https://doi.org/10.46389/rjd-2021-1020
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2021; volume 28, issue 2, pages 126-130

Original Research

Peculiarities of carbacetam effect on the processes of fibrinolysis and proteolysis in the brain of rats with neurodegeneration induced by type 2 diabetes mellitus

Olga Kmet*

Department of Pharmacology at Higher State Educational Establishment of Ukraine, «Bukovinian State Medical University», Chernivtsi, Ukraine

*Correspondence to: Kmet Olga, PhD, Associate Professor, Department of Pharmacology at Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», 2, Teatralna Sq., Chernivtsi, 58002, Ukraine, E-mail id: kmet.olga@bsmu.edu.ua, Phone: (+38 0372) 55-37-54; Cellphone: 095-863-41-11; ORCID authors: https://orcid.org/0000-0003-0336-1103

Received: 4 October 2020 / Accepted: 16 November 2020

Abstract

Introduction: Diabetic neurodegeneration is formed with underlying type 2 diabetes mellitus (T2DM) which is an independent factor promoting development of cognitive disorders. Considering an important role of GABA receptors in the development of neurodegenerative processes, the issue concerning carbacetam effect produced on proteolytic and fibrinolytic mechanisms of the cerebral cortex and hippocampus with experimental simulation of T2DM has become of a certain interest. **Materials and Methods:** Type 2 diabetes mellitus was simulated by streptozotocin in the dose of 30 mg/kg injected through the peritoneum of rats kept during 30 days on a high-fat diet with a free access to fructose solution. **Results:** Under conditions of pathological process development the activity of the main groups of proteolytic enzymes intensify in the cerebral cortex and hippocampus. Carbacetam administration to rats with diabetes mellitus promotes reduction of proteolysis processes which might be associated with pharmacodynamics of the drug. **Conclusions:** The data obtained experimentally substantiate reasonability of pathogenic correction by means of carbacetam in case of disorders of proteolytic and fibrinolytic systems activity in the cerebral cortex and hippocampus induced by T2DM.

Keywords: carbacetam, fibrinolysis, proteolysis, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is one of the most common endocrine pathologies in the world. The disease is characterized by a chronic course and development of complications in various organs and systems resulting in disability of capable of working individuals [1]. Diabetic neurodegeneration is formed with underlying type 2 diabetes mellitus (T2DM) which is an independent factor promoting development of cognitive disorders.

Brain activity considerably depends on the balance between neural activity and its metabolic requirements. Neurodegenerative processes are associated with these mechanisms respectively [2]. Considering a dynamic character of the brain activity and considerable metabolic requirements of the bioelectric-active nervous tissue, microcirculation of the brain must be very sensitive to the tissue it supplies. A direct innervation of the microvascular endothelium by means of the receptors of gamma amino butyric acid (GABA) neurons is confirmed anatomically [3]. GABA activity enables to maintain and improve metabolic, neurotrophic and energy supply of the brain by means of control over the nervous-vascular units [4], which dysfunction promotes disturbance of the hematoencephalic barrier integrity. Damages or poorly functioning barrier is more susceptible to inflammatory reactions including microglia and astrocytes which can promote advance of neurodegenerative processes.

Moreover, decreased GABA concentration in the brain will promote increasing pathological role of other neurotransmitters, glutamate with calcium compromised neurons [5], oxidative stress, disturbance of the membrane integrity in particular, resulting in swelling and activation of intracellular protease.

Under hyperglycemia conditions free radicals are known to be formed in the process of glucose "self-oxidation" during formation of the final products of accelerated glycosylation. Further they react with unsaturated compounds disturbing the structure of enzymatic proteins and lipids of the cellular membranes [6]. These processes activate proteolysis and lipid peroxide oxidation resulting in changes of physical-chemical properties of the biological membranes. It leads to destruction of membranous lipids where hydrolytic enzymes play the main role. An excessive formation of free radicals followed by the damage of the neuron membranous structures and DNA results in functional disorders of the nerve cells [7].

Considering the importance of GABA role in the receptors development of neurodegenerative processes, the issue concerning carbacetam effect produced on proteolytic and fibrinolytic mechanisms of the cerebral cortex and hippocampus with experimental simulation of T2DM has become of a certain interest.

Objective

To study carbacetam effect, as a modulator of GABA-receptors, produced on the fibrinolysis and proteolysis in the brain of rats with neurodegeneration induced by T2DM.

