoxant of benzimidazole structure, in a dose of 25 mg/kg produced a cerebroprotective effect and normalized individual behavioral characteristics, parameters of energy metabolism, and state of the antioxidant system in the brain of highly and low resistant rats. The effect of bemithyl was most pronounced in highly resistant animals. During combined treatment, pyrazidol and bemithyl had an additive effect in animals of both groups. They normalized behavioral reactions and prevented the development of metabolic disturbances in the brain.

◆ **Keywords:** resistance to hypoxia; antihypoxants; craniocerebral trauma; ethomerzol; bemithyl; pyrazidol; behavior, metabolism.

INTRODUCTION

Practical medicine needs in defense of the organism from oxygen insufficiency. In this case, the use of antihypoxants is rather effective. The concept of antihypoxants and search of new drugs from this group are developing at the Department of Pharmacology of the Military Medical Academy, Saint Petersburg, Russia. Antihypoxants are the compounds of different chemical structure (aliphatic and cyclic aminothiols, benzimidazoles) with common effects (neither transmitter nor tissue specific and systemic), possessing both energy stabilizing and antioxidant actions and enhancing the resistance of the hole organism to oxygen deficit. Besides, these drugs support physical endurance in extreme conditions of

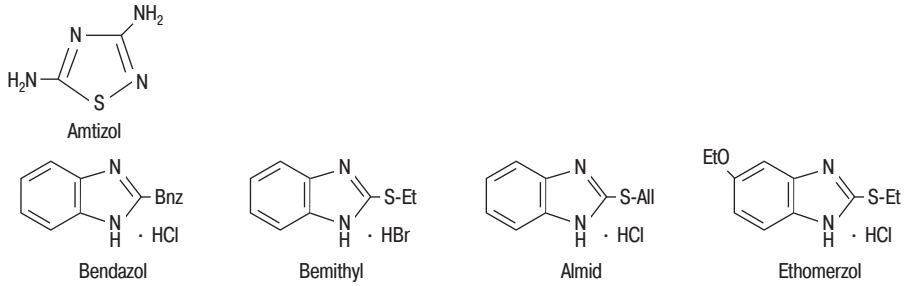

Fig. 1. Structures of some antihypoxants

environment (oxygen deficit, overheating, intoxications, exhaustion) and accelerate the recovery of physical and psychical status after extreme loads, intoxications with phosphorogranic compounds, in stress etc. [1]. Such actoprotective activity of antihypoxants is due to their ability to accelerate the reparative and adaptive synthesis of RNA, enzymes, structural proteins following the action of different aggressive agents [10]. The defensive properties of antihypoxants vary, it allows to profile each drug in different clinical situations and in healthy beings [3]. For example, bemithyl (Fig. 1), a benzimidazole derivative, was used to support physical activity in military people in Afghanistan (1979–1989), in liquidators of disaster subsequences at the Chernobyl atomic station (1986, Ukraine), earthquake in Armenia (1988), railway catastrophe in Ufa (Russia) and in other extreme situations. Bemithyl was used by polar investigators in Antarctica expeditions, by recruits in severe situations etc. In clinical practice, bemithyl is used as an antiasthenic drug, when weakness, rapid fatiguability and decreased availability are observed. Aminothiol antihypoxants can be prescribed as supplementary drugs in intensive care and in urgent medicine [12]. Amtizol is assigned in common hypoxia due to blood insufficiency, critical hemodynamic disorders, asphyxia, resection of the lung. In cardiological practice, amtizol enhanced the efficacy of traditional therapy of heart arrhythmias, arrhythmogenic and cardiogenic collapse. Therefore, antihypoxants are new original basic and high effective pharmacological drugs perspective for the use both in pharmacology of healthy human beings and in general practice.

ANTIHYPOXANTS, NOOTROPICS AND BRAIN INJURY

The pathogenetic factors in acute craniocerebral trauma include oxygen starvation of the brain. The individual resistance of the body to acute hypoxia is of particular significance in the post-traumatic period, and its relationship with the typological characteristics of higher nervous activity and brain metabolism determines the direction of pharmacological correction of the consequences of craniocerebral trauma. Selection of the optimum route to restoring central nervous system function and brain metabolism, with consideration of the risks of forced normalization and delays in the various stages of rehabilitation after trauma, remains a current problem.

Effective reversal of behavioral abnormalities in animals and elimination of metabolic disturbances during the post-traumatic period can be addressed using antihypoxic agents, which increase the body's stability to the action of acute or chronic hypoxia. Effective antioxidants include ethomersol (5-ethoxy-2-ethylthiobenzimidazole), synthesized in the Department of Pharmacology of the Military Medical Academy; the biochemical and physiological effects of this agent are mediated by activation of protein synthesis and enzymes involved in energy metabolism and antioxidant systems [14]. The metabolic type of action of ethomersol is responsible for its significant protective properties in cerebrovascular disturbances of hypoxic origins [4]. The fact that ethomersol has actoprotective properties of nonexhausting nature leads us to expect its use to produce effective restoration of functional and metabolic lesions of the brain in the post-traumatic period. The aim of the present work was to study the role of resistance to acute hypoxia in rats in the realization of the protective effects of ethomersol after craniocerebral trauma.

The experiments were performed using white male Wistar rats weighing 160–180 g (groups of 8–10 animals). Before trauma, all animals were classified according to their resistance to acute hypoxia by elevating them in a barochamber to a height of 12,000 m at a rate of 50 m/sec, and keeping them at that altitude untill the onset of agonal respiration. Animals tolerating hypoxia for 5–10 min were regarded as low-resistant (LR) animals, those tolerating more than 10 min were regarded as high-resistant (HR) animals. Closed craniocerebral trauma of moderate severity was inflicted 24 h later, using a weight of 64 g in free fall within a hollow tube of height 80 cm and diameter 1.3 cm, landing on the parietal area of the head [7]. With the aim of avoiding fracturing the jaw, animals' heads were fixed on a soft support. When depressed fractures to the parietal bone occurred, animals were subjected to euthanasia within a minute of craniocerebral trauma and their brains were not studied further.

Ethomersol (25 mg/kg) was given intraperitoneally (i. p.) three times, once daily, for three days. The reference preparation consisted of 20% piracetam solution from ampules (ICN-Oktyabr', Russia) given at a dose of 60 mg/kg by the same schedule as used for ethomersol. The positive effects of treatment on the course of craniocerebral trauma were assessed in terms of the survival of the animals, measurements of body temperature, respiration rate, measures of cere-

■ **Table 1. Effects of piracetam and ethomersol on changes in physiological measures in rats after craniocerebral trauma (n = 8)**

Measure	Group	Before CCT	Immediately after CCT	One day after CCT	Three days after CCT	Three days after CCT + + piracetam	Three days after CCT + + ethomersol
Body weight, g	HR	185.1 ± 3.3	161.2 ± 2.2*	176.2 ± 4.4*	152.2 ± 2.5*	167.3 ± 3.4**	178.1 ± 2.2*
	LR	183.5 ± 2.2	163.1 ± 2.3*	177.4 ± 4.1*	155.1 ± 2.3*	171.2 ± 3.2**	181.2 ± 2.1**
Respiratory rate, per min	HR	124 ± 3	146 ± 3*	163 ± 3*	141 ± 2*	137 ± 3**	131 ± 3**
	LR	128 ± 2	137 ± 3.3*	153 ± 2*	129 ± 4*	132 ± 1**	130 ± 3**
Body temperature, °C	HR	38.55 ± 0.12	38.12 ± 0.11*	37.53 ± 0.11*	37.76 ± 0.11*	38.17 ± 0.11**	38.37 ± 0.11**
	LR	38.48 ± 0.13	38.10 ± 0.11*	37.13 ± 0.11*	37.58 ± 0.14*	38.46 ± 0.12**	38.52 ± 0.13**

Note. * $p < 0.05$ compared with rats without craniocerebral trauma; ** $p < 0.05$ compared with rats with craniocerebral trauma.
CCT – craniocerebral trauma; HR = rats with high and LR = rats with low resistance to hypoxia.

■ **Table 2. Effects of Ethomersol on Survival of Rats Three Days after Craniocerebral Trauma**

CCT	CCT + + piracetam	CCT + + ethomersol			
Surviving/total	%	Surviving/total	%	Surviving/total	%
HR17/23	72.7	16/20	80	15/18	83.3
LR14/24	60	17/24	70.8	16/19	84.2

Note. * $p < 0.05$ compared with rats without craniocerebral trauma; ** $p < 0.05$ compared with rats with craniocerebral trauma.
CCTCCT – craniocerebral trauma; HR = rats with high and LR = rats with low resistance to hypoxia.

bral edema, the behavior of the animals, and biochemical investigations.

Assessment of the presence and severity of cerebral edema was performed after decapitating the animals three days after craniocerebral trauma; brains were removed with the olfactory lobes and weight was measured wet and after drying to constant weight. Calculations were made using calibration plots. Overall measures of physiological responses to craniocerebral trauma were obtained from rats with different levels of resistance to acute hypoxia using an open field test and an elevated plus maze test to address the orientational-investigative, emotional, stereotypic, and motor components according to a behavioral atlas for rodents. Rats were placed in the open field for 5 min and measurements were made of the spontaneous horizontal and vertical movement activity (square crossings, rearings), investigative behavior (numbers of glances into openings and excursions into the center), and emotionality (numbers of defecatory boluses and grooming acts). The elevated plus maze test involved measurements of the time spent in the closed and open arms of the maze, the number of excursions into the closed arms, the number of excursions into the open arms, the number of hangings from the open arms, and the number of glances out of the closed arms. The level of anxiety was assessed in terms of the percentage ratio of excursions into the open arms to the total number of excursions [6].

The state of brain energy metabolism was assessed in terms of the contents of creatine phosphate, ATP, and lactic and pyruvic acids [14, 15] in tissue frozen in liquid nitrogen. Lipid peroxidation processes were studied in terms of the contents of the lipoperoxidation products diene conjugates and malonic dialdehyde. The state of the antioxidant system was evaluated in terms of the level of reduced glutathione and superoxide dismutase activity [13]. Enzyme activity was related to the protein content of samples as estimated by the Lowry method. Data were analyzed statistically using Student's t-test.

Immediately after infliction of craniocerebral trauma, high-resistance and low-resistance individuals showed transient tonic and clonic convulsions (2–4 sec), loss of responses to the environment, stupefaction, lying on the side, these manifestations lasting 10–20 sec. Rats showed increases in respiratory rate, decreased body temperature and weight, and involuntary micturition and defecation. Changes in these measures persisted throughout the observation period and were more marked in individuals with low resistance to hypoxia (Table 1).

