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Antioxidant effects of 2-ethylthiobenzimidazole and a succinic acid salt complex in hypoxia-trained rats during acute oxygen deprivation

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ABSTRACT

BACKGROUND: The body's response to hypoxia is largely determined by individual sensitivity. Available data show that both animal and human subjects with high resistance to hypoxia are less susceptible to its damaging effects on the brain, myocardium, liver, and kidneys.

AIM: To conduct an experimental study of the antioxidant effects (lipid peroxidation markers and antioxidant system activity in the brain) of 2-ethylthiobenzimidazole, a succinic acid salt complex (amosuccinate) and their combination to increase the individual resistance of the rat brain to hypoxia during interval hypoxic hypobaric training.

MATERIALS AND METHODS: Acute hypoxic hypobaric hypoxia in rats was induced in a flow pressure chamber. Animals were categorized according to their resistance to acute hypoxia by ascending them to an altitude of 11,000 m at a rate of 50 m/s and exposing them to this altitude until agonal respiration occurred. Rats withstanding hypoxia for 5–10 min were considered low-resistant, while those surviving for more than 10 min were high-resistant. The interval hypoxic training lasted three days and consisted of one-day training cycles with six ascends to an altitude of 5000 m (with 20-minute interval between ascends) at a rate of 15 m/s and exposure to this altitude for 30 min. A synthetic adaptogen, ethylthiobenzimidazole (Metaprot), at a dose of 25 mg/kg, and a succinic acid salt complex (amosuccinate), at a dose of 50 mg/kg, were administered intraperitoneally for three days immediately after each training cycle. The control group consisted of trained and untrained rats receiving 0.9% sodium chloride solution. In the brain, the concentration of lipid peroxidation products (diene conjugates, malondialdehyde) and the state of antioxidant systems (reduced glutathione, catalase and superoxide dismutase activity) were determined.

RESULTS: Acute hypoxia led to excessive lipid peroxidation and reduced activity of antioxidant systems. Ethylthiobenzimidazole and amosuccinate in combination with hypoxic training inhibited lipid peroxidation in the rat brain. The concentration of diene conjugates in the rat brain decreased by 12–26%, and malondialdehyde by 13–58%. The studied agents increased the levels of reduced glutathione by 42–76%, catalase by 1.5 times, and superoxide dismutase by 1.5–2.2 times. The combined effect of these agents was greater than their individual effects.

CONCLUSIONS: High-altitude training in combination with synthetic adaptogens (ethylthiobenzimidazole and amosuccinate) increases the adaptive capacity of the brain, as evidenced by longer survival at altitude, decreased excessive lipid peroxidation, and restored antioxidant systems.

Keywords: ethylthiobenzimidazole; amosuccinate; succinic acid salts; high-altitude hypoxia; adaptation; lipid peroxidation; antioxidant systems; brain, rats.

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Антиоксидантные эффекты 2-этилтиобензимидазола и комплекса солей янтарной кислоты у предварительно тренированных к гипоксии крыс при остром кислородном голодании

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АННОТАЦИЯ

Актуальность. Реакция организма на гипоксию в значительной степени определяется индивидуальной чувствительностью к ней. Показано, что субъекты с высокой устойчивостью к гипоксии (человек и животные) менее подвержены повреждающему действию гипоксии на мозг, миокард, печень, почки.

Цель — экспериментальное изучение антиоксидантных эффектов (показатели перекисного окисления липидов и активность антиоксидантных систем в головном мозге) 2-этилтиобензимидазола и комплекса солей янтарной кислоты (амосукцината) и их сочетания для повышения индивидуальной устойчивости мозга крыс к гипоксии в процессе интервальной гипоксической гипобарической тренировки.

