THE EFFECT OF ERASTIN AND G3139 ON RAT LIVER MITOCHONDRIA IN CHRONIC ALCOHOL INTOXICATION

Yu.L. Baburina*, A.I. Zvyagina, I.V. Odinokova, O.V. Krestinina

Institute of Theoretical and Experimental Biophysics of the Russian Academy of Science,
3 Institutskaya str., Pushchino, 142290 Russia; *e-mail: byul@rambler.ru

The effect of modulators of VDAC channels — G3139 and erastin on the mitochondrial permeability transition pore (mPTP) functioning and changes in the content of proteins involved in regulation of mPTP (VDAC, CNPase, and TSPO) has been investigated in liver mitochondria of rats with chronic alcohol intoxication. It was shown that the mitochondria of rats treated with ethanol were more sensitive to mPTP induction. Moreover, ethanol induced changes in the expression of mPTP regulator proteins. G3139 and erastin were also able to influence the studied mitochondrial parameters, and they increased their effect in the liver mitochondria of rats treated with ethanol, as compared to the mitochondria of control rats. We hypothesize that the results of this study may help to elucidate the mechanisms of chronic action of ethanol on mitochondria and contribute to the development of new therapeutic strategies for treating the consequences of ethanol-related diseases.

Key words: mitochondrial nonspecific pore (mPTP); voltage-dependent anion channel (VDAC); chronic alcohol intoxication; erastin

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INTRODUCTION

Treatment of diseases caused by chronic alcohol abuse still represents an important problem in the world. Since the main metabolism of ethanol in the body occurs in the liver, this organ that primarily suffers from the consequences of chronic alcohol intoxication. Ethanol-induced damage leads to various diseases such as steatosis, cirrhosis, alcoholic steatohepatitis, fibrosis, and hepatocellular carcinoma. Many aspects of the mechanisms of action of chronic ethanol consumption on the liver remain unclear. However, there is evidence that mitochondria play a key role in these processes [1]. Chronic alcohol consumption leads to impairments in mitochondrial functions and mechanisms of antistress protection and activation of proapoptotic signaling pathways [2-4]. Mitochondrial dysfunction is associated primarily with changes in the permeability of mitochondrial membranes. Since the inner membrane is impermeable to water-soluble metabolites, specific transport proteins are involved in the transmembrane transport through the inner mitochondrial membrane. The outer membrane is permeable to water-soluble metabolites, which enter the intermembrane space through voltage dependent anion channels (VDACs), also known as porins. VDAC activity is regulated by various factors, including proteins that bind to these channels [5-7]. In the VDAC-associated state, these proteins are able to switch the channels to a closed state. In this state of VDAC channels, the flow of metabolites decreases, the permeability for calcium ions increases, and, thus, mitochondria become more sensitive to apoptotic signals [8, 9]. It has been shown that the action of ethanol causes a change in the activity of VDAC and a change in the permeability of mitochondrial membranes, which leads to the formation of mPTP [10, 11].

The pore functioning leads to collapse of the membrane potential, of mitochondrial swelling, impairments of ion homeostasis, in particular calcium induced calcium release, and release of apoptogenic factors, inducing a cascade of reactions in the cytoplasm that initiate programmed cell death. Until recently, VDAC has been considered a structural component of mPTP; currently, discussions about its involvement in mPTP continue, but it remains undoubted that VDAC is involved in pore regulation [8, 12, 13]. There is growing evidence for mPTP dysfunction in alcohol intoxication [14-16]. In this context, it is relevant to study interaction of VDAC with mPTP in order to search for new targets susceptible to the effects of alcohol and ways to protect against the consequences of alcohol poisoning.

The aim of this study was to investigate the effect of modulators of VDAC channels, G3139 and erastin, on the functioning of rat liver mitochondria under conditions of chronic alcohol intoxication.

MATERIALS AND METHODS

Animals and their Treatments

The effect of chronic alcohol intoxication on mitochondrial function was investigated in Wistar rats. Animals were exposed the Lieber-DeCarli alcoholic

Abbreviations used: ANT – adenine nucleotide translocase; mPTP – mitochondrial permeability transition pore); TSPO – translocation protein; VDAC – voltage-dependent anion channel.

liquid diet; using this model it is possible to achieve alcohol consumption in high doses [17]. Ingredients for the liquid diet were produced by "BioServ" (USA). The control diet contained fats, proteins, carbohydrates, micronutrients, and vitamins, with 18% of total calories were from proteins, 35% from fats, and 47% from carbohydrates. In the alcoholic diet, 36% of calories from carbohydrate components were replaced by calories from ethanol; the ethanol concentration in the final diet was 5%. This model uses isocaloric paired feeding of animals. For this purpose, male rats of the same age and weight, divided into pairs, were kept in separate cages equipped with special graduated drinkers, without access to water and solid food. Eight rats (aged two months) were used in the experiments, four in each group. The duration of the experiment was 2 months. Rats fed the Lieber-DeCarli ethanol diet had free access to food throughout the day, and control rats received an amount of food equivalent to the amount of food consumed by their paired rats; food intake was measured daily. During the 10 day habituation period, the rats received gradually increasing amounts of ethanol (0.1%, 2%, 3%, 4% and 5%) in the diet and then received a food containing 5% ethanol for 8 weeks. At the beginning of the experiment, the average weight of rats was 167.87 g. During the experiment, weight gain was 182.54±9.65 g for control rats and 163.12±11.55 g for rats with alcohol intoxication. Rats consumed an average of 60-80 calories per day, and ethanol-consuming rats received 14.86-16.25 g of ethanol per 1 kg of rat weight, which was consistent with published data [17].

