POLYAMINES AND CEREBRAL ISCHEMIA

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The group of polyamines (PA) comprises putrescine [NH2(CH2)4NH2], spermidine [NH2(CH2)4NH(CH2)3NH2] and spermine [NH2(CH2)3NH(CH2)4NH(CH2)3NH 2], and their derivatives. PA are present in all tissues of eukaryotes; they are essential for the processes of cell proliferation, growth and development. Since their primary and secondary amino groups are connected with the N* at physiological pH, PA interacts electrostatically with negatively charged molecules, primarily such as DNA and RNA, as well as protein's complexes [9]. In living organisms, PA are presented in the free (protonated) and related forms. The interaction of PA with nucleic acids and acidic macromolecules are more stable than with inorganic cations Mg2+ and Ca2+.

The metabolism of PA in mammals. PA can mutually transform each other in their biosynthesis and breakdown [1, 2]. Putrescine is synthesized from ornithine in the formation of which involved the enzyme arginase. The rate limiting enzyme in the biosynthesis of PA is ornitine decarboxilase (ODC) [23]. Spermidine synthase is an enzyme that carries out the transfer of NH2(CH2)3-fragment from S-adenosylmethionine to the amino group of \ putrescine, which leads to the formation spermidine. The spermine is formed by adding NH2(CH2)3-fragment to spermidine with the participation of the enzyme sperimine sintetase. The spermine and spermidine converted back to putrescine as a result of the catabolism of the enzymes of polyaminoacids, spermine oxidase (SMO), acetyl polyamine oxidase (AcPAO). SMO carries out the oxidative transition of spermine in spermidine [24].

Acetylated spermine and spermidine subsequently subjected to oxidative cleavage between C3 and N4 with the formation of more low molecular weight polyamines. Products of the oxidation of PA are low molecular weight PA, H2O2, 3-aminopropanal (3-AP) and 3-acetylaminophenol. These aldehydes are unstable and spontaneously pass in acrolein after deamination. Acrolein, an unsaturated aldehyde, easily reacts with lysine residues of proteins with the formation of protein-conjugated acrolein (PCAcrolein) [22] (Fig. 1). Acrolein, a highly toxic compound,

when interacting with proteins, lipids, and nucleic acids, exerts systemic damage that leads to cell death.