Материалы и методы. Острую гипоксическую гипобарическую гипоксию у крыс вызывали в проточной барокамере. Животных разделяли по устойчивости к острой гипоксии, поднимая их в барокамере на высоту 11 000 м со скоростью 50 м/с и экспозицией на высоте до возникновения агонального дыхания. Крысы, выдерживающие воздействие гипоксии в течение 5–10 мин, считались низкоустойчивыми, более 10 мин — высокоустойчивыми. Курс интервальной гипоксической тренировки составлял 3 дня. Однодневный цикл тренировки состоял из 6-кратного подъема крыс со скоростью 15 м/с на высоту 5000 м и экспозицией на высоте в течение 30 мин. Интервал между подъемами — 20 мин. В работе использовали синтетический адаптоген этилтиобензимидазол (Метапрот) 25 мг/кг и комплекс солей янтарной кислоты (амосукцинат) 50 мг/кг, которые вводили внутрибрюшинно на протяжении 3 дней сразу после окончания однодневного цикла тренировки. Контрольную группу составляли тренированные и нетренированные крысы, получавшие 0,9 % раствор натрия хлорида. В головном мозге определяли содержание продуктов липопероксидации (диеновые конъюгаты, малоновый диальдегид) и оценивали состояние антиокислительных систем (содержание восстановленного глутатиона, активность каталазы и супероксиддисмутазы).

Результаты. Острая гипоксия вызывала чрезмерную липопероксидацию и снижение активности антиокислительных систем. Этилтиобензимидазол и амосукцинат в сочетании с гипоксической тренировкой препятствовали липопероксидации в головном мозге крыс. Содержание диеновых конъюгатов в головном мозге крыс снижалось на 12–26 %, малонового диальдегида — на 13–58 %. Препараты повышали содержание восстановленного глутатиона на 42–76 %, каталазы — в 1,5 раза, супероксиддисмутазы — в 1,5–2,2 раза. Эффект сочетанного применения этих препаратов был больше, чем у препаратов по отдельности.

Выводы. Высотные тренировки в сочетании с синтетическими адаптогенами (этилтиобензимидазол и амосукцинат) повышают адаптивные возможности мозга, что подтверждается как увеличением времени выживания на высоте, так и снижением чрезмерной липопероксидации и восстановлением антиокислительных систем.

Ключевые слова: этилтиобензимидазол; амосукцинат; соли янтарной кислоты; высотная гипоксия; адаптация; перекисное окисление липидов, антиокислительные системы; головной мозг; крысы.

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BACKGROUND

Oxygen deficiency (hypoxia) as one of the main typical pathogenic processes is considered to occur mainly in healthy people who are exposed to certain environmental conditions where the partial pressure of oxygen decreases, e.g., mountaineering, air travel, underwater, disaster recovery, etc. Hypoxia is known to affect all types of metabolism in the body, usually temporarily disrupting the functioning of its organs and systems and subsequently affecting a person's well-being. In hypoxic conditions, the brain, heart, kidneys, and liver suffer the most because of their high oxygen and glucose requirements, high levels of various lipids, and intensive organ metabolism. Brain hypoxia significantly reduces the body's resistance to oxygen deprivation and restructures all of its physiological and biochemical systems into a gentler (less intensive) mode of functioning [1]. The body's response to hypoxia is largely determined by an individual's sensitivity, which can be high and low. Subjects with high hypoxia tolerance (humans and animals) are shown to be less susceptible to the damaging effects of hypoxia on the brain, heart, liver, and kidneys than those with low tolerance [2].

AIM: To experimentally evaluate the antioxidant effects (lipid peroxidation and activity of brain antioxidant systems) of 2-ethylthiobenzimidazole and a complex of succinic acid salt (amosuccinate) and their combinations to increase individual brain tolerance to hypoxia during Interval hypoxic/hyperoxic hypobaric training in rats.

MATERIALS AND METHODS

Animal selection. The experiments were performed on 114 sexually mature white male crossbreed rats, weighing 160–180 g, obtained from the Rappolovo husbandry (Leningrad region, Russia). Rats were maintained in a housing room with standard lighting and food and ad libitum access to water and food.