Piracetam and ethomersol given for three days during the post-traumatic period maintained the respiratory rate and body temperature at the levels seen in intact animals. One day after craniocerebral trauma, rat brains showed sharp congestion of the cerebral vessels, with punctate or microfocal hemorrhages into the soft and hard meninges, along with hematomas in the cerebral cortex and cerebellum, which were most marked in individuals with low resistance. These changes were seen in 40% of cases three days after craniocerebral trauma. In rats given piracetam and ethomersol, pathomorphological changes in the brain were milder. Piracetam and ethomersol increased the survival time of the rats after craniocerebral trauma (Table 2).

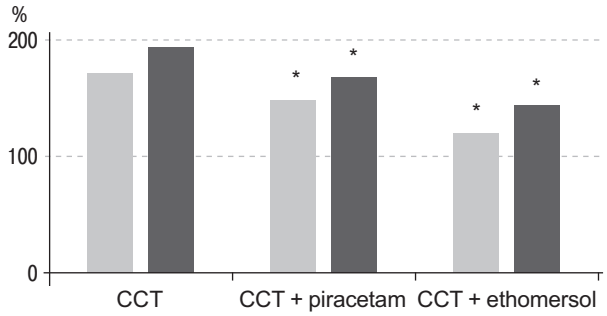

Fig. 2. Effects of ethomersol on cerebral edema in rats 3 days after craniocerebral trauma (CCT). The horizontal axis shows animal groups; the vertical axis shows cerebral edema (%), $p < 0.05$. White columns are high-resistance and shaded columns are low-resistance rats, * $p < 0.05$

■ **Table 3. Effects of Ethomersol on Measures of Rat Behavior in the Open Field Test Three Days after Craniocerebral Trauma (n = 10)**

Group		Types of movement activity				
		Horizontal	Vertical	Searching	Grooming	Emotional
Intact	HR	55.2 ± 6.2	8.2 ± 2.2	5.0 ± 0.9	2.6 ± 0.7	1.5 ± 0.6
	LR	65.2 ± 5.2	10.2 ± 3.2	7.0 ± 0.5	3.6 ± 0.2	2.5 ± 0.3
CCT	HR	35.4 ± 4.3*	6.4 ± 3.4*	2.0 ± 0.3*	1.1 ± 0.2*	0.5 ± 0.2*
	LR	25.2 ± 4.2*	3.2 ± 2.4*	2.0 ± 0.6*	0.6 ± 0.2*	0.7 ± 0.6*
CCT + piracetam	HR	39.9 ± 4.2	7.2 ± 3.2**	3.0 ± 0.9**	1.6 ± 0.7	0.7 ± 0.1**
	LR	29.7 ± 4.2	4.2 ± 2.2**	3.2 ± 0.6**	0.8 ± 0.4**	0.7 ± 0.3
CCT + ethomersol	HR	45.2 ± 6.2**	8.9 ± 3.2**	4.0 ± 0.2**	2.2 ± 0.7**	1.2 ± 0.2**
	LR	56.5 ± 4.2**	9.2 ± 4.2**	6.0 ± 0.9**	2.9 ± 0.6**	1.9 ± 0.8**

Note. Data are compared with 3 days after craniocerebral trauma (CCT). * $p < 0.05$ compared with rats without craniocerebral trauma; ** $p < 0.05$ compared with rats with craniocerebral trauma. HR = rats with high and LR = rats with low resistance to hypoxia.

The most widespread and severe consequences of traumatic head injury was cerebral edema, whose development is mostly responsible for the appearance of neurological complications of craniocerebral trauma. Administration of ethomersol for three days decreased the severity of the signs of edema, preventing the increase in brain weight due to increased water content (Fig. 2).

Decreases in cerebral edema after treatment with ethomersol may result from its vasodilatory actions resulting from blockade of potential-dependent and particularly receptor-controlled Ca^{2+} channels [11]. Craniocerebral trauma, along with local brain damage, produced a universal generalized body reaction, with the features of stress, with classical signs of the state of tension and neuroendocrine and neurohumoral impairments. An integral measure of functional-metabolic changes arising after craniocerebral trauma was provided by changes in the behavior of the animals. Immediately after craniocerebral trauma, rats of both groups showed decreases in spontaneous movement activity. In the subsequent hours (up to a day), there were episodes of increased excitability and aggression alternating with a state of inhibition, which persisted to three days in control animals not treated with pharmacological agents. Moderately severe craniocerebral trauma suppressed motor and orientational-investigative activity for three days in rats with high and low resistance to hypoxia. After trauma, animals of both groups demonstrated different patterns of behavior. In low-resistance rats, the main manifestation was psychomotor inhibition, with a decrease in the volume of movement patterns, decreased autonomic manifestations of emotionality, and increased anxiety (Table 3).

Administration of ethomersol had psychoactivating effects, expressed as decreases in psychopathological symptomatology and restoration of the structure of individual behavior. The less marked effects of piracetam were probably due to the short course of treatment, as piracetam is characterized by a more gradual onset of therapeutic changes. After treatment with ethomersol, high-resistance and especially low-resistance rats showed recovery of orientational-investigative behavior within one day of craniocerebral trauma, with increases in the connectedness of movement and investigative activity. The restitution time after craniocerebral trauma in highly resistant individuals was shorter than that in animals with low resistance to hypoxia. Analysis of the behavioral reactions of the rats in the elevated plus maze after administration of ethomersol showed in-