Histological Analysis

For histological analysis, fragments of the periportal zone of the liver lobules were quickly cut with a scalpel from the whole liver immediately after the organ removal from the abdominal cavity and quickly washed with cold phosphate-buffered saline to remove blood. The samples were then fixed in neutral buffered formalin for 24 h at room temperature according to the standard method. After fixation, the fragments were washed three times in distilled water to remove excess phosphates and then immersed in O.C.T. Compound (Optimal cutting temperature compound, "Compound Tissue Tek", Japan) for 12 h at 4°C. Sets of three successive 9 μm thick transverse sections were prepared using a Shandon CRYOTOME 620E ("Thermo Fisher Scientific", USA) with a 30 μm step. Each set of three adjacent sections were stained with hematoxylin and eosin (H&E) and two differential trichrome staining methods. To obtain a general picture of alcoholic liver damage, histotopograms were taken using a Nikon Eclipse Ti-E microscope station ("Nikon", Japan) and Nis Elements AR4.13.05 (Build933) software.

Fibrosis as an indicator of alcoholic liver injury was assessed by two methods: Mallory's trichrome staining and Lilly's trichrome staining. The percentage of fibrotic changes in the periportal zone of the liver was assessed from digitized images (at least five areas of analysis from each section) using the non-commercial ImageJ software [18]. The degree of fibrosis was assessed by the increase in deposited collagen (blue areas on the section) in the tissue and was calculated as the area occupied by collagen, expressed as a percentage of the total area of the analyzed area. Data are presented as mean ± standard deviation.

Isolation of Rat Liver Mitochondria

Rat liver mitochondria were isolated from Wistar rats by the standard method using a homogenization medium containing 210 mM mannitol, 70 mM sucrose, 1 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 0.05% bovine serum albumin (BSA) fraction V, and 10 mM Tris (pH 7.3). The homogenate was centrifuged at 800 *g* for 10 min to precipitate nuclei and damaged cells. The supernatant containing mitochondria was centrifuged for 10 min at 9000 *g*. The mitochondrial pellet was washed twice in the medium without EGTA and BSA for 10 min at 9000 *g* and resuspended in the same medium. The protein concentration was determined by the Bradford method. The protein concentration in the suspension of isolated liver mitochondria was 45-50 mg/ml.

Determination of Mitochondrial Functions

The mitochondrial suspension (1 mg protein/ml) was incubated at 25°C in a medium containing 125 mM KCl, 10 mM Tris (pH 7.4), and 2 mM K_2HPO_4. In experiments, 5 mM glutamate and 5 mM malate were used as respiratory substrates. Calcium ion fluxes in mitochondria were determined using Ca^{2+}-sensitive electrodes ("Niko Analit", Russia) in a 1-ml thermostated chamber [19]. mPTP opening was induced by a threshold concentration of Ca^{2+} (each Ca^{2+} addition contained 50 nmol Ca^{2+} per mg of protein). Mitochondria were preincubated with the test substances (G3139, erastin) for 10 min.

Mitochondrial swelling was determined by change in the light scattering in the mitochondrial suspension at 540 nm on a Tecan I-Control Infinite 200 spectrophotometer "TECAN" (Austria). The standard incubation medium contained 125 mM KCl, 10 mM Tris, 2 mM KH_2PO_4, 5 mM glutamate, and 5 mM malate. Swelling was initiated by adding a threshold concentration of Ca^{2+} (250 μM). The protein concentration in the cuvette was 0.35 mg/ml, the measurements were carried out at 25°C.

Electrophoresis and Western Blot Analysis

Samples for determination of changes in the levels of mitochondrial proteins (VDAC, TSPO and CNPase) were prepared using 50 μl-aliquots taken from the thermostated chamber during measurements

of the mitochondrial function under various conditions (control and Ca^{2+}, in the presence/absence of inhibitors). The aliquots were centrifuged for 5 min at 10000 *g*, the mitochondrial pellet was separated, and Laemmli's buffer ("Bio-Rad", USA) was added to solubilize mitochondrial proteins. The samples were heated to 95°C for 5 min. The mitochondrial lysate (20 μg) was loaded onto each track of the gel. The kits from "Bio-Rad", containing marker proteins from 10 kDa to 250 kDa, were used as molecular mass markers. The transfer of proteins from the gel onto a nitrocellulose membrane (0.2 μm, "Bio-Rad") was carried out using a semi-dry transfer apparatus ("Bio-Rad") by the Western blot method.

We used antibodies to VDAC (1:1000, "Calbiochem", USA), polyclonal antibodies to TSPO (1:1000, "Abcam", USA). Monoclonal anti-CNP antibodies (anti-CNP Ab) were obtained as described in [20], and used at a dilution of 1:10000. Polyclonal antibodies to Tom20 (1:1000, "Santa Cruz", USA) were used as a protein load control. Immunoreactivity was detected with the corresponding secondary antibodies conjugated with horseradish peroxidase ("Jackson Immuno Research", USA). Peroxidase activity was determined using ECL (Enhanced chemiluminescence, "Bio-Rad") and the ChemiDoc Touch Imaging System ("Bio-Rad"). Quantitative analysis was performed using densitometry (Image Lab software, "Bio-Rad").