Modeling of acute hypoxic hypobaric hypoxia and pulsed hypoxia training in rats. Acute hypoxic hypobaric hypoxia was induced by simulating conditions of high altitude exposure in a hyperbaric chamber for laboratory animals. Rats were classified according to their tolerance to acute hypoxia by being elevated to 11,000 m altitude in a hyperbaric chamber at a rate of 50 m/s and exposed to these conditions until agonal breathing occurred. Rats that could withstand hypoxia for 5–10 min were considered to have low hypoxia tolerance (LHT), and those that could withstand hypoxia for more than 10 min were considered to have high hypoxia tolerance (HHT). Moderate hypoxia was used to evaluate the metabolic effects of study agents and their combinations in acute oxygen deprivation, with no animal mortality observed in the hyperbaric chamber. For this purpose, rats were put to conditions of 8,000-meter altitude at 50 m/s and exposed for 30 minutes. This model was chosen due to its convenience in assessing the antihypoxic activity of pharmacological agents and its ability to analyze a wide range of hypoxic effects [2].

A special interval hypoxic training program was developed. To acclimate rats to hypoxia, interval training in a flow-through hyperbaric chamber was used for 3 days. The one-day training cycle consisted of 6 elevations to 5,000 meters at 15 m/s with 30 minutes of exposure. The interval between elevations was 20 minutes. At the middle and the end of the elevations, the rats were additionally elevated to 6,500 m and then lowered to 5,000 m.

Characteristics of pharmacological agents. The study used the synthetic adaptogen 2-ethylthiobenzimidazole hydrobromide (Metaprot) and a complex of succinic acid salts (Amosuccinate*). Metaprot is structurally similar to the purine bases of nucleic acids such as adenine and guanine, and has properties typical of adaptogen, antihypoxant, and actoprotector agents [3, 4]. A complex of succinic acid salts (laboratory code: amosuccinate) was chosen because, among the natural substrate metabolites, acid succinates are potent modulators of endogenous succinate receptors (SUCNR1), L-type calcium channels, and formation of active forms of steroids [5, 6].

The agents were administered intraperitoneally at the optimal effective dose (Metaprot 25 mg/kg, amosuccinate 50 mg/kg b.w.) for 3 days immediately after the end of the one-day training cycle. The control group included trained and untrained rats that received an equivalent volume of 0.9% normal saline.

Rats that received pharmacological support during training were exposed to acute hypoxia 1 week after the end of the training cycle to evaluate efficacy of synthetic adaptogens combined with the proposed interval training. Untrained rats exposed to acute hypoxia served as the control group.

Biochemical analysis of lipid peroxidation products (LPPs). Intensity of free radical processes in brain was assessed by concentrations of primary (diene conjugates of unsaturated fatty acids) and secondary (malondialdehyde) LPPs. The content of thiobarbituric acid (TBA)-binding products, as measured by malondialdehyde concentration, was determined after preparation of 10% homogenates of cerebral hemispheres in 25 mM Tris-HCl with 175 mM KCl buffer (pH 7.4) and protein precipitation in these homogenates. Diene conjugates were extracted from a 100 mg sample of brain tissue with 2 mL of a 1 : 1 mixture of heptane and isopropanol using the technique of Stalnaya [7, 8].

Methods for determination of antioxidant enzyme activity. The status of the brain's antioxidant system was assessed by the activity of catalase and superoxide dismutase (SOD). These enzymes prevent the excessive generation of hydrogen peroxide and superoxide radicals. Catalase activity was determined by the hydrogen peroxide decomposition reaction according to the Bergmeyer technique [9].

* The drug is not approved in Russia.

SOD activity was assessed by inhibition of nitroblue tetrazolium reduction in the presence of phenazine methosulfate [9]. The status of glutathione as the brain's antioxidant system was assessed by the level of reduced glutathione [10]. The activities of all the study enzymes were attributed to the protein content of the samples, determined by the standard technique of Lowry et al. [10].

Statistical analysis of the study results. Graph Pad Prizm v.6 software was used for statistical processing of the quantitative data. All data are presented as mean ± standard error of the mean ($M \pm m$). The Kolmogorov–Smirnov test was used to test the normality of the data distribution. In the case of normal distribution, one-way ANOVA analysis of variance was used to identify statistical differences between multiple groups. For comparisons between only two groups, paired Student's *t*-test for independent samples was used. If the distribution was not normal, a non-parametric equivalent of analysis of variance was used. In this case, the non-parametric Mann–Whitney test was used for pairwise comparisons. Differences were considered significant at the 95% significance level ($p < 0.05$).

RESULTS

The rat experiment used the method of short-term pulsed non-damaging hypoxic exposure of moderate intensity. Rats were preliminarily divided into HHT and LHT groups according to their tolerance to acute hypoxia, and survived an average of 12.15 min (HHT) and 5.12 min (LHT) at 11,000 m altitude. After interval hypoxic training, survival time at 11,000 m increased to 14.61 min in HHT rats and 6.79 min in LHT rats.

A course of treatment with ethylthiobenzimidazole 25 mg/kg increased survival time at altitude to 16.27 ± 0.27 min (+11%) in HHT rats and 8.98 ± 0.28 min (+32%, $p < 0.05$) in LHT rats compared to trained rats that did not receive treatment. A complex of succinic acid salts (amosuccinate) 50 mg/kg had little effect on survival which increased to 15.56 ± 0.28 min (+7%) in HHT rats and to 7.74 ± 0.22 min (+14%) in LHT rats. The combined use of ethylthiobenzimidazole with a complex of succinic acid salts provided a more significant increase in survival time at altitude to 20.46 ± 0.27 min (+40%) in HHT rats and to 15.28 ± 0.25 min (+125%) in LHT rats.

Ethylthiobenzimidazole and, to a lesser extent, succinic acid salts increased survival of HHT and LHT rats at 11,000 m with 30 min exposure. The combined use of ethylthiobenzimidazole and amosuccinate caused a greater and more significant increase in survival in terms of antihypoxic effect potentiation, which was more significant in LHT rats, and the results in LHT rats were similar to those in HHT rats.

After 7 days, surviving rats were subjected to repeated acute hypoxia to evaluate residual antihypoxic effects of the study agents. The agents and their combinations remained effective, as evidenced by survival time at altitude of HHT rats receiving ethylthiobenzimidazole and a two-fold increase in survival time of LHT rats compared to rats trained without the use of agents. In LHT rats, ethylthiobenzimidazole showed a more significant prolonged effect, increasing survival time by 27% compared to the first hypoxic episode. When exposed to repeated hypoxia, HHT rats trained with the use of a complex of succinic acid salts survived 8% longer at altitude than during the first hypoxic episode, while LHT rats survived 32% longer.

Therefore, the combined use of interval hypoxic training with the benzimidazole derivative ethylthiobenzimidazole enhances the antihypoxic effect of training, increases individual tolerance to hypoxia, and provides higher hypoxia tolerance in LHT rats. However, ethylthiobenzimidazole and, to a lesser extent, amosuccinate show a longer and more significant effect on LHT animals. This effect is enhanced by the combined use of ethylthiobenzimidazole and succinic acid salts.

Hypoxia is characterized by excessive lipid peroxidation [2]. Therefore, in order to understand this antihypoxic mechanism, synthetic adaptogens (ethylthiobenzimidazole and amosuccinate) were evaluated as pharmacological agents to enhance effectiveness of physiological methods in improving tolerance of rats to free radical processes of hypoxic origin. The study found that these agents, when combined with hypoxic training, prevented excessive lipid peroxidation in the rat brain. Ethylthiobenzimidazole decreased the brain levels of diene conjugates (the primary LPPs) in HHT and LHT rats by 14% and 12%, respectively ($p < 0.05$). Amosuccinate significantly decreased the brain levels of diene conjugates by 17% in HHT rats and by 26% in LHT rats. Hypoxic training with ethylthiobenzimidazole decreased the levels of malondialdehyde (the secondary LPP) by 13% and 56% in HHT and LHT rats, respectively, and hypoxic training with amosuccinate decreased the levels of malondialdehyde by 22% and 58%, respectively (Table 1).

