PATHOGENETIC ROLE OF DISORDERS OF INSULIN SIGNALING PATHWAYS AND DIABETES-ASSOCIATED HYPERGLYCEMIA IN THE MECHANISMS OF DIABETIC ENCEPHALOPATHY FORMATION AND HYPERSENSITIVITY OF THE BRAIN TO ISCHEMIA-REPERFUSION

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Abstract. The aim of the study was to analyze the literature data regarding the pathogenetic role of insulin signaling disorders and diabetes-associated hyperglycemia in the mechanisms of diabetic encephalopathy and hypersensitivity of the brain to ischemia-reperfusion.

Conclusion. Analysis of the literature data shows a number of common links between the pathogenesis of diabetic encephalopathy and ischemic-reperfusion brain injury, which may, to some extent, explain the predisposition of diabetics to acute cerebral circulatory disorders and their adverse course. However, it is clear that the severity of such comorbid pathology cannot be explained only by the additive effect of individual links in the pathogenesis, which indicates the necessity for further in-depth study of its molecular-genetic aspects.

Key-words: insulin, hyperglycemia, diabetic encephalopathy, cerebral reperfusion ischemia.

Background. According to the International Diabetes Federation, there are currently about 382 million people in the world with diabetes mellitus (DM) [1], and experts estimate that over the next 25 years their number will increase up to 642 million [2]. According to the World Health Organization, in 2030, due to numerous life-threatening complications, diabetes will be the seventh leading cause of death [3]. Traditionally, diabetic complications are classified as micro- and macrovascular ones [4, 5]. One of the most dangerous manifestations of the latter ones is acute cerebrovascular disorder of ischemic origin [1, 4, 6, 7]. According to various authors, the frequency of such complications in patients with DM is 2 to 5 times higher than in the population without this background disease [8, 9]. Taking into account that more than 30% of all strokes are reported in patients with DM, and the mortality rate is significantly higher than in the general population [10-12], the mechanisms responsible for increasing the frequency and severity of ischemic brain damage in DM are very actively studied recently, which indicates the relevance of generalizing the results of scientific research in this area.

The aim of the study was to analyze the literature data regarding the pathogenetic role of the disorder of insulin signaling pathways and diabetes-associated hyperglycemia in the mechanisms of diabetic encephalopathy and hypersensitivity of the brain to ischemia-reperfusion.


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Disorder of insulin signaling pathways in diabetes leads to a number of complications. Although daily use of insulin is the key to the life of patients with DM type I, exogenous insulin cannot fully reproduce the subtle regulation of its production by a normally functioning pancreas. Therefore, in these patients during the day there are fluctuations in glucose levels from hyper- to hypoglycemia, which has an equally negative effect on the morphofunctional state of nerve cells. As the normal cerebral function depends on continuous and relatively stable circulating glucose level, these fluctuations affect brain function, leading to changes in its electrophysiological properties, cognitive functions deficiency, neuronal degeneration, and ultimately – the death of neurons and atrophy of the brain [13-15].

In hyperglycemia the effects of insulin deficiency are exacerbated by the high sensitivity of nervous tissue to excess glucose, which is due to the peculiarities of its intracellular metabolism. Most cells of the organism have mechanisms to remove excess glucose and restore intracellular homeostasis. A peculiarity of Schwann neurons and cells, as well as the capillary endothelium of organs prone to diabetic complications, is the lack of ability to remove excess glucose leading to a sharp increase in its concentration inside the cell [16]. In most of these cells, glucose uptake is regulated by insulin-independent transporters, so their intracellular glucose concentration is proportional to the level of hyperglycemia. In hypoglycemia, the amount of GLUT1 in the epithelial cells of the blood-brain barrier increases. This is a compensatory response to support the brain in conditions of deficiency of its main energy substrate. When the level of glucose in the blood exceeds the norm, the number of GLUT1 remains constant creating the conditions for the implementation of both acute toxic effects of hyperglycemia and neurodegenerative processes caused by chronic hyperglycemia [17, 18].

The basis of morphofunctional destabilization of the CNS structures in diabetes mellitus includes multiple mechanisms. Chronic hyperglycemia has been shown to disrupt brain energy. Decreased cellular ATP content in the brain of animals with diabetes has been demonstrated in various models of diabetes (streptozotocin model, spontaneously diabetic Goto-Kakizaki rats, etc.) [16, 19], which indicates the universal nature of such changes.