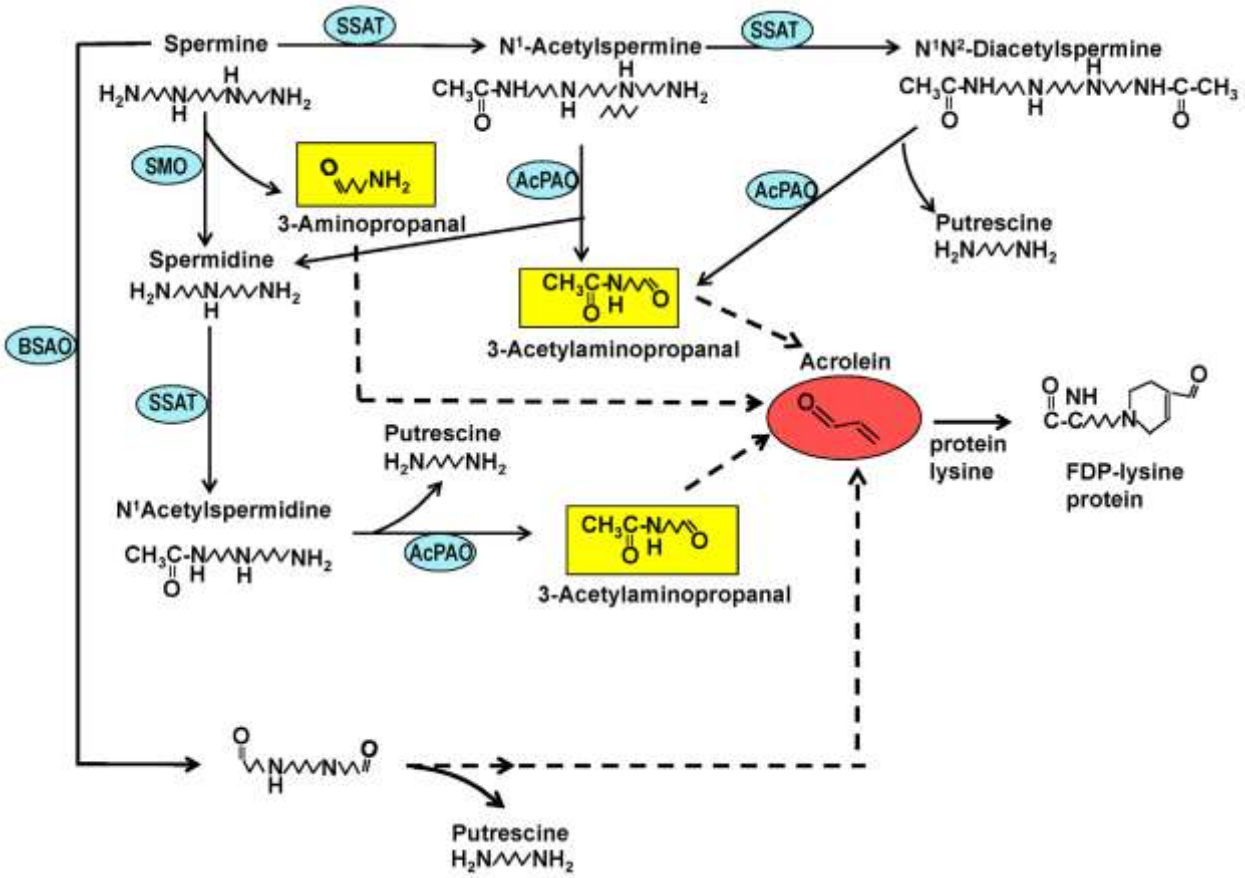

Fig. 1. Polyamine catabolism (*cited by Park M.H., Igarashi K., 2013*).

Metabolic control of the PA. The activity of enzymes of biosynthesis and disintegration of the PA (ODC, SAMDC and SSAT1) is regulated by changes in the concentrations of PA, as well as by a variety of compounds: growth factors, hormones and others [23, 24]. At high concentrations of spermidine and spermine in the cells, the activity of enzymes of PA synthesis (ODC and SAMDC) is suppressed, while the activity of enzymes of decay (SSAT1) is increased. Conversely, when the cellular content of PA is reduced, ODC and SAMDC are positively regulated and SSAT1 is suppressed. Regulation of synthesis and decay of the PA occurs at the level of transcription and translation of the biosynthesis of these enzymes [12]. With increasing content of PA inside the cell, the activity of ODC is inhibited by induction of protein, antienzyme, that forms a complex with ODC monomer leading to inactivation of its enzyme activity [2]. Degradation of this complex occurs in the 26S proteasomes. More complex regulation of ODC activity is represented by a protein inhibitor antienzyme [23]. Products of the oxidation of PA represent low molecular weight PA, H2O2, 3-

aminopropanal (3-AP) and 3-acetylaminophenol. These aldehydes are unstable and spontaneously pass in the acrolein after deamination. Acrolein, an unsaturated aldehyde, easily reacts with lysine residues of proteins with the formation of protein-conjugated acrolein (PCAcrolein) [22] (see Fig. 1). Acrolein, a highly toxic compound, when interacting with proteins, lipids and nucleic acids, exerts systemic damage that leads to cell death.

Normal homeostasis of PA is supported by a complex multiple feedback mechanisms at the level of biosynthesis, decomposition, absorption and release from cells [2, 8]. Considering the role of polyamines in the pathogenesis of certain diseases, many authors stick to the term "system of polyamines", which means the diversity of derivatives of polyamines (acetylpytrescine, acetylspirmidine, acetylspirmine, cadaverine and its derivatives), enzymes for their synthesis and degradation, as well as their transmembrane transporters.

General patterns of physiological and pathophysiological effects of the PA. It is shown that manifestations of excessive accumulation of PA in the cell are associated with the transformation of tissues and/or apoptosis, while a decrease leads to inhibition of cell growth, migration, or blocking the development of biosynthesis of nucleic acids and proteins. A sharp increase in the content of PA and activation of enzymes of their synthesis (ODC and SAMDC) associated with hyperproliferative and development of oncologic process is observed [19]. Activation of the collapse of the PA causes oxidative stress (OS) in all body tissues of animals that makes a significant contribution in molecular mechanisms of aging and pathogenesis of diseases associated with the development of OS [1, 2, 19] (Fig. 2).

PAS are involved in such processes as the increase of life expectancy, regulation of the activity of receptors and ion channels; they have a positive impact on behavior, learning and memory [2, 8]. Discoveries that the content spermidine and putrescine decreases with aging of all living organisms, combined with a decrease in the ability to memorize, provided the molecular basis for the role of the PA in autophagy of cells [17].