After activation of brain antioxidant systems, changes in LPP processes were observed in both groups. Hypoxic training with ethylthiobenzimidazole significantly increased brain levels of reduced glutathione by 19% and 36% in HHT and LHT rats, respectively, and hypoxic training with amosuccinate increased brain levels of reduced glutathione by 22% and 60%, respectively. Interval hypoxic training with synthetic adaptogens was associated with an increase in the brain SOD activity in both groups. Ethylthiobenzimidazole increased the brain SOD activity in HHT and LHT rats by 52% and 159%, respectively, and amosuccinate increased the brain SOD activity by 45% and 187%, respectively ($p < 0.05$).

Synthetic adaptogens improved the activity of catalase, decreasing its brain activity in HHT rats and increasing its brain activity in LHT rats compared with hypoxic training without pharmacological support. In addition, ethylthiobenzimidazole

Table 1. Effect of ethylthiobenzimidazole and amosuccinate on lipid peroxidation and antioxidant system activity in the brain of rats trained to hypoxic hypoxia ($M \pm m$, $n = 10$)

Таблица 1. Влияние этилтиобензимидазола и амосукцината на процессы перекисного окисления липидов и активность антиоксидантных систем в головном мозге крыс, тренированных к гипоксической гипоксии ($M \pm m$, $n = 10$)

Parameters	Rat groups	High hypoxia tolerance	Low hypoxia tolerance
Diene conjugates, μmol/g	Training	23.61 ± 0.22	28.54 ± 0.21
	Training + ethylthiobenzimidazole	20.15 ± 0.19*	25.61 ± 0.19*#
	Training + ammosuccinate	21.11 ± 0.16*	26.52 ± 0.22*
	Training + ethylthiobenzimidazole + ammosuccinate	19.21 ± 0.21*	26.27 ± 0.22*
Malondialdehyde, μmol/g	Training	12.24 ± 0.17	15.12 ± 0.18
	Training + ethylthiobenzimidazole	9.12 ± 0.21*	10.71 ± 0.15*#
	Training + ammosuccinate	7.25 ± 0.17*	8.82 ± 0.17*
	Training + ethylthiobenzimidazole + ammosuccinate	6.89 ± 0.21*	7.99 ± 0.14*
Reduced glutathione, μmol/g	Training	35.17 ± 0.16	29.12 ± 0.19
	Training + ethylthiobenzimidazole	38.71 ± 0.15*	32.24 ± 0.17*#
	Training + ammosuccinate	36.55 ± 0.14*	32.51 ± 0.16*
	Training + ethylthiobenzimidazole + ammosuccinate	40.72 ± 0.14*	36.24 ± 0.16*
Superoxide dismutase, A/mg protein	Training	2.24 ± 0.05	1.89 ± 0.07
	Training + ethylthiobenzimidazole	2.45 ± 0.03*	2.24 ± 0.06*
	Training + ammosuccinate	2.34 ± 0.04*	2.11 ± 0.05*
	Training + ethylthiobenzimidazole + ammosuccinate	2.72 ± 0.04*	2.37 ± 0.06*
Catalase, μmol H_2O_2/min × mg protein	Training	8.32 ± 0.19*	2.13 ± 0.17*
	Training + ethylthiobenzimidazole	7.04 ± 0.13*	2.56 ± 0.18*#
	Training + ammosuccinate	6.43 ± 0.16*	4.12 ± 0.16*
	Training + ethylthiobenzimidazole + ammosuccinate	5.75 ± 0.15*	7.71 ± 0.14*

*$p < 0.05$ compared to the hypoxia-trained rats; #$p < 0.05$ compared to intact highly resistant rats.
*$p < 0,05$ по сравнению с группой тренированных к гипоксии крыс; #$p < 0,05$ по сравнению с интактными высокоустойчивыми крысами.

restored the catalase activity in LHT rats to levels typical of intact LHT animals. After the use of amosuccinate, the catalase activity was not significantly different from that found in brains of intact HHT rats.