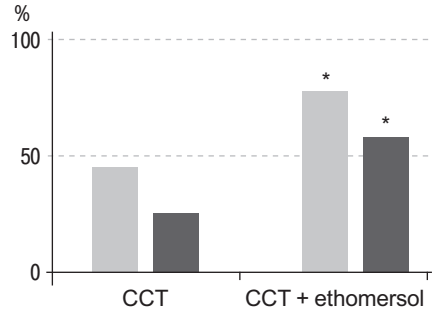

Fig. 3. Effects of ethomersol on measures of anxiety in the elevated maze test in rats. The vertical axis shows the number of excursions into the open arms as a proportion of all excursions. CCT – craniocerebral trauma, *$p < 0.05$ to CCT group

■ **Table 4. Effects of Ethomersol on Measures of Energy Metabolism, Lipid Peroxidation, and Antioxidant Systems in the Brains of Rats after Craniocerebral Trauma (n = 8)**

Measure	Group	Intact	CCT, 1 day	CCT, 3 days	CCT, 3 days + piracetam	CCT, 3 days + ethomersol
Diene conjugates, mmol/g	HR	21.69 ± 0.28	45.05 ± 1.36[a]	47.73 ± 0.51[a]	35.59 ± 0.46[b]	28.21 ± 0.31[b]
	LR	24.09 ± 0.32	61.19 ± 0.78[a]	51.14 ± 0.24[a]	43.33 ± 0.23[b]	31.11 ± 0.22[b]
Malonic dialdehyde, mmol/g	HR	6.56 ± 0.17	19.17 ± 0.64[a]	10.33 ± 0.16[a]	8.48 ± 0.15[b]	7.24 ± 0.37[b]
	LR	7.66 ± 0.16	23.58 ± 0.15[a]	16.16 ± 0.13[a]	9.74 ± 0.12[b]	8.52 ± 0.17[b]
Reduced glutathione, mmol/g	HR	42.09 ± 0.69	18.62 ± 0.40[a]	21.79 ± 0.40[a]	33.53 ± 0.41[b]	35.91 ± 0.77[b]
	LR	31.12 ± 0.19	12.23 ± 0.62[a]	18.09 ± 0.53[a]	22.32 ± 0.34[b]	28.49 ± 0.24[b]
Superoxide dismutase, Act/mg protein	HR	3.11 ± 0.09	0.95 ± 0.04[a]	1.07 ± 0.05[a]	2.34 ± 0.08[b]	2.59 ± 0.08[b]
	LR	2.09 ± 0.05	0.71 ± 0.03[a]	1.11 ± 0.07[a]	1.86 ± 0.03[b]	2.18 ± 0.04[b]
Creatine phosphate, mmol/g	HR	4.12 ± 0.05	1.89 ± 0.03[a]	2.92 ± 0.12[a]	3.18 ± 0.08[b]	3.89 ± 0.07[b]
	LR	3.72 ± 0.04	1.12 ± 0.05[a]	1.87 ± 0.03[a]	2.92 ± 0.05[b]	3.12 ± 0.02[b]
ATP, mmol/g	HR	3.53 ± 0.12	2.12 ± 0.13[a]	2.06 ± 0.08[a]	3.78 ± 0.32[b]	3.27 ± 0.07[b]
	LR	2.78 ± 0.15	1.32 ± 0.08[a]	1.89 ± 0.11[a]	2.12 ± 0.07[b]	2.98 ± 0.05[b]
Lactate, mmol/g	HR	2.13 ± 0.05	7.46 ± 0.13[a]	6.26 ± 0.18[a]	3.78 ± 0.32[b]	3.27 ± 0.07[b]
	LR	3.75 ± 0.09	9.63 ± 0.07[a]	5.52 ± 0.06[a]	4.31 ± 0.09[b]	3.52 ± 0.07[b]
Pyruvate, mmol/g	HR	0.36 ± 0.01	0.15 ± 0.03[a]	0.08 ± 0.01[a]	0.22 ± 0.01[a]	0.32 ± 0.01[b]
	LR	0.27 ± 0.02	0.16 ± 0.01[a]	0.06 ± 0.01[a]	0.18 ± 0.01[a]	0.26 ± 0.01[b]

Note. [a] $p < 0.05$ compared with rats without craniocerebral trauma; [b] $p < 0.05$ compared with rats with craniocerebral trauma at 3 days. CCT – craniocerebral trauma; HR = rats with high and LR = rats with low resistance to hypoxia.

creases in the time spent by the animals in the open arms, in the frequency of hanging from the edges of the maze, and the number of vertical rearings. There was an increase in the percentage ratio of the number of excursions into the open arms to the total number of excursions. Overall, those measures sensitive to anxiotropic factors and used for evaluating anxiety [6] demonstrated that ethomersol released the state of anxiety in animals subjected to craniocerebral trauma (Fig. 3). The most positive changes were seen in animals with low resistance to hypoxia.

Thus, administration of ethomersol to rats for three days after moderately severe craniocerebral trauma prevented disintegration of the individual components of overall behavioral reactions and facilitated the rapid recovery of the structure of individual behavior in animals with both high and low resistance to hypoxia. The extent of changes in the reflex-behavioral status of the rats during the post-traumatic period was most marked in animals with low resistance to hypoxia; the protective effects of ethomersol were also most marked in this group of animals.