The introduction of the PA experimental animals to the area of the hippocampus significantly increases their ability to learn, while the introduction of inhibitors of the binding of polyamines with the NR2B subunit of NMDA receptors (arkain, ifenprodil and traxoprodil) causes the violation of the processes of memorization and reproduction of the conditioned reflex in animals [20]. Negative modulators of NMDA receptors disrupt cognitive activity, and positive (such as glycine, spermidine and spermine) may compensate dysfunction of cholinergic

and glutamatergic transmission, repair the impaired memory and learning [13]. On the model of focal cerebral ischemia in rats it was shown that under ischemia caused by disorders of memory and learning (deterioration of the formation and reproduction of conditioned reflexes) glycine facilitates the formation, but does not improve the preservation of acquired skill [5].

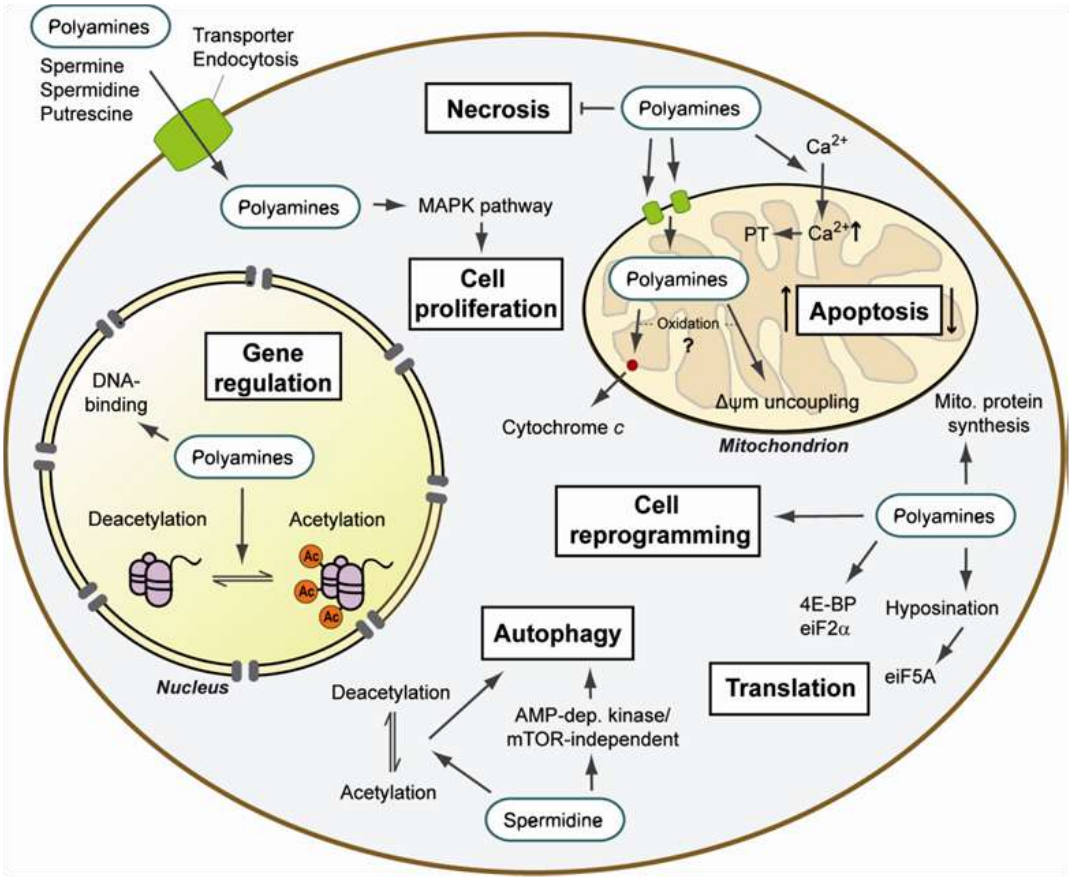

Fig. 2 Summary of the cellular metabolism of action of polyamines (cited by Minois N., Carmona-Gutierrez D., Madeo F. Polyamines in aging and disease. AGING. 2011. V. 3. N 8. P. 1-17.).

For PA, antioxidant, anti-inflammatory, neuroprotective, antidepressant, and anticonvulsