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The study of enalapril effect on the functional-metabolic parameters of the cerebral mitochondria in rats with type 2 diabetes mellitus

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ABSTRACT

The results of experimental and clinical studies do not exclude interrelations between diabetes mellitus and local renin-angiotensin system of the brain. Considering an important role of the system in development of neurodegenerative processes we have become

interested in the issue concerning enalapril effect on the mitochondrial state of the cerebral cortex and hippocampus in experimental simulation of type 2 diabetes mellitus. Objective of the study is to learn enalapril effect on the functional-metabolic parameters of mitochondria in the cerebral cortex and hippocampus of rats under neurodegeneration conditions stipulated by type 2 diabetes mellitus. The experiments were conducted on laboratory nonlinear albino male rats with the body weight 0.18-0.20 kg. Type 2 diabetes is modeled on streptozotocin and a high-fat diet. Enalapril was administered intraperitoneally at a dose of 1 mg/kg, once daily for 14 days. The administration of 14 days of enalapril was found in the dose of 1 mg/kg during 14 days produces a positive effect on the functional-metabolic state of the mitochondria in the cerebral cortex and hippocampus in rats with type 2 diabetes mellitus, which is indicative of decreased relative rate of mitochondrial swelling and increased intensity of light dispersion, and increased activity of α-ketoglutarate dehydrogenase and succinate dehydrogenase. The use of enalapril rats with type 2 diabetes improves the state of the prooxidant-antioxidant system in the cerebral cortex and hippocampus by the degree of reduced markers of protein and lipid modification oxidation (reduced content of carboxyl phenylhydrazine and products reacting with 2-thiobarbituric acid) and increased catalase activity. The data obtained present experimental substantiation of enalapril protective effects in case of mitochondrial dysfunction in the cerebral cortex and hippocampus of rats induced by type 2 diabetes mellitus.

Keywords: enalapril, type 2 diabetes mellitus, functional state of the mitochondria.

1. INTRODUCTION

Scientific studies of the recent years have found certain associations between type 2 diabetes mellitus (DM) and development of neurodegenerative disorders. Pathogenic basis of these mechanisms is formed by considerable changes in metabolic processes due to disturbed mitochondrial functions. It is these organelles that play a dominating role in supplying energy to the organs, tissues and cells of the body for their normal functioning. Pathologic increase in the production of oxygen active forms by mitochondria initiates' excessive opening of the mitochondrial pore accompanied by Ca^{2+}-overload and development of mitochondrial swelling (Tykhonenko et al., 2016).

Under conditions of development of neurodegenerative processes the activity of NADPH-oxidase is known to increase. Its main function is generation of oxygen active forms. It is a marker of damage with ischemic-reperfusion injuries of the central nervous system (Hiroshi et al., 2017). The activity of the enzyme is directly affected by the local renin-angiotensin system of neurons through the activation of angiotensin receptors of the first type. Clinical studies evidenced that certain inhibitors of angiotensin-converting enzyme, angiohypertensive drugs, reduce the risk and severity of hemorrhagic stroke development (Estato et al., 2013). Moreover, certain data are indicative of the improvement of neurologic motor activity and concomitant decrease of brain edema in the treatment of normotensive rats with non-hypotensive dose of enalapril (Hamdollah and Gholam et al., 2012). The results of experimental and clinical studies (Junker et al., 1985; Estato et al., 2013) do not exclude interrelations between DM and local renin-angiotensin system of the brain. Considering an important role of the system in development of neurodegenerative processes we have become interested in the issue concerning enalapril effect on the mitochondrial state of the cerebral cortex and hippocampus in experimental simulation of type 2 DM.

Objective of the study is to learn enalapril effect on the functional-metabolic parameters of mitochondria in the cerebral cortex and hippocampus of rats under neurodegeneration conditions stipulated by type 2 diabetes mellitus.