Therefore, the addition of these agents to interval hypoxic training improves effects of training, enhances adaptive metabolic brain changes in rats with various individual tolerance to hypoxia, and increases the proportion of HHT animals in a mixed population.

Acute hypoxia prevented excessive lipid peroxidation in brain tissue in trained rats protected by synthetic adaptogens. For example, after the use of ethylthiobenzimidazole, brain levels of diene conjugates were 17% lower in HHT rats and 15% lower in LHT rats compared to the control group. In acute hypoxia, prior hypoxic training with amosuccinate decreased brain levels of dienes in HHT and LHT rats by 22% and 25%, respectively, and prior use of ethylthiobenzimidazole and amosuccinate decreased brain levels of dienes by 28% and 39%, respectively (Table 2).

However, the level of malondialdehyde (the secondary LPP) decreased. In acute hypoxia, prior interval hypoxic training with ethylthiobenzimidazole decreased brain malondialdehyde levels in HHT and LHT rats by 33% and 22%, respectively, and prior hypoxic training with amosuccinate decreased brain malondialdehyde levels by 42% and 47%, respectively, and the use of ethylthiobenzimidazole and amosuccinate decreased brain malondialdehyde levels by 54% and 56%, respectively ($p < 0,05$).

In acute hypoxia, prior pulsed hypoxic training with pharmacological support using synthetic adaptogens maintained the higher activity of brain antioxidant systems in rats with various tolerance to hypoxia than in the control group. In acute hypoxia, after the use of ethylthiobenzimidazole, brain levels of reduced glutathione were 26% and 15% higher in HHT and LHT rats, respectively, than in untrained rats.

In acute hypoxia, after the use of ethylthiobenzimidazole, the brain levels of reduced glutathione were 26% and 15% higher in HHT and LHT rats, respectively, than in untrained rats. In acute hypoxia, after pre-training with ethylthiobenzimidazole and amosuccinate, brain glutathione levels in HHT and LHT rats were 71% and 83% higher, respectively, than in untrained rats.

Table 2. Effect of acute hypoxia on lipid peroxidation and antioxidant system activity in the brain of rats trained with synthetic adaptogens ($M \pm m$, $n = 10$)

Таблица 2. Влияние острой гипоксии на процессы перекисного окисления липидов и активность актиоксидантных систем в головном мозге крыс, тренированных на фоне синтетических адаптогенов ($M \pm m$, $n = 10$)