Metabolic changes in the brains of high-resistance and low-resistance animals after craniocerebral trauma were also different. Low-resistance animals, which represent a “risk group” for trauma, showed a more marked energy deficit, greater lactic acidosis, more extensive activation of lipid peroxidation, and greater suppression of antioxidant systems (see Table 4). The properties of ethomersol prevented restriction of the

rate of NAD-dependent respiration demonstrated in the model of cerebral circulatory hypoxia [10], suggesting that it should stabilize energy metabolism in the brain after craniocerebral trauma. In fact, during the post-traumatic period, ethomersol and piracetam facilitated normalization of the pool of macroergic substances in the brains of rats with high and low resistance to hypoxia, eliminating metabolic acidosis. The two agents produced similar decreases in lactic acidosis, though ethomersol yielded larger pools of creatine phosphate and ATP in the brain, due to its ability to prevent uncoupling of phosphorylation from oxidation.

Treatment of post-traumatic rats with piracetam and ethomersol help prevent extreme lipid peroxidation and stabilized the activity of brain antioxidant systems. Ethomersol was more effective than piracetam in preventing the accumulation of primary and secondary products of lipid peroxidation, decreasing reduced glutathione levels and superoxide dismutase activity in the brain. As compared with piracetam, the positive metabolic changes seen in rats given ethomersol were more marked in the group with low resistance to acute hypoxia. The similarity of the structure of ethomersol and most of its protective effects in hypoxic damage to those of bemethyl, another member of the pharmacological antioxidant class, suggests that the antioxidant effects of ethomersol may result from its energy-stabilizing actions and its separate antiradical and antioxidant activities [14]. Thus, the individual resistance of the body to acute hypoxia is of decisive importance in the early recovery period following craniocerebral trauma, while treatment with ethomersol prevents the behavioral and metabolic abnormalities occurring after cerebral trauma.

ANTIHYPOXANTS, ANTIDEPRESSANTS AND BRAIN INJURY

The postcommotional period of craniocerebral trauma (CCT) is characterized by long-term rehabilitation of patients. The pathogenesis of CCT includes neurodynamic and metabolic disorders of the central nervous system that are primarily related to hypoxic injury of the nervous tissue and results in the development of post-hypoxic encephalopathy. Psychopathological syndrome is a common delayed consequence of CCT manifested in asthenic, neurotic, and neurosis-like disorders [5]. The severity of psychovegetative syndrome and emotional-and-intellectual disturbances progressively increases after CCT. Pathological changes in the brain develop in the delayed period after CCT and are similar to those observed in depressive patients [2].

The general principle of pharmacotherapy for CCT is combined treatment with considering pathogenetic characteristics of the disease. The standard therapy for posttraumatic disorders should include pharmacological preparations with nootropic and antihypoxic activity. To avoid overuse of medications for combined treatment of patients with CCT, it is necessary to synthesize new preparations with specific pharmacological activity. Antihypoxant bemithyl (2-ethylthio-benzimidazole hydrobromide) possesses nootropic and mild psychostimulant activity and fully conforms these requirements [4, 8, 9]. Here we studied the cerebroprotective effect of individual and combined treatment with the antihypoxant bemithyl and antidepressant pyrazidol in animals with CCT characterized by different resistance to hypoxia.

The experiments were performed on male Wistar albino rats weighing 160–180 g. Each group included 8–10 animals. The animals were divided into groups by the resistance to acute hypoxia. Hypoxia was produced in an altitude chamber. The rats were elevated to a height of 12,000 m (50 m/sec) and were maintained under these conditions until the appearance of agonal breathing. The animals that survived for 5–10 and more than 10 min were considered to be low resistant (LR) and highly resistant (HR), respectively. Mild closed CCT was produced 24 h after hypoxia. The weight of 64 g fell freely from a hollow tube (height 80 cm, diameter 1.3 cm) to the parietal region of the head [7]. The head was fixed on a soft pad to prevent jaw fracture. The animals with depressed fracture of the parietal lobe died over the first minutes after CCT (not more than 5% rats). The brains from died animals were not examined. The rats were divided into groups. Group 1 animals received intraperitoneal injections of bemithyl in a dose of 25 mg/kg (ICN-Oktyabr', Russia) for 20 days. Pyrazidol in a dose of 1 mg/kg (Farmakon, Russia) was injected intraperitoneally to group 2 rats. Combined treatment with 1 mg/kg pyrazidol and 25 mg/kg bemithyl was applied to group 3 animals. Control rats received an equivalent volume of physiological saline. The conclusion about the positive effect of drugs on animals with CCT was based on survival rate, behavioral reactions, and biochemical assays. The physiological reaction of rats to CCT was studied in the open field and elevated plus maze tests. We recorded orientation-and-exploratory, emotional, stereotypic, and locomotor activity of animals. The state of energy metabolism in the brain was determined by the contents of creatine phosphate, ATP, lactic acid, and pyruvic acid [14] in brain tissues pre-frozen in liquid nitrogen. The intensity of lipid peroxidation (LPO) was estimated by the concentrations of conjugated dienes and malonic dialdehyde (MDA) [15]. The state of the antioxidant system was evaluated by the amount of reduced glutathione and superoxide dismutase (SOD) activity [13]. Enzyme activity was calculated per protein content in samples measured by the method of Lowry. The results were analyzed by Student's t test.

The rats phenotypically differing in the resistance to hypoxia before CCT exhibited various behavioral reactions. HR animals were characterized by "smooth" behavior in the open field and elevated plus maze. LR rats exhibited pronounced locomotor and exploratory activity and high level of anxiety. CCT was followed by inver-

sion of individual behavioral characteristics. LR rats were sluggish and did not display exploratory activity. These changes reflect disintegration of individual components in the general behavioral reaction. HR rats retained such behavioral reactions as sniffing, and motion. Exploratory activity decreased in animals of both groups. However, HR animals demonstrated behavioral and autonomic signs of increased emotional reactivity. The number and duration of grooming reactions and defecation rate increased. These changes reflected psychomotor excitation of animals. In control rats the observed behavioral reactions persisted for 20 days after trauma.