Statistical Analysis

The mean value of the parameters calculated from the results of five-six experiments (± SD) for statistical analysis. The statistical significance of differences between pairs of values was assessed using ANOVA type 2 (the Student-Newman-Keuls test). The figures show the results of typical experiments, the diagrams show the mean values from at least 5 experiments. The *p*-value less than 0.05 (* – $p<0.05$) indicates a statistically significant difference in the level of collagen versus the control.

RESULTS AND DISCUSSION

Since the liver is the main barrier to toxic substances in the body, the main metabolism of ethanol occurs in it during alcohol intoxication. The metabolism of ethanol in the liver leads to formation of various hepatotoxic by-products and significant oxidative stress in the liver, and, as a result, to the death of hepatocytes [21].

First, we evaluated the degree of liver tissue damage in alcohol intoxication. We analyzed histotopograms of samples of the periportal zones of the liver tissue in two groups of animals to assess the degree and nature of alcoholic damage to the liver tissue and determine its localization. In addition, the deposition of collagen in the walls of the central vessels (Fig. 1a) and in the parenchyma (Fig. 1b) was evaluated. In fragments of the liver tissue of rats subjected to alcohol intoxication, collagen accumulation was observed in the walls of large vessels and the liver parenchyma (Fig. 1c). In addition, signs of the development of parenchymal fibrosis and thickening of the vascular walls with the formation of fibrous septa were noted. The increase in fibrotic changes in this group of rats, estimated using macros from ImageJ, was significantly greater than in control samples (mean value ± standard deviation 38.8±3.9% versus 10.0±3.0%, $p<0.001$; n=10) (Fig. 1b). A comparative study of the cellular structure of the tissue also showed that in the group of rats with alcohol intoxication, there were signs of swelling and necrosis of hepatocytes, accompanied by fragmentation and/or complete destruction of the nuclei (Fig. 1c).

Diseases related to ethanol consumption are primarily associated with impaired structure and functions of mitochondria; in particular, this includes altered functioning of pro-apoptotic signaling pathways, oxidative stress and, as a consequence, impaired functioning of anti-stress protective mechanisms [3, 22, 23]. Since one of the key characteristics of the mitochondrial functioning is the mPTP opening, we have investigated the effect of chronic ethanol consumption on the parameters of its functioning. There is evidence that alcohol intoxication leads to disturbances in mPTP functioning [14-16]. Chronic alcohol consumption leads to an increase in the sensitivity of mitochondria to mPTP induction, and, consequently, to increased permeability of the inner mitochondrial membrane, its depolarization, swelling, and damage of the outer membrane [15]. At present, the study of the composition and structure of the pore continues. Currently, certain evidence exists that mPTP does not have permanent structural components, and in different situations, different protein components and channels may be involved in its formation [24-26]. Based on results of recent studies conducted in our laboratory, we have hypothesized the existence of a compensatory system formed by mPTP regulatory proteins (CNP, VDAC, TSPO) in the liver mitochondria under conditions of chronic alcoholism [14, 27, 28].

Initially, VDAC was considered a structural component of the pore; later, experiments with knockout mice showed that knockout of all three VDAC isoforms did not interfere with mPTP functioning [29]. However, a large number of reports provided new evidence for the involvement of VDAC in mPTP [8, 13, 30, 31].

We have studied the effect of two modulators of VDAC channels, erastin (2 μM) and G3139 (5 μM). Erastin is a VDAC-binding small molecule that reduces the rate of NADH oxidation in isolated yeast mitochondria expressing a single VDAC2 isoform [32] and increases NADH penetration into liposomes containing human VDAC2 [33]. Erastin interaction with VDAC causes mitochondrial dysfunction, release of reactive oxygen species (ROS) and ultimate cell death [32].

