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Original Article

Correlation between cognitive disorders in rats with type 2 diabetes mellitus and biochemical markers of oxidative stress

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Abstract

In recent decades, the prevalence of type 2 diabetes mellitus (T2D) has risen due to population aging. T2D is closely linked to nervous system pathology, with metabolic disorders affecting biochemical pathways crucial for neural function and cognitive processes. Scientific evidence indicates that patients with T2D exhibit increased brain GABA levels, correlating with cognitive impairment. This study aimed to examine the relationship between cognitive disorders, oxidative stress markers in the cerebral cortex and hippocampus, and the modulation of GABA receptors by carbacetam. Experiments were conducted on male albino rats, with T2D induced by streptozotocin (30 mg/kg) and a high-fat, high-fructose diet. Carbacetam (5 mg/kg) was administered for 14 days. Findings revealed significant correlations between cognitive dysfunction and oxidative stress, highlighting the need for novel neurodegenerative treatment strategies. These results provide an experimental basis for future clinical research on carbacetam as a potential therapeutic agent for T2D-related cognitive decline.

Keywords: type 2 diabetes mellitus, carbacetam, oxidative stress, cognitive ability of rats.

Introduction

In recent decades, due to the aging of the population, the sickness rate of type 2 diabetes (T2D) has increased. The World Health Organization estimates approximately 422 million people in the world with the diagnosis of DM, and about 1,5 million die annually [1]. An increased number of evidence from epidemiological studies indicates that T2D produces a negative effect on the structure and functions of the brain and promotes the progress of cognitive dysfunctions starting from a mild decrease of cognitive abilities and resulting in apparent dementia [2, 3]. At the same time, scientific studies confirm that the disease is associated with greater cerebral atrophy with age, and there is suggestion that changes can occur early in adulthood and correlate with the duration of the disease [4]. Moreover, metabolic disorders associated with T2D affect various biochemical ways responsible for neural function and decrease cognitive processes [5].

Therefore, early diagnostics of accelerated aging of the brain can promote finding people with a higher risk of dementia development in time [6]. Unfortunately, due to the lack of reliable and sensitive biomarkers and accurate diagnosis of diabetic lesions of the brain, it is mostly based on psychological and clinical signs. According to the scientific evidence, patients with T2D present an increased concentration of GABA in the brain, which correlates with the degree of cognitive function disorder [7]. Therefore, it is a serious barrier to self-sufficiency and a growing social burden for the aging population. In total, adjuvant precognitive therapy to alleviate cognitive decline in patients with diabetes is nowadays an unsatisfied medical need. Underlying pathophysiological mechanisms are unclear, but insulin signalization modulates the activity

of neuromodulators, including inhibitory receptors of γ-aminobutyric acid (GABA) and excitant glutamate receptors. Therefore, we were interested in learning correlations between cognitive disorders in rats with T2D and biochemical markers of oxidative stress after modulation of GABA receptors. For our research, we chose a new modulator of the GABA-ergic system, a derivative of β-carbolines – carbacetam, which was synthesized at the L. M. Lytvynenko Institute of Physical-Organic Chemistry and Coal Chemistry of the National Academy of Sciences of Ukraine under the supervision of Doctor of Chemical Sciences S. L. Bogza [8].

The objective was to study correlations between cognitive disorders according to behavioral response data, biochemical markers of oxidative stress in the cerebral cortex and hippocampus of rats with T2D, and carbacetam modulation of GABA receptors.

Material and methods

The experiments were conducted on laboratory nonlinear albino male rats, following the main principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (18.03.1986), the EU Directives No. 609 of 24.11.1986, and the Order of the Ministry of Health of Ukraine No. 690 of 23.09.2009.

T2D was simulated by means of streptozotocin (Stz) injected into the peritoneum of the rats in the dose of 30 mg/kg on the citrate buffer (pH=4,5). The animals were kept on a high-fat diet and free access to fructose solution (200 g/L). The citrate buffer was injected into the peritoneum of the control group of rats with a standard diet and free access to water. Simulated T2D was confirmed on the seventh day after Stz administration by means of the determination of glucose in the blood plasma on an empty stomach. The rats with hyperglycemia lower than 10 mmol/L were removed from the experiment. On the 10th week after Stz administration, T2D was verified according to the main indicators of carbohydrate and lipid metabolism, positive glucose tolerance test and morphological changes.

