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FUNCTIONAL DISORDERS OF THE ANTIOXIDANT PROTECTION GLUTATHIONE COMPONENT IN THE BRAIN OF RATS WITH EXPERIMENTAL TYPE 2 DIABETES MELLITUS AND CARBACETAM AND ENALAPRIL EFFECT PRODUCED ON IT

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Abstract

Changes of glutathione component of the antioxidant system in the central nervous system with experimental type 2 diabetes mellitus are investigated under effect of Carbacetam and Enalapril. The experiments were conducted on nonlinear laboratory albino male rats with the body weight of 0,18–0,20 kg with type 2 diabetes mellitus simulated by Streptozotocin and high-fat diet.

Under conditions of simulation of type 2 diabetes mellitus in the cerebral cortex and hippocampus of rats the content of reduced glutathione, SH-groups, activity of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase are found to decrease. The data obtained are indicative of decrease of the antioxidant system in the examined structures of the brain.

After administration of Carbacetam and Enalapril during 14 days to rats with diabetes mellitus the content of SH-groups and activity of glutathione-dependent enzymes in the cerebral cortex and hippocampus increase. Enalapril administration promotes increase of reduced glutathione in the cerebral cortex only, which is indicative of a higher sensitivity of this structure to the action of the examined drug. Improvement of the state of antioxidant protection glutathione component in the cerebral cortex and hippocampus after Carbacetam and Enalapril administration under conditions of simulation of type 2 diabetes mellitus is indicative of the ability of the drugs to activate the state of glutathione chain of the antioxidant protection in the central nervous system; mostly in case of Carbacetam administration than Enalapril.

Keywords: *type 2 diabetes mellitus, neurodegeneration, Carbacetam, Enalapril, glutathione component of antioxidant protection.*

Introduction

The sickness rate of diabetes mellitus (DM) today is characterized as a growing pandemic promoting continuous improvement of the disease and its complications. One of the target organs of diabetes is the central nervous system (CNS). Its hyperglycemic lesions are manifested by progressing cognitive disorders and reduced quality of life for patients. A considerable pathogenic component of DM is activation of processes of free radical oxidation of biomolecules resulting in pro-antioxidant imbalance [1].

Since diabetes is a metabolic disease characterized by hyperglycemia, insulin resistance or reduced insulin secretion, free radical increase in proportion to hyperglycemia, non-enzymatic glycosylation and oxidative destruction of proteins. Extremely high levels of free radicals and simultaneous decrease of antioxidant protective mechanisms accelerate damage of cellular organelles, lipid peroxide oxidation and development of DM complications respectively [1].

A universal neuromediator of the CNS providing the balance of inhibition and simulation, energy requirements and brain resistance to hypoxia is gamma-aminobutyric acid (GABA). It should be noted that GABA functional cycle is closely associated with glucose metabolism: its transport and utilization [2]. Moreover, it participates in many metabolic processes: increases oxygen supply to the cells, ATP formation, that is, it increases cellular resistance of the brain to oxygen insufficiency, activates protein synthesis, energy processes, and improves blood supply to the brain. Considering mediator and metabolic properties of this acid we became interested in investigation of a new GABA modulator - Carbacetam [3] produced on the state of glutathione component in the antioxidant system under conditions of simulated diabetes mellitus in rats.

The results of experimental and clinical studies in recent years are indicative of a considerable role of renin-angiotensin system (RAS) in the development of DM complications from the side of the CNS. First of all, it is excessive effects of angiotensin II (A II) induced by hyperglycemia, resulting in mitochondrial dysfunction, energy deficiency, oxidative stress and death of neurons [4, 5]. The brain activity is known to be regulated by both the circulating (systemic) RAS and tissue (local) RAS directly involved in the development of DM complications [4]. Correspondingly, RAS blockers by means of activation of antioxidant enzymes and vasodilation effects increase tissue resistance to hyperglycemia damaging effects. Considering the mentioned above we are interested in investigation of RAS blocker Enalapril on the glutathione component in the brain of rats with diabetes mellitus.

Materials and methods

The experiments were conducted on male rats with the body weight of 0,18–0,20 kg, kept under conditions of natural changes of light regimen, temperature and air humidity according to vivarium standards. The study was conducted keeping to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific (Strasburg, 1986).

Type 2 DM was induced by intraperitoneal (i/p) injection of Streptozotocin (Stz) in the dose of 30 mg/kg to rats fed for 30 days on high-fat diet with free access to fructose solution (200 g/L) [6-8]. The control group of rats with a standard diet and free access to water was i/p injected with citrate buffer only (pH=4,5). On the 7th day after Stz injection type 2 DM was evidenced by detection of glucose concentration in the blood plasma on an empty stomach. The rats with hyperglycemia lower than 10 mmol/L were excluded from the experiment.

