ORIGINAL ARTICLE

CONTENTS

Assessment of carbacetam effect on the mitochondria of hippocampal neurons in rats of different sexes with experimental metabolic syndrome

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ABSTRACT

Aim: To study carbacetam effect on the mitochondria of hippocampal neurons in rats of different sexes simulating metabolic syndrome.

Materials and Methods: The experiments were conducted on non-linear laboratory albino male and female rats with the body weight of 0,220-0,250 kg. To create the pattern, the rats were kept (60 days) on a high-fat diet (fat enrichment was provided by the addition of solid pork lard) with free access to fructose solution (100 g/L). Carbacetam was injected into the peritoneum in the dose of 5 mg/kg once a day during 14 days.

Results: Simulated metabolic syndrome was found to manifest by a decreased light scattering and an increased relative rate of mitochondrial swelling in the hippocampal mitochondrial fraction; increased free radical lipid and protein oxidation with more marked changes in males. When rats with metabolic syndrome receive carbacetam during 14 days, in their mitochondrial fraction light scattering and relative rate of mitochondrial swelling decrease, both in males and females. The content of products reacting with 2-thiobarbituric acid and protein oxidative modification decrease, and catalase activity in males and females increases, superoxide dismutase activity increases in males only.

Conclusions: Thus, a decreased intensity of mitochondrial swelling and improved condition of the antioxidant system of the hippocampal mitochondria of rats with metabolic syndrome irrespective of their sex is indicative of the effective correction of GABA receptors by means of carbacetam under conditions of the experiment.

KEY WORDS: metabolic syndrome, carbacetam, lipid and protein peroxide oxidation, mitochondrial functional state

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INTRODUCTION

Urbanization, associated sedentary lifestyle, and overeating are the main cause of current health epidemic – metabolic syndrome [1, 2]. Excessive caloric intake and reduced energy loss lead to lipid accumulation and obesity. Accumulated toxic lipids inhibit insulin signaling in the liver and skeletal muscles provoking insulin resistance [3, 4]. Metabolic syndrome is a complex and multifactorial disorder affecting a large part of the world's population and is characterized by a group of interrelated conditions that increase the risk of cardiovascular diseases, stroke and type 2 diabetes mellitus. Although definite etiology of the syndrome requires further investigation, the recent researches have determined a crucial role performed by mitochondria in its pathogenesis.

According to science mitochondria that today are considered as keys to solve numerous mysteries play an important role in energy metabolism and oxidative stress, which is one of the molecular mechanisms of damaging different organs and tissues with metabolic syndrome [5, 6, 7]. Since the neurons are restricted in glycolytic abilities, their functional activity depends on mitochondrial energy production more than other body cells. Accumulation of mitochondria in the synapses is a well-known fact. They provide the mechanisms of transmission of nerve impulses [8]. Therefore, mitochondrial dysfunction is one of the pathogenic links of metabolic disorders. At the same time, mitochondria are known to contain gamma-aminobutyric acid (GABA). Its functional cycle is closely associated with glucose metabolism [9]. Considering cerebroprotective effects of GABA agents with functional and organic disorders of the CNS, learning carbacetam effect as a modulator of GABA-ergic system with metabolic disorders taking into account sexual differences is of certain interest.

AIM

To study carbacetam effect on the mitochondria of hippocampal neurons in rats of different sexes simulating metabolic syndrome.

MATERIALS AND METHODS

The experiments were conducted on non-linear laboratory albino male and female rats with the body weight of 0,220-0,250 kg, kept under standard vivarium conditions with a natural change of day and night at a temperature of 18-22 °C and relative humidity of 40-60 %. The study was conducted keeping to the main principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986); the EU Directives № 609 of 24.11.1986 and the Order of the Ministry of Health of Ukraine № 690 of 23.09.2009.

All the rats were divided into two groups by random sampling method: 1 – control group; 2 – group with simulated metabolic syndrome. To create the pattern, the rats were kept (60 days) on a high-fat diet (fat enrichment was provided by the addition of solid pork lard) with free access to fructose solution (100 g/L) [10]. The reproduction of the syndrome was confirmed by determining the fasting blood glucose concentration in the blood plasma and positive glucose tolerance test. Rats with hyperglycemia lower than 7,0 mmol/L were isolated from the experiment. After that, the group of rats with syndrome (7 rats) was injected with carbacetam into the peritoneum in the dose of 5 mg/kg during 14 days. The groups of comparison including the control one and rats with simulated pathology (7 ratsin each group) 0,9 % NaCl solution was injected in the same way.

Euthanasia of rats was conducted under chloroform. The brain was removed in the cold and carefully washed with cool 0,9 % NaCl solution. The hippocampus was isolated according to a stereotaxic atlas [11]. Then it was washed with cool (2-4°C) 0,9 % KCl solution, grinded, homogenized in a 10-fold volume of pH 7,4 buffer (saccharose 250 mM, EDTA 1 mM, tris-HCl 10 mM) [12].

The mitochondrial fraction was isolated by means of differential centrifugation of the examined structure homogenates: 700 g during 10 min (4°C), supernatant –11 000 g during 20 min (4°C). The sediment was re-suspended in 5 ml of pH 7,4 buffer (without EDTA), then it was centrifuged again under similar conditions. The mitochondrial fraction (the sediment obtained) was re-suspended in the same buffer and immediately taken for the examination.

Mitochondrial swelling was registered according their ability to extension-contraction and optic density changes. Mitochondrial suspension was placed into the incubation pH 7,4 buffer (mmol/L): saccharose – 150, KCl – 50, KH_2PO_4 – 2, succinate – 1, tris-HCl – 5 (final volume of 3 ml). The optic density level of the mitochondrial suspension with λ 520 nm was registered during 60 min of swelling with Ca^{2+} inductor (50 mcmol/L) available. The change in the level of mitochondrial swelling was determined as the difference between the rate of organelle swelling at 5, 10, 15, 20, 30, 40, 60 min relative to the initial value. The mitochondrial suspension in the incubation medium without the inductor was used as the control followed by further registration of the optic density during 60 min. Protein concentration in the incubation medium was 0,4 mg/ml. The change of E520 parameters in the incubation medium was used to calculate a relative rate of mitochondrial swelling [12].

Lipid peroxide oxidation (LPO) in the mitochondria was assessed according to the levels of thiobarbituric acid active products (TBAAP) [13]; carbonylation of the mitochondrial proteins – by the amount of 2,4-dinitrophenylhydrazone derivatives with the formation of carboxylphenylhydrazone (CPH). It was presented in nmol of carbonyl derivatives per 1mg of protein [14]. The state of the antioxidant defense system in the mitochondria was estimated according to the enzymatic activity of superoxide dismutase (SOD) and catalase [12]. Protein content in the mitochondria was determined by Lowreh method [15].

The results of the study was statistically processed by means of Student criterion. To confirm reliability of the conclusions the non-parametric Mann-Whitney comparison test was used that demonstrated similar results of the calculations obtained by the Student criterion concerning p value. Differences were statistically reliable with $p \leq 0,05$.

RESULTS

Participation of the mitochondria in various critical processes within the cell is a well-known fact. These are oxidative phosphorylation, tricarboxylic acid cycle, fatty acid oxidation, calcium ion homeostasis, and apoptosis regulation [16]. Although, their main function is ATP production in order to obtain energy. Disorders of the main mitochondrial functions is usually characterized as mitochondrial dysfunction. Recently the scientists have associated it with numerous diseases. Therefore, many studies are being carried out in order to find how mitochondrial dysfunction is connected with pathology of different diseases, for example, with metabolic syndrome and GABA-receptors.