2. MATERIAL AND METHODS

In conducting the experimental studies we kept to «General Ethic Principles of Experiments Conducted on Animals» (Kyiv, 2001), which agrees with the European Union directives 2010/10/63 on protection of animals used for scientific purposes. In addition, they were confirmed by the Board on Biomedical Ethics Issues at the Higher State Educational Establishment of Ukraine «Bukovinian State Medical University». During the experiment conducted on laboratory nonlinear albino male rats with the body weight of 0.18-0.20 kg, the animals were kept under standard vivarium conditions, were in quarantine during 7 days with natural day and night changes on appropriate dietary intake.

First all the rats were divided into two groups: 1 – control one (7 rats); 2 – rats with simulated type 2 DM. The pathology was simulated by streptozotocin (Stz) injected through the peritoneum in the dose of 30 mg/kg on citrate buffer (pH=4.5). For 30 days the rats were kept on high-fat diet with free access to fructose solution (200 g/L) (Kmet et al., 2019). On the 11th day after Stz administration the rats were divided into two groups (7 rats each): the 1st group – simulated pathology with injection of 0.9 % NaCl solution through the peritoneum; the 2nd one – DM with enalapril injection through the peritoneum in the dose of 1 mg/kg of the body weight during 14 days.

Euthanasia of rats was conducted under light ether narcosis. The brain was removed cold and washed thoroughly with cool 0.9 % NaCl solution. The cerebral cortex and hippocampus were isolated according to the stereotaxic atlas (Paxinos and Watson, 2013). Mitochondrial fraction homogenates of the structures examined was isolated by means of the differentiation centrifugation method in homogenized medium: 250 mM of sucrose, 1 mM EDTA, 10 mM tris-HCl, pH 7.4 at a temperature of 0–3°C (Shabalina et al., 1995). Opening of the mitochondrial pore was examined by means of spectrophotometric registration of mitochondria swelling and changes of the suspension optic density with λ=520 during 60 minutes. A relative arte of mitochondria swelling was determined as difference between the rate of organelle swelling on the 60th minute concerning the initial value (Vadzyuk, 2015; Eisenhofer et al., 2010). Lowry protein assay determined the protein concentration in the incubation medium to be 0.4 mg/ml (Ceban et al., 2016). The intensity of lipid peroxide oxidation (LPO) in the mitochondria was evaluated by the levels of active products of 2-thiobarbituric acid (AP TBA) (Kushnir et al., 2018). Free radical protein oxidation processes were assessed by the content of carboxyl phenylhydrazine (CPH) (Kopylchuk and Voloshchuk, 2016). The state of the antioxidant protection system in the mitochondria was evaluated by the activity of superoxide dismutase (SOD) enzymes [EC 1.15.1.1] and catalase [EC 1.11.1.6] (Feysa, 2019). The activity of enzymes of α-ketoglutarate dehydrogenase (α-KGDH) [EC 1.2.4.2] and succinate dehydrogenase (SDH) [EC 1.3.5.1] was determined by means of spectrophotometric method (Prohorova, 1982).

The results of the study were statistically processed by means of parametric t-Student criterion. In case normal distribution was lacking Mann-Whitney U-criterion was used. Differences were considered statistically valuable with p≤0.05. Point estimate of the results was presented in the form of mean values and mean value standard error (M±m).

3. RESULTS

The results of the studies conducted (Fig. 1, 2) showed that under conditions of simulated type 2 DM the processes of mitochondria swelling in the cerebral cortex and hippocampus intensified in comparison with that of the control. The results obtained are characterized by a reduced intensity of light absorption in the organelles, which is indicative of increase of their volume at the expense of an intensified permeability of the mitochondrial membranes. Thus, the intensity 41.1% decreased in the cerebral cortex and 42.3% decreased in the hippocampus. Therefore, development of neurodegenerative processes caused by type 2 DM, stipulate the loss of ability of the mitochondrial neurons to regulate their volume in comparison to that of the control rats. At the same time, administration of enalapril during 14 days caused partial restoration of the mitochondrial functional activity consisting of the ability to contract. Its intensity compared with the group of the simulated pathology 50.3% increases in the cerebral cortex and 57.9% – in the hippocampus.

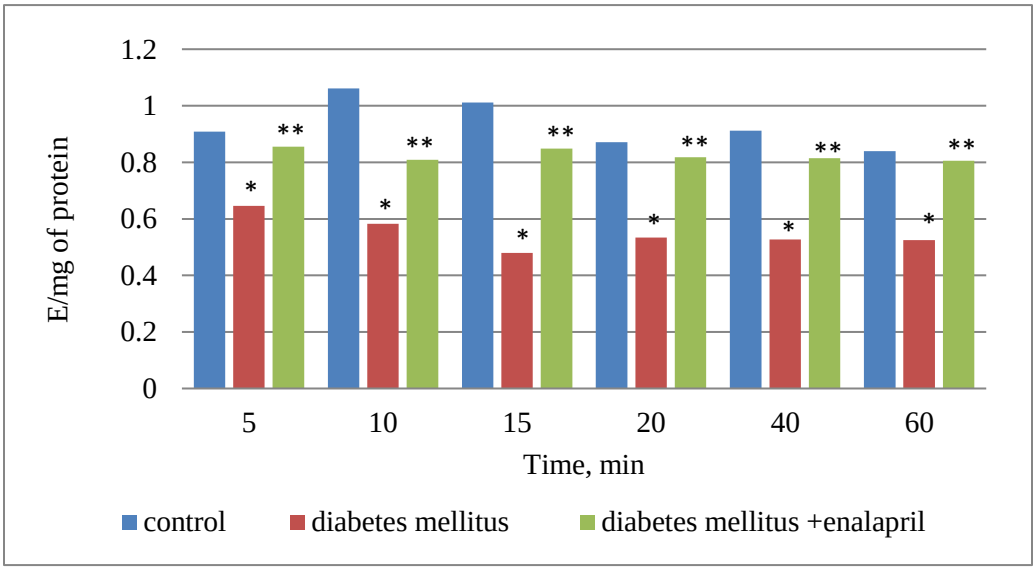

Figure 1 Intensity of mitochondrial swelling in the cerebral cortex of rats with type 2 diabetes mellitus after enalapril administration during 14 days in the dose of 1 mg/kg. (M±м, n=7)

Notes:* – reliability of difference compared with the control group of rats, ** – reliability of difference compared with the group of rats with type 2 diabetes mellitus.

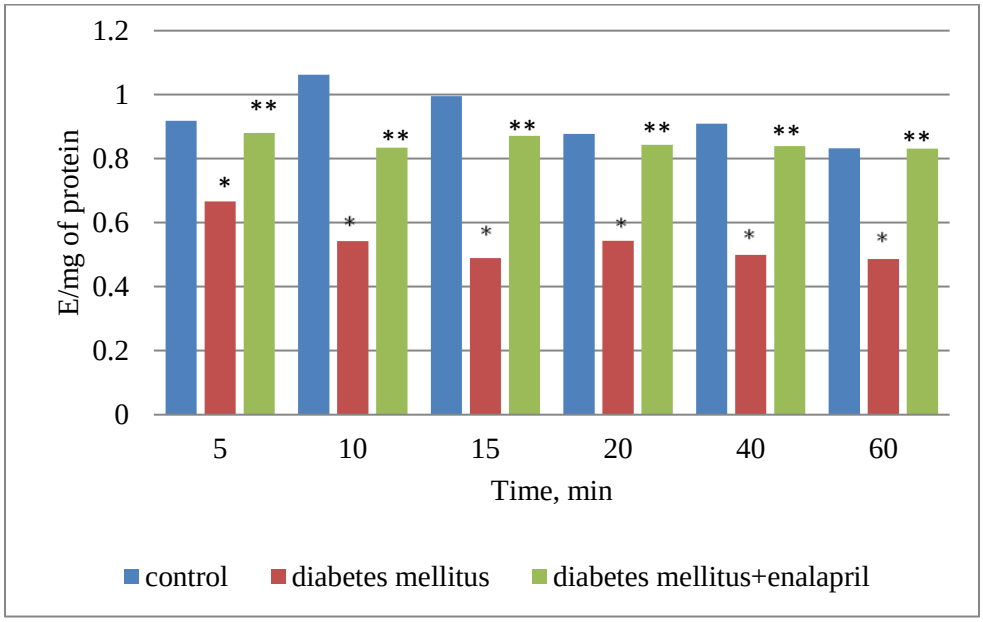

Figure 2 Intensity of mitochondrial swelling in the hippocampus of rats with type 2 diabetes mellitus after enalapril administration during 14 days in the dose of 1 mg/kg. (M±м, n=7)

Notes:* – reliability of difference compared with the control group of rats, ** – reliability of difference compared with the group of rats with type 2 diabetes mellitus.