On the 11th week after Stz injection the rats were randomize into three groups: I – with Carbacetam i/p injection in the dose of 5 mg/kg; II – with Enalapril i/p injection in the dose of 1 mg/kg, III – with injection of normal saline solution. The rats from the control group during the whole period of correction (14 days) received physiological solution (saline).

Euthanasia of rats was performed under light ether narcosis. The brain was removed at cold temperature, washed carefully with cool 0,9 % NaCl solution, and the cerebral cortex and hippocampus were isolated by the stereotaxic atlas [9]. Cytoplasmic fraction was isolated by means of the differential centrifugation method of homogenate of the examined structures on the refrigerator centrifuge at 1000 g 10 min, later – 1400 g 10 min at

the temperature of 4 °C. To evaluate the antioxidant system state of the cerebral cortex and hippocampus the content of the following substances was determined: reduced glutathione (G-SH), sulfhydryl (SH-) groups and activity of glutathione reductase (GR) [enzyme code (EC) 1.6.4.2], glutathione peroxidase (GP) [EC 1.11.1.9], and glucose-6-phosphate dehydrogenase (G-6-PDG) [EC 1.1.1.49] by means of certain methods [10, 11]. The amount of protein in specimens was determined by Lowry method [12].

The results were statistically processed by means of Student t-criterion. Mann-Whitney criterion was applied parallel that demonstrated similar results. Therefore, confidence level was considered sufficient with $p \leq 0,05$.

Results and Discussion

Since glutathione is a central component of antioxidant systems, its content in the cerebral cortex and hippocampus was examined (Table). Thus, in rats with simulated DM the content of G-SH 48,4 % decreased in the cortex and 48,1 % - in hippocampus as compared with the parameters of the control group of rats. Injection of Carbacetam and Enalapril resulted in an increased content of G-SH 1,9 and 1,5 times in the cerebral cortex. In the hippocampus under effect of Carbacetam the content of G-SH 1,5 times increased, and with injection of Enalapril it became close to the level of that of the control group. Increase of G-SH content is likely to occur at the expense of its intensified regeneration from the oxidized form in the tissues of the cerebral cortex and hippocampus.

Further analysis of the results demonstrated a decreased activity of enzymes participating in the process of antioxidant protection in rats with DM. GP activity that uses G-SH to neutralize hydrogen peroxide and other hydroperoxides was 39,6 % lower in the cerebral cortex and 36,2 % lower in the hippocampus of rats with DM than those in the control group. At the same time, GR activity decreased in both examined structures on an average 42,5 % as much concerning the control parameters. Reduced activity of enzymes is most likely stipulated by their intensified use for neutralization of excessive amounts of oxygen active forms in the cerebral tissues. Moreover, G-6-PDG activity decreased in the group of a simulated pathology both in the cerebral cortex and hippocampus: on an average 1,6 times as much in comparison with the control. Reduced activity of this enzyme decreases energy supplies of cells at the expense of inhibition of pentose-phosphate glycolysis, resulting in decreased amount of G-SH, which neutralizes oxygen active forms. At the same time, the content of SH-groups contained in glutathione and providing biochemical metabolic reactions and maintenance of the membrane functional characteristics, 35,7 % decreased in the cerebral cortex and 38,0 % – in the hippocampus.

Administration of Carbacetam and Enalapril to rats with DM promoted increased antioxidant protection both in the cerebral cortex and hippocampus. In particular, in comparison with the parameters of rats with control pathology, the content of G-SH after Carbacetam injection in the cerebral cortex 1,6 times increased and 1,5 times – under Enalapril effect. After administration of Carbacetam GP activity increased both in the cerebral cortex and hippocampus: 52,2 % and 43,1 % respectively. Though after administration of Enalapril to diabetic rats there was no reliable difference found in GP activity.

Though GR activity increased both after administration of Carbacetam and Enalapril in both examined structures 1,7 and 1,6 times respectively. Carbacetam indication promoted increased activity of G-6-PDG both in the cerebral cortex and hippocampus: 47 % and 45 % respectively. At the same time, after Enalapril indication a tendency was observed to increase of this enzyme activity in both examined structures. The content of SH-groups after Carbacetam administration in the cerebral cortex 41,6 % increased and 32,1 % – in the hippocampus. Under effect of Enalapril the content of SH-groups in the cerebral cortex 36,6 % increased and 16,6 % – in the hippocampus.

Thus, in the course of the conducted experimental investigations we have determined that Carbacetam and Enalapril increase activity of the antioxidant system of the brain under conditions of simulated type 2 DM in rats. The results obtained are indicative of availability of their administration in order to increase neuron resistance to the action of

free radical compounds under conditions of diabetes.

Activation of lipid peroxidation reaction is known to be one of the fundamental biological mechanisms to damage biological structures and promote development of cellular pathology under effect of harmful factors of various genesis. Lipid peroxide oxidation mechanism in the cells of the CNS is similar to the mechanisms in other tissues, though intensity of the process here is considerably higher. It is mainly determined by a high content of polyunsaturated fatty acids in the brain, which are a substrate of lipid peroxide oxidation, high concentrations of metal ions with changeable valence essential for functioning of enzymes, low activity of antioxidant enzymes and other components of antioxidant protection. On the whole, it is the deficiency of the antioxidant system in the cerebral tissue that explains its special sensitivity to the production of free radical substances [13], and in case of pathogenesis of DM and its neurological complications in particular. Under conditions of diabetes hyperglycemia induces formation of free radicals at the expense of accelerated auto-oxidation of glucose and non-enzymatic glycosylation of proteins [14]. Highly reagent oxygen-containing free radicals formed during these processes cause intensification of lipid and protein peroxide oxidation processes with formation of toxic products.

Increase of antioxidant protection under effect of Carbacetam is most likely caused by a simulating effect on GABA-receptors [15]. Since GABA modulators are known to prevent a destructive action of lipid peroxidation products, promote normalization of qualitative and quantitative content of phospholipids, they produce a protector effect on the membrane structures of the nerve cell. At the expense of affinity of nerve cells to GABA-benzodiazepin-receptor complex Carbacetam decreases hyperirritability of glutamate receptors, and glutamate exciting toxicity respectively [16]. Due to this fact the content of reduced glutathione and its enzymes (GP, GR) increases, which is an important constituent of a regulatory and protective action of the drug [2].

Increase of the antioxidant system against the ground of Enalapril administration is likely associated with inhibition of RAS excessive activity under conditions of hyperglycemia. Since angiotensin II in an excessive amount is neurotoxic, it promotes production of oxygen active forms resulting in intensification of oxidative stress [5]. Correspondingly, Enalapril administration blocks angiotensin II synthesis resulting in spasmolytic and vasodilation effects. It leads to the improvement of blood supply of the cerebral tissues and protection of its functional state against hypoxia development due to activation of glutathione component of the antioxidant protection of the nerve cells in the cerebral cortex and hippocampus.

Conclusions

1. Under conditions of simulated type 2 diabetes mellitus the content of reduced glutathione, SH-groups, activity of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase decrease, which is indicative of inhibition of the antioxidant system.

2. After administration of Carbacetam and Enalapril during 14 days to rats with diabetes mellitus the content of SH-groups and activity of glutathione-dependent enzymes in the cerebral cortex and hippocampus increase. The content of reduced glutathione and activity of glucose-6-phosphate dehydrogenase increase in both examined structures after Carbacetam administration. Enalapril administration promotes increase of reduced glutathione in the cerebral cortex only, which is indicative of a higher sensitivity of this structure to the action of the examined drug.

3. Improvement of the state of antioxidant protection glutathione component in the cerebral cortex and hippocampus after Carbacetam and Enalapril administration under conditions of simulation of type 2 diabetes mellitus is indicative of the ability of the drugs to activate the state of glutathione chain of the antioxidant protection in the central nervous system; mostly in case of Carbacetam administration than Enalapril.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. de Souza Bastos A, Graves DT, de Melo Loureiro AP, Júnior CR, Corbi SCT, Frizzera F, Scarel-Caminaga RM, Câmara NO, Andriankaja OM, Hiyane MI, Orrico SRP. Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients. J Diabetes Complications. 2016;30(8):1593-9. doi: 10.1016/j.jdiacomp.2016.07.011.
2. Ziablitsev SV, Starodubska OO, Bogza SL. Carbacetam influence on cognitive disturbances in experimental brain injury, possible vasopressin role. Trauma. 2017;18(2):53-8. doi: 10.22141/1608-1706.2.18.2017.102559.
3. Li Y, Sun H, Chen Z, Xu H, Bu G, Zheng H. Implications of GABAergic Neurotransmission in Alzheimer's Disease. Front Aging Neurosci. 2016;8(31):1-12. doi: 10.3389/fnagi.2016.00031.
4. Nakagawa P, Sigmund CD. How is the Brain Renin-Angiotensin System Regulated? Hypertension. 2017;70(1):10-8. doi: 10.1161/HYPERTENSIONAHA.117.08550.
5. Jackson LD, Eldahshan W, Fagan SC, Ergul A. Within the Brain: The Renin Angiotensin System. Int J Mol Sci. 2018;19(3):1-23. doi.org/10.3390/ijms19030876.
6. Guo X, Wang Y, Wang K, Ji B, Zhou F. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. J Zhejiang Univ Sci B. 2018;19(7):559-569. doi: 10.1631/jzus.B1700254.
7. Jurgoński A, Juśkiewicz J, Zduńczyk Z. A High-Fat Diet Differentially Affects the Gut Metabolism and Blood Lipids of Rats Depending on the Type of Dietary Fat and Carbohydrate. Nutrients. 2014;3;6(2):616-626. doi: 10.3390/nu6020616.
8. Ruiza GA, Gracia FY. The Role of Fructose in Type 2 Diabetes and Other Metabolic Diseases. Journal of Nutrition & Food Sciences. 2018;8:659. DOI: 10.4172/2155-9600.1000659.
9. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 7th Edition. Academic Press; 2013.472 p.
10. Gerush IV, Bevzo VV, Ferenchuk YeO. The effect of melatonin on lipid peroxide oxidation, oxidative modification of proteins and mitochondria swelling in the skeletal muscle tissue of rats under alloxan diabetes. The Ukrainian Biochemical Journal. 2018;90(3):62–9.
11. Kushnir OYu, Yaremii IM, Shvets VI, Shvets NV. Influence of melatonin on glutathion esystem in rats skeletal muscle under alloxan induced diabetes. Fiziolohichnyi zhurnal. 2018;64(5):54–62.
12. Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. Journal of Medicine and Life. 2016;9(3):259-62.
13. Garbarino VR, Orr ME, Rodriguez KA, Buffenstein R. Mechanisms of Oxidative Stress Resistance in The Brain: Lessons Learned From Hypoxia Tolerant Extremophilic Vertebrates. Arch Biochem Biophys. 2015;15:8-16. doi: 10.1016/j.abb.2015.01.029.
14. Sadri H, Goodarzi MT, Salemi Z, Seifi M. Antioxidant Effects of Biochanin A in Streptozotocin Induced Diabetic Rats. Brazilian Archives of Biology and Technology. 2017;60:1-10. doi.org/10.1590/1678-4324-2017160741.
15. Kmet OG, Ziablitsev SV, Filipets ND, Kmet TI, Slobodian XV. Carbacetam effect on behavioral reactions in experimental Alzheimer's disease Archives of the Balkan Medical Union. 2019;54(1):124-9.
16. Moncada S, Nisticò G, Bagetta G, Higgs EA. Nitric Oxide and the Cell: Proliferation, Differentiation, and Death. – Princeton: Princeton University Press, 2017. 328 p.

Table: Carbacetam and Enalapril effect on the indices of glutathione system in cytosolic fraction of rats with type 2 diabetes mellitus (M±m, n=7)

Indices	Brain structures	Control	Diabetes mellitus	Diabetes mellitus + Carbacetam	Diabetes mellitus + Enalapril
Reduced glutathione (mcmol/(g of tissue))	Cerebral cortex	7,37±0,60	3,80±0,55*	6,20±0,34**	5,56±0,37*,**
	Hippocampus	6,84±1,02	3,55±0,41*	5,50±0,32**	5,18±0,78
Glutathione peroxidase (nmol GSSG/(min of mg of protein))	Cerebral cortex	143,17±13,99	86,48±15,09*	131,58±11,62**	121,88±10,55
	Hippocampus	131,46±15,55	83,82±18,39*	119,97±6,57**	110,52±10,07
Glutathione reductase (nmol NADPH /(min of mg of protein)	Cerebral cortex	3,71±0,49	2,23±0,44*	3,64±0,13**	3,48±0,27**
	Hippocampus	3,81±0,64	2,09±0,41*	3,53±0,23**	3,32±0,22**
Glucose-6-phosphate dehydrogenase (nmol/(min of mg of protein)	Cerebral cortex	6,29±0,11	3,94±0,26*	5,81±0,41**	5,20±0,68
	Hippocampus	4,83±0,37	2,88±0,55*	4,19±0,27**	3,59±0,27*
Sulfhydryl groups (nmol/(min of mg of protein)	Cerebral cortex	72,81±2,36	46,83±2,98*	66,48±2,74**	63,95±3,61**
	Hippocampus	70,58±3,80	50,83±3,56*	67,13±2,78**	59,28±1,35**

Notes: * – statistical significance in comparison of mean values with the control group; ** – statistical significance in comparison with the group of rats with diabetes mellitus.