



Changes in the respiratory function of the heart and brain mitochondria of animals after chronic alcohol intoxication affected by a new GABA derivative

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Academic editor: Mikhail Korokin ♦ Received 6 November 2020 ♦ Accepted 27 January 2021 ♦ Published 23 March 2021

Citation: Popova TA, Kustova MV, Khusainova GK, Perfilova VN, Prokofiev II, Smolnyakova YA, Borodkina LE, Tyurenkov IN, Ostrovskiy OV, Vasil'eva OS (2021) Changes in the respiratory function of the heart and brain mitochondria of animals after chronic alcohol intoxication affected by a new GABA derivative. *Research Results in Pharmacology* 7(1): 33–40. <https://doi.org/10.3897/rrpharmacology.7.60469>

Abstract

Introduction: Chronic **ethanol** consumption leads to significant functional and structural changes in the mitochondria of the heart and brain, increasing generation of reactive **oxygen** species. Therefore, the search for substances, which improve the functional state of the mitochondria and, meantime, reduce the oxidative stress, is relevant.

Materials and methods: 10-months-old Wistar female rats were used in the experiments. Chronic alcohol intoxication (CAI) was modelled by replacing drinking **water** with a 10% **ethanol** solution containing **sucrose** (50 g/L) for 24 weeks. Four groups were formed: 1 – intact animals; 2 – animals after chronic alcohol consumption; 3 – rats after CAI which were administered RSPU-260 (25 mg/kg); 4 – rats after CAI which were administered the reference drug **Mildronate** (50 mg/kg). The intensity of lipid peroxidation (LPO) and the rate of **oxygen** consumption in various metabolic states were determined.

Results and discussion: Administration of the compound RSPU-260 to the animals exposed to alcohol over a long period of time resulted in an increase in both the rate of **oxygen** consumption (state 3) and the respiratory control ratio (RCR) of the mitochondria of heart and brain cells. The use of a GABA derivative promoted a decrease in **malonic dialdehyde** in the mitochondria of the heart and brain. Total **SOD** activity in the mitochondria of heart cells was significantly increased in the groups of rats treated with RSPU-260. In terms of efficiency, the compound RSPU-260 was comparable to the reference drug **Mildronate**.

Conclusions: The compound RSPU-260, and the reference drug **Mildronate** improve mitochondrial oxidative phosphorylation in heart and brain cells, the functioning of antioxidant enzymes in animals after CAI, and can be used to correct alcoholic damage to these organs.

Keywords

chronic alcohol intoxication, mitochondrial dysfunction, a GABA-derivative, **Mildronate**.

Introduction

There are many medical problems associated with alcohol consumption, among which are acute **ethanol** poisoning, chronic alcoholism leading to the development of arrhythmias, alcoholic heart damage, increased risk of sudden cardiac death, damage to the central nervous system, liver and other systems (Fernández-Solà and Planavila Porta 2016). Globally, 3 million people die from alcohol abuse every year, accounting for 5.3% of all deaths (World Health Organization 2019).

Ethanol and its metabolite **acetaldehyde** are capable of penetrating cell membranes and triggering many pathological changes in body tissues, including metabolic disorders (Shokri-Kojori et al. 2017) and lipid peroxidation. Chronic consumption of **ethanol** leads to significant functional and structural changes in the cardiac mitochondria, decreasing the activity of all complexes of the mitochondrial respiratory chain, except for the second complex, which leads to a decrease in the synthesis of ATP. Besides, in heart cells, ethyl alcohol increases the expression of CYP2E1 (Zhang et al. 2011), which is involved in the oxidation of **ethanol** to **acetaldehyde**. This leads to increased generation of reactive **oxygen** species (ROS) (Manzo-Avalos and Saavedra-Molina 2010), which oxidize mitochondrial proteins and cause the degradation of mitochondrial DNA, exacerbating mitochondrial dysfunction. Moreover, intracellular **calcium** redistribution can cause the opening of the mitochondrial pore, the release of **cytochrome C**, mitochondrial swelling, the formation of abnormal cristae (Laurent and Edwards 2014), and apoptosis (Hajnóczky et al. 2005, Ren and Wold 2008). Dead cardiomyocytes are replaced by connective tissue resulting in fibrosis, which leads to a significant deterioration in the myocardial function.