As it is known, the main source of energy in neurons is mitochondria. Studies of the diabetes effect on brain mitochondria have shown that these organelles isolated from the brain of male rats with streptozotocin diabetes had reduced respiration rate/oxygen consumption ratio [20] and impaired activity of complexes III, IV and V of the electron transport chain [21]. According to other researchers, streptozotocin diabetes leads to a decrease in the coefficient of respiratory control – the rate of outflow from the electronic transport chain, as well as a decrease in the ADP/O ratio (phosphorylation efficiency of mitochondrial ADP relative to oxygen consumption) in mitochondria of hippocampus, which indicates the structural dependence of the cerebral effects of diabetes on the brain mitochondria [22]. Similar effects were found in mitochondria isolated from the brain of spontaneously diabetic Goto-Kakizaki rats. Moreover, the additional load of beta-amyloid introduction (taking into account the connection between Alzheimer’s disease and DM) potentiates mitochondrial dysfunction [21]. The combination of these data indicates a violation of the efficiency of the electron transfer chain in the brain mitochondria in diabetes mellitus.

Due to the important role of mitochondrial dysfunction in the pathogenesis of ischemic-reperfusion brain injury, the pre-existing dysfunction and lower ATP availability in the brain of patients with DM may contribute to more significant damaging effects of cerebral ischemia.

In the pathogenesis of diabetic encephalopathy, as a secondary complication of DM, an important role belongs to the increased formation of ROS and insufficiency of the endogenous antioxidant system [22, 23]. An important contribution to the strengthening of free radical processes is the activation of the mitochondrial source of ROS [14, 21]. Hyperglycemia enhances the flow of electron donors from the tricarboxylic acids cycle to the mitochondrial electron transport chain, which blocks the transfer of electrons to complex III due to high mitochondrial membrane potential. Electrons then accumulate on coenzyme Q, after which they are converted into molecular oxygen, which enhances the conversion of oxygen molecules into superoxide free radicals [24]. The role of coenzyme Q in increasing ROS levels in diabetes is confirmed by changes in its content in mitochondria derived from brain tissue of rats with streptozotocin-induced diabetes [25]. In other studies, the appearance of a significant amount of ROS of mitochondrial origin is associated with the activation of complexes I and III [26].

Streptozotocin-induced diabetes in laboratory animals leads to increased mitochondrial nitric oxide synthase production, oxidized/reduced glutathione ratio and hydrogen peroxide content, and decreased mitochondrial superoxide dismutase (SOD) levels [22, 27]. Other researchers [28] observed a significant decrease in mitochondrial catalase activity, Mn-SOD and reduced glutathione level, as well as an increase in LPO intensity and glutathione peroxidase activity in the brain of diabetic animals. These studies demonstrate an imbalance between the production and neutralization of ROS. It has also been determined that the brain mitochondria of rats with Goto-Kakizaki diabetes are more susceptible to OS [29], and this increased susceptibility is inversely correlated with antioxidant levels. An imbalance between the pro-oxidant and
antioxidant systems of mitochondria has also been demonstrated in rats with alloxan-induced diabetes. This effect varies in different parts of the brain [30]. Chronic imbalance between the formation of free radicals and their neutralization by endogenous antioxidants in the presence of DM leads to the damage of important biochemical components of neurons, such as proteins, lipids and nucleic acids [28, 31].

Thus, the data of literature suggest that diabetes alters the balance between pro- and antioxidant systems in the brain, leading to OS. This increase in baseline of OS in the amount of post-ischemic OS can lead to the activation of numerous mechanisms leading to cell death, thereby exacerbating cerebral ischemic damage in diabetes.

As it was mentioned above, due to hyperglycemia, the Krebs cycle is overloaded with excess glucose, as a result of which in the electron transport chain the formation of NADH and FADH2 – donors of free electrons increases, which transfer them to oxygen molecules with the formation of large superoxide-anion [25, 27, 28]. The latter activates poly-ADP-ribos polymerase, which inhibits the activity of the enzyme glyceraldehyde-3-phosphate dehydrogenase – a key enzyme of the glycolytic cascade, as a result of which excess of glucose is directed through the alternative pathways of metabolism, which leads to the activation of the polyol pathway of metabolism of glucose and other sugars, enhanced by intracellular formation of glycosylation end products and their receptors and ligands, activation of protein kinase C isoforms, hexosamine pathway hyperactivity. Activation of these pathways, in turn, enhances OS through conversion of superoxide anion to other, more active forms of ROS and reactivity capable NO derivatives, causing damage to both neurons themselves and the development of micro- and macro-angiopathies and completes the formation of diabetic encephalopathy [31-33].

Another key factor in brain tissue damage in diabetes is a violation of calcium homeostasis [34]. It is known that due to the presence of Ca2+-transport mechanism that controls the normal ratio of accumulated and released Ca2+, mitochondria play an important role in buffering the cellular content of Ca2+ [35, 36]. Insufficient mitochondrial buffering of Ca2+ in the brain in diabetes can lead to a decrease in the threshold of activation of pro-apoptotic pathways, which leads to the increased cell death after cerebral ischemia against the background of DM [21, 37, 38].