Individual or combined administration of pyrazidol and bemithyl increased the survival rate of rats with CCT (Table 5). The number of HR and LR rats receiving pyrazidol and surviving 3 days after CCT was 10% higher compared to untreated animals. Bemithyl increased the number of survived HR and LR rats by 44 and 52%, respectively. After combined treatment with pyrazidol and bemithyl the survival rate of HR and LR rats surpassed the control by 50 and 66%, respectively. Behavioral reactions of rats with CCT underwent changes after 20-day therapy (Table 6). Locomotor and exploratory activity of LR animals receiving pyrazidol increased, which was

■ **Table 5. Animal Survival 3 Days after CCT**

Group	CCT (n = 30)		CCT and pyrazidol (n = 30)		CCT and bemithyl (n = 19)		CCT and pyrazidol + bemithyl (n = 30)	
	abs.	%	abs.	%	abs.	%	abs.	%
HR	14	40	15	50	16	84	27	90
LR	8	27	11	37	15	79	28	93

Note. CCT – craniocerebral trauma; HR = rats with high, LR = rats with low resistance to hypoxia.

■ **Table 6. Effect of Individual or Combined Treatment with Pyrazidol and Bemithyl on Locomotor Activity, Emotional Reactivity, and Level of Anxiety in Rats with CCT ($M \pm m$, n = 10)**

Index		Intact	CCT	CCT and pyrazidol	CCT and bemithyl	CCT and Pyrazidol + bemithyl
Locomotor activity						
Number of crossed squares	HR	55.2 ± 6.2	38.2 ± 4.2*	25.2 ± 2.1#	48.3 ± 2.6#	58.2 ± 4.7#
	LR	65.2 ± 4.3	21.2 ± 2.2*	26.5 ± 3.3#	59.5 ± 3.4#	64.9 ± 3.3#
Latency of the first movement, sec	HR	25.2 ± 4.1	30.1 ± 4.2*	10.3 ± 3.2#	19.3 ± 5.4#	26.1 ± 2.4#
	LR	15.0 ± 2.2	40.1 ± 2.3*	29.2 ± 2.5#	12.3 ± 1.4#	14.2 ± 3.4#
Number of entries into the center	HR	10.0 ± 1.3	5.2 ± 2.2*	4.1 ± 1.1#	8.2 ± 2.3#	11.2 ± 4.3#
	LR	7.2 ± 1.2	3.1 ± 1.2*	4.3 ± 2.1#	5.6 ± 1.9#	8.4 ± 2.6#
Number of rearing postures	HR	20.0 ± 2.2	14.2 ± 3.4*	12.1 ± 1.4#	15.2 ± 2.7#	19.2 ± 5.1#
	LR	42.2 ± 3.2	2.2 ± 1.2*	3.5 ± 2.3#	35.4 ± 2.3#	45.2 ± 2.7#
Number of explored holes	HR	5.0 ± 0.9	1.0 ± 0.3*	0.6 ± 1.3#	4.1 ± 1.3#	5.5 ± 1.3#
	LR	8.2 ± 4.2	0.5 ± 0.2*	1.2 ± 1.4#	7.4 ± 2.3#	7.9 ± 4.2#
Emotional reactivity						
Number of grooming reactions	HR	2.0 ± 0.1	3.2 ± 0.3*	0.8 ± 0.2	1.6 ± 0.3#	1.8 ± 0.2
	LR	3.5 ± 0.2	0.9 ± 0.2*	1.4 ± 0.4#	2.0 ± 0.2#	2.6 ± 0.3#
Duration of grooming reactions, sec	HR	25.3 ± 4.2	29.2 ± 2.3*	8.1 ± 3.3	18.3 ± 4.1#	20.2 ± 2.4#
	LR	32.3 ± 2.0	5.2 ± 2.4*	8.3 ± 4.5#	19.2 ± 2.5#	28.3 ± 4.3#
Defecation rate	HR	2.0 ± 0.3	3.1 ± 0.3*	1.3 ± 0.4	1.6 ± 0.2#	1.8 ± 0.2#
	LR	2.8 ± 0.2	0.8 ± 0.2*	1.2 ± 0.2#	2.2 ± 0.2#	2.6 ± 0.4#

■ **Table 6. (continued)**

Index		Intact	CCT	CCT and pyrazidol	CCT and bemithyl	CCT and Pyrazidol + bemithyl
Anxiety						
Time spent in the open arm, sec	HR	53 ± 3	22 ± 5*	29 ± 4	45 ± 6#	56 ± 4
	LR	78 ± 4	17 ± 5*	42 ± 6#	48 ± 3#	73 ± 3#
Time spent in the closed arm, sec	HR	222 ± 6	278 ± 5*	271 ± 3	255 ± 3#	244 ± 4#
	LR	247 ± 7	283 ± 4*	258 ± 2#	252 ± 4#	227 ± 5#
Looking out from the closed arm	HR	7.4 ± 0.3	2.2 ± 0.4*	2.7 ± 0.4	4.8 ± 0.3#	6.9 ± 0.4#
	LR	5.2 ± 0.2	1.2 ± 0.2*	2.6 ± 0.6#	4.5 ± 0.2#	5.6 ± 0.2#
Number of entries into the center	HR	4.4 ± 0.2	1.2 ± 0.3*	1.4 ± 0.3	2.2 ± 0.4#	4.6 ± 0.4#
	LR	2.3 ± 0.3	0.4 ± 0.2*	0.8 ± 0.1#	1.6 ± 0.2#	2.5 ± 0.1#
Number of overhanging postures	HR	6.2 ± 0.2	1.4 ± 0.3*	1.7 ± 0.2	3.4 ± 0.3#	5.9 ± 0.2#
	LR	4.6 ± 0.3	0.9 ± 0.5*	2.4 ± 0.4#	3.2 ± 0.2#	4.4 ± 0.3#