Chronic alcohol intoxication causes an increase in vascular permeability in the brain, circulation and neurotransmitter disorders. Oxidation of **ethanol** to **acetaldehyde** occurs with the help of alcohol dehydrogenase, CYP2E1, and **catalase**; the activity of xanthine oxidase and iNOS increases, which leads to the production of a large number of ROS and reactive nitrogen species (RNS) (Reddy et al. 2013). Dead neurons are replaced by glial cells (Sokolova 2017). All of the above causes neurological and cognitive impairments. Thus, oxidative stress develops in both the heart and the brain (Hernández et al. 2016), and the conditions can result in the apoptosis. All of these problems are aggravated during the alcohol withdrawal period (Parthasarathy et al. 2015).

Certainly, the search for molecular targets involved in the development of oxidative stress and bioenergetic disorders could be promising for the development of a new pharmacological strategy for correcting the alcohol-related negative consequences. Gamma-aminobutyric acid (GABA) and its derivatives can increase the efficiency of the respiratory chain (Perfilova et al. 2017). Therefore, it can be assumed that the compound RSPU-260 (a two-component composition of **methyl-4-amino-3-phenylbutanoate hydrochloride** and **L-arginine hydrochloride** in a 1:1 ratio

Fig. 1) will improve the functional state of the mitochondria and reduce manifestations of oxidative stress.

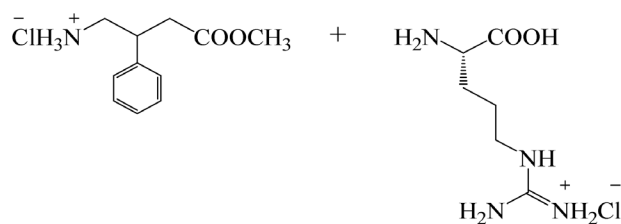


Figure 1. Chemical structure of the compound RSPU-260.

Materials and methods

Experimental animals

The experiments were performed on 10-month-old Wistar female rats (280–320 g), obtained from the Stolbovaya Nursery (Moscow region, Russia). The animals were maintained in standard vivarium conditions under GLP rules (Order of the Ministry of Health of the Russian Federation dated 01.04.2016 No. 199n “On the Approval of the Rules of Good Laboratory Practice”), the international provisions of The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986). The animals had free access to food and **water**. The experimental procedures on the animals were conducted under the Local Ethics Committee of Volgograd State Medical University, Volgograd, Russia (Protocol No. 2034-2017 dated 15.09.2017).

Drugs and treatment

Compound RSPU-260 was synthesized at the Department of Organic Chemistry of Herzen State Pedagogical University (St. Petersburg, Russia). **Mildronate** (active ingredient – **meldonium**) was used in the form of a ready-to-use solution (100 mg/ml) (Grindex, Latvia). **Physiological saline** and the drugs were administered intra-abdominally once for 14 days (RSPU-260 at the dose of 25 mg/kg, **Mildronate** at the dose of 50 mg/kg), starting from the next day after cessation of CAI.

Experimental design

All the animals were divided into groups: 1 – intact animals (n = 9); 2 – animals after chronic alcohol intoxication (n = 8); 3 – rats after CAI which were administered RSPU-260 (n = 8); 4 – rats after CAI which were administered the reference drug **Mildronate** (n = 8). Chronic alcohol intoxication was modelled by replacing drinking **water** with a 10% (by volume) **ethanol** solution (95% **ethanol** solution, RFK, Russia) sweetened with **sucrose** (50 g/L) for 24 weeks (Kryzhanovskii et al. 2015). The replacement and measurement of the amount of the consumed solution were performed once a day at the same time.