Parameters	Rat groups	High hypoxia tolerance	Low hypoxia tolerance
Diene conjugates, μmol/g	Hypoxia – control	25.75 ± 0.66	32.12 ± 0.25
	Training + ethylthiobenzimidazole + hypoxia	21.42 ± 0.19*	27.23 ± 0.16*
	Training + ammosuccinate + hypoxia	20.11 ± 0.18*	24.17 ± 0.21*
	Training + ethylthiobenzimidazole + ammosuccinate + hypoxia	18.52 ± 0.19*	19.54 ± 0.23*
Malondialdehyde, μmol/g	Hypoxia – control	16.69 ± 0.24	19.47 ± 0.21
	Training + ethylthiobenzimidazole + hypoxia	11.14 ± 0.23*	15.23 ± 0.19*
	Training + ammosuccinate + hypoxia	9.76 ± 0.19*	10.24 ± 0.16*
	Training + ethylthiobenzimidazole + ammosuccinate + hypoxia	7.64 ± 0.21*	8.57 ± 0.15*
Reduced glutathione, μmol/g	Hypoxia – control	23.10 ± 0.23	18.15 ± 0.21
	Training + ethylthiobenzimidazole + hypoxia	29.16 ± 0.17*	20.94 ± 0.18*
	Training + ammosuccinate + hypoxia	34.47 ± 0.18*	25.79 ± 0.16*
	Training + ethylthiobenzimidazole + ammosuccinate + hypoxia	39.58 ± 0.19*	33.25 ± 0.15*
Superoxide dismutase, A/mg protein	Hypoxia – control	1.20 ± 0.05	0.86 ± 0.07
	Training + ethylthiobenzimidazole + hypoxia	1.85 ± 0.04*	1.83 ± 0.06*
	Training + ammosuccinate + hypoxia	2.64 ± 0.06*	2.35 ± 0.05*
	Training + ethylthiobenzimidazole + ammosuccinate + hypoxia	2.86 ± 0.05*	2.79 ± 0.04*
Catalase, μmol H_2O_2/min × mg protein	Hypoxia – control	12.36 ± 0.15	1.46 ± 0.19
	Training + ethylthiobenzimidazole + hypoxia	10.12 ± 0.14*	1.69 ± 0.17*
	Training + ammosuccinate + hypoxia	8.32 ± 0.17*	3.52 ± 0.18*
	Training + ethylthiobenzimidazole + ammosuccinate + hypoxia	6.53 ± 0.15*	4.18 ± 0.15*

*$p < 0.05$ compared to the control group (acute hypoxia).
*$p < 0,05$ по сравнению с контрольной группой (острая гипоксия).

In acute hypoxia, after hypoxic training with ethylthiobenzimidazole, the brain SOD activity was shown to be 54% and 11% higher in HHT and LHT rats, respectively, than in untrained rats. After hypoxic training with amosuccinate, the brain SOD activity was 120% and 173% higher in HHT and LHT rats, respectively, than in controls ($p < 0.05$). In acute hypoxia, after hypoxic training with this combination of agents, the brain SOD activity remained 138% and 224% higher in HHT and LHT rats, respectively, than in untrained rats ($p < 0.05$).

After pulsed hypoxic training with synthetic adaptogens, acute hypoxia caused less significant suppression of the brain catalase activity in LHT rats and activated the brain catalase activity in HHT rats. After prior hypoxic training with ethylthiobenzimidazole, the brain catalase activity was 18% lower in HHT rats and 16% higher in LHT rats compared to the control group ($p < 0.05$). Hypoxic training with amosuccinate decreased brain catalase activity by 33% in HHT rats and increased it by 141% in LHT rats. The combination of these agents showed a more significant effect, reliably decreasing brain catalase activity by 47% in HHT rats and increasing it by 186% in LHT rats.

Therefore, the study of potential pharmacological improvement of physiological ways to increase individual tolerance of rats to hypoxia using synthetic adaptogens revealed the effectiveness of hypoxic training combined with the study agents in improving the energy disorders and the processes of excessive lipid peroxidation in the brain of rats with different hypoxia sensitivity.

DISCUSSION

When evaluating metabolic changes during hypoxia under the action of synthetic adaptogens (ethylthiobenzimidazole and amosuccinate), it should be noted that each of them is characterized by a certain profile of pharmacological activity, despite significant differences in increasing individual tolerance to hypoxic conditions.

First of all, as for the selection of pharmacological substances to be evaluated, 2-ethylthiobenzimidazole

hydrobromide, bemethyl, is currently widely used in pharmacology and medicine as an antihypoxant and actoprotector. For a long time it was one of the authorized agents of the Ministry of Defense of the USSR and the Russian Federation as an agent that increases physical and, to a lesser extent, mental performance in humans [2–4]. Ethylthiobenzimidazole has been shown to have typical antihypoxic properties that are more significant under chronic hypoxia. Amosuccinate is a complex of succinic acid salts. In medicine, succinic acid salts (succinates) are found in agents such as Mexidol (3-oxypyridine succinate) or Cytoflavin (a compound based on succinic acid salts). They are used as biologically active dietary supplements due to their long history of use in food [5, 6, 11]. Pharmacologically, succinates have antihypoxic and adaptogenic effects. Doses of succinate in pharmacological agents and dietary supplements vary, reaching gram concentrations per single dose in some supplement formulations. The developers of such agents believed that exogenous succinate could partially restore the body's impaired glucose metabolism (glycolysis) that occurs during hypoxia. According to the bioenergetic concept of hypoxia [1], during oxygen deprivation, glycolysis does not proceed properly, under-oxidized products such as lactate and pyruvate accumulate, and the Krebs cycle, which ensures the formation of ATP as the universal energy source, fails. Therefore, high doses of succinate are suggested to optimize the Krebs cycle, acting as a kind of energy donor. However, it should be mentioned that since all salts of succinic acid in a liquid medium dissociate into a cation and a succinate anion, succinate does not easily penetrate biological barriers (stomach walls, capillary walls, cell membranes, mitochondria, etc.), and exogenous succinate has an extremely low potential to enter mitochondria and, more importantly, the Krebs cycle. With the discovery of succinate-sensitive receptors (SUCNR1), it became possible to explain the effects of succinate as a signaling molecule that triggers a cascade of intracellular mechanisms activating the nuclear apparatus and mitochondrial function [1, 6]. The question then arose as to what doses of succinate and what types of succinic acid salts were most preferable to ensure this function. When asked about doses, one can confidently answer that the doses of oral succinic acid salts should be low, approximately 25–100 mg/kg. As for salts, they should preferably be formed by combining a weak acid (succinate) and a weak base, such as ammonia (NH_4OH). It should be noted that succinic acid obtained from natural amber showed the greatest pharmacological activity. Natural substrate metabolites in the form of acidic succinate salts are shown to be potent modulators of orphan receptors and SUCNR1 receptors. They are also capable of activating L-type calcium channels, promoting intracellular Ca^{2+} accumulation across the endoplasmic and sarcoplasmic reticulum and mitochondria, and regulating the limiting step in cholesterol metabolism, entry into mitochondria and subsequent biotransformation into active forms of steroids [5]. Therefore, as demonstrated in this paper, the choice of amosuccinate in the form of a complex of succinic acid salts at 50 mg/kg as a potential antihypoxant fits well within this concept.

In addition, the aim of the study was not only to evaluate the antihypoxic activity of ethylthiobenzimidazole and amosuccinate, but also their potential to modulate the adaptive effects of interval hypoxic training (preconditioning), which we have addressed in previous papers [7, 12]. In fact, low doses of the agents (ethylthiobenzimidazole 25 mg/kg and amosuccinate 50 mg/kg) had a potentiating effect on the adaptive effects of pulsed interval hypoxic training. This indicates the correct choice of hypoxic training conditions and pharmacological support, which made it possible to obtain synergistic effects of both, known as summation and potentiation.

CONCLUSION

1. Interval hypobaric training induces an appropriate metabolic response to hypoxia in rat brain tissue, preventing excessive lipid peroxidation and activating antioxidant defense enzymes.

2. Synthetic adaptogens (ethylthiobenzimidazole and amosuccinate) enhance antihypoxic effects of interval hypobaric training, increasing survival time of trained rats in acute hypoxia and adaptive metabolic brain changes in HHT and LHT animals.

3. The beneficial effects of synthetic adaptogens (ethylthiobenzimidazole and oxyethylammonium methylphenoxyacetate) are more significant in LHT rats, which contributes to an increase in the proportion of HHT rats in the general population.

4. The agents can be ranked as follows, in increasing order of effectiveness: Ethylthiobenzimidazole < Amosuccinate < Ethylthiobenzimidazole + Amosuccinate.

ADDITIONAL INFO

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