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Efficacy of ethylmethylhydroxypyridine succinate derivative in a model of intermittent hypoxia in comparison with the reference drug

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Abstract

Introduction. Intermittent hypoxia (IH) promotes oxygen free radical oxidation, which may precede many diseases. Decreased physical activity, ischaemic processes in organs and disturbances at the cellular level, may be a consequence of intermittent hypoxia. It is important to search for potential drugs to correct this process.

Aim. Comparative study of the efficacy of the active metabolite of ethylmethylhydroxypyridine succinate (EMHPS), ethylmethylsulfapyridine (EMSP), with a native molecule in a model of IG in mice.

Materials and methods. Test subjects were administered intraperitoneally for 14 days – EMSP was administered at a dose of 85 mg/kg, Mexidol® – at a dose of 100 mg/kg. Prolonged intermittent hypoxia was reproduced by placing animals in a membrane hypoxifier. The following conditions have been set for 14 days: 6 % – oxygen content in the hypoxic chamber, duration – 6 hours. The effect of the drug on dynamic load (grip strength test), respiratory parameters (plethysmograph parameters), behavioral and cognitive parameters (open field and elevated plus maze tests), heart rate and venous oxygen saturation were evaluated, and the potential mechanism of action was studied by real-time PCR.

Results and discussion. It was found that EMSP was effective in terms of plethysmography parameters, in particular, it helped the body adapt to chronic hypoxic effects, which resulted in significant differences in inhalation and exhalation parameters from the control group. The study of behavioral and cognitive states revealed the presence of anxiety, decreased exploratory activity and increased mobility of animals in all groups. These parameters were less pronounced for animals treated with EMSP and Mexidol® than in the control group. There was a tendency to increase the expression of a gene affecting the ubiquinol-cytochrome c-reductase complex, which is a part of mitochondrial respiration.

Conclusion. According to the results of the study, EMSP showed comparable protective properties with a native molecule EMHPS. There was also a tendency to increase the stimulation of UQCRC2 gene against the background of EMSP administration compared with EMHPS.

Keywords: intermittent hypoxia, 3-oxypyridine, ethylmethylsulfopyridine, mice, UQCRC2

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Эффективность производного этилметилгидроксипиридина сукцината на модели интермиттирующей гипоксии в сравнении с референтным препаратом

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Резюме

Введение. Интермиттирующая гипоксия (ИГ) способствует свободнорадикальному окислению кислорода, что может предшествовать многим заболеваниям. Снижение физической активности, ишемические процессы в органах и нарушения на клеточном уровне, могут быть следствием прерывистой гипоксии. Актуальным является поиск потенциальных ЛС для коррекции данного процесса.

Цель. Сравнительное изучение эффективности активного метаболита этилметилгидроксипиридина сукцината (ЭМГПС) – этилметилсульфапиридина (ЭМСП), с нативной молекулой на модели ИГ у мышей.

Материалы и методы. Введение исследуемых объектов осуществляли внутрибрюшинно в течение 14 дней – ЭМСП вводили в дозе 85 мг/кг, Мексидол® – в дозе 100 мг/кг. Длительную интермиттирующую гипоксию воспроизводили путем помещения животных в мембранный гипоксикатор. Устанавлен следующий режим в течение 14 дней: 6 % – содержание кислорода в гипоксической камере, продолжительность – 6 часов. Оценивали влияние препарата на динамическую нагрузку (тест «сила хвата»), параметры дыхания (показатели плетизмографа), поведенческие и когнитивные показатели (тесты «открытое поле» и «приподнятый крестообразный лабиринт»), частоту сердечных сокращений и насыщение венозной крови кислородом, а также изучали потенциальный механизм действия методом ПЦР realtime.

Результаты и обсуждение. Было выявлено, что ЭМСП проявлял эффективность по параметрам плетизмографии, в частности помогал адаптироваться организму к хроническому гипоксическому воздействию, что выражалось в значимых отличиях по показателям вдоха и выдоха от контрольной группы. Исследование поведенческих и когнитивных состояний выявили наличие тревожности, снижение исследовательской активности и увеличение подвижности животных во всех группах. У животных, которым вводили ЭМСП и Мексидол® данные параметры были менее выражены, чем в контрольной группе. Была отмечена тенденция к увеличению экспрессии гена, влияющего на комплекс убихинол-цитохром с-редуктазы, являющимся частью митохондриального дыхания.
Заключение. Согласно результатам исследования, ЭМСП продемонстрировал защитные свойства, сравнимые с нативной молекулой ЭМГПС. Также была выявлена тенденция к усилению стимуляции гена UQCRC2 на фоне введения ЭМСП по сравнению с ЭМГПС.

Ключевые слова: интермиттирующая гипоксия, 3-оксипирид, этилметилсульфопиридин, мыши, UQCRC2

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. С. В. Оковитый – идея и планирование эксперимента. Е. М. Евгеньева – моделирование интермиттирующей гипоксии. Е. М. Евгеньева – введение препаратов. Г. А. Плиско – обработка данных. Е. М. Евгеньева – проведение тестов «ПКЛ» и «ОТ». Е. М. Евгеньева – измерение ЧСС, сатурации и функции дыхания. Н. С. Курмазов, Т. А. Пятиизбянцев – измерение экспрессии генов методом ПЦР в реальном времени. Д. Ю. Ивкин, Г. А. Плиско, В. Ц. Болотова, Е. М. Евгеньева – подготовка и редактура рукописи. М. В. Журавлева, М. А. Тихая, Е. Е. Падерина – перевод статьи на английский язык.

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INTRODUCTION

Intermittent hypoxia (IH) is a pathological factor that contributes to the generation of oxygen free radicals that precede many pathological processes. One of the most frequent manifestations of IH is obstructive sleep apnea syndrome [1, 2], that can be a predictor of such diseases as: diabetes mellitus, hypertension and heart failure [3, 4]. Mostly, sleep apnea is caused by mechanical obstruction of the upper airways. Lack of oxygen leads to IH, which is terminated due to reflex respiratory movements. However, the quality of sleep is impaired, resulting in sleepiness, cognitive decline and stress-induced activation of the sympathetic nervous system [3]. The latter leads to the release of adrenaline and cortisol, which can cause decreased insulin sensitivity [5]. IH provokes neuroinflammation with subsequent behavioral and neuropsychological disorders in the central nervous system (CNS) [6].

IH is one of the pathophysiological processes of chronic cerebral ischemia, causing structural damage to neurons and CNS dysfunction [7]. Brain tissue damage in CH is most likely due to changes in the functioning of ion channels, glutamate excitotoxicity, increased production of proinflammatory cytokines, and the formation of chronic neuroinflammation [8, 9].

Mainly, IH can initiate a cascade of pathological conditions characterised by sympathetic activation, mitochondrial dysfunction, inflammation, oxidative stress, endothelial dysfunction and metabolic disorders [10].

Due to the prevalence of IH influence on various pathological processes, the study of antihypoxants and antioxidants is relevant [11]. Among substances possessing these properties are 3-oxypyridine derivatives, including: ethylmethylhydroxypyridine succinate (EMHPS), ethylmethylhydroxypyridine malate, etc. [12–14].

In this paper we studied antihypoxant properties of the metabolite of EMHPS – ethylmethylsulfapyridine (EMSP) on the model of IH in mice. In previous studies, EMSP showed antihypoxant and antioxidant properties [15, 16]. In addition, the effects on the central nervous system and dynamic load, as well as the possible mechanism of action of EMSP were evaluated.

The aim was to compare the efficacy of the active metabolite of ethylmethylhydroxypyridine succinate (EMHPS), ethylmethylsulfapyridine (EMSP), with a native molecule in a model of IG in mice.

MATERIALS AND METHODS

The screening study was conducted on a total of 18 white laboratory outbred male mice weighing 20–22 g, obtained from the laboratory animal nursery of FGUP PLJ "Rappolovo".

Animals were kept in polysulfone type IV cages with steel lattice lids with a feeding hollow and a steel feed divider. The air temperature throughout the experiment in the animal housing was 22 ± 2 °C, the light regime was 12 h of light and 12 h of darkness. The animals were provided free access to feed and water throughout the experiment. The feed for keeping laboratory animals, corresponded to ISO 34566-2019 "Complete feeds for laboratory animals". Animals were given purified and normalized by organoleptic properties water based on SanPiN (Sanitary Regulations and Standarts) 2.1.3684-21 "Sanitary and epidemiological requirements for the maintenance of urban and rural settlements, water bodies, drinking water and drinking water supply, air, soil, living quarters, operation of industrial and public premises, organization and conducting of sanitary and anti-epidemic (preventive) measures".

The animals were kept in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes.

Randomisation was performed according to premeasured saturation indices (Table 1). Three groups were formed in the study: Group No. 1 – control ($n = 6$), animals were modelled IG and intraperitoneal injected with physiological solution (NaCl 0.9 %); Group No. 2 – experimental ($n = 6$), animals were modelled IH and intraperitoneal administered the studied compound – EMSP at a dose of 85 mg/kg for 14 days; Group No. 3 – experimental ($n = 6$), animals were modelled IH and intraperitoneal administered a comparison drug – Mexidol® (active ingredient – EMHPS) at a dose of 100 mg/kg for 14 days.

Table 1. Group distribution

Group No. 1 (Control), %	Group No. 2 (EMSP), %	Group No. 3 (EMHPS), %
78.1 ± 11,4	78.0 ± 4,3	78.2 ± 4,04

The doses of EMSP and Mexidol® were taken based on the results of previous studies [15].

Long-term intermittent normobaric hypoxia was modelled by placing animals in a 75-litre membrane hypoxicator – BIO-NOVA-2004 (Russia) adapted for rodents. The following conditions were set: oxygen content in the hypoxic chamber – 6 %, duration of a single hypoxic cycle – 6 hours. The duration of daily hypoxic exposure –14 days. Oxygen content in the chamber was monitored using a PKG-4 series gas analyzer (CJSC "EXIS", Russia). The investigated substances were administered 30 min before placing the animals in the hypoxic chamber.

Before the administration of the studied compounds and modeling of IH, the animals were assessed for dynamic load, as well as respiratory parameters, behavior parameters, heart rate (HR) and venous oxygen saturation (SpO_2) were measured. These parameters were also measured after the end of administration of the study compounds. The clinical condition of the animals was checked daily and body weight was measured weekly. After euthanasia in animals of all groups, the expression of mitochondrial complex genes in the liver was determined by real-time polymerase chain reaction (real-time PCR) and blood biochemical examination was performed.

Dynamic load was measured in the "grip strength" test. The study was performed using the Grip Strength Meter system manufactured by TSE Systems (Germany), which is suitable for conducting experiments on small rodents. Muscle grip strength of the forelegs was measured by recording the force required for the animal to unclench its fingers (gram – power) in three consecutive determinations. The measurement was performed on both forelegs separately and simultaneously.

To assess respiratory function, animals were placed one at a time in a single-chamber barometric thoracic plethysmograph (EMKA Technologies, France) ventilated with a continuous flow of air at 2 L/min (EMKA Technologies, France) in which they were allowed to move freely. A differential pressure sensor was connected to the chamber and the pressure signals were amplified, digitized and sampled at a rate of 100 Hz using validated and dedicated software (IOX version 1.7.0, EMKA Technologies, France). Before each measurement, calibration was performed according to the manufacturer's recommendations by injecting 20 ml of room temperature air into the chamber. The following parameters were analyzed (ANALYST version 1.49, EMKA Technologies, France): inspiratory duration (Ti), expiratory duration (Te), maximal inspiratory flow rate (PIF), maximal expiratory flow rate (PEF), inspiratory volume (TV), expiratory volume (EV), relaxation time (RT), minute volume (MV), respiratory movement rate (RR) (f), end-inspiratory pause (EIP), end-exhalation pause (EEP), estimated pause (Penh), mid-expiratory flow rate corresponding to 50 % of the exhaled volume (EF50).

The behavioral assessment was performed using the Open Field Test (OFT) and the Elevated Plus Maze (EPM).

The OFT setup consisted of a circular platform with a diameter of 63 cm, containing 13 holes in the floor and divided into 19 equal sections with a designated central zone. Each animal was placed in the center of the platform, facing away from the experimenter. During a three-minute observation period, the following parameters were recorded using video tracking: the number of crossed sectors, the number of explored sectors, rearings, burrow pokeings, grooming episodes, total distance traveled, velocity and the percentage of time spent in motion.