Isolation of mitochondria

The hearts and brains were rapidly removed from the narcotized rats (**chloral hydrate**, 350 mg/kg) and homogenized (at 4 °C) in a Potter-Elvehjem homogenizer in isolation medium, containing 220 mmol of **mannitol** (Sigma, USA), 100 mmol of **sucrose** (Sigma, USA), 1 mmol of **EDTA** (Fluka Analytical, Czech Republic), 4 mmol of **KH₂PO₄** (Sigma, USA), and 20 mmol of **HEPES** (Sigma, USA), pH = 7.3. Mitochondria were obtained by the standard method of differential centrifugation (Lanza et al. 2009). The homogenates were centrifuged at 600 G for 10 minutes to sediment debris and intact cells. The supernatant was centrifuged again for 20 minutes (8000 G). The sediment was resuspended and used as a mitochondrial fraction, in which respiration intensity, the concentration of lipid peroxidation products and antioxidant enzyme activities were determined.

Assessment of mitochondrial function

Respiration intensity was assessed by the polarographic method with a Clark-type **oxygen** electrode (Oxytherm System, Hansatech instruments, UK). The functional state of mitochondria was studied according to the protocol described by Lanza (Lanza et al. 2009). Into a thermostatted cell with polarography medium (0.5 mmol of **EDTA**, 3 mmol of **MgCl₂ * 6H₂O**, 20 mmol of **taurine**, 10 mmol of **KH₂PO₄**, 20 mmol of **HEPES**, 110 mmol of **sucrose**, 1 g/L of **BSA**, pH = 7.4), while permanently stirring with a magnetic stirring bar, the following substances were sequentially introduced by 100 µl: 1) a suspension of mitochondria containing 0.5–1 mg of protein/ml; 2) substrate of complex I of the respiratory chain – **malate/glutamate** (at the concentration of 5 mmol/5 mmol); 3) **ADP** (200 µmol); 4) substrate of complex II of the respiratory chain – **succinate** (5 mmol); 5) inhibitor of complex I – **rotenone** (0.5 µmol); 6) ATP synthase inhibitor – **oligomycin** (2.5 mmol); 7) uncoupler of oxidative phosphorylation – proton ionophore carbonylcyanide-4-(trifluoromethoxy) phenylhydrazone (**FCCP**) (0.05 mmol). All solutions were preliminarily heated for 20 min at 33 °C. The reagents required for the preparation of the solutions were manufactured by Sigma-Aldrich (USA).

The mitochondrial respiration rate was expressed in nmol **O₂**/min/mg of protein and calculated in the following metabolic conditions: V_1 – the rate of endogenous respiration; V_2 – the rate of substrate-dependent respiration; V_3 – the rate of **oxygen** consumption in the presence of oxidation substrates and **ADP** (oxidative phosphorylation); V_4 – the respiration rate after the addition of **Oligomycin**, an inhibitor of mitochondrial ATP synthase; V uncoupling – after adding **FCCP** proton transporter across membranes bypassing the ATP synthase channel. The respiratory control rate (RCR) was calculated as a V_3/V_4 ratio (Brand et al. 2011).

Determination of the level of lipid products oxidation and antioxidant enzyme activities

To assess the activity of antioxidant enzymes the mitochondrial suspensions were frozen to destroy mitochondria. After thawing the suspensions, the concentration of **malonic dialdehyde (MDA)** (Oliveira et al. 2007), the activity of **catalase** (Oliveira et al. 2007), **glutathione peroxidase (GP)** (Oliveira et al. 2007), and superoxide dismutase (**SOD**) (Kostyuk et al. 2009) were determined.

