PHARMACOLOGY AND TOXICOLOGY

Bemithyl Potentiates the Antioxidant Effect of Intermittent Hypoxic Training

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The rats were adapted to hypoxic hypoxia by intermittent training in a flow pressure chamber for 3 days. The course of bemithyl treatment (25 mg/kg intraperitoneally, 3 days) started immediately after the 1st day of training. Bemithyl potentiated the adaptive metabolic changes in rat brain induced by repeated hypoxic hypoxia, increased the individual resistance to hypoxia, and produced a long-lasting effect.

Key Words: *intermittent hypoxic hypoxia; lipid peroxidation; antioxidant system; bemithyl*

Oxygen deficiency requires strong mobilization and strain of adaptive capacities of the organism. In contrast to chronic treatment, intermittent hypoxia considerably modulates homeostasis. It is directed toward the maintenance of stable state and optimal level of functional activity under changed living conditions [6]. Genetically and phenotypically determined individual differences in the resistance to hypoxia contribute to variations in the mechanisms of urgent and long-term adaptation. Oxygen deficiency training increases the resistance to hypoxia and is accompanied by adaptive changes. However, adaptation is a complex multilevel and long-term process [1]. High-altitude training in combination with administration of potent and rapidly acting pharmacological stimulators of adaptation (*e.g.* bemithyl, 2-ethylthio-benzimidazole hydrobromide) can potentiate and strengthen the effect of physiological mechanisms increasing the resistance to hypoxia and ensures optimal adaptive response (hypoxia-trained subjects included). The adaptive effect of bemithyl is associated with nonspecific stimulation of protein synthesis and activation of cell genome. These changes are required for long-term adaptation to high-altitude hypoxia [4]. The effect of high-altitude training can be increased and strengthened by combined treatment with bemithyl [5]. Energy metabolism and intensity of free radical processes determine the initial resistance of experimental animals to hypoxia and contribute to its improvement during adaptation. Previous studies showed that bemithyl inhibits nonenzymatic and enzymatic Fe^{2+}-induced lipid peroxidation (LPO) in metabolizing and non-metabolizing model systems. The existence of primary antioxidant activity of bemithyl was experimentally substantiated [3].

Here we studied whether bemithyl can potentiate the antioxidant effect of intermittent hypoxic training in rats with different resistance to hypoxia.

MATERIALS AND METHODS

Experiments were performed on male rats weighing 160-180 g. They were divided by the resistance to acute hypoxia in an altitude chamber. The rats were elevated to a simulated altitude of 12,000 m (50 m/sec) and were maintained under these conditions until the appearance of agonal breathing. The animals surviving for 5-10 and more than 10 min were considered to be low resistant (LR) and highly resistant (HR) to hypoxia, respectively.

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The rats were adapted to hypoxic hypoxia by intermittent training in a flow altitude chamber for 3 days. The course of one-day training included 6-fold ascent to 5000 m (15 m/sec) over 30 min. The interval between repeated sessions was 20 min. In the middle and before the end of each session, the animals were additionally elevated to an altitude of 6500 m and then descended to 5000 m. Bemithyl in an optimal effective dose of 25 mg/kg was injected intraperitoneally over 3 days. Bemithyl treatment started immediately after the 1st day of training. The control group included trained and untrained animals receiving an equivalent volume of physiological saline. The intensity of peroxidation was estimated by the content of conjugated dienes and malonic dialdehyde (MDA) in brain samples frozen in liquid nitrogen. The antioxidant system was studied by measuring activities of catalase [9] and superoxide dismutase (SOD) [2] and concentration of reduced glutathione [7].

The effectiveness of combined exposure to bemithyl and intermittent training was studied in 2 series. In series I the animals were exposed to acute hypoxia in the pressure chamber (altutide 8000 m, 50 m/sec, 30 min) 1 week after the end of training and bemithyl treatment. Untrained rats maintained under conditions of acute hypoxia served as the control. In series II closed craniocerebral trauma of moderate severity was produced 1 week after the end of training and bemithyl treatment. The weight of 64 g fell freely in a hollow tube (height 80 cm, diameter 1.3 m) to the parietal region of the head [8]. Untrained rats with craniocerebral trauma served as the control.

The results were analyzed by Student's *t* test.

RESULTS

Intermittent hypoxic training was accompanied by a decrease in the amount of LPO products in the brain of HR and LR rats (compared to the group of acute hypoxia). The concentration of conjugated dienes in the brain of HR and LR animals decreased by 14 and 16%, respectively (Table 1). The content of MDA in HR and LR rats was lower than in animals exposed to acute hypoxia (by 45 and 12%, respectively, $p<0.05$).