Note. * $p < 0.05$ compared to intact animals; # $p < 0.05$ compared to craniocerebral trauma. CCT – craniocerebral trauma; HR = rats with high and LR = rats with low resistance to hypoxia.

manifested in shortened of the latency of the first movement and increased number of crossed squares, vertical rearing postures, entries into the center of the open field, and explored holes. Pyrazidol had a mild cataleptic effect in HR rats. This drug decreased vertical and horizontal activity of HR animals. After treatment with pyrazidol shortening of the latency of the first movement in HR rats was more pronounced than in LR animals. Behavioral changes in HR rats reflect the sedative effect of pyrazidol. Bemithyl significantly increased locomotor and exploratory activity of HR and LR rats. The observed changes were most pronounced in LR animals. However, these indexes in treated rats did not attained the control level. Combined administration of pyrazidol and bemithyl practically did not modulate locomotor and exploratory activity of LR and HR rats.

Emotional reactivity of rats treated in the posttraumatic period underwent considerable changes. Pyrazidol had a sedative effect on HR rats, which was manifested in a decrease in the rate and duration of grooming reactions (see Table 6). By contrast, pyrazidol produced a stimulating effect and increased emotional reactivity of LR animals. Bemithyl acted as a psychostimulant in HR and, especially, in LR rats. Combined treatment with pyrazidol and bemithyl more significantly improved emotional reactivity of animals, which did not differ from normal. In animals receiving the test preparations the indexes of anxiety corresponded to the emotional state. Pyrazidol increased the time spent in the open arms of the elevated plus maze, number of entries into the center of the maze, and count of overhanging postures. We observed an increase in the ratio between the count of entries into open arms and total number of transitions. These indexes illustrate the influence of anxiotropic factors and are used for evaluation of anxiety [6]. The observed changes indicate that pyrazidol relieves symptoms of anxiety in animals with CCT. It should be emphasized that these indexes only slightly changed in HR rats. However, changes in the degree of anxiety were significant in LR animals and reflected a potent antidepressant effect of the preparation.

Bemethyl therapy alleviated symptoms of anxiety in LR and HR rats with CCT. The level of anxiety after monotherapy with bemithyl did not reach normal. The psychostimulant action of bemithyl was most pronounced in LR rats. Combined administration of bemithyl and pyrazidol reduced the degree of anxiety in animals of both groups.

Our results show that individual or combined treatment with the test preparations produces various effects on behavioral reactions of rats with CCT. Pyrazidol had a typical “balanced” effect, increased locomotor activity, and reduced anxiety and emotional reactivity in LR rats. However, the preparation produced sedative and mild cataleptic effects in HR animals. As distinct from pyrazidol, bemithyl did not decrease locomotor and emotional activity in HR rats. Moreover, bemithyl had a psychostimulant action and increased locomotor and emotional activity in LR animals. During combined treatment, pyrazidol and bemithyl had an additive effect on behavioral reactions of animals phenotypically differing by the resistance to hypoxia. These rats did not differ from intact animals in locomotor and orientation-and-exploratory activity, emotional reactivity, and level of anxiety. Behavioral reactions serve as a general criterion for functional and metabolic changes. It can be hypothesized that bemithyl possessing metabolic activity produces a strong therapeutic effect during treatment of CTT consequences. Bemithyl possesses energy-stimulating and antioxidant properties. The protective effect of this preparation during CCT requires further investigations. We studied the metabolic effect of individual or combined treatment

■ **Table 7. Effect of pyrazidol, bemithyl, and their combination on parameters of energy metabolism, lpo, and antioxidant systems in the brain of rats after CCT ($M \pm m$, $n = 10$)**