The method for determining an **MDA** level is based on measuring the amount of TBA-reactive products, formed by boiling a 0.7% solution of **thiobarbituric acid (TBA)** with isolated mitochondria in an acidic medium (with the addition of 1.3% **H₃PO₄**). The optical density of the resulting solution was determined at 532 nm on a Helios γ spectrophotometer (Thermo Electron Corporation, UK). The **MDA** level was expressed in µmol MDA/mg of protein.

Catalase activity was determined by a decrease in **hydrogen peroxide (H₂O₂)** in the reaction mixture as compared to a blank sample (without mitochondrial suspension). The enzyme reduced **H₂O₂** to **water** and **oxygen** for 10 minutes in phosphate buffer at pH 6.8, after which, the reaction was stopped by adding a 4% solution of **ammonium molybdate**. Later the mixture was centrifuged for 20 min at 8000 rpm in a CM-50 centrifuge (Elmi, Latvia). The absorbance of the supernatant was measured at 410 nm on a Helios γ spectrophotometer (Thermo Electron Corporation, UK). The enzyme activity was expressed in mg **H₂O₂**/min/mg of protein.

The determination of **glutathione peroxidase** activity was based on the oxidation of **glutathione** by **tert-butyl hydroperoxide**. After a 5-minute incubation of a mixture of 40 mM of **glutathione (GSH)**, 10 mM **tert-butyl hydroperoxide**, and a suspension of mitochondria in Tris-HCl buffer, the oxidation of **glutathione** was stopped by adding a 20% solution of **trichloroacetic acid**. After that, it was centrifuged for 10 minutes at 3000 rpm in a CM-6M centrifuge (Elmi, Latvia). The level of reduced **glutathione** in the supernatant was determined on a Helios γ spectrophotometer (Thermo Electron Corporation, UK) at 412 nm using 0.4% **5,5'-dithiobis (2-nitrobenzoic acid)**. The specific activity of the enzyme was expressed in µmol **GSH**/min/mg of protein.

The **SOD** activity was evaluated by the rate of decrease in the optical density of a solution of quercetin (1.4 µM) in phosphate buffer (pH 7.8) in the presence of 0.8 mM of **tetramethylethylenediamine (TMED)**. The optical density was measured immediately and 20 minutes after the addition of the mitochondrial suspension. **SOD** inhibits the quercetin oxidation by **TMED**, therefore the optical density of the reaction mixture decreased at a lower rate. **SOD** activity was expressed as per cent of inhibition/mg of protein.

The protein concentration in the samples was determined using a Pierce BCA Protein Assay Kit (Thermo Scientific, USA).

Statistical analysis

A statistical data analysis was carried out using Statistica 12.5 software package (StatSoft, USA). The normality was checked using the Shapiro-Wilk test. The Student's t-test and Newman-Keuls test were used for comparisons of the normally distributed data. The findings are presented as the mean \pm standard error of the mean ($M \pm SEM$). A p-value of less than 0.05 ($p \leq 0.05$) was considered statistically significant.

Results and discussion

Oxygen consumption in the presence of oxidation substrates of complex I of electron transport chain (ETC) and ADP in rats with CAI was significantly lower than in intact females: in the heart – by 36% ($p < 0.05$), in the brain – by 42% ($p < 0.05$). The decrease in the V_3 during activation of the first and second mitochondrial complexes in rats of the control group was more pronounced in the mitochondria of the brain than of the heart compared with the animals of the intact group, and was amounted to 38% ($p < 0.05$) and 40% ($p < 0.05$) respectively. Oxygen uptake after inhibition of the first complex by rotenone was also significantly lower than in intact rats – by 36% ($p < 0.05$) and by 44% ($p < 0.05$) in the heart and brain, respectively. There was no significant difference in mitochondrial respiration in state 4 for these organs in the animals of the control and intact groups (tables 1, 2). This may be explained by a decrease in the activity of enzymes of the citric acid cycle and complexes I, II, IV of ETC in the cardiac mitochondria under the influence of alcohol, leading to decline of V_3 (Jing et al. 2011).