Materials and Methods

The experiments were conducted on non-linear laboratory albino male rats with their body weight of 0.18–0.20 kg, kept under standard vivarium conditions with natural alternation of day and night, according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986); European Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions regarding the protection of animals used for experimental and other scientific purposes, and the Order of the Ministry of Health of Ukraine № 690 of 23 September 2009.

Type 2 diabetes mellitus was simulated by streptozotocin (Stz) in the dose of 30 mg/kg on the citrate buffer (pH = 4.5) injected through the peritoneum of rats kept during 30 days on a high-fat diet with a free access to fructose solution (200 g/L) [8]. On the 11th week after Stz injection the group of rats with DM (7 rats) received a course (14 days) of carbacetam injections through the peritoneum in the dose of 5 mg/kg in the volume 1 mL of 0.9% NaCl solution/0.1 kg of the body weight. The groups of comparison, that is, control group and the group of rats with simulated pathology (7 rats in each) received a solvent through the peritoneum in the similar regimen.

Euthanasia of rats was performed under light ether narcosis. The brain was removed cool and carefully washed with cool 0.9% NaCl solution. The hippocampus and cerebral cortex were removed according to the stereotaxic [9]. Brain homogenates were prepared in 0.05 M tris-HCl buffer (pH 7.4).

Proteolysis/fibrinolysis was investigated according to the method using the set of reagents Simko Ltd. The state of proteolytic activity was determined on the basis of reaction with azoalbumin (low molecular proteinolysis), azocasein (high molecular proteinolysis) and azocollagen (collagenolysis). Fibrinolytic activity was examined evaluating the degree of homogenate staining due to plasmin formation in the presence of ε-aminocapronic acid (non-enzymatic fibrinolytic activity [NFA]) or without it (total fibrinolytic activity [TFA]). Enzymatic fibrinolytic activity (EFA) was determined by the difference between NFA and NFA. Proteolysis and fibrinolysis were estimated in E440/hour/mg of tissue, where E440 – extinction index for activity [10].

The results of the study were statistically processed applying parametric Student t-criterion. In case normal distribution was lacking Mann-Whitney U-criterion was applied. The differences were considered reliable with $p < 0.05$. Point

estimate of the results was represented in the form of mean values and standard mean error (M ± m).

Results

The studies conducted demonstrated (Figures 1, 2) that under conditions of neurodegeneration induced by T2DM proteolytic activity of the cerebral cortex was characterized by 22.1% increase of enzymatic azoalbumin breaking up, 6.7% increase of azocaseinolysis and 65.5% increase of azocol. A similar tendency was observed while studying proteolytic activity of the hippocampus (Figures 3, 4) of rats with diabetes mellitus. Thus, the index of azoalbuminolysis did not differ reliably from that of the control, azocaseinolysis 26.8% increased and azocollagen – 67.5%. Fibrinolysis in the cerebral cortex of rats with DM (Figure 5) was characterized by the increase of total fibrinolytic activity (TFA) at the expense of enzymatic fibrinolytic activity (EFA). At the same time, TFA 9.9% increased and EFA 14.2% increased concerning the control values. Fibrinolysis in the hippocampus (Figure 6) differed from that of the control by 26.7% increase of EFA index.

Carbacetam administration to rats with DM promoted reduction of the proteolytic processes in the cerebral cortex according to the indices of azoalbumin enzymatic breaking out (6.1% decrease) and azocollagenolysis (8.5% decrease) in comparison with the values of the simulated pathology. Azocaseinolysis and azocololysis in the hippocampus of rats 7.3% and 9.6% decreased, respectively. And carbacetam administration in this case did not change reliably the indices of fibrinolysis in the cerebral cortex and hippocampus of rats with T2DM.

Discussion

Under conditions of pathological process development the activity of the main groups of proteolytic enzymes and synthesis of protein peroxide oxidation products intensify in the cerebral cortex and hippocampus, which is indicated in our previous publications [8]. Carbacetam administration to rats with DM promotes reduction of

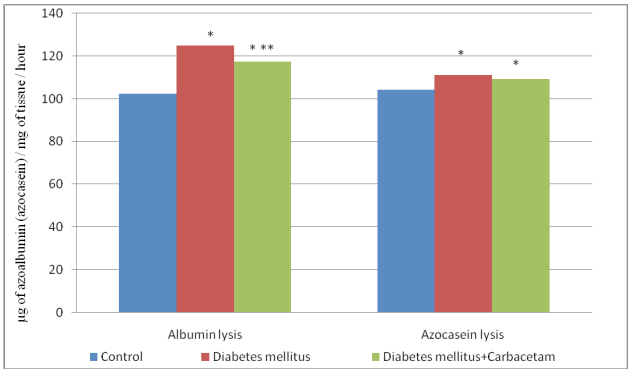

Figure 1: The effect of carbacetam on the rates of proteolysis of the cerebral cortex of rats under conditions of neurodegeneration induced by T2DM (M ± m, n = 7).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

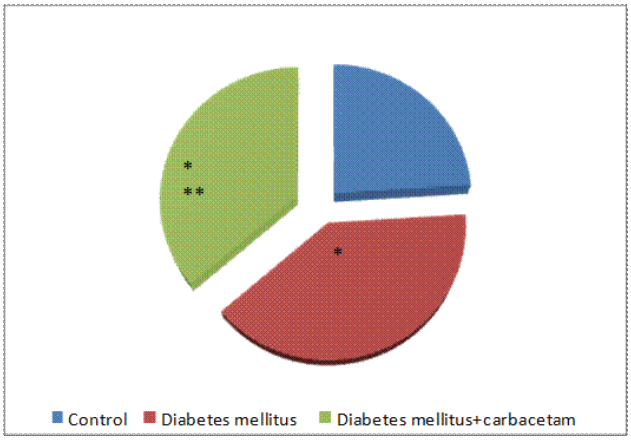

Figure 2: The effect of carbacetam on azocollagen lysis of the cerebral cortex of rats under conditions of neurodegeneration induced by T2DM (M ± m, n = 7).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

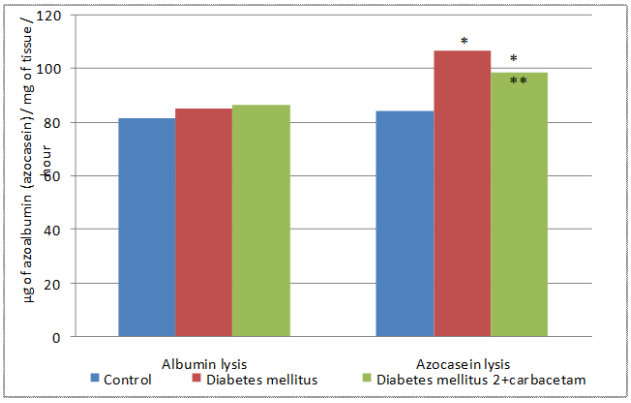

Figure 3: The effect of carbacetam on the proteolysis of the rat hippocampus under conditions of neurodegeneration induced by T2DM (M ± m, n = 7).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

https://doi.org/10.46389/rjd-2021-1020

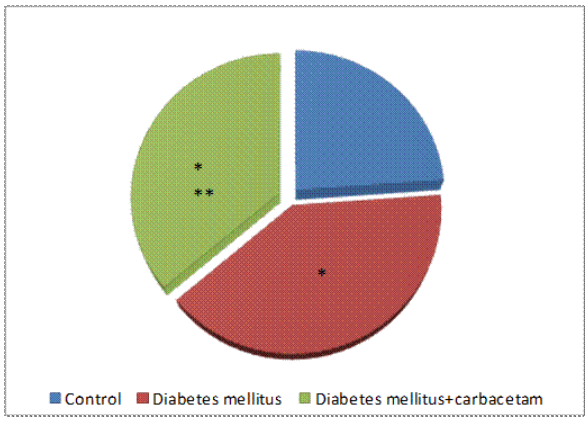

Figure 4: The effect of carbacetam on azocollagen lysis of the rat hippocampus under conditions of neurodegeneration induced by T2DM (M ± m, n = 7).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

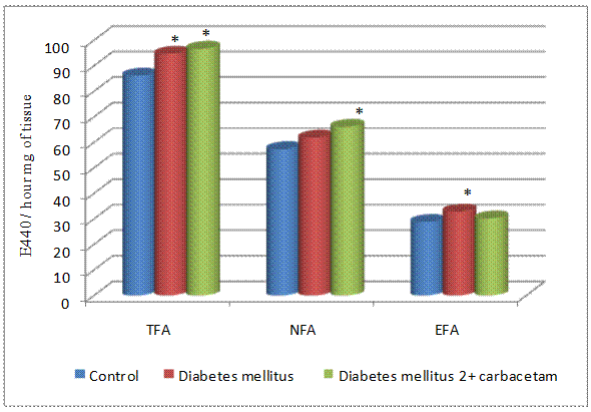

Figure 5: The effect of carbacetam on fibrinolysis of the cerebral cortex of rats under conditions of neurodegeneration induced by T2DM (M ± m, n = 7).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

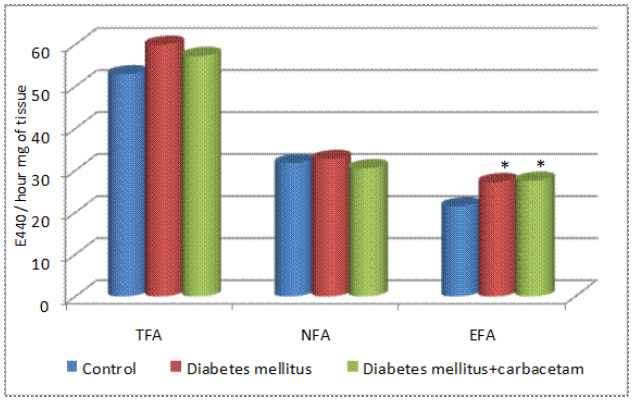

Figure 6: The effect of carbacetam on fibrinolysis of the hippocampus of rats under conditions of neurodegeneration induced by T2DM (M ± m, n = 7).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

proteolysis processes which might be associated with pharmacodynamics of the drug, since modulation of GABA receptors controls both direct and indirect penetration of calcium ions into the neurons through the astrocytes and glial network. The neurons become hyperpolarized. Their hyperpolarization plays a crucial role in a long inhibition of the synaptic transmission [11, 12]. During hyperpolarization phase, calcium channels, protected by energy, are blocked and therefore protecting neurons against toxicity of calcium ions and giving a chance for them to eliminate their excessive amount [13, 14]. The processes of mitochondria oxidation slow down respectively (lipid and protein peroxide oxidation) [15] resulting in decrease of proteolytic mechanisms [16]. Thus, due to removal of toxic calcium excess from the cells mitochondrial production of adenosine triphosphate increases quickly. An excessive irritability of neurons decreases. The processes leading to neuron apoptosis become slower.

One more probable carbacetam effect is regulation of neurotransmitter level and maintaining of metabolism in neurons [8]. The brain activity considerably depends on the balance between neuron activity and its metabolic requirements. At the same time, a great amount of lactate is released into the extracellular space which is utilized through the astrocyte network and saves neuron activity during glucose deficiency [17]. First of all, due to metabolic disorders the structure and functions of erythrocytes change – they become spherical in shape, their ability to deformity decreases resulting in reduced blood flow and endothelial damage [18]. Erythrocytes adsorb plasma proteins, fibrinogens and fibrin on their surface decreasing superficial negative charge. It results in intensification of adhesion and aggregation of erythrocytes. Metabolic disorders produce a negative effect of the platelets as well. The effect of long hyperglycemia on the endothelial cells results in increased production of fibrinolytic factors, intensification of thrombotic activity (reduced synthesis of prostacyclin and increased synthesis of thromboxane A2 by platelets) [19]. It promotes greater aggregation of platelets, vascular spasm and damage of the capillary epithelium. All the above processes disturb the integrity of the hematoencephalic barrier, which functioning

considerably depends on the activity of GABA-receptors [4, 20]. Thereafter, modulation with carbacetam will promote retention of the barrier integrity and reduction of inflammatory processes. Due to these mechanisms both fibrinolysis and proteolysis slow down. This is what we have observed in our studies.

Therefore, administration of carbacetam to rats with neurodegeneration induced by T2DM is pathogenically substantiated by the results of the studies conducted.

Conclusions

Development of neurodegeneration induced by T2DM is characterized by considerable changes of fibrinolytic and proteolytic activity of the cerebral cortex and hippocampus tissues leading to imbalance between the tissue indices. Administration of carbacetam to rats with diabetes mellitus during 14 days decreases collagenolysis, enzymatic fibrinolytic activity in the cerebral cortex, low molecular proteinolysis in the hippocampus; the values of proteolysis in the cerebral cortex decrease according to the findings of enzymatic breaking up of azoalbumin and azocollagenolysis, azocasinolysis and azocololysis in the hippocampus of rats. The data obtained experimentally substantiate reasonability of pathogenic correction by means of carbacetam in case of disorders of proteolytic and fibrinolytic systems activity in the cerebral cortex and hippocampus induced by T2DM.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Chentli, F., Azzoug, S., Mahgoun, S. (2015). Diabetes mellitus in elderly. *Indian J Endocrinol Metab*. 19(6): 744–752.
2. Vergara, R. C., Jaramillo-Riveri, S., Luarte, A. et al. (2019). The energy homeostasis principle: neuronal energy regulation drives local network dynamics generating behavior. *Front Comput Neurosci*. 13: 1–18.
3. Vaucher, E., Tong, X. K., Cholet, N., et al. (2000). GABA neurons provide a rich input to microvessels but not nitric oxide neurons in the rat cerebral cortex: a means for direct regulation of local cerebral blood flow. *J Comp Neurol*. 421(2): 161–171.
4. Roth, F. C., Draguhn, A. (2012). GABA metabolism and transport: effects on synaptic efficacy. *Neural Plast*. ID 805830: 1–12.
5. Meyerhoff, D. J., Murray, D. E., Durazzo, T. C., Pennington, D. L. (2018). Brain GABA and glutamate concentrations following chronic gabapentin administration: a convenience sample studied during early abstinence from alcohol. *Front Psychiatry* 9: 1–8.
6. Kmet, O. G., Filipets, N. D., Kmet, T. I., et al. (2020). Experimental evaluation of enalapril on the antioxidant protection and nitrogen oxide system of the brain in rats with type 2 diabetes mellitus. *Med Sci*. 24(104): 2732–2738.
7. Roman, F., Olaf, M. (2015). Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. *Oxidative Med Cell Long*. ID 610813: 1–19.
8. Kmet, O. G., Filipets, N. D., Hrachova, T. I., et al. (2019). 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. *Arch Balk Med Union* 54(3): 431–437.
9. George, P., Charles, W. (2013). The rat brain in stereotaxic coordinates. 7th Edition. Academic Press, 1-472.
10. Mahalyas, V. M., Mikheev, A. O., Rohoviy, Yu. Ye., et al. (2001). Modern methods of experimental and clinical studies of the central research laboratory of the Bukovina State Medical Academy. Chernivtsi, BSMA. 1–42.
11. Lorenz-Guertin, J. M., Jacob, T. C. (2018). GABA type a receptor trafficking and the architecture of synaptic inhibition. *Dev Neurobiol*. 78(3): 238–270.
12. Mele, M., Leal, G., Duarte, C. B. (2016). Role of $GABA_A$ R trafficking in the plasticity of inhibitory synapses. *J Neurochem*. 139(6): 997–1018.
13. Cataldi, M. (2013). The changing landscape of voltage-gated calcium channels in neurovascular disorders and in neurodegenerative diseases. *Curr Neuropharmacol*. 11(3): 276–297.
14. Zamponi, G. (2016). Targeting voltage-gated calcium channels in neurological and psychiatric diseases. *Nat Rev Drug Discov*. 15: 19–34.
15. Kmet, O. G., Filipets, N. D., Kmet, T. I., Vepriuk, Y. M. (2020). The study of enalapril effect on the functional-metabolic parameters on the cerebral mitochondria in rats with type 2 diabetes mellitus. *Med Sci*. 24(104): 2089–2095.
16. Cenini, G., Voos, W. (2019). Mitochondria as potential targets in Alzheimer disease therapy: an update. *Front Pharmacol*. 10: 1–20.
17. Falkowska, A., Gutowska, I., Goschorska, M., et al. (2015). Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. *Int J Mol Sci*. 16(11): 25959–25981.
18. Kosenko, E. A., Tikhonova, L. A., Montoliu, C., et al. (2017). Metabolic abnormalities of erythrocytes as a risk factor for Alzheimer's disease. *Front Neurosci*. 11: 728.
19. Raminderjit, K., Manpreet, K., Jatinder, S. (2018). Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. *Front Neurosci*. 11: 1–17.
20. Vazana, U., Veksler, R., Pell, G. S., et al. (2016). Glutamate-mediated blood-brain barrier opening: implications for neuroprotection and drug delivery. *J Neurosci*. 36(29): 7727–7739.

 https://doi.org/10.46389/rjd-2021-1020