Results of our studies demonstrated in Fig.1 and Fig.2 show the dynamics of intensity changes of the hip-

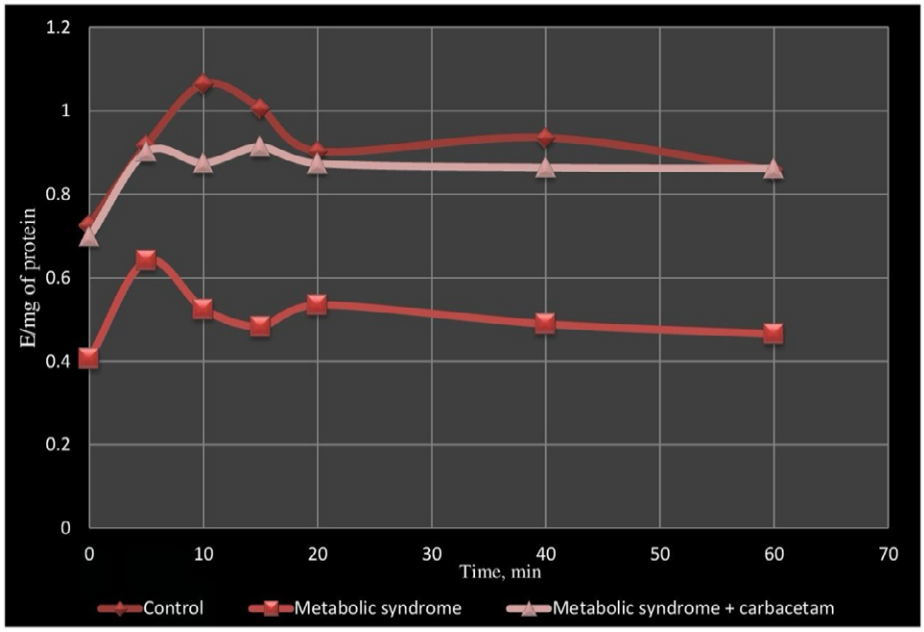

Fig. 1. Intensity of mitochondrial swelling in the hippocampus of male rats with metabolic syndrome after carbacetam administration during 14 days in the dose of 5 mg/kg
Picture taken by the authors

pocampal mitochondrial swelling in male and female rats. Thus, light scattering level of the mitochondrial suspension in the control group of male and female rats from the 1st to the 15th minute increased, and after the 20th to 60th – decreased. Thereby, our results obtained coordinate with literature data concerning physiological role of the mitochondria in maintenance of their own homeostasis due to their ability to maintain Ca2+ balance in the matrix.

Rats with metabolic syndrome in comparison with those from the control group presented decreased light scattering of the mitochondrial suspension after60 minutes of incubation by 45,71 % in males and 39,2 % – in females (Fig. 1, 2). After a 14-day administration of carbacetam rats with metabolic syndrome presented gradual increase of light scattering both in males and females – by 85,3 and 60,8 % respectively compared to the simulated pattern of the pathology. Thus, simulation of metabolic syndrome provoke more pronounced mitochondrial lesions in male rats, but they respond to carbacetam administration better.

Results of our studies showed (Fig. 3) that a relative rate of mitochondrial swelling in rats with metabolic syndrome decreased in comparison with the control group both in males and females – by 50,0 % and 25,0 % respectively. It should be noted that after carbacetam administration, compared to the results obtained from the group of simulated pathology, a relative rate of mitochondrial swelling increased in both research groups: by 59,5 % – in males and 16,0 % – in females.

That is, inn rats of both sexes with metabolic syndrome, the sensitivity of the mitochondrial pore to the action of calcium ions increases, which is probably related to the overload of these ions under pathological conditions. A probable cause of these processes is a disturbance of the membrane permeability. However, administration of carbacetam to rats caused a decrease in the level of mitochondrial swelling. It is possible that the mechanism of carbacetam action is associated with prevailing intensification of NAD-dependent oxidation, which is one of the ways to increase the resistance of the mitochondrial respiratory link [17].

In this respect, the next stage of our research was to study the processes of lipid and protein oxidation in the mitochondria. The results of our experiment are presented in the Table 1. As we can see, the content of products of lipid and protein peroxidation increases in rats with metabolic syndrome. Thus, in rats with simulated pathology the content of TBAAP increased in male and female rats by 81,0 % and 97,6 % respectively. At the same time, CPH content in these groups increased as well. It was 56,3 % in males, and 41,9 % in females compared to the control group of rats.