The RCRs for the first complex of cardiac and brain mitochondria in the females after CAI were lower than in the intact animals by 37% ($p < 0.05$) and 38% ($p < 0.05$), respectively. These parameters for the second complex were lower by 37% ($p < 0.05$) for the cardiac mitochondria and by 33% ($p < 0.05$) for the brain mitochondria. There was a pronounced decrease in the RCR of the combined activation of the first and second ETC complexes in females of the control group, which amounted to 39% ($p < 0.05$) for the heart mitochondria and 36% ($p < 0.05$)

for the brain mitochondria (tables 1, 2). This indicates a significant decrease in the efficiency of the respiratory chain and a significant leakage of electrons, which is known to lead to the uncoupling of the oxidative phosphorylation (Piano and Phillips 2014), to reduce processes of biogenesis, and to develop oxidative stress (Matyas et al. 2016). Some studies demonstrate a decrease in the activity of mitochondrial ATP synthase, the content of ATP, as well as the mRNA of adenine nucleotide translocator 1 (ANT1) in rats after 2, 4 or 6 months of alcohol intake (Jing et al. 2011). Ethanol increases the conductance of chloride ions, inhibits neurotransmission and reduces neuronal activity (Wilcox et al. 2014). An increase in mitochondrial permeability due to calcium overload, oxidative stress, and pH changes facilitate the opening of the mitochondrial pore (Tapia-Rojas et al. 2017). This leads to the release of proapoptotic factors that cause cell death (Steiner and Lang. 2017).

In the rats with CAI treated with compound RSPU-260, the respiration rate in state 3 upon activation complex I was significantly higher than in animals of the control group by 42% ($p < 0.05$) in the heart mitochondria (Table 1) and by 24% ($p < 0.05$) in the brain mitochondria (Tables 1, 2). The administration of RSPU-260 led to a significant increase in the V_3 during activation of the first and second mitochondrial complexes of the heart – by 47% ($p < 0.05$) and of the brain – by 29% in the rats exposed to alcohol. In the females with CAI treated with compound RSPU-260, the respiration rate in state 3 after the addition of succinate increased by 26% ($p < 0.05$) in heart cells and by 37% ($p < 0.05$) in brain cells compared with rats of the control group. There were no significant differences in the rates of oxygen consumption after the addition of Oligomycin (Tables 1, 2).

In the rats treated with compound RSPU-260, a significant increase in the RCR of cardiac and brain mitochondria was observed: by 32% ($p < 0.05$) and 33% ($p < 0.05$) when activating complex I, by 32% ($p < 0.05$) and 29% ($p < 0.05$) when activating both complexes I and II, and by 6% and 37% ($p < 0.05$) when activating complex II, respectively, compared with the females after CAI without any pharmacological correction (tables 1, 2).

In the rats after CAI treated with Mildronate, the rate of oxygen consumption in the presence of substrates of

Table 1. Influence of RSPU-260 Compound on Functional State of Rat Heart Mitochondria After CAI