The EPM apparatus contains two open arms and two enclosed arms of equal dimensions, elevated 75 cm above the floor. During testing, each animal was placed in the central platform facing an open arm. The 5-minute test session was followed by returning the animal to its home cage. The following parameters were recorded: time spent in open and closed arms, number of crossed squares, number of rearing, head-dipping behavior and grooming episodes. Following each trial, the maze surfaces were thoroughly cleaned with Lysaxin spray to eliminate olfactory cues.

Heart rate (HR) and venous oxygen saturation (SpO_2) were monitored using a pulse oximeter developed at Saint Petersburg Electrotechnical University «LETI» (V. I. Ulyanov). Animals were temporarily restrained while a clip sensor was attached to the tail. The sensor has two light sources: 660 nm (red) and 940 nm (infrared). The photodetector registers the light level after partial absorption of the flow by tissues and blood components and the microprocessor analyzes the obtained results and determines SpO_2 and HR.

The animals were euthanized by decapitation. Due to the influence of other euthanasia methods (a CO_2 box with carbon dioxide air replacement or the administration of high doses of narcotic drugs) on the postmortem study results.

Mitochondrial gene expression in liver tissue was analyzed using real-time polymerase chain reaction (real-time PCR). Following euthanasia, liver samples were collected for total RNA extraction using the "Extract-RNA" (JSC "Evrogen", Russia). The RNA concentration in the final solution was measured immediately after extraction using a "NanoPhotometer N50" spectrophotometer (Implen GmbH, Germany). Next, complementary DNA (cDNA) was synthesized from the RNA template using the "MMLV RT kit" (JSC "Evrogen", Russia). Finally, a reaction was performed to synthesize complementary DNA strands from the cDNA template at primer annealing sites. Fluorescence of the product was provided by the fluorescent dye SYBR Green (JSC "Evrogen", Russia). Each sample was measured in duplicate alongside no-template controls. Amplification data was analyzed using the $2^{-\Delta\Delta CT}$ method to determine fold changes in gene expression between experimental and control samples. Melting curve analysis was conducted to identify nonspecific amplification products. Samples showing multiple peaks were excluded from further analysis. Target genes included: NDUFS1 – encodes the largest subunit of Complex I responsible for electron transfer from NADH to the respiratory chain; UQCRC2 – encodes a mitochondrial protein that forms part of Complex III, essential for complex assembly and stabilization; COX1 – encodes a subunit of Complex IV involved in aerobic metabolism (electron transfer in oxidative phosphorylation); SDHA – encodes a protein component of SDH complex located on the inner mitochondrial membrane, participating in both the TCA cycle and respiratory chain.

Statistical analysis was performed using STATISTICA v10.0 (StatSoft, Tulsa, OK, USA). Intergroup comparisons were analyzed using the Kruskal-Wallis test, while intragroup baseline assessments were performed using pairwise t-tests. Statistical significance was set at $p \leq 0.05$. Results are presented as mean (M) ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

No animal mortality occurred in any group throughout the study duration. Body weight measurements and biochemical parameters showed no significant intergroup or intragroup differences.

According to the table 2, a decrease in grip strength was noted in all groups, which is consistent with the data obtained in the study of the effect of chronic intermittent hypoxia on neuromuscular joints [17]. However, the Control group demonstrated a more pronounced tendency toward reduction ($p \leq 0.07$) compared to the EMSP group ($p \leq 0.3$). And in the EMGPS group, a statistically significant difference between the final values and the background values was revealed.

Table 2. Grip Strength Test

Group	Baseline, N	Final, N
Control	98,2 ± 11,5	86,8 ± 7,0
EMSP	95,2 ± 16,0	84,5 ± 18,1
EMHPS	92,6 ± 9,3	77,7 ± 3,6*

Note. * $p \leq 0,05$, Student›s t-test (Baseline vs. Final); N – Newton.

It can be concluded that the investigated compound EMSP helps to more effectively prevent a decrease in grip strength against the background of chronic IH.