Analyzing the data obtained (Fig. 3) we have found that relative rate of mitochondria swelling in rats with DM compared with the control group 25% increased in the cerebral cortex and 27% – in the hippocampus. At the same time, 14-day administration of enalapril reduced the examine parameter in comparison with the DM group of rats in the following way: 25.0% lower – in the cerebral cortex and 21.7% – in the hippocampus. According to present literary data one of the important links in pathogenesis of type 2 DM and its complications is free radical lipid and protein oxidation (Popova et al., 2013). Since functioning of any cell and its organelles is closely associated with the structural state of the membrane provided by their lipid surrounding, investigation of the state of the mitochondrial prooxidant-antioxidant system of the examined structures in the brain is of certain interest.

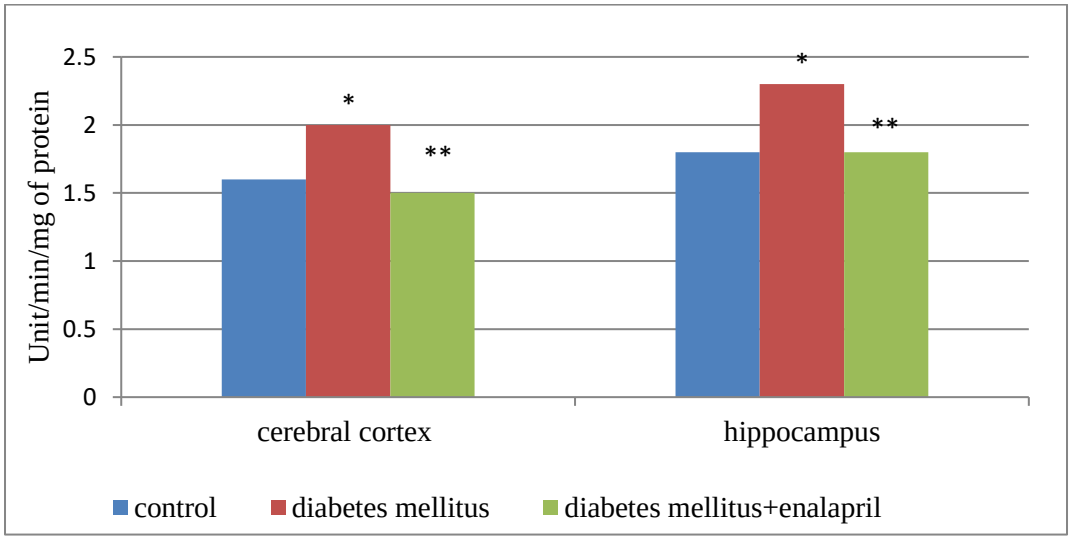

Figure 3 Relative rate of mitochondrial swelling in the cerebral cortex and hippocampus of rats with type 2 diabetes mellitus after enalapril administration during 14 days in the dose of 1 mg/kg (M±м, n=7)

Notes: – reliability of difference compared with the control group of rats;*

*** – reliability of difference compared with the group of rats with type 2 diabetes mellitus.*

Thus, according to the data in the Table 1, the content of AP TBA in the mitochondria of the cerebral cortex and hippocampus of rats with type 2 DM 82.8% and 106% increased respectively in comparison with the control group. And CPH content increased the

control parameters as well: 37.7% in the cerebral cortex and 43.2% – in the hippocampus. The data obtained are indicative of intensification of the processes of lipid and protein peroxidation, and characterize a damaging effect on the structural-functional state of the mitochondrial membranes in the brain.