Indicators	Experimental groups			
	Intact group (n = 9)	CAI + Saline solution (n = 8)	CAI + RSPU-260 (25 mg/kg) (n = 8)	CAI + Mildronate (50 mg/kg) (n = 8)
V_1 , nmol O_2 /min/mg of protein	30.9 \pm 0.7	21.0 \pm 1.4*	27.8 \pm 0.7#	28.3 \pm 0.5#
V_2 , nmol O_2 /min/mg of protein	37.1 \pm 1.2	26.3 \pm 1.1*	36.6 \pm 1.2#	37.7 \pm 0.8#
V_3 (I), nmol O_2 /min/mg of protein	57.8 \pm 1.9	37.2 \pm 1.4*	53.0 \pm 2.2#	53.6 \pm 1.7#
V_3 (I + II), nmol O_2 /min/mg of protein	69.6 \pm 2.3	42.2 \pm 2.1*	62.1 \pm 2.5#	63.7 \pm 1.8#
V_3 (II), nmol O_2 /min/mg of protein	47.1 \pm 2.3	30.1 \pm 1.8*	37.9 \pm 2.5#	38.4 \pm 1.6#
V_4 , nmol O_2 /min/mg of protein	19.4 \pm 1.0	19.4 \pm 1.0	21.6 \pm 0.7	20.7 \pm 0.6
V (FCCP), nmol O_2 /min/mg of protein	42.7 \pm 1.7	35.6 \pm 2.2	42.3 \pm 3.6	42.7 \pm 1.7
RCR (I)	3.0 \pm 0.1	1.9 \pm 0.1*	2.5 \pm 0.03#	2.6 \pm 0.1#
RCR (I + II)	3.6 \pm 0.1	2.2 \pm 0.1*	2.9 \pm 0.1#	3.1 \pm 0.1#
RCR (II)	2.4 \pm 0.1	1.6 \pm 0.1*	1.7 \pm 0.1	1.9 \pm 0.1

Note: * – in relation to the indices of the intact animal group ($p < 0.05$, Student's t-test); # – in relation to the indices of the control group of animals after CAI ($p < 0.05$, Newman-Keuls test).

Table 2. Influence of RSPU-260 Compound on Functional State of Rat Brain Mitochondria After CAI

Indicators	Intact group (n = 9)	Experimental groups		
		CAI + Saline solution (n = 8)	CAI + RSPU-260 (25 mg/kg) (n = 8)	CAI + Mildronate (50 mg/kg) (n = 8)
V ₁ , nmol O ₂ /min/mg of protein	34.5 ± 0.9	22.1 ± 0.8*	25.7 ± 0.9#	28.1 ± 1.3#
V ₂ , nmol O ₂ /min/mg of protein	41.2 ± 1.4	26.1 ± 1.4*	31.6 ± 1.0#	36.0 ± 1.9#
V ₃ (I), nmol O ₂ /min/mg of protein	56.7 ± 2.2	33.1 ± 1.2*	41.0 ± 1.8#	46.9 ± 2.8#
V ₃ (I + II), nmol O ₂ /min/mg of protein	64.4 ± 2.2	39.8 ± 2.2*	46.2 ± 1.9	54.9 ± 3.5#
V ₃ (II), nmol O ₂ /min/mg of protein	55.0 ± 2.2	30.6 ± 1.1*	37.9 ± 1.9#	45.0 ± 2.8#
V ₄ , nmol O ₂ /min/mg of protein	19.5 ± 0.5	18.6 ± 0.7	17.4 ± 1.0	20.9 ± 1.6
V (FCCP), nmol O ₂ /min/mg of protein	54.1 ± 5.2	34.2 ± 6.0*	32.9 ± 4.5	51.6 ± 5.2#
RCR (I)	2.9 ± 0.1	1.8 ± 0.1*	2.4 ± 0.1#	2.3 ± 0.1#
RCR (I + II)	3.3 ± 0.1	2.1 ± 0.1*	2.7 ± 0.1#	2.6 ± 0.1#
RCR (II)	2.8 ± 0.1	1.6 ± 0.04*	2.2 ± 0.1#	2.2 ± 0.03#

Note: * – in relation to the indices of the intact animal group (p < 0.05, Student’s t-test); # – in relation to the indices of the control group of animals after CAI (p < 0.05, Newman-Keuls test).