On the 11th week after Stz administration rats were blindly distributed into the following groups: I – with administration of carbacetam into the peritoneum in the dose of 5 mg/kg; II – with administration of physiological solution. The rats from the control group received only physiological solutions during the whole period of correction (14 days) [6]. Euthanasia of rats was conducted under ether narcosis. The brain was removed in the cold and carefully washed with cool 0.9% NaCl solution. The hippocampus and cerebral cortex were isolated according to a stereotaxic atlas [7].

The cognitive ability of rats was assessed by the indices of "open field" and conditioned passive avoidance reflex (CPAR) tests [9]. Lipid peroxide oxidation (LPO) was assessed according to the levels of thiobarbituric acid active products (TBA AP). Proteins were assessed by the number of products of protein oxidative modification (POM) by means of the spectrophotometric method [10]. The state of the antioxidant defense system was estimated according to the enzymatic activity of superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GP), and glucose-6-phosphate dehydrogenase (G-6-PDH) [11]. Protein content in the samples was determined using the Lowreh method.

The correlation method was applied to analyze the factors influencing on the indicators considering the direction, power and value of the connection, and calculation of the Spearman correlation index (r). Only reliable indicators were considered. The indicator p assessed the power of correlation from three levels: weak – under 0,33, moderate – from 0,34 to 0,66, and strong – more than 0,67.

Results and discussion

Performing correlation analysis, we found a number of correlations between the functional state of the central nervous system and the indicators of the prooxidant-antioxidant system of the cerebral cortex and hippocampus of rats with T2D (Table 1). Thus, the index of the latent period of rats was negatively correlated with glutathione peroxidase activity and was positively correlated with the POM of this structure. We have found positive correlations between motor activity and the activity of antioxidant enzymes: SOD and hippocampal catalase.

Examination of holes by rats had a positive, reliable correlation with glutathione peroxidase, catalase of the cerebral cortex and SOD, hippocampal catalase. It had a negative correlation with POM, TBA AP of the cortex and POM of the hippocampus. Analysis of the correlation between the vegetative activity of rats and the prooxidant-antioxidant system has found a negative correlation with the TBA AP of the hippocampus. At the same time, a positive correlation was found between the vegetative activity and SOD activity of the cortex and glutathione peroxidase, and hippocampal glutathione reductase.

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Table 1: Pairs of correlations between the indicators of the functional state of the central nervous system and certain indices of the prooxidant-antioxidant system of the cortex and hippocampus of rats with type 2diabetes.

Pairs of correlations		Correlation coefficient, r	Reliability of correlation, p
Latent period	Cortical glutathione peroxidase	-0.709	<0.01
Latent period	Cortical POM	0.791	<0.01
Motor activity	Hippocampal SOD	0.792	<0.01
Motor activity	Hippocampal catalase	0.826	<0.01
	Cortical glutathione peroxidase	0.710	<0.01
	Cortical catalase	0.800	<0.01
	Cortical POM	-0.848	<0.01
Examination of holes	Cortical TBA AP	-0.843	<0.01
	Hippocampal SOD	0.736	<0.01
	Hippocampal catalase	0.727	<0.01
	Hippocampal POM	-0.801	<0.01
Vegetative functions			
Fecal bolus	Cortical glutathione reductase	0.749	<0.01
Urination	Cortical glutathione reductase	0.739	<0.01
Grooming	Cortical glutathione reductase	0.839	<0.01
Vegetative functions			
Fecal bolus	Cortical SOD	0.838	<0.01
Urination	Cortical SOD	0.842	<0.01
Grooming	Cortical SOD	0.883	<0.01
Vegetative functions			
Fecal bolus	Hippocampal TBA AP	-0.749	<0.01
Urination	Hippocampal TBA AP	-0.843	<0.01
Grooming	Hippocampal TBA AP	-0.812	<0.01
Vegetative functions			
Fecal bolus	Hippocampal glutathione peroxidase	0.768	<0.01
Grooming	Hippocampal glutathione peroxidase	0.752	<0.01
Vegetative functions			
Fecal bolus	Hippocampal glutathione reductase	0.721	<0.01
Grooming	Hippocampal glutathione reductase	0.814	<0.01

A group of weak correlations was found between the indices of the functional state of the central nervous system and hippocampal G-6-PDH activity. A negative, weak correlation was detected between the indicator of the latent period and TBA AP of the hippocampus. At the same time, a direct moderate correlation was found between oriental-learning activity and glutathione reductase, glutathione peroxidase of the cortex and hippocampus of rats.

Analysis of the kinds of correlation makes a basis to understand the value of correct interpretation of the results obtained. The results of the correlation analysis between the indicators of the functional state of the central nervous system and certain indices of the prooxidant-antioxidant system of rats with T2D and carbacetam correction revealed substantial links (Table 2).

The highest values of the linear correlation coefficients were detected in the pair of vegetative functions

Table 2: Pairs of correlations between the indicators of the functional state of the central nervous system and certain indices of the prooxidant-antioxidant system of the cortex and hippocampus of rats with type 2diabetes after carbacetam correction.

Pairs of correlations		Correlation coefficient, r	Reliability of correlation, p
Latent period	Cortical glutathione peroxidase	-0.709	<0.01
Motor activity	Cortical glutathione reductase	-0.741	<0.01
Motor activity	Hippocampal SOD	0.780	<0.01
Motor activity	Hippocampal G-6-PDH	-0.692	<0.01
Vegetative functions			
Grooming	Cortical SOD	0.776	<0.01
Grooming	Cortical TBA AP	-0.798	<0.01
Grooming	Hippocampal POM	-0.842	<0.01

(grooming) and cortical SOD. At the same time, the latent period had a negative correlation with cortical glutathione peroxidase, and motor activity had a negative correlation with cortical glutathione reductase and hippocampal G-6-PDH. The vegetative functions (grooming) had a negative correlation with cortical TBA AP hippocampal POM.

The correlation analysis performed enabled the determination of certain relations between cognitive disorders and examined biochemical indices of the cerebral cortex and hippocampus of rats. The results obtained are confirmed and correlate with biochemical markers of oxidative stress induced by pathogenic mechanisms of T2D in the examined cerebral structures of rats. At the same time, carbacetam's effectiveness as a GABA-receptor modulator is demonstrated in the evaluation of its effect on cognitive processes and oxidant-antioxidant balance.

In our opinion, the data obtained concerning anti-amnestic effects are stipulated by carbacetam nootropic activity, which is associated with the influence on the GABAergic system [9]. First, it is caused by the system of GABA synthesis and degradation in the walls of cerebral vessels, which plays a considerable role in the regulation of cerebral circulation. It includes dilation of the cerebral vessels, increased volume of the blood flow, oxygenation and improvement of the brain energy [10]. Moreover, carbacetam modulates GABA A-receptors, which are chlorine channel permeability regulators in the central nervous system [11]. When intracellular chlorine anion increases, hyperpolarization happens, neural communication and synchronization of the neural population improves, and cognitive processes activate [12]. One more important factor is that carbacetam promotes restoration of vasopressin secretion, which produces anti-stress action and a positive effect on cognitive and mnestic functions of animals [13].

The processes described above explain the mechanism of enhancing antioxidant protection in neurons of the examined structures under the influence of carbacetam as GABA-receptor modulator. Since our studies, we have found intensification of the processes of free radical oxidation of lipids and proteins and damage of biological membranes in rats with T2D. Accordingly, these mechanisms affect various functions, resulting in increasing the stiffness of the membrane, reduced activity of enzymes associated with the membrane, disturbance of the membrane receptors and permeability changes. In addition to phospholipid damage, radicals are able to attack membrane proteins directly and induce the binding of lipids-proteins and proteins-proteins that affect membrane integrity.

A probable protective effect of carbacetam is its binding with GABA A-receptors. It provokes conformation changes in the ion channels of the cellular membranes, due to which the 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, the 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 the increasing affinity of the nerve cells to the GABA-ben-

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zodiazepine receptor complex, which decreases the hyperexcitability of glutamate receptors and glutamate excitotoxicity, respectively [14]. It results in decreased activity of NO-synthase, reduced production of NO, and increased content of reduced glutathione and its enzymes. As a result, the functional stability of neurons increases, which is confirmed by a positive structural rebuilding of the cerebral cortex of rats in the experiments conducted [14, 15].

Therefore, the established mechanisms of cerebral cells protection specific for carbacetam as a new derivative of β-carboline, which are supplemented by the correlation between cognitive disorders in rats with T2D and biochemical markers of oxidative stress, make an experimental basis for further clinical studies of this promising neuroprotector.