The control group, there was a significant increase in Ti, EM, and UM values, as well as a decrease in f and an increase in AP compared to the background values. It is important to note, although not statistically significant, a 35 % increase in Te compared to the background data. A change in these parameters shows the presence of compensatory and pathological changes against the background of IG, which indicates successful modeling of pathology (Table 3).

Amid the introduction of EMSP, a change in respiratory parameters was also observed after the end of the IG simulation. There was a significant decrease in PEF, *f*, and EF50 values compared to the background values. A significant change in the background readings of only these parameters may indicate a less pronounced lesion of the respiratory system of animals against the background of the introduction of EMS than in the control group. The values of Ti, EV, and PEF had a significant difference from the control group, which also indicates less pronounced pathological processes against the background of IH, as well as a possible predominant effect on the inspiratory mechanisms of EMF.

Table 3. **Plethysmography parameters**

Group	Control		EMPS		EMHPS	
	Baseline	Final	Baseline	Final	Baseline	Final
Ti (s)	58,5 ± 6,9	75,9 ± 13,1[B]	62,9 ± 6,1	64,6 ± 4,3[A]	65,9 ± 9,8	74,2 ± 5,7
Te (s)	177,9 ± 58,1	240,3 ± 125,6	207,5 ± 86,0	219,8 ± 27,3	240,4 ± 74,9	180,4 ± 51,9
PIF (mL/s)	3,7 ± 0,3	3,9 ± 0,2	3,7 ± 0,4	3,9 ± 0,16	3,6 ± 0,2	3,7 ± 0,2
PEF (mL/s)	3,4 ± 0,3	3,3 ± 0,3	3,5 ± 0,2	3,0 ± 0,1[A,B]	3,3 ± 0,2	3,3 ± 0,2
TV (mL)	0,13 ± 0,02	0,17 ± 0,02[B]	0,14 ± 0,04	0,15 ± 0,01	3,1 ± 0,2[B]	3,1 ± 0,2[B]
EV (mL)	0,12 ± 0,02	0,17 ± 0,02[B]	0,14 ± 0,04	0,14 ± 0,01[A]	0,14 ± 0,02	0,15 ± 0,01
RT	257,3 ± 189,9	244,9 ± 234,4	193,2 ± 195,2	146,9 ± 11,9	267,5 ± 203,0	146,1 ± 59,8
MV (mL)	39,7 ± 10,3	48,2 ± 5,9	46,4 ± 7,40	42,0 ± 5,3	38,0 ± 9,1	41,8 ± 0,9
t between 2 breaths (s)	329,4 ± 150,5	353,6 ± 179,0	287,2 ± 91,5	319,3 ± 33,7	372,7 ± 106,8	345,6 ± 12,7
f (bpm)	335,5 ± 44,3	280,4 ± 41,7[B]	342,6 ± 30,2	285,2 ± 11,0[B]	305,0 ± 47,7	287,3 ± 3,4
EIP	1,5 ± 0,4	2,0 ± 0,5[B]	1,7 ± 0,3	1,9 ± 0,5	2,0 ± 0,9	2,1 ± 0,5
EEP	12,7 ± 3,7	12,2 ± 1,6	12,2 ± 2,2	11,6 ± 1,6	12,1 ± 1,6	10,9 ± 1,6
Penh	0,54 ± 0,1	0,40 ± 0,1	0,52 ± 0,1	0,40 ± 0,05	0,42 ± 0,2	0,42 ± 0,2
EF50 (mL/s)	2,5 ± 0,3	2,4 ± 0,3	2,5 ± 0,3	2,1 ± 0,1[B]	2,4 ± 0,3	2,2 ± 0,3

Note. A – $p \leq 0,05$, Kruskal-Wallis test (EMPS vs. Control; EMHPS vs. Control); B – $p \leq 0,05$, Student's t-test (Baseline vs. Final); *t* – time.

The reference compound EMGPS during the pathology showed no significant differences from the indicators in the control group. In comparison with the background values, only a decrease in PEF was observed, as in the EMF group.

Based on the data obtained, it can be concluded that the studied compound EMSP contributed to the normalization of respiration compared with the control group against the background of IG. EMSP and EMGPS showed comparable effectiveness in terms of plethysmography parameters against the background of IG. The positive effect of the studied EMSP and reference EMGPS on respiratory parameters in IH corresponds to the data given in the article by N. N. Andreeva. The article contains both experimental and clinical results showing that EMHPS increases the body's resistance to oxygen deficiency and reduces the risk of developing post-ischemic disorders [18].

In the control group under IH, a significant decrease in the time spent in the open arm (35 %) and on the central platform (59 %) was observed compared to baseline values. Additionally, a more than twofold reduction (55 %) in the number of entries into the open arm and a significant decrease in the number of times the animals peeked from the closed arm were recorded. All these parameters, as well as a significant increase in grooming behaviors, indicate anxiety in the animals following the IH modeling (Table 4).

In the EMSP group, a decrease in time spent in the open arm (78 %) and an increase in time spent in the closed arm (67 %) were also observed, suggesting anxiety in the animals. Anxiety was further indicated by a fourfold increase in the time of the first entry into the open arm (400 %) and a significant decrease in peeking out from it. Moreover, a significant increase in grooming behavior was noted. However, it is worth mentioning the more than twofold increase (134 %) in time spent on the central platform and a 77 % reduction in defecation compared to baseline data. These results suggest that the animals experienced anxiety under pathology modeling, but compared to the control group, anxiety was less pronounced in the EMSP group.

In the groups with the introduction of EMSP and EMGPS, the presence of anxiety in animals was also observed. This resulted in a decrease in the time spent by animals in the open arm by 78 and 44 %, respectively, as well as an increased time spent in the closed arm by 67 and 27 %, respectively, compared with the background values. However, it is worth noting that these differences had no statistical significance. The presence of anxiety in animals can be determined by a significant increase in the number of grooming behaviors in the EMSP group. At the same time, an increase in the time spent on the central platform was also noted in the EMSP group, which corresponds to the data obtained in the study by T. A. Voronina [19]. When comparing the data in the experimental groups with the data in the control group, it can be noted that the introduction of EMSP and EMGPS contributed to an increase in the time spent in the open arm. The paper of N. V. Avdeeva also revealed an increase in the time spent by animals in the open arm against the background of the introduction of EMGPS [20].

In the control group, the OFT showed a decrease in exploratory activity. The number of sectors explored and peeks into burrows were significantly lower than baseline values. An increase in motor activity was also observed, with significant differences in movement and

Table 4. **Effects of prolonged intermittent hypoxia on laboratory animal behavior in the EPM Test**

Group	Control		EMSP		EMHPS	
	Baseline	Final	Baseline	Final	Baseline	Final
Time spent on open arms (s)	25,0 ± 23,4	9,0 ± 11,6	91,5 ± 78,1	20,2 ± 18,3	28,0 ± 32,9	18,5 ± 19,4
Time spent on closed arms (s)	139,5 ± 33,3	163,8 ± 24,8	83,8 ± 72,2	140,2 ± 27,9	129,5 ± 31,7	165,2 ± 26,3
Time spent on central platform (s)	20,5 ± 12,1	12,2 ± 14,1	9,7 ± 9,5	22,7 ± 13,5	26,7 ± 9,0	1,8 ± 3,3[B]
Latency for first open arm entry (s)	88,3 ± 79,8	103,3 ± 89,8	20,0 ± 31,1	81,7 ± 83,1	59,5 ± 70,3	94,0 ± 116,0
Number of open arm entries (arb. units)	2,2 ± 1,8	1,0 ± 1,3	2,0 ± 1,5	1,7 ± 1,9	2,7 ± 2,6	1,5 ± 1,6
Number of closed arm entries (arb. units)	4,8 ± 2,9	3,2 ± 2,7	3,7 ± 3,9	4,5 ± 3,6	5,2 ± 1,6	1,8 ± 1,7[B]
Head dips from closed arms (arb. units)	8,3 ± 1,5	4,8 ± 3,3[B]	3,7 ± 3,0	4,7 ± 3,5	7,2 ± 3,2	4,0 ± 2,2
Head dips from open arms (arb. units)	9,2 ± 5,9	3,0 ± 3,6	10,8 ± 5,2	2,8 ± 1,9[B]	10,8 ± 6,1	2,5 ± 3,3
Grooming (arb. units)	0,8 ± 1,2	2,5 ± 1,1[B]	0,8 ± 0,8	2,3 ± 0,8[B]	0,8 ± 0,8	1,7 ± 1,0
Rearing with wall support (arb. units)	8,8 ± 5,5	5,0 ± 4,7	7,8 ± 7,1	7,2 ± 3,9	8,8 ± 5,2	4,3 ± 2,7
Fecal boli count (arb. units)	0,3 ± 0,5	0,8 ± 1,2	1,3 ± 1,2	0,3 ± 0,5	0	1,8 ± 1,7[B]