Table 1 Enalapril effect on free radical lipid and protein oxidation, energy supply in the mitochondria of the cerebral cortex and hippocampus of rats with type 2 diabetes mellitus (M±m, n=7)

Indices	Examined structures cerebral	Control	Diabetes mellitus	Diabetes mellitus + Enalapril
The content of AP TBA, nmol/mg of protein	cortex	12.8±1.25	23.4±1.23*	15.4±0.78**
	hippocampus	11.6±0.65	23.9±0.86*	15.1±0.61**
The content CPH, nmol/mg of protein	cortex	24.7±1.39	34.0±0.40*	26.7±1.06**
	hippocampus	18.3±1.10	26.2±1.03*	19.4±0.79**
The activity of SOD, units/mg of protein	cortex	0.43±0.027	0.33±0.017*	0.40±0.052
	hippocampus	0.38±0.045	0.26±0.038	0.31±0.038
The activity of catalase, mcmol H_2O_2/min of mg of protein	cortex	175.9±10.58	124.2±11.72*	174.2±12.83**
	hippocampus	170.2±10.99	82.5±11.28*	139.1±17.54**
The activity of α-KGDH, nmol/min of mg of protein	cortex	40.4±2.23	26.5±1.01*	33.3±2.14**
	hippocampus	43.5±2.24	26.8±1.52*	35.3±3.39**
The activity of SDH nmol/min of mg of protein	cortex	6.5±0.57	2.2±0.15*	3.5±0.30* **
	hippocampus	7.2±0.32	2.4±0.27*	3.6±0.24* **

Notes: – reliability of difference compared with the control group of rats,*
*** – reliability of difference compared with the group of rats with type 2 diabetes mellitus.*

At the same time, enalapril administration to rats with type 2 DM during 14 days promoted decrease of AP TBA and CPH content in the mitochondria of the cerebral cortex 34.3% and 21.5% as much, and in the hippocampus – 36.8% and 25.9% respectively, in comparison with animals with simulated pathology. The results are indicative of the fact that the drug decreases activity of the prooxidant membranous system of the organelles in the examined structures. The state of the antioxidant protection of the mitochondrial membrane was assessed by the activity of such enzymes as SOD and catalase (Table 1). 23.3% decreased SOD activity was found in the mitochondria of the cerebral cortex in rats with type 2 DM as compared with the control group parameters. It should be noted that only a tendency to decrease the given enzyme activity was found in the hippocampus. At the same time, catalase activity decreased in both structures: 29.4% in the cerebral cortex and 51.5% – in the hippocampus. Analyzing the results obtained we can suggest that the hippocampus is more susceptible to damaging diabetes effects which might be associated with phylogenetic properties of the structure (Norman et al., 2000).

Enalapril administration to rats during 14 days promoted 40.3% increase of catalase activity in the cerebral cortex and 68.6% increase in the hippocampus compared with the group of modeled pathology. Reliable changes of SOD activity in the mitochondria of the examined structures were not found, which most likely was caused by an excessive formation of hyperoxides of fatty acids producing a direct effect on the activity of the enzyme.

According to the present hypothesis of oxidative stress it is disorder of the mitochondrial redox-signal ways – balance regulators between accumulation and utilization of energy – which is the main cause stipulating the advance of diabetic vascular complications (James et al., 2012; Sena and Chandel, 2012). Considering this fact we have examined α-KGDH and SDH activity in the mitochondria of the examined structures of the brain.

Our study has determined that α-KGDH activity in the group of rats with type 2 DM 34.4% decreased in the cerebral cortex and 38.4% – in the hippocampus. SDH activity decreased as well: 66.2% in the cerebral cortex and 66.7% in the hippocampus. After enalapril administration, activity of these enzymes increased. Thus, α-KGDH activity 25.7% increased in the cerebral cortex and 31.7% - in the hippocampus. SDH activity increased both in the cerebral cortex and hippocampus –59.1 and 50.0% respectively.