Conclusions

Correlation indices of the functional state of the central nervous system with the indicators of the prooxidant-antioxidant system of the cortex and hippocampus of rats with type 2 diabetes characterize their value in the functional-metabolic continuum.

The obtained data on the correlation between cognitive disorders and biochemical markers of oxidative stress in rats with T2D confirm the necessity to develop new methods of therapy for neurodegenerative processes. It is an experimental base for further clinical studies of carbacetam.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Baduini IR, Vildosola JCE, Kavehmoghaddam Sh, et al. Type 2 diabetes mellitus and neurodegenerative disorders: The mitochondrial connection. Pharmacological Research. 2024; 209: 107439. DOI: 10.1016/j.phrs.2024.107439
2. Madhusudhanan J, Suresh G, Devanathan V. Neurodegeneration in type 2 diabetes: Alzheimer's as a case study. Brain Behav. 2020;10(5):e01577. doi: 10.1002/brb3.1577.
3. Grasset L, Frison E, Helmer C, et al. Understanding the relationship between type-2 diabetes, MRI markers of neurodegeneration and small vessel disease, and dementia risk: a mediation analysis. Eur J Epidemiol. 2024;39:409–417 https://doi.org/10.1007/s10654-023-01080-7
4. Zhang T, Shaw M, Cherbuin N. Association between Type 2 Diabetes Mellitus and Brain Atrophy: A Meta-Analysis. Diabetes Metab J. 2022;46(5):781-802. doi: 10.4093/dmj.2021.0189.
5. Antar SA, Ashour NA., Sharaky M, et al. Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. Biomedicine & Pharmacotherapy. 2023;168:115734. https://doi.org/10.1016/j.biopha.2023.115734.
6. Baduini IR, Vildosola CJE, Kavehmoghaddam Sh, et al. Type 2 diabetes mellitus and neurodegenerative disorders: The mitochondrial connection. Pharmacological Research. 2024;209:107439. https://doi.org/10.1016/j.phrs.2024.107439.
7. Van Bussel FC, Backes WH, Hofman PA, et al. Increased GABA concentrations in type 2 diabetes mellitus are related to lower cognitive functioning. Medicine. 2016;95:e4803. doi: 10.1097/MD.0000000000004803.
8. Bogza SL. New heterocyclic systems for new drugs. Nauka inov. 2015;11(6):85-93. https://doi.org/10.15407/scin11.06.085.
9. Li Y, Sun H, Chen Z, et al. Implications of GABAergic Neurotransmission in Alzheimer's Disease. Front. Aging Neurosci. 2016;8(31):1 – 12. doi: 10.3389/fnagi.2016.00031.
10. Salvagno M, Sterchele ED, Zaccarelli M, et al. Oxidative Stress and Cerebral Vascular Tone: The Role of Reactive Oxygen and Nitrogen Species. Int. J. Mol. Sci. 2024;25:3007. https://doi.org/10.3390/ijms25053007
11. Wang Z, Choi K. Pharmacological modulation of chloride channels as a therapeutic strategy for neurological disorders. Frontiers in Physiology. 2023;14:1122444. DOI: 10.3389/fphys.2023.1122444.
12. Cherubini EDiCG, Avoli M. Dysregulation of GABAergic Signaling in Neurodevelomental Disorders: Targeting Cation-Chloride Co-transporters to Re-establish a Proper E/I Balance. Front Cell Neurosci. 2022;5:15:813441. doi: 10.3389/fncel.2021.813441.
13. Zyablitsev S, Starodubska A, Bogza S. The effect of carbacetam on cognitive impairment in experimental brain injury, the possible role of vasopressin. TRAUMA. 2022;18(2):53 – 8. https://trauma.zaslavsky.com.ua/index.php/journal/article/view/515
14. Khaitovich MV. GABA-ergic neuroprotection: clinical application. Medicines of Ukraine. 2016;1(197-198):22 – 26.
15. Kmet OG, Filipets ND, Hrachova TI, Vepriuk YM, Vlasova KV. Protein peroxide oxidation and morphological condition of the cerebral cortex and the hippocampus of rats with neurodegeneration with type 2 diabetes mellitus under carbacetam effect. Archives of the Balkan Medical Union. 2019;54(3): 431 – 437. https://doi.org/10.31688/ABMU.2019.54.3.05