Note. B – $p \leq 0,05$, Student's t-test (Baseline vs. Final).

rest parameters compared to baseline values. Additionally, grooming behavior increased by 113 %, compared to baseline data, indicating anxiety under pathological conditions (Table 5).

In the EMSP group, the number of sectors explored was also significantly reduced compared to baseline values. The number of peeks into burrows did not show a significant difference compared to baseline values, indicating a less pronounced reduction in exploratory activity compared to the control group. The introduction of EMSP increased animal mobility, as reflected in significant differences in speed, movement, and rest parameters from baseline values.

The EMGPS compound did not show significant differences but exhibited a tendency toward reduced exploratory activity, with a 20 % decrease in sectors explored (compared to 26 % in EMSP and 38 % in the control group) and a 43 % reduction in burrow peeks (compared to 15 % in EMSP and 71 % in the control group) compared to baseline values. A trend toward increased motor activity was observed, with movement percentage increased by 24 % and rest decreased by 23 %, though these changes were not statistically significant.

The data obtained in the OFT correlate with the previously described anxiolytic effect of EMHPS in the paper of T. A. Voronina [12]. In particular, both studied compounds did not have a sedative effect and did not reduce research activity in comparison with the control group.

Table 5. **The effect of prolonged intermittent hypoxia on laboratory animals' behavior in the OFT**

Group	Control		EMSP		EMGPS	
	Baseline	Final	Baseline	Final	Baseline	Final
Crossed sectors (u.a.)	190,0 ± 57,8	185,3 ± 96,1	198,5 ± 45,3	187,0 ± 56,7	218,7 ± 70,8	195,5 ± 36,2
Explored sectors (u.a.)	28,5 ± 4,4	17,8 ± 6,9[B]	29,8 ± 2,9	22,2 ± 4,9[B]	27,5 ± 7,6	22,2 ± 7,2
Rearing without support (u.a.)	1,2 ± 2,9	2,5 ± 4,2	1,2 ± 1,8	1,3 ± 1,2	0	2,0 ± 2,5
Rearing with support (u.a.)	6,7 ± 7,1	8,3 ± 7,6	11,3 ± 3,9	11,2 ± 6,0	8,7 ± 6,8	9,8 ± 6,9
Holeboard head-dips (u.a.)	7,8 ± 4,4	2,3 ± 1,2[B]	6,8 ± 3,5	5,8 ± 3,4	7,0 ± 3,2	4,0 ± 1,8
Grooming (u.a.)	1,5 ± 0,8	3,2 ± 1,7	1,5 ± 0,8	1,8 ± 0,8	1,2 ± 0,9	1,8 ± 1,2
Distance traveled (m)	12,8 ± 6,2	15,9 ± 7,6	15,2 ± 4,1	19,8 ± 3,9	16,8 ± 8,9	20,9 ± 4,9
Speed (m/s)	0,2 ± 0,1	0,2 ± 0,03	0,2 ± 0,03	0,2 ± 0,02[B]	0,2 ± 0,1	0,2 ± 0,01
Movement (%)	33,8 ± 8,6	62,1 ± 16,5[B]	41,5 ± 8,9	53,4 ± 9,1[B]	41,7 ± 13,4	52,0 ± 12,9
Rest (%)	64,2 ± 8,4	35,5 ± 16,6[B]	56,3 ± 8,9	44,0 ± 9,2[B]	57,7 ± 14,6	45,5 ± 13,0

Note. B – $p \leq 0.05$, difference according to Student's t-test from baseline values.