The results obtained are indicative of the activation of lipid and protein peroxidation processes in both sexes of rats with metabolic syndrome. At the same time, in the research groups the activity of the major enzymes of the antioxidant defense decreases. Thus SOD activity 32,6 % decreased in male rats with simulated pathology, and the same tendency to decrease is found among females. Catalase activity 21,5 % decreased in male rats and 18,1 % in females.

Administration of carbacetam to rats promoted a decrease in the content of TBAAP and CPH in both groups, in comparison with the rats with simulated pathology. Thus, inn male rats the content of TBAAP and CPH decreased by 31,9 %, 21,3 %, and in female rats by 27,3 %, 17,6 %, respectively. At the same time,

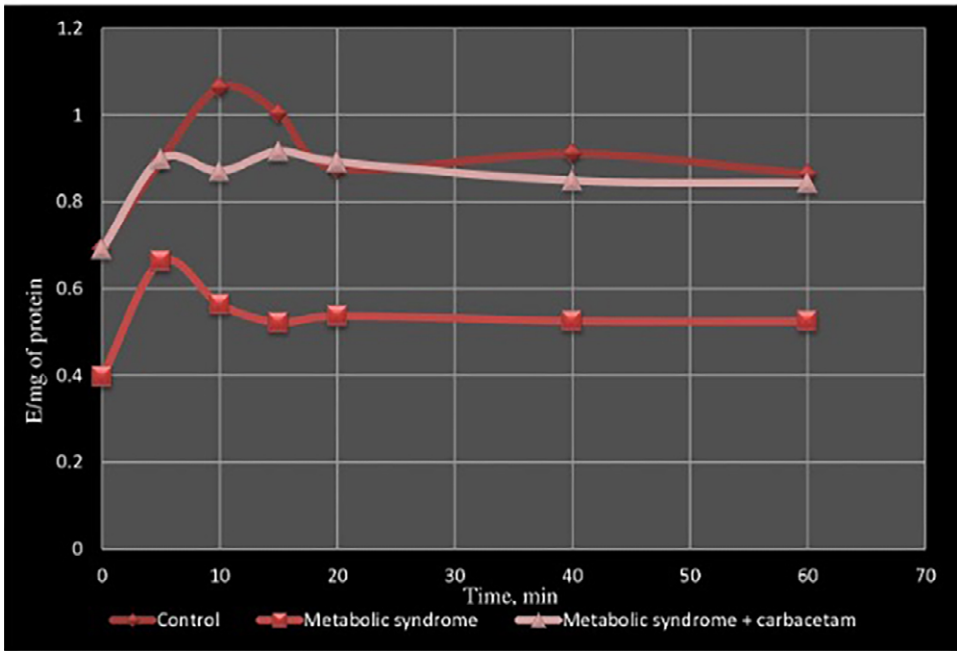

Fig. 2. Intensity of mitochondrial swelling in the hippocampus of female rats with metabolic syndrome after carbacetam administration during 14 days in the dose of 5 mg/kg
Picture taken by the authors

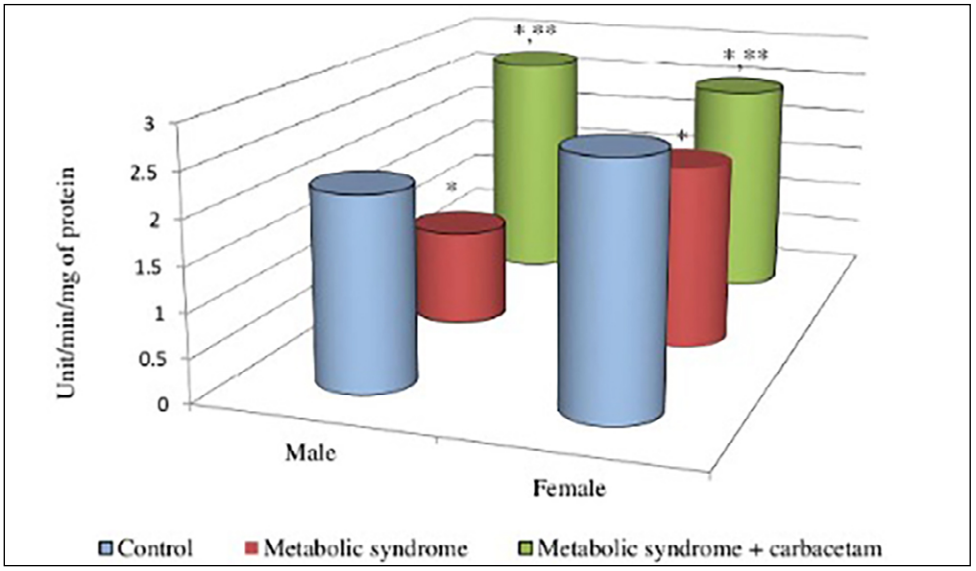

Fig. 3. Relative rate of mitochondrial swelling in the hippocampus of rats with metabolic syndrome after carbacetam administration during 14 days in the dose of 5 mg/kg (M±м, n=7)
Notes:* – reliability of differences compared with the control group of rats,
** – reliability of differences compared with the group of rats with metabolic syndrome
Picture taken by the authors

the activity of the antioxidant defense enzymes increased. SOD activity increased by 24,1 % in males, but among females only a tendency to increase the enzymatic activity was found. Meanwhile, catalase activity increased both in males and females – by 14,8 % and 11,2 % respectively.

Therefore, disorders of the mitochondrial functional state and prooxidant-antioxidant imbalance is found in both sexes with metabolic syndrome, but more pronounced in males. Nevertheless, modulation of GABA-receptors with carbacetam promotes improved mitochondrial functional state and decreased processes of lipid and protein peroxidation with activation of the antioxidant defense enzymes. Male rats are exposed to the modulator effect better than females.

DISCUSSION

Our experimental data are consistent with the publications of other scientists. In particular, it is believed that males are more sensitive to oxidative stress than females [18]. First, this is due to increased leakage of superoxide anion in the mitochondrial chain. In addition, they have lower expression and activity of important antioxidant enzymes, such as SOD and glutathione peroxidase. These facts contribute to a greater accumulation of oxidative damage over time in DNA, proteins and lipids, disrupting the proper function of cells and tissues. Therefore, taking into account sex differences in metabolic syndrome will improve the understanding of pathogenesis and contribute to the development of personalized medical approaches to more effectively address the problems of metabolic disorders.

Table 1. Carbacetam effect on free radical lipid and protein oxidation in the mitochondria of the hippocampus of rats with metabolic syndrome (M±m, n=7)

Indices	Research groups	Control	Metabolic syndrome	Metabolic syndrome + carbacetam
The content of TBAAP, nmol/mg of protein	Males	11.6±0.66	21.0±1.14*	14.3±0.69* **
	Females	12.4±0.91	24.5±0.99*	17.8±0.59* **
The content CPH, nmol/mg of protein	Males	18.3±0.89	28.6±1.31*	22.5±0.79* **
	Females	22.4±0.66	31.8±0.73*	26.2±0.88* **
The activity of SOD, units / mg of protein	Males	0.43±0.039	0.29±0.008*	0.36±0.015**
	Females	0.37±0.050	0.28±0.023	0.32±0.024
The activity of catalase, mcmol H2O2/ min of mg of protein	Males	191.8±7.97	150.5±4.31*	172.7±3.17**
	Females	180.4±5.62	147.9±2.89*	164.4±2.21* **

Notes:* – reliability of differences compared with the control group of rats,
** – reliability of differences compared with the group of rats with metabolic syndrome.
Source: compiled by the authors of this study

