Drug Correction of Behavioral Reactions and Metabolic Disorders in Rats with Craniocerebral Trauma

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Intraperitoneal injection of bemithyl in a dose of 25 mg/kg for 3 days after craniocerebral injury reduced psychopathological symptoms in rats with different resistance to acute hypoxia, restored the structure of individual behavior, and prevented metabolic disorders in the brain.

Key Words: *craniocerebral injury; bemithyl; functional and metabolic disorders*

Delayed effects of craniocerebral injury (CCI) appear as changes in the metabolism and functional activity of higher nervous activity, while their recovery depends, among other things, on individual resistance to acute hypoxia. Antihypoxic agent bemithyl (2-ethylthiobenzimidasole hydrobromide) characterized by a wide spectrum of actions rapidly and effectively eliminates psychopathological symptoms and metabolic disorders. Metabolic and physiological effects of bemithyl are mediated through stimulation of protein synthesis, energy metabolism and antioxidant systems. The effects of this drug on the basic cellular processes determine its use in the treatment and rehabilitation as the agent accelerating repair and recovery and correcting metabolic processes [3]. The psychotropic effect of bemithyl is used in the treatment of asthenic disorders [1].

We studied the effects of bemithyl on behavioral reactions and metabolic changes in the brain of rats with different resistance to acute hypoxia after CCI.

MATERIALS AND METHODS

Experiments were carried out on male albino rats (160-180 g). All animals were divided into groups of 8-10 rats with different resistance to acute hypoxia before the injury. For evaluation of the hypoxic resistance the animals were "elevated" in a pressure chamber to a simulated altitude of 12,000 m (50 m/sec assent rate) and exposed to these conditions until the appearance of agonal respiration. In low-resistant (LR) and highly resistant (HR) animals agonal respiration appeared after 5-10-min of >10-min, respectively. After 24 h closed CCI of medium severity was inflicted by dropping a 64-g block on the parietal area through a 80-cm tube with 1.3-cm diameter [8]. In order to rule out fractures of the jaws, the head was fixed on a soft pad. In case of depressed fracture of the parietal bone the animals died within the first minutes after injury and their brain was not examined. In experimental groups bemithyl was injected intraperitoneally once a day throughout the observation period (3 days) in a dose of 25 mg/kg. Control animals received 60 mg/kg piracetam (reference drug). The effect of the drug on the course of CCI was evaluated by animal survival and behavior, changes in body temperature, respiration rate, and biochemical values. Physiological reaction of rats with different hypoxic resistance to CCI was studied using the open field and elevated plus-maze tests. The orientation, exploratory, emotional, stereotypical, and motor components were taken into consideration in accordance with the behavioral atlas for rodents [6].

Energy metabolism in the brain was evaluated by the content of creatine phosphate [2], ATP, lactate, and pyruvate [9], and by activities of lactate dehydrogenase [5] and SDH [5] in brain tissue frozen in liquid nitrogen. LPO processes were evaluated by the content of LPO products (dienic conjugates and MDA) [8],

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TABLE 1. Effects of Piracetam and Bemithyl on Changes in Some Physiological Parameters in Rats after CCI ($M \pm m$, n=8)

Parameter		Before CCI	Immediately after CCI	24 h after CCI	3 days after CCI	3 days after CCI+ piracetam	3 days after CCI+ bemithyl
Body weight, g	HR	188.1±4.3	164.2±3.2*	177.2±4.4*	152.2±2.5*	168.3±4.9$^+$	179.1±3.2$^+$
	LR	185.8±3.2	166.1±3.3*	178.5±4.1*	156.1±3.3*	171.2±3.2$^+$	182.5±2.3$^+$
Respiration rate, min	HR	125±4	148±4*	168±3*	142±2*	138±3$^+$	132±4$^+$
	LR	127±3	139±5.3*	155±2*	128±5*	133±1$^+$	131±3$^+$
t, °C	HR	38.60±0.13	38.15±0.12*	37.63±0.12*	37.86±0.11*	38.19±0.11$^+$	38.48±0.11$^+$
	LR	38.54±0.11	38.10±0.12*	37.23±0.11*	37.69±0.14*	38.48±0.13$^+$	38.63±0.15$^+$

Note. $p<0.05$ compared to *rats without CCI; $^+$rats immediately after CCI.

and antioxidant system status was evaluated by the content of reduced glutathione [5] and SOD activity [4]. The enzyme activities were standardized by protein content in samples (Lowry method).

The data were processed statistically using Student's t test.

RESULTS

Short-term (2-4 sec) tonic and clonic convulsions and loss of reactions to all stimuli were observed immediately after infliction of CCI in both LR and HR animals; torpidity and lateral posture persisted for 10-20 sec. Respiration rate increased, body temperature and weight decreased because of spontaneous urination and defecation. Changes in these parameters were observed during the entire period of observation and were more pronounced in LR animals (Table 1). Treatment with piracetam and bemithyl for 3 days maintained the respiration rate and body temperature at the same level as in intact animals.

Cerebral vessels were sharply plethoric in 40% rats sacrificed 3 days after CCI; there were petechial or small focal hemorrhages in the dura mater and/or pia mater, hematomas in the cortex and cerebellum, most pronounced in LR animals. Pathomorphological changes in the brain were less pronounced in rats treated with piracetam and bemithyl. Piracetam and bemithyl therapy prolonged the life span of animals after CCI (Table 2).

Zoosocial behavior of animals is an integrative reflection of the functional and metabolic changes after CCI. Spontaneous motor activity decreased immediately after CCI. In subsequent hours (up to 3 days) episodes of high anxiety and autoaggression followed by stupor were observed in untreated animals. Inhibition of motor and orientation and exploratory activities were observed for 3 days after medium-severe CCI in both LR and HR rats. The open field test revealed decreased horizontal (number of crossed squares) and vertical activities (rearings). Testing in an elevated plus-maze revealed incomplete exits from sleeves with demonstrations of "risk evaluation" (stretched posture and hanging off), which attested to increased anxiety [10]. The behavior of HR and LR animals was different after the injury. Psychomotor inhibition, decreased volume of movement patterns, decreased autonomic manifestations of emotions, and higher anxiety were more pronounced in LR rats. The metabolic "portrait" of HR and LR animals after CCI was also different. Energy deficiency, lactate acidosis, enzymopathy, LPO activation, and inhibition of antioxidant systems were more pronounced in LR animals, which can be regarded as a risk group in case of injury (Table 3).

Treatment with bemithyl had a psychoactivating effect, which manifested in a decrease of psychopathological symptoms and recovery of reflexes and behavioral status of animals. Zoosocial realization of the orientation and exploratory reflex recovered and the coordination of motor and exploratory activities increased as soon as on day 1 after CCI in HR and even more so in LR rats treated with bemithyl. The number of rearings and duration of stay on the central platform of the elevated plus-maze attested to decreased anxiety

TABLE 2. Animal Survival on Day 3 after CCI

Group	HR			LR		
	survived	total	%	survived	total	%
CCI	18	24	72.7	15	25	60
CCI+piracetam	17	20	84.2	17	24	70.8
CCI+bemithyl	16	18	89.5	16	19	84.2

TABLE 3. Effects of Piracetam and Bemithyl on Energy Metabolism, LPO, and Antioxidant Systems in the Brain of Rats after CCI ($M \pm m$, n=8)

Parameter		Intact	3 days after CCI		
			without treatment	piracetam	bemithyl
Dienic conjugates, μmol/g	HR	21.58±0.26	47.62±0.31*	35.32±0.24+	28.11±0.21+
	LR	24.03±0.23	52.01±0.21*	43.43±0.23+	31.23±0.22+
MDA, μM/g	HR	6.35±0.17	10.13±0.12*	8.53±0.13+	7.14±0.17+
	LR	7.68±0.16	16.26±0.13*	9.82±0.12+	8.64±0.17+
Reduced glutathione, μmol/g	HR	42.02±0.19	21.64±0.30*	33.43±0.31+	35.86±0.27+
	LR	31.22±0.19	18.19±0.33*	22.42±0.34+	28.56±0.24+
SOD, A/mg protein	HR	3.11±0.09	1.08±0.05*	2.24±0.06+	2.45±0.03+
	LR	2.09±0.05	1.14±0.07*	1.92±0.04+	2.28±0.04+
Creatine phosphate, μmol/g	HR	4.11±0.05	2.91±0.02*	3.12±0.08+	3.83±0.04+
	LR	3.82±0.04	1.93±0.03*	2.99±0.05+	3.54±0.02+
ATP, μmol/g	HR	3.48±0.12	2.08±0.08*	3.83±0.11+	3.16±0.07+
	LR	2.83±0.14	1.94±0.10*	2.22±0.07+	2.88±0.05+
Lactate, μmol/g	HR	2.11±0.05	6.15±0.08*	3.67±0.05+	3.16±0.07+
	LR	3.72±0.06	5.41±0.06*	4.21±0.07+	3.42±0.06+
Pyruvate, μmol/g	HR	0.36±0.01	0.07±0.01*	0.21±0.01+	0.31±0.01+
	LR	0.27±0.02	0.05±0.01*	0.17±0.01+	0.25±0.01+
Lactate dehydrogenase, μmol NADH/min/mg protein	HR	1.08±0.04	3.13±0.08*	2.53±0.07+	1.53±0.03+
	LR	1.27±0.05	4.06±0.07*	3.06±0.05+	1.57±0.03+
SDH, nmol succinate/min/mg protein	HR	8.45±0.20	3.71±0.12*	4.14±0.09+	6.21±0.05+
	LR	7.22±0.24	3.72±0.13*	4.65±0.07+	6.29±0.05+

Note. $p<0.05$ compared to *intact animals, +day 3 after CCI.

in rats of both groups. The time of restitution after CCI was shorter in HR rats compared to LR animals.

In addition, piracetam and bemithyl promoted normalization of the macroerg pool, activation of SDH, elimination of metabolic acidosis, prevention of excessive lipoperoxidation, and stabilization of the antioxidant systems activation in the brain of both LR and HR rats. The metabolic changes were more pronounced in rats treated with bemithyl than in those treated with piracetam. Bemithyl more effectively prevented accumulation of primary and secondary LPO products, decrease in reduced glutathione content and SOD activity in the brain. Both drugs similarly reduced the level of lactate acidosis, but bemithyl better maintained the levels of creatine phosphate and ATP in the brain. Positive metabolic changes in rats treated with bemithyl were more pronounced in LR animals.

Hence, individual resistance to acute hypoxia is an important factor during the early period after CCI. Bemithyl therapy promoted harmonic correction of psychopathological and metabolic disorders after brain trauma. Bemithyl reduces the strain in the functioning of metabolic pathways in LR animals, which allows us to recommend it as an effective adaptogene improving individual resistance after CCI.

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