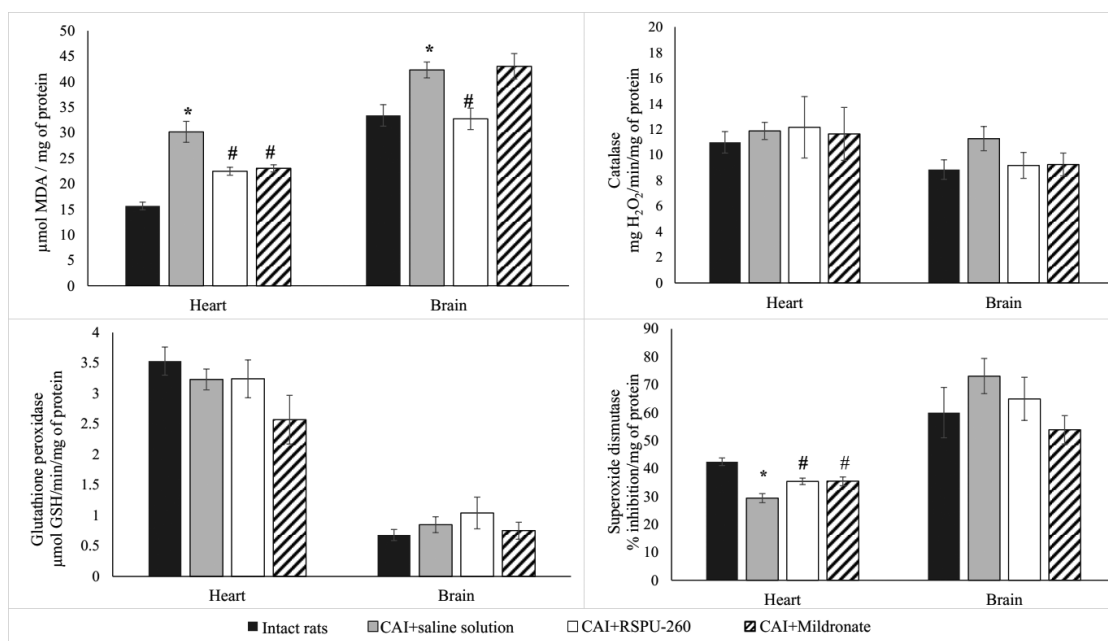


Figure 2. Influence of compound RSPU-260 on the MDA concentration and antioxidant enzymes activity of mitochondria of heart and brain cells in rats after CAI. **Note:** * – in relation to the indices of the intact animal group (p < 0.05, Student’s t-test); # – in relation to the indices of the control group of animals after CAI (p < 0.05, Newman-Keuls test).

oxidation of the mitochondrial complex I, I + II, II and ADP was significantly higher than in the control group. RCRs for the heart mitochondria of the rats after CAI treated with Mildronate were significantly higher than in animals of the control group: by 37% (p < 0.05) when activating complex I, by 41% (p < 0.05) when activating complexes I + II, and by 19% when activating complex II. At the same time, an increase in RCR of the brain mitochondria was more pronounced: by 28% (p < 0.05), 24% (p < 0.05), and 37% (p < 0.05), respectively (Tables 1, 2).

Chronic alcohol intoxication caused an increase in the level of lipid peroxidation products compared with the intact group (Fig. 2), which was much more pronounced in the cardiac mitochondria – by 93% (p < 0.05), than in the brain mitochondria – by 27% (p < 0.05). There was a decrease in glutathione peroxidase activity by 8.5% and SOD by 31% (p < 0.05) in the heart mitochondria of the control rats. In the brain mitochondria of animals of the same group, there was a tendency to an increase in the ac-

tivity of catalase by 27%, glutathione peroxidase by 25%, and SOD by 22% in comparison with intact rats (Fig. 2).

The administration of compound RSPU-260 contributed to a decrease in the concentration of MDA in the cardiac and brain mitochondria in the rats with CAI compared with the animals of the control group – by 26% (p < 0.05) and 23% (p < 0.05), respectively. At the same time, the activity of catalase and glutathione peroxidase did not significantly change. In the rats treated with Mildronate after alcohol intoxication, there was a decrease in the concentration of LPO products in the heart by 24% (p < 0.05) (Fig. 2). The total SOD activity increased in the heart mitochondria of the rats with CAI treated with compound RSPU-260 and Mildronate by 20% (p < 0.05) and 21% (p < 0.05), respectively (Fig. 2).