Insufficient glycemic control in patients with DM leads to ketoadiposis and cerebral edema. Acidosis is also associated with the loss of activity of mitochondrial electron transfer chain complexes mentioned above [21]. The degree of loss of enzymatic activity of mitochondria correlates with the severity of acidosis. These disorders of mitochondrial function lead to a decrease in energy production during acidosis. In addition, acidosis reduces the ability of mitochondria to buffer cellular Ca2+ content [38, 39]. Decreased extracellular pH leads to depolarization of intra-synaptosomal mitochondria and increased oxidative stress in isolated synaptosomes of rat brain [40]. Acid-induced neuronal damage in diabetes and cerebral ischemia is mediated by acid-sensitive ion channel 1a (ASIC1a), present on the plasma membrane as well as in mitochondria [41]. ASIC1a plays a certain role in regulating mitochondrial pore permeability by influencing the mechanisms that initiate mitochondrial cell death pathways [42, 43].

Under conditions of cerebral ischemia, hyperglycemia leads to increased lactate and H+ formation and, thus, to intra- and extracellular acidosis [21, 34] with a simultaneous increase in cerebral ischemic injury outside the “anaerobic threshold” [44]. On the other hand, acidosis reduces the rate of ATP hydrolysis, which may be useful during ischemia/reperfusion when energy requirements are high [45]. However, the detrimental effects of acidosis on brain functions outweigh its beneficial effects.

Mitochondria play a vital role in mediating cell death pathways. Prolonged stimulation of these pathways and mitochondrial destabilization leads to mitochondrial damage, mitochondrial pore opening, and increased membrane permeability [46, 47]. Further destabilization of the mitochondrial membrane results in the release of mitochondrial components such as cytochrome c and apoptosis-inducing factor (AIF), which in turn regulate the internal pathways of programmed cell death [48, 49]. In the process of cell death based on caspase cascade activation, released cytochrome c activates caspases, which in turn cause the breakdown of many cellular proteins and DNA [50, 51]. Other pathways of ischemic cell death independent of caspase activation, include calpain- and poly (ADP-ribose) polymerase-1 (PARP1)-mediated release of AIF from mitochondria with subsequent translocation to the nucleus [21, 37, 52]. Proteins of the Bcl-2 family, namely, Bax, Bak, Bid, Bad, Bim and PUMA are included into the terminal effects of mitochondrial membrane disorder, leading to ischemic death of neurons through the formation of pores in the outer membrane of mitochondria [48, 49, 53]. The biochemical sequence of involvement of mitochondrial mechanisms that induce neuronal death includes activation of mitogen-activated protein kinase signaling pathways, c-JNK, modulation of various proteins such as Bcl-2, Bax, Bad, Bim, Bcl-xL, Bcl-2 and c-Jun [54, 55]. During an ischemic attack, activation of these pathways leads to calcium-mediated swelling and lysis of mitochondria. Such mitochondrial disorders cause a massive release of biochemical stressors that are known to activate certain critical pathways of cell death [56]. Activation of these pathways is especially significant in...
diabetes. Studies of the mechanisms by which diabetic cell death pathways are enhanced in cerebral ischemia have shown that cytochrome c release into the cytosol and increased cell death pathway activity (caspase-3 activation and PARP1 breakdown) were markedly higher in the brain of experimental diabetic rats [21, 57, 58]. These facts demonstrate that increased activity of cell death pathways in rats with diabetes mellitus may play a key role in increasing the damaging effects of cerebral ischemia. It has been determined that diabetes increases the activity of caspase-9 in the hippocampus [13, 22,59], long-term streptozotocin diabetes causes morphological and immune-histochemical disorders in the mitochondria of dorsal root ganglion neurons [60], hyperglycemia in spinal ganglia culture leads to the increased fragmentation of mitochondria, which at later stages causes apoptosis [61]. Thus, diabetes-related pathways of increased ischemic neuronal death can be attributed primarily to excessive activation of mitochondrial cell death pathways. According to the literature, diabetes-associated hyperglycemia enhances the activation of major mediators of cell death, namely cytochrome c, poly-(ADP-ribose)-polymerase and caspase, creating a mechanistic basis for hypersensitivity to ischemia.

Thus, the scientific evidence accumulated to date leaves no doubt about the role of diabetic encephalopathy in the susceptibility of the brain to ischemic-reperfusion injury.

**Conclusion**

Analysis of the literature data shows a number of common links between the pathogenesis of diabetic encephalopathy and ischemic-reperfusion brain injury, which may, to some extent, explain the predisposition of diabetics to acute cerebral circulatory disorders and their adverse course. However, it is clear that the severity of such comorbid pathology cannot be explained only by the additive effect of individual links in the pathogenesis, which indicates the necessity for further in-depth study of its molecular-genetic aspects.

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