TABLE 1. Effect of Combined Exposure to Intermittent Training and Bemithyl Administration on LPO in Rat Brain (n=8, $M\pm m$)

Parameter, group		HR	LR
Conjugated dienes, μmol/g	intact	18.33±0.13	24.09±0.12
	hypoxia	25.75±0.26*	32.12±0.25*
	training	22.13±0.22*+	27.14±0.21*+
	training+bemithyl	19.11±0.17+	23.86±0.19+
MDA, μmol/g	intact	6.56±0.17	7.66±0.16
	hypoxia	16.69±0.24*+	19.47±0.21*+
	training+bemithyl	8.02±0.21+	7.47±0.13+

Note. Here and in Table 2: $p<0.05$: *compared to intact animals; +compared to acute hypoxia.

TABLE 2. Effect of Combined Exposure to Intermittent Training and Bemithyl Administration on the Antioxidant System in Rat Brain (n=8, $M\pm m$)

Parameter, group		HR	LR
Reduced glutathione, μmol/g	intact	42.09±0.69	31.12±0.19
	hypoxia (control)	23.10±0.23*	18.15±0.21*
	training	33.12±0.14*+	22.18±0.18*+
	training+bemithyl	39.57±0.15+	30.19±0.16+
SOD, A/mg protein	intact	3.11±0.09	2.09±0.05
	hypoxia	1.20±0.05*	0.86±0.07*
	training	2.22±0.04*+	1.11±0.06*+
	training+bemithyl	3.38±0.02+	2.87±0.04+
Catalase, μmol H_2O_2/mg protein/min	intact	5.91±0.52	3.19±0.33
	hypoxia	12.36±0.59*	1.46±0.19*
	training	8.32±0.19*+	2.13±0.17*+
	training+bemithyl	7.04±0.13+	2.56±0.18+

TABLE 3. Effect of Bemithyl on the Lifespan (min) of Rats during Acute Hypoxia (Altitude 11,000 m)

Group	HR	LR
Untrained	12.54±0.45	4.15±0.35
Trained	13.06±0.34*	4.97±0.27*
Training+bemithyl	14.78±0.35^{+}	7.92±0.28^{+}
Acute hypoxia, training+bemithyl	14.74±0.57	10.12±0.63^{x}

Note. $p<0.05$: *compared to untrained rats; $^{+}$compared to trained rats; ^{x}compared to the 1st episode of hypoxia.

Activity of the antioxidant system increased after intermittent hypoxic training (Table 2). The content of reduced glutathione in HR and LR rats increased by 45 and 22%, respectively. SOD activity in these animals increased by 85 and 29%, respectively. Acute hypoxia led to hyperactivation of cerebral catalase. After hypoxic training catalase activity decreased by 34% in HR rats, but increased by 46% in LR animals ($p<0.05$).

As differentiated from acute hypoxia, intermittent hypoxic training prevented accumulation of LPO products and inhibition of the antioxidant system in HR and LR rats. It should be emphasized that activation of catalase and SOD in the brain of trained rats was accompanied by an increase in the concentration of conjugated dienes and MDA. The content of reduced glutathione in these rats was lower than in intact animals. The observed changes were most pronounced in LR rats.

Administration of bemithyl in combination with hypoxic training modulated hyperactivation of LPO in the brain of HR and LR rats. The concentration of primary LPO products (conjugated dienes) in the brain of HR and LR rats decreased by 14% compared to trained animals not receiving bemithyl ($p<0.05$). The concentration of secondary LPO products (MDA) in HR and LR rats decreased by 13 and 56%, respectively. In HR and LR animals receiving bemithyl and exposed to hypoxic training, changes in LPO were accompanied by activation of the brain antioxidant system. The content of reduced glutathione in the brain of HR and LR rats increased by 19 and 36%, respectively ($p<0.05$). SOD activity in the brain of HR and LR rats increased by 52 and 159%, respectively. Administration of bemithyl in combination with hypoxic training prevented changes in catalase activity. Enzyme activity decreased by 15% in HR rats, but increased by 20% in LR animals (compared to hypoxic training without pharmacological treatment). It is important that brain catalase activity in LR rats reached the level typical of intact animals.

Our results show that bemithyl has a strong antioxidant effect under conditions of LPO hyperactivation. Administration of bemithyl in combination with intermittent hypoxic training increases its effectiveness and contributes to adaptive metabolic changes in the brain of rats with different resistance to hypoxia.

The role of bemithyl in adaptation to hypoxia was studied immediately after the last training session with acute hypoxia at an altitude of 11,000 m. We recorded the lifespan of animals. Untrained HR and LR rats survived at this altitude for 12.54 and 4.15 min, respectively (Table 3). After intermittent training the lifespan of HR and LR animals increased to 13.06 and 4.97 min, respectively. Combined exposure to training and bemithyl administration increased the lifespan of HR and LR rats by 13 and 59%, respectively (compared to trained animals not receiving bemithyl).

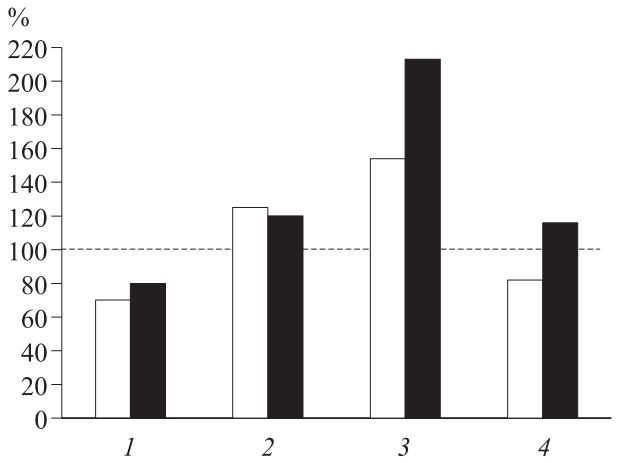

Fig. 1. Effect of preliminary exposure to intermittent hypoxic training and bemithyl administration on LPO and activity of the antioxidant system in rat brain during acute hypoxia. 100%, hypoxia. Here and in Fig. 2: MDA (*1*), glutathione (*2*), SOD (*3*), and catalase (*4*). Light bars: animals highly resistant to stress. Dark bars: animals low resistant to stress.

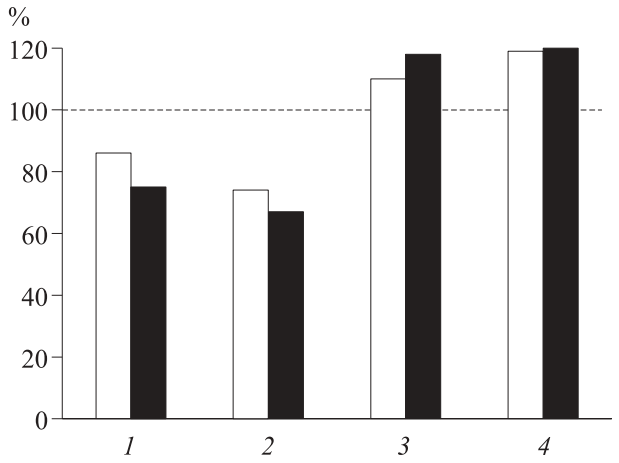

Fig. 2. Effect of preliminary exposure to intermittent hypoxic training and bemithyl administration on lipid peroxidation and activity of the antioxidant system in rat brain during craniocerebral trauma. 100%, untrained animals with craniocerebral trauma.

We measured the duration of the antihypoxic effect observed after combined exposure to hypoxic training and bemithyl administration. Survived rats were repeatedly exposed to acute hypoxia after 1 week. After bemithyl administration and hypoxic training the high-altitude lifespan remained unchanged in HR rats, but increased by 2 times in LR animals (compared to training without bemithyl administration). The preparation had a more pronounced and prolonged effect in LR rats. Bemithyl increased the lifespan of LR animals by 7% compared to that observed in the 1st hypoxic episode.

Acute hypoxia produced less significant metabolic changes in the brain of HR and LR rats exposed to training and bemithyl administration (compared to untrained animals not receiving the test preparation, Fig. 1). The content of conjugated dienes in the brain of HR and LR rats was lower than in control animals (by 17 and 15%, respectively). MDA concentration in HR and LR rats decreased by 30 and 20%, respectively. The amount of reduced glutathione in the brain of HR and LR rats receiving bemithyl was higher compared to untrained animals exposed to acute hypoxia (by 26 and 20%, respectively). After acute hypoxia SOD activity in the brain of HR and LR rats was higher than in untrained animals (by 54 and 113%, respectively). Catalase activity in HR and LR rats receiving bemithyl and exposed to hypoxic training exceeded the control by 18 and 16%, respectively.

We studied whether the proposed method of intermittent hypoxic training and pharmacological treatment with bemithyl can improve the resistance of rats with craniocerebral trauma. Metabolic changes in the brain of trained rats receiving bemithyl were less pronounced compared to control untrained animals with craniocerebral trauma (Fig. 2). The concentration of conjugated dienes in the brain of trained HR and LR rats receiving bemithyl was lower than in the control (by 25 and 33%, respectively, $p<0.05$). MDA concentration in the brain of HR and LR rats decreased by 14 and 24%, respectively. The content of reduced glutathione in the brain of HR and LR rats receiving bemithyl and exposed to training exceeded the control by 10 and 18%, respectively ($p<0.05$). SOD activity in the brain of HR and LR rats was higher than in control animals (by 19 and 20%, respectively).

Our findings indicate that the proposed method of intermittent hypoxic training produces adequate metabolic response of brain tissue in HR and LR rats to hypoxia. However, activity of the antioxidant system in trained rats is lower than in control animals. Bemithyl potentiates the adaptive effect of intermittent hypoxic training. The positive effect of bemithyl is more pronounced in rats low resistant to hypoxia, which contributes to an increase in the ratio of HR animals. Bemithyl should be considered as an adaptogen, which holds much promise for increasing individual resistance to free radical oxidation during hypoxia.

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