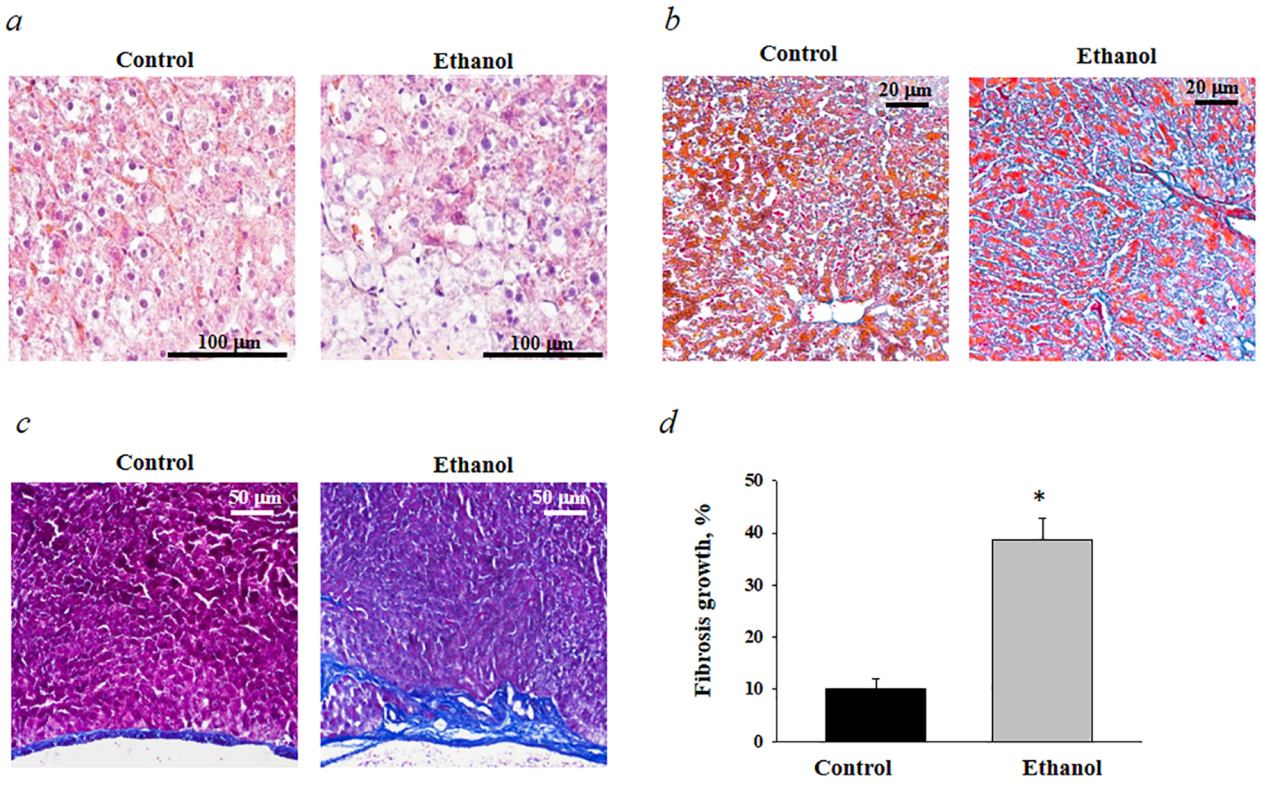

Figure 1. Fragments of histotopograms of periportal areas of the rat liver tissue. Light microscopy: (**a**) – Masson trichrome stain (collagen/fibrosis is stained blue; non-collagen components are stained pink; cell nuclei are stained dark crimson), ruler 100 μm. (**b**) – Mallory trichrome stain (collagen/fibrosisis stained blue; non-collagen components are stained orange/red; cell nuclei are stained red), ruler 20 μm. Photomicrographs of perivascular hepatocytes in rat liver: (**c**) – H&E (cell nuclei are in purple, erythrocytes are in red, cell cytoplasm is in pink), ruler 50 μm. (**d**) – diagram showing the increase in deposited collagen in the periportal region of the liver of each group of rats. Data are presented as the mean ± standard deviation of five independent experiments. * – $p<0.05$ indicates a significant difference in the level of collagen in relation to the control. The statistical significance of differences between pairs of means was assessed using ANOVA type 2 (Student-Newman-Keuls). A color version is available in the electronic version of the article.

The other test substance was G3139 (Oblimersen Genasense, Bcl-2 antisense), a synthetic DNA oligodeoxyribonucleotide phosphorothioate designed to reduce *bcl-2* mRNA expression. It is widely used as an inhibitor of VDAC channels [34]. The ability of G3139 to stimulate the mPTP opening in rat brain and liver mitochondria was previously shown [35].

In order to reveal the effect of erastin and G3139 on the mPTP functioning in liver mitochondria of rats exposed to chronic alcohol intoxication, we measured one of the key parameters of the pore, Ca^{2+} capacity, the amount of calcium required to reach the threshold concentration and mPTP opening. Figure 2 shows that the addition of erastin and G3139 caused a decrease in the Ca^{2+} capacity, corresponding to the initiation of pore opening. However, in liver mitochondria of rats with alcohol intoxication (Fig. 2b, grey columns), both substances enhanced their effect. For example, the Ca^{2+} capacity of mitochondria of control animals in the presence of G3139 was 15% lower compared to the corresponding control (without additions, white column 1 vs white column 2), while in the mitochondria of rats with alcohol intoxication, the Ca^{2+} capacity in the presence of G3139 was reduced by 2 times as compared to the control without additions (grey column 1 vs grey column 2). The same trend was observed in the presence of erastin: the effect of erastin increased from 30% to 45% in liver mitochondria of rats with alcohol dependence compared to controls. Thus, we have found that erastin and G3139 enhance their effect in the liver mitochondria of rats with alcohol intoxication compared to the effect in the liver mitochondria of control animals.

To confirm this effect, we have investigated another parameter that characterizes the mPTP opening, calcium-induced swelling of mitochondria. Addition of Ca^{2+} at a threshold concentration to a suspension of mitochondria incubated in a standard medium caused a decrease in light scattering. This indicates swelling: mitochondrial membranes become permeable to substances with a low molecular weight. Quantitatively, the swelling process was characterized by the time to reach the half-maximal light scattering signal ($T_{1/2}$). Figure 3 shows results of these expeirments. In general, they repeat the data obtained earlier, confirming the enhancement of the effect of modulators of VDAC channels under the influence of ethanol. At the same time, it should be noted

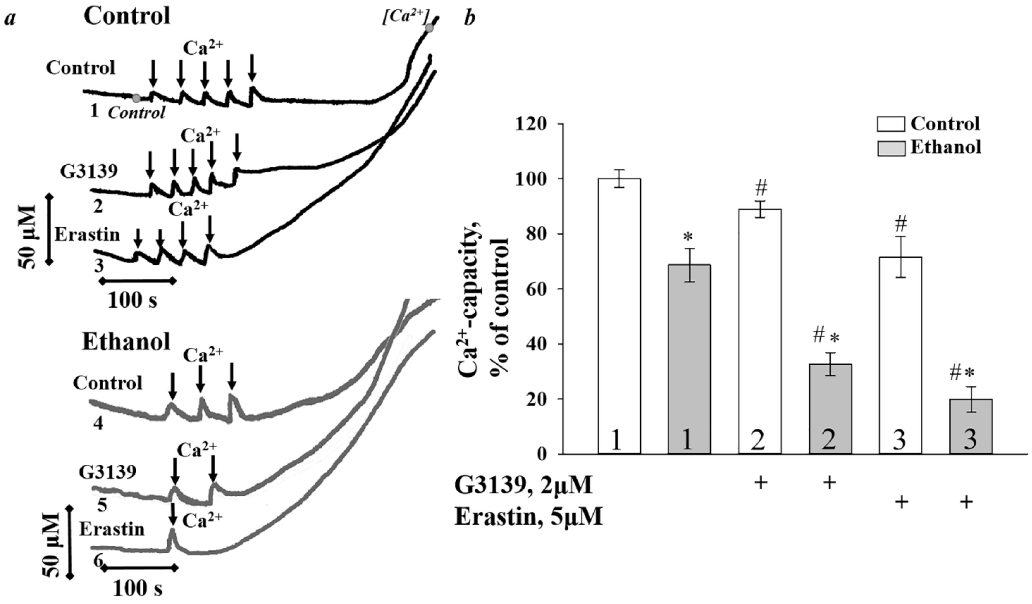

Figure 2. The effect of G3139 and erastin on the Ca^{2+} capacity of rat liver mitochondria: (**a**) Ca^{2+} transport in mitochondria, (**b**) quantitative analysis of threshold [Ca^{2+}]. Values are shown in % relative to control, the reference value of Ca^{2+} capacity (in control animals without supplementation) is 150 nmol Ca^{2+} per mg of protein. The values of three independent experiments are presented. * – $p<0.05$ comparison of the values of the ethanol group relative to the corresponding parameter of the control group (gray vs white bars). # – $p<0.05$ comparison of values in the presence of additions (G3139 and erastine) vs. values in the absence of additions (columns 3 and 2 vs. 1). The grey dots indicate the sampling sites for Western blot analysis (results are shown in Figs. 3, 4).

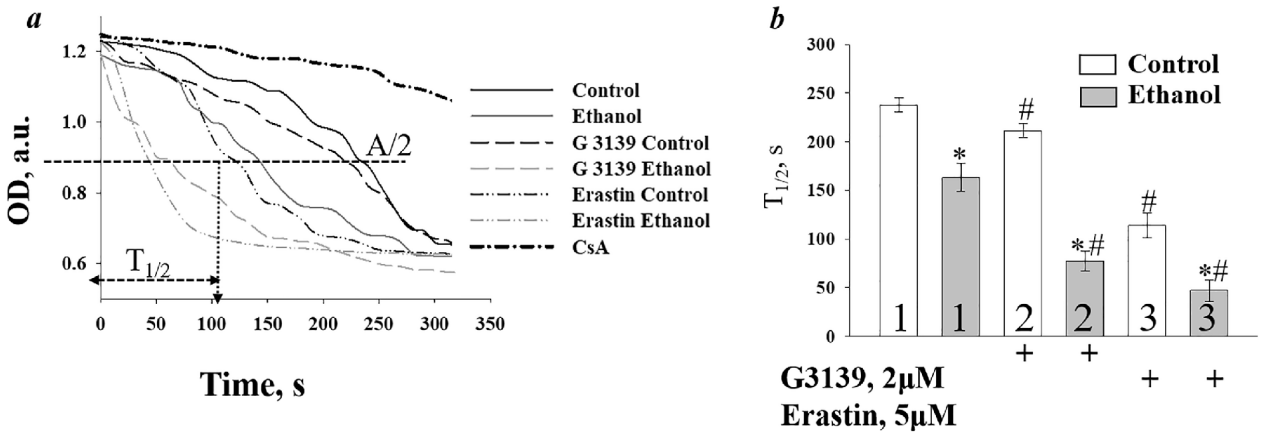

Figure 3. The effect of G3139 and erastin on swelling of rat liver mitochondria. Swelling was initiated by the addition of Ca^{2+} corresponding to a threshold concentration of 250 nmol Ca^{2+} per mg of protein. The values of three independent experiments are presented. (**a**) – swelling curves of liver mitochondria, (**b**) – swelling half-time (time to reach 1/2 of the swelling amplitude, $T_{1/2}$) of mitochondria. * – $p<0.05$ comparison of the values of the ethanol-treated rats versus the corresponding parameter of the control animals (grey vs white bars). # – $p<0.05$ comparison of values in the presence of additions (G3139 and erastine) vs. values in the absence of additions (columns 3 and 2 vs. 1).