Thus, the results of this study are consistent with the literature data. Our findings indicate the development of mitochondrial dysfunction in the cells of the heart and the brain. This is evidenced by a decrease in the activity of

complexes I and II of the mitochondrial respiratory chain in animals after chronic alcohol intoxication. It was also demonstrated by the developing oxidative stress, which resulted in an increased level of the LPO product – MDA. At the same time, the activity of antioxidant enzymes in the mitochondria of the heart decreased, while in the brain there was a tendency to increase. According to the results of studies carried out in other laboratories, the activities of catalase, glutathione peroxidase and SOD can change in different ways: decrease, increase, or remain unchanged. It depends on a dose of alcohol, duration of consumption, etc. (Haorah et al. 2011). In the brain, SOD and catalase activities can increase as a protective reaction against oxidative stress (Haorah et al. 2011).

Compound RSPU-260 (a new GABA derivative) helps to limit the effects of alcoholic damage to the mitochondria of heart and brain cells by increasing the coupling between respiration and phosphorylation, decreasing in the MDA level, and increasing the SOD activity in heart cells. Such effects of the compound may be due to the combined action of its components – mefebut and L-arginine. Mefebut is a methyl ester of phenibut, and, according to previous studies, has an antihypoxant activity. L-arginine can be used as a substrate for the synthesis of nitric oxide by eNOS in blood vessels and by nNOS in the brain, the activity of which decreases in chronic alcohol intoxication (Karadayian et al. 2015). An increase in nitric oxide production can improve systemic and coronary hemodynamics, correct metabolic disorders in cells, inhibit the calcium-dependent mechanism of mitochondrial pore opening (Reddy et al. 2013), and prevent apoptosis. Mefebut, contained in the composition, can prevent disturbance in the synthesis and release of GABA in neurons after alcohol withdrawal, at the same time preventing neuronal death as a result of excitotoxicity and preserving the functioning of mitochondria, as evidenced by a more pronounced improvement in the coupling between oxidation and phosphorylation than in the animals treated with Mildronate. A lower SOD activity in the brain mitochondria of the rats treated with RSPU-260 compound as compared to the

control animals, along with a lower MDA concentration, may indicate a decrease in the manifestations of oxidative stress. For the mitochondria of the heart, the effect of the test compound was comparable to the reference drug Mildronate. Mildronate limits the development of oxidative stress in cells by correcting metabolic disorders: it competitively inhibits the synthesis of carnitine, which leads to a decrease in the carnitine-dependent transport of long-chain free fatty acids in mitochondria, in brain cells, and it helps inhibit the glutamate cascade and, therefore, prevents the development of apoptosis. It is successfully used in the complex therapy of ischemic heart and brain lesions, and correction of alcohol withdrawal symptoms. Mildronate can indirectly increase the production of NO (Kalvin'sh 2002) without inducing nNOS, while reducing the absorption of carnitine, which stabilizes the work of SOD in the cells of the endothelium of the brain (Haorah et al. 2011), which may be due to the low activity of this enzyme and which, in combination with a significant concentration of MDA, indicates less limited development of oxidative stress in the mitochondria of the brain cells of animals treated with the drug after alcohol intoxication.

Conclusion

Chronic alcohol intoxication with 10% ethanol solution for 24 weeks leads to the development of mitochondrial dysfunction and oxidative stress in the heart and brain of rats. A GABA derivative, compound RSPU-260, and the reference drug Mildronate improve the coupling of oxidation and phosphorylation, the functioning of antioxidant enzymes in animals after CAI, and can be used to correct alcoholic damage to these organs after long-term alcohol consumption.

Conflict of interests

The authors declare no conflict of interests.

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