4. DISCUSSION

Therefore, the study conducted sowed that against the ground of brain damage under conditions of type 2 DM, enalapril in the dose of 1 mg/kg stipulates a protective action on the mitochondria of the cerebral cortex and hippocampus. Relying on the scientific

data concerning the interrelations between functioning of the enzymes of the mitochondrial electron-transport chain and structural state of the membranes provided by their lipid content, a positive effect of the drug obtained is likely to be associated with blockade of the first type angiotensin receptors. It is worth noting that the effect of renin-angiotensin system blockers is realized through peroxisome proliferator-activated receptor-γ (PPARγ receptors), which are the central regulation of insulin and glucose metabolism, which suppresses the progression of neurodegeneration, mediated by hyperphosphorylation and tauphosphorylation is a mechanism of the neuroprotective action of enalapril (Luppi et al., 2019).

Accordingly, the activity of NADPH oxidase, proinflammatory and proapoptic vicious chains is reduced: the decrease in the production of reactive oxygen species in mitochondria. As a result of the decrease in the content of AP TBA and CPH - markers of the state of the prooxidant system. In addition, the systemic effects of enalapril and, accordingly, an increase in cerebral blood flow and oxygen content in neurons, a decrease in the formation of reactive oxygen species, and, accordingly, the damaging effects of AP TBA and CPH are not excluded. At the same time, the enzymes of the antioxidant protection of mitochondria are activated: the activity of catalase increases, which allows to judge the antioxidant effect of the drug.

Bioenergetics processes provide improvement of the structural-functional state of the mitochondrial membranes. This fact is indicative of an increased activity of α-KGDH and SDH. After all, SDH, an enzyme that promotes mitochondrial oxidative phosphorylation by participating in the citric acid cycle, modulates mitochondrial transport of K^+ by participating in the formation of an intramembrane multiprotein complex that exhibits sensitivity to ATP. Succinate - an intermediate in the citric acid cycle acts as a signaling molecule through binding to G-protein coupled receptors (Inmaculada and Navdeep, 2020). All of the above contributes to the increase in the number of ATP in neurons due to the balance of oxidation and phosphorylation processes, the intensification of the functioning of ion pumps and, accordingly - the restoration of functional and metabolic parameters of mitochondria (Pastorino et al., 1993).

Therefore, under conditions of mitochondrial dysfunction after enalapril administration energetic processes in the cerebral cortex and hippocampus improve, which is indicative of an increased resistance of neurons to pathological effect on the central nervous system caused by type 2 DM.

5. CONCLUSION

Enalapril administration in the dose of 1 mg/kg during 14 days produces a positive effect on the functional-metabolic state of the mitochondria in the cerebral cortex and hippocampus in rats with type 2 diabetes mellitus, which is indicative of decreased relative rate of mitochondrial swelling and increased intensity of light dispersion, and increased activity of α-ketoglutarate dehydrogenase and succinate dehydrogenase. Enalapril administration to rats with type 2 diabetes mellitus improves the state of the prooxidant-antioxidant system in the cerebral cortex and hippocampus by the degree of reduced markers of protein and lipid modification oxidation (reduced content of carboxyl phenylhydrazine and products reacting with 2-thiobarbituric acid) and increased catalase activity. The data obtained present experimental substantiation of enalapril protective effects in case of mitochondrial dysfunction in the cerebral cortex and hippocampus of rats induced by type 2 diabetes mellitus.

List of abbreviations

DM – diabetes mellitus
Stz – streptozotocin
AP TBA – active products of 2-thiobarbituric acid
CPH – carboxyl phenylhydrazine
SOD – superoxide dismutase
α-KGDH – α-ketoglutarate dehydrogenase
SDH – succinate dehydrogenase

Conflicts of Interest:

The authors declare no conflict of interest.