that, as on, the effect of erastin on Ca^{2+} capacity and mitochondrial swelling was more potent than the effect of G3139. In the presence of G3139 the half-life time ($T_{1/2}$) decreased by 10% in the control mitochondria, while erastin caused almost a 2-fold decrease in the parameter. In the mitochondria of rats with alcohol intoxication, G3139 caused a 2-fold increase in the rate of swelling of mitochondria, while erastin caused a 3-fold increase in the parameter. Thus, it was found that mitochondria isolated from the liver of rats with chronic alcoholism were more sensitive to the induction of mPTP caused by various agents, compared with mitochondria isolated from control animals. In all cases mPTP induction was cyclosporine-sensitive. The difference in the effect of G3139 and erastin on the initiation of mRTP opening and stimulation of high-amplitude mitochondrial swelling can be explained by the difference in the sites of action of these compounds on VDAC. The data obtained suggest that under pathological conditions of chronic alcohol exposure, changes in the structure of mPTP or in the mechanisms

of its regulation can occur in mitochondria. These changes may represent a compensatory response to the damage that occurs during alcogol intoxication. We suggest that VDAC is involved in the functioning of this system, and VDAC modulators can be used to regulate it. There is information in the literature about the role of VDAC in the mPTP functioning in other pathologies [8, 12, 30]. Probably, this system arises in response to damage to mitochondria of various etiologies.

Next, we have tested the effect of the studied modulators of VDAC channels on the level of the VDAC protein in the mitochondria of control rats and rats with chronic alcohol intoxication. The studies were carried out under conditions of closed and opened mPTP. We found no change in the protein level under conditions of mPTP opening as compared with mitochondria with closed mPTP. G3139, like erastin, increased the content of VDAC in liver mitochondria of control rats by ~50-60% both in open and closed pores (Fig. 4a,b, white columns 1 and 2 vs 3 and 4). It should be noted here that similar changes were observed by us earlier, for example, when studying the effect of TSPO ligands on rat liver mitochondria during alcohol intoxication. We suggest that preincubation of mitochondria with modulators of VDAC channels leads, along with pore initiation, to some conformational changes in protein complexes; this reflects changes in the level of proteins. It is important that the proteins in which changes occur are located on the outer membrane of mitochondria. Moreover, the activity of proteases in the control, depending on the type of protein being hydrolyzed, may be higher or lower than in other conditions. Elucidation of the mechanism of the detected changes is the goal of our further research. Under the influence of chronic ethanol consumption, the protein level increased approximately 2-fold (Fig. 4a,b, without additions, grey columns 1 and 2 vs white columns 1 and 2). In the mitochondria of rats with chronic consumption of ethanol in the presence of G3139, the content of VDAC increased by 1.5 times, and in the presence of erastin by 2 times compared with the control (grey bars 3 and 4 vs gray bars 1 and 2). Thus, the influence of VDAC channel modulators on the distribution of this protein in mitochondria is enhanced in disorders associated with alcohol intoxication.

Since we found changes in the content of VDAC, we decided to test whether the content of mitochondrial proteins, putative partners of VDAC in mitochondria, considered as regulators of mPTP, also changed. One of these proteins is the translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor (PBR), which, according to our studies, may be involved in the regulation of mitochondrial membrane permeability and mPTP regulation [14, 35]. We have previously shown that TSPO ligands (protoporphyrin IX and PK 11195) may be involved in the regulation of mPTP in liver mitochondria during chronic alcohol intoxication [14]. Since the studies of our group, as well as literature data, indicate a close relationship between TSPO and VDAC [14, 36, 37], we also investigated the effect of VDAC modulators