In addition, the more pronounced sensitivity of GABA receptors to the action of carbaracetam in males due to the increase in the activity of antioxidant defense enzymes is probably due to a certain ratio of sex hormones. For example, it is known that significant differences in GABA concentrations are observed during the estrous cycle in females. This indicates the relationship between the neural system and sex hormones and their significant role in the expression of feedback [19]. This assumption is based on the data of other scientists, since in our experiment we did not determine the levels of hormones, which is a prospect for further research. After all, hormonal differences are the main cause of behavioral and physiological changes observed between individuals. At the same time, there are few studies that show sex-related differences in the functioning of the GABA-ergic system. Sex bias is observed in many pathological mechanisms [19]. Taking into account all the above, it confirms the importance of this study to determine the relationship between gender and GABAergic receptors observed in metabolic syndrome. Understanding these processes will provide an opportunity to influence the prevalence and progression of the disease, and will serve as a basis for the development of new preventive and therapeutic strategies.

Modulation of type A GABA-receptors, which regulate permeability of chlorine channels, is a probable mechanism of correction of the obtained changes [20]. According to the science, it is this type of receptors located in the walls of the cerebral vessels. Their stimulation promotes vasodilation and improvement of blood supply to the brain [21]. Therefore, these processes improve blood flow volume and brain oxygenation. In its turn, it slows the intensity of lipid and protein peroxidation processes. It results in a decreased generation of oxygen active forms and insulin resistance [22, 23]. Accordingly, the balance between energy production and its use is restored leading to better cellular metabolism. Since disturbed metabolism is the main cause of metabolic syndrome, it is one of the possible mechanisms of its prevention and treatment.

In addition, in our studies, we observe an increase in the activities of antioxidant defense enzymes - SOD and catalase when using carbaracetam. This indicates the ability of this drug, through the modulation of GABA receptors, to restore antioxidant defense when it is reduced, in particular - metabolic syndrome. Its binding to these type A receptors causes conformational changes in ion channels of cell membranes, due to which the permeability of the central part of the channel for chloride ions increases [24]. It provokes conformation changes in the ion channels of the cellular membranes due to which permeability of a central part of the channel for chlorine ions increases. Increased entrance of chlorine ions causes hyperpolarization and metabolism correspondence to the functional requirements of the cells. At the same time, glutamate-calcium excitotoxicity cascade is modulated and calcium-dependent pathologic reactions decrease. The changes indicated result in decreased formation of oxygen active forms, LPO, and increased activity of the antioxidant protection enzymes.

One of the probable protective mechanisms of carbacetam action is increasing affinity of the nerve cells to GABA-benzodiazipine receptor complex, which decreases hyperexcitability of glutamate receptors and glutamate excitotoxicity respectively [25]. It results in decreased activity of NO-synthase, reduced production of NO, increased content of reduced glutathione and its enzymes. As a result, functional stability of neurons increases [25].

Scientific literary sources report that oxidative stress and mitochondrial dysfunction participate in the pathology of metabolic diseases, which is evidenced in our studies [26]. Based on the obtained results, we observe improvement of the functional state of mitochondria in the hippocampus of rats after carbacetam administration, which is indicative of an important value of GABA-receptors in metabolic disorders. Meanwhile, further studies are required to learn the role of GABA-receptors in metabolic disorders and the ways responsible for the mitochondrial function and sensitivity to insulin in the human body.

CONCLUSIONS

1. When simulating metabolic syndrome in the mitochondrial fraction of the hippocampus of rats, light scattering decreases and a relative rate of mitochondrial swelling increases; free radical oxidation of lipids and proteins increases with more pronounced changes in males.
2. 14-day administration of carbacetam to rats with metabolic syndrome promotes a decrease in light scattering and a relative rate of mitochondrial swelling in both males and females observed in the mitochondrial fraction. The content of products reacting with 2-thiobarbituric acid and protein oxidative modification decrease. Catalase activity in both males and females increases, and superoxide dismutase increases in males only.
3. A decreased intensity of mitochondrial swelling and improved condition of the antioxidant system of the hippocampal mitochondria of rats with metabolic syndrome irrespective of their sex is indicative of the effective correction of GABA receptors by means of carbacetam under conditions of the experiment.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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