Table 6. **The effect of IH on heart rate and SpO_2 in the test animals**

Group	Control		EMSP		EMGPS	
	Baseline	Final	Baseline	Final	Baseline	Final
HR, bpm	511,3 ± 55,2	463,3 ± 44,6	469,3 ± 40,6	535,0 ± 70,9	495,2 ± 54,0	492,3 ± 32,8
SpO_2, %	78,1 ± 11,4	74,4 ± 7,9	78,0 ± 4,3	77,8 ± 4,4	78,2 ± 4,0	80,1 ± 3,4

Note. HR – heart rate; SpO_2 – oxygen saturation level.

As shown in Table 6, the SpO_2 levels did not differ between groups or within groups. HR also showed no differences, but a trend toward changes was observed in the control and EMSP groups. In the control group, a 10 % decrease in HR from baseline was noted. In the EMSP group, a 14 % increase in HR compared to baseline was observed, indicating a possible effect of EMSP on HR under IH conditions. The reference compound EMGPS did not affect HR.

The ability to increase HR under IH conditions is an advantage of the EMSP compound. Since hypoxic states cause oxygen deficiency in the blood, an increase in HR can contribute to more rapid oxygen delivery to organs due to increased blood flow.

No statistically significant differences were found in gene expression analysis. The gene UQCRC2, examined in the EMSP group, showed the most significant difference when compared to the control group ($p \leq 0.1$). In the EMGPS group, this value was less significant ($p \leq 0.5$).

Table 7 shows a clear expression of the UQCRC2 gene in the EMSP and EMGPS groups, with the expression being more pronounced in the EMSP group. An increase in the UQCRC2 gene expression indicates that the investigated compound influences the ubiquinol-cytochrome c-reductase complex (complex III), part of the mitochondrial respiratory chain. Furthermore, since the protein encoded by this gene is necessary for the assembly and stabilization of complex III, EMSP under pathological conditions contributes to more efficient protection and stable functioning of the mitochondrial respiratory chain. Despite the absence of significant differences, the tendency to increase the expression of the UQCRC2 gene in the EMSP and EMGPS groups corresponds to the described mechanism of the antihypoxic effect of EMGPS described by T. A. Voronina [12].

Table 7. **The effect of the tested drugs on gene expression**

Groups	Control	EMSP	EMGPS
Gene NDUFS1	1,3 ± 0,8	0,9 ± 0,5	0,9 ± 0,6
Gene UQCRC2	1,1 ± 0,6	2,5 ± 2,1	1,8 ± 0,9
Gene COX1	1,7 ± 2,0	1,0 ± 0,7	1,0 ± 0,8
Gene SDHA	1,2 ± 0,9	1,0 ± 0,9	0,9 ± 0,4

The selected genes are integral components of the electron transport chain in mitochondrial respiration. The SDHA gene encodes subunit A of the succinate dehydrogenase complex, expressed in most tissues, involved in metabolism, and participates in electron transfer from succinate to coenzyme Q [21]. The UQCRC2 gene encodes a protein found in mitochondria, part of the ubiquinol-cytochrome c reductase complex (also known as complex III), and essential for the assembly and stabilization of the mitochondrial respiratory chain [22]. The NDUFS1 gene encodes a protein part of complex I with NADH dehydrogenase and oxidoreductase activity, transferring electrons from NADH into the respiratory chain [23]. The COX1 gene (cyclooxygenase-1) is a key enzyme in the inflammatory pathway, converting arachidonic acid to prostanoids, which have different effects depending on the effector mechanism in each cell [24].

CONCLUSION

In the grip strength test, the reduction in this parameter in the EMSP group was much less shown compared to the EMGPS or control groups.

Plethysmography data revealed that EMSP normalized the measured parameters under hypoxia, compared to the control group, and showed higher effectiveness than the reference compound.

The EPM and OFT showed that EMSP and the reference EMGPS reduced anxiety and increased exploratory activity compared to the control group. EMSP outperformed EMGPS in some parameters.

Gene expression analysis revealed a trend towards increased stimulation of the UQCRC2 gene in the EMSP group. Expression in the EMSP group was higher than in the control and EMGPS groups.

The investigated EMSP compound showed protective properties under IH, comparable to the reference drug.

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