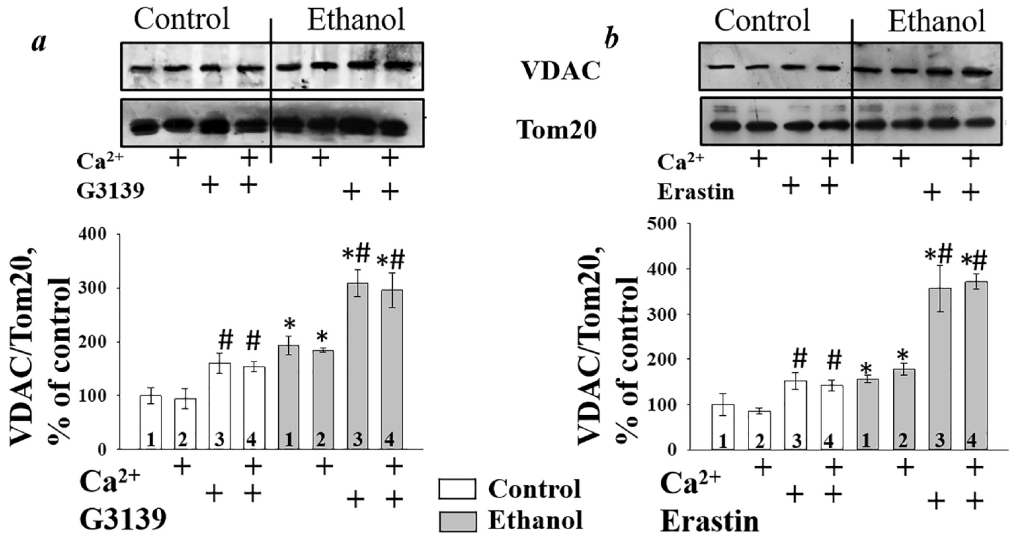

Figure 4. Changes in the content of VDAC in the presence of 5 μM G3139 (a) and 2 μM erastin (b) in rat liver mitochondria in the absence/presence of the threshold Ca^{2+} concentration. Anti-Tom20 antibodies were used as a protein load control. The values of three independent experiments are presented The upper part of the figure shows immunoblots stained with the corresponding antibodies, the lower part shows quantitative changes in the content of VDAC as a percentage relative to the control (white column 1, mitochondria of control rats without additions), normalized to Tom20. * – $p<0.05$ comparison of the values of animals treated with ethanol versus the corresponding parameter of the control rats (grey columns vs white columns). # – $p<0.05$ comparison of values in the presence of additions (G3139 and erastin) versus values in the absence of additions (column 3 vs. column 1 and column 4 vs. column 2).

on TSPO levels in the mitochondria of control animals and animals with chronic alcohol intoxication. Results of these experiments indicate that under the influence of G3139 and erastin, the content of TSPO, similarly to VDAC, increased in control animals and decreased in animals with chronic alcohol intoxication. The level of TSPO in liver mitochondria of rats with chronic alcohol intoxication significantly increased as compared with control regardless of closed and opened mPTP. Figure 5a,b shows quantitative characteristics of these effects.

Another protein partner of VDAC is cyclic nucleotide phosphodiesterase (2′,3′-cyclic nucleotide-3′-phosphodiesterase; CNPase), a myelin sheath protein found by us in mitochondria of unmyelinated tissues [38]. Earlier we have also shown that CNPase in mitochondria co-localizes with VDAC and is involved in the regulation of mPTP [39]. The results of the work have shown that the CNPase content decreases during alcohol intoxication, and the observed decrease is potentiated by the action of G3139 and erastin in the liver mitochondria of animals subjected to chronic alcohol intoxication (Fig. 5c,d).

Thus, we have found that in liver mitochondria of rats with chronic alcohol intoxication G3139 and erastin decreased the level of CNPase, and simultaneously increased TSPO and VDAC. These concerted changes may indicate existence of a relationship between the studied proteins under these conditions.

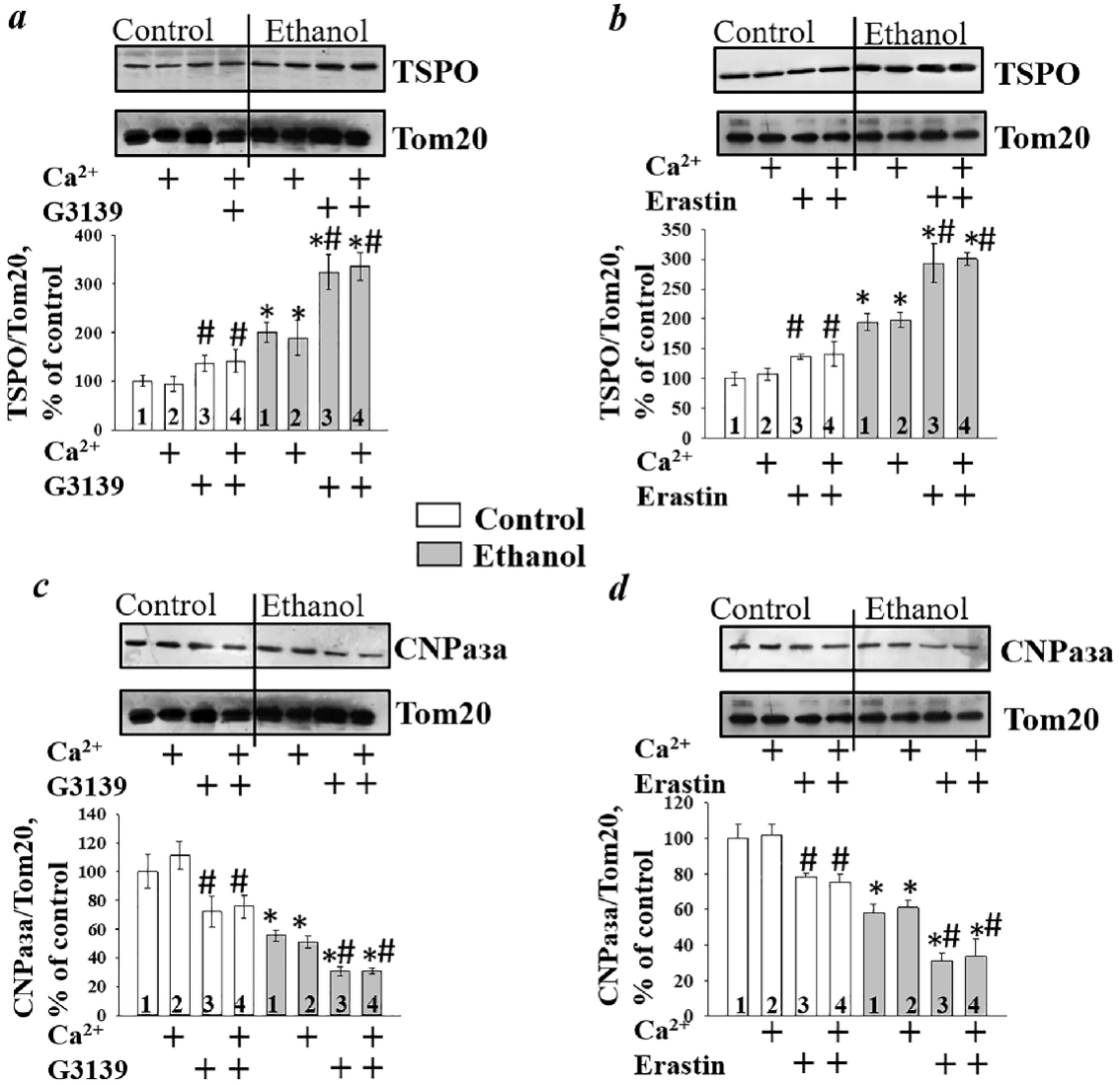

Figure 5. Changes in the content of TSPO (**a**, **b**) and CNPase (**c**, **d**) in the presence of G3139) and erastin in rat liver mitochondria in the absence/presence of the threshold Ca^{2+} concentration. Anti-Tom20 antibodies were used as a protein load control. The values of three independent experiments are presented The upper part of the figure shows immunoblots stained with the corresponding antibodies, the lower part shows quantitative changes in the content of VDAC as a percentage relative to the control (white column 1, mitochondria of control rats without additions), normalized to Tom20. * – $p<0.05$ comparison of the values of animals treated with ethanol versus the corresponding parameter of the control rats (grey columns vs white columns). # – $p<0.05$ comparison of values in the presence of additions (G3139 and erastin) versus values in the absence of additions (column 3 vs. column 1 and column 4 vs. column 2).

Certain evidence exists in the literature that VDAC plays an important role in various mitochondrial pathologies [8, 12, 40, 41]; however, mechanisms of these processes remain unclear. We propose a functional interaction between TSPO, CNPase, and VDAC in mitochondria in response to ethanol-induced degenerative changes. Additional studies are needed to elucidate the mechanism of this interaction and its functional significance. Nevertheless, results of our study indicate that these proteins are potential targets for therapeutic regulation. Erastine and G3139 are able to influence the function of this system and thus can be considered as pharmacological agents for targeted treatment of the consequences of alcohol intoxication.

CONCLUSIONS

In this study we have shown that modulators of the VDAC channels erastin and G3139 are able to initiate the mPTP opening in the mitochondria of animals with alcohol intoxication. In addition, we have found that their presence changes the level of mPTP regulator proteins (CNPase, VDAC, TSPO), and these changes are synchronous. These results suggest the presence of a complex of these proteins in mitochondria. The mechanism of actio of this complex is currently unknown and is the subject of our further research. Nevertheless, our results suggest that the complex plays a key role in the regulation of the conductance of VDAC channels in rat liver mitochondria under chronic alcohol intoxication and, consequently, in the regulation of mitochondrial membrane permeability and programmed cell death during ethanol-induced damage.

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COMPLIANCE WITH ETHICAL STANDARDS

The studies were carried out in accordance with generally accepted international standards for the treatment of animals (Directive 2010/63/EU of the European Parliament and the Council of the European Union on the protection of animals used for scientific purposes of September 22, 2010) and approved by the Commission on Biological Safety and Ethics of the Institute of Theoretical and Experimental Biophysics RAS (protocol no. 05/2022 dated March 05, 2022).

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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ВЛИЯНИЕ ЭРАСТИНА И G3139 НА МИТОХОНДРИИ ПЕЧЕНИ КРЫС ПРИ ХРОНИЧЕСКОЙ АЛКОГОЛЬНОЙ ИНТОКСИКАЦИИ

Ю.Л. Бабурина*, А.И. Звягина, И.В. Одинокова, О.В. Крестинина

Институт теоретической и экспериментальной биофизики Российской академии наук, 142290, Московская обл., Пущино, ул. Институтская, 3; *эл. почта: byul@rambler.ru

Исследовано влияние модуляторов VDAC-каналов — G3139 и эрастина — на функционирование митохондриальной поры (mitochondrial permeability transition pore, мPTP), а также на изменения в содержании белков, регулирующих работу мPTP (VDAC, CNPаза и TSPO) в митохондриях печени крыс с хронической алкогольной интоксикацией. Показано, что митохондрии печени крыс, получавших этанол, более чувствительны к индукции мPTP. Более того, под воздействием этанола происходят изменения в экспрессии белков-регуляторов мPTP. G3139 и эрастин также способны влиять на исследуемые митохондриальные параметры, причём их действие в митохондриях печени крыс, получавших этанол, более выражено по сравнению с митохондриями печени контрольных крыс. Мы предполагаем, что результаты этого исследования могут помочь выяснить механизмы хронического действия этанола на митохондрии и внести вклад в разработку новых терапевтических стратегий для лечения патологий, связанных с потреблением этанола.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: митохондриальная неспецифическая пора (мPTP); потенциал-зависимый анионный канал (VDAC); хроническая алкогольная интоксикация; эрастин

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