Index		Intact	CCT	CCT and pyrazidol	CCT and bemithyl	CCT and pyrazidol + bemithyl
Conjugated dienes, μmol/g	HR	21.58 ± 0.26	45.12 ± 0.32*	37.25 ± 0.21	27.89 ± 0.16	20.56 ± 0.21
	LR	25.02 ± 0.22	56.14 ± 0.82*	41.35 ± 0.22#	29.35 ± 0.13	23.22 ± 0.23
MDA, μmol/g	HR	6.18 ± 0.15	15.14 ± 0.12*	10.27 ± 0.12#	7.72 ± 0.11	7.01 ± 0.17
	LR	7.34 ± 0.14	20.82 ± 0.14*	12.12 ± 0.11#	8.13 ± 0.12	8.14 ± 0.14
Reduced glutathione, μmol/g	HR	42.12 ± 0.43	22.62 ± 0.40*	29.79 ± 0.21	37.15 ± 0.21	39.85 ± 0.27
	LR	30.52 ± 0.22	15.23 ± 0.62*	21.02 ± 0.23#	24.12 ± 0.14	28.87 ± 0.24
SOD, U/mg protein	HR	3.21 ± 0.07	1.25 ± 0.06*	1.57 ± 0.04	2.04 ± 0.07	2.89 ± 0.06
	LR	2.02 ± 0.06	0.88 ± 0.03*	1.22 ± 0.03#	1.97 ± 0.04	2.18 ± 0.04
Creatine phosphate, μmol/g	HR	4.25 ± 0.04	1.12 ± 0.04*	2.28 ± 0.12	3.18 ± 0.04	3.69 ± 0.04
	LR	3.24 ± 0.05	0.87 ± 0.05*	1.98 ± 0.03#	2.87 ± 0.05	2.98 ± 0.03
ATP, μmol/g	HR	3.68 ± 0.14	2.12 ± 0.9*	2.45 ± 0.12	3.18 ± 0.12	3.78 ± 0.05
	LR	2.43 ± 0.15	1.29 ± 0.05*	1.81 ± 0.11#	2.11 ± 0.07	2.12 ± 0.02
Lactate, μmol/g	HR	2.11 ± 0.04	6.24 ± 0.03*	5.12 ± 0.12#	2.88 ± 0.22	3.12 ± 0.05
	LR	3.89 ± 0.11	8.43 ± 0.06*	6.52 ± 0.09#	4.54 ± 0.05	4.12 ± 0.06
Pyruvate, μmol/g	HR	0.38 ± 0.01	0.14 ± 0.02*	0.19 ± 0.01#	0.25 ± 0.01	0.32 ± 0.01
	LR	0.25 ± 0.02	0.09 ± 0.01*	0.14 ± 0.01#	0.19 ± 0.01	0.22 ± 0.01

Note. * $p < 0.05$ compared to intact animals; # $p < 0.05$ compared to CCT – craniocerebral trauma.

with pyrazidol and bemithyl in rats with CCT. The test preparations produced various cerebroprotective effects (Table 7).

Pyrazidol was more potent in preventing the development of metabolic disturbances in the brain of LR rats. This preparation decreased the contents of conjugated dienes and MDA by 26 and 42%, respectively. Moreover, pyrazidol increased the content of reduced glutathione and SOD activity by 38 and 39%, respectively. Stabilization of the energy potential in the brain of LR rats receiving pyrazidol manifested in increased contents of creatine phosphate and ATP (by 128 and 40%, respectively). The preparation prevented accumulation of excess lactate, but increased pyruvate content by 56%. The metabolic state in the brain of HR rats receiving pyrazidol tended to normal. It should be emphasized that bemithyl was more potent than pyrazidol during the therapy of animals with CCT. In animals of both groups bemithyl prevented accumulation of LPO products in the brain, suppression of the antioxidant system, and development of metabolic acidosis and energy deficit. The therapeutic effect of bemithyl was more pronounced in LR rats. However, metabolic indexes in bemithyl-treated rats did not reach the normal. After combined administration of pyrazidol and bemithyl, the metabolic state of the brain in treated rats did not differ from that in intact animals. Combined treatment with the test preparations most significantly normalized the content of LPO products, SOD activity, and amount of macroergic compounds in LR rats.

These data indicate that the phenotypic resistance to hypoxia plays a role in the formation of behavioral reactions and metabolic changes in the brain after CCT. Therefore, individuals differing in the resistance to hypoxia should receive individual pharmacological correction. Pyrazidol monotherapy for CCT consequences produced a “balanced” effect in HR rats. This preparation had an anxiolytic effect and reduced psychomotor excitation of HR rats in the posttraumatic period. However, pyrazidol produced an activating effect in LR animals. Bemithyl had the same effects in HR and LR rats with CCT. The preparation normalized individual behavioral characteristics and metabolism in the brain. However, bemithyl therapy was more preferable to LR animals. The mild psychostimulant effect of bemithyl in LR rats was more pronounced than in HR animals. During combined treatment, pyrazidol and bemithyl produced an additive corrective effect in HR and LR rats with CCT consequences. This treatment normalized behavioral reactions and prevented the

development of metabolic disturbances in the brain. Taking into account the additive effect of drugs, it cannot be excluded that the antidepressant pyrazidol can be used in a lower dose during combined treatment with bemithyl. Sometimes, it is impossible to evaluate phenotypic characteristics of the organism by the resistance to hypoxia. Then, the correction of disturbances in higher nervous activity and metabolic changes in the brain during CCT should involve combined treatment with the antidepressant pyrazidol and antihypoxant bemithyl.

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◆ Информация об авторах

Петр Дмитриевич Шабанов — д-р мед. наук, профессор, заведующий кафедрой фармакологии. ФГБВОУ ВО «Военно-медицинская академия им. С.М. Кирова», Санкт-Петербург. E-mail: pdshabanov@mail.ru.

Ирина Викторовна Зарубина — д-р биол. наук, профессор, старший преподаватель кафедры фармакологии. ФГБВОУ ВО «Военно-медицинская академия им. С.М. Кирова», Санкт-Петербург. E-mail: i.v.zarubina@inbox.ru.

◆ Information about the authors

Petr D. Shabanov — Dr. Med. Sci., Professor and Head, Department of Pharmacology. S.M. Kirov Military Medical Academy, Saint Petersburg, Russia. E-mail: pdshabanov@mail.ru.

Irina V. Zarubina— PhD, Dr. Biol. Sci. (Pharmacology), Professor, Senior Lecturer, Department of Pharmacology. S.M. Kirov Military Medical Academy, Saint Petersburg, Russia. E-mail: i.v.zarubina@inbox.ru.