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REFERENCE

1. 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.
2. Eisenhofer S, Toókos F, Hense BA, et al. A mathematical model of mitochondrial swelling. BMC RES Notes 2010;3:67.
3. Estato V, Obadia N, Carvalho-Tavares J, et al. Blockade of the renin–angiotensin system improves cerebral microcirculatory perfusion in diabetic hypertensive rats. Microvascular Research 2013;87:41-9.
4. Estato V, Obadia N, Carvalho-Tavares J, et al. Blockade of the renin-angiotensin system improves cerebral microcirculatory perfusion in diabetic hypertensive rats. Microvascular Research 2013;87:41-9.
5. Feysa SV. Lipid peroxidation and antioxidant defense status in patients with non-alcoholic fatty liver disease and concomitant hypothyroidism. Fiziolohichnyi zhurnal 2019;65(2):89-96.
6. Hamdollah Panahpour, Gholam Abbas Dehghani. Attenuation of focal cerebral ischemic injury following post ischemic inhibition of angiotensin converting enzyme (ACE) activity in normotensive rat. Iranian Biomedical Journal 2012;16(4):202-8.
7. Hiroshi Yao, Tetsuro Ago, Takanari Kitazono, Toru Nabika. NADPH oxidase-related pathophysiology in experimental models of stroke. Int J Mol Sci 2017;18(10):1-17.
8. Inmaculada Martínez-Reyes, Navdeep S. Chande. Mitochondrial TCA cycle metabolites control physiology and disease. Nature Communications 2020;102:11.
9. James AM, Collins Y, Logan A, Murphy MP. Mitochondrial oxidative stress and the metabolic syndrome. Trends Endocrinol Metab 2012;23(9):429-34.
10. Junker U, Jaggi C, Bestetti G, Rossi GL. Basement membrane of hypothalamus and cortex capillaries from normotensive and spontaneously hypertensive rats with streptozotocin-induced diabetes. Acta Neuropathol 1985;65(3-4):202-8.
11. Kmet OG, Filipets ND, Kmet TI, et al. Pharmacological correction of cognitive disorders in experimental neurodegeneration caused by 2 type diabetes mellitus. Problems of endocrine pathology 2019;4:52-9.
12. Kopylchuk GP, Voloshchuk OM. Peculiarities of the free radical processes in rat liver mitochondria under toxic hepatitis on the background of alimentary protein deficiency. Ukr Biochem J 2016;88(2):66-72.
13. Kushnir OYu, Yaremii IM, Shvets VI, Shvets NV. Influence of melatonin on glutathione system in rats skeletal muscle under alloxan induced diabetes. Fiziolohichnyi zhurnal 2018;64(5):54-62.
14. Luppi M, Hitrec T, Di Cristoforo A, et al. Phosphorylation and dephosphorylation of tau protein during synthetic torpor. Front. Neuroanat 2019;13(57):1-13.
15. Norman Kreisman, Soheil Soliman, David Gozal. Regional differences in hypoxic depolarization and swelling in hippocampal slices. The American Physiological Society 2000;83(2):1031-38.
16. Pastorino JG., Snyder JW, Serroni A. Biological effect of cadmium and response of the adult rat. J Biol Chem 1993;268(2):13791-98.
17. Paxinos George, Watson Charles. The Rat Brain in Stereotaxic Coordinates. 7th Edition. Academic Press, 2013. 472 p.
18. Popova TN, Agarkov AA, Verevkin AN. Intensity of free radical processes in rat liver with type 2 diabetes mellitus and introduction of epifamin. Acta naturae 2013;5(4):118-22.
19. Prohorova MI. Methods of biochemical studies (lipid and energy metabolism). L.: Izd-vo Leningr. Un-ta, 1982. 272 p.
20. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. Molec Cell 2012;48(2):158-67.
21. Shabalina IG, Kolosova NG, Grishanova AYu. et al. Oxidative phosphorylation, F_oF_1-ATFase activities and cytochromes levels in liver mitochondria of rats with inherited free radical hyperproduction. Biochemistry 1995;60(12):2045–52.
22. Tykhonenko T, Dyakun K, Sergiichuk Iu. Mitochondrial dehydrogenase activity of rat brain nerve endings. Visnyk of the Lviv University. Series Biology 2016;73:203-7.
23. Vadzyuk OB. Protective effects of potassium transport in mitochondria from rat myometrium under activation of mitochondrial permeability transition pore. Ukr Biochem J 2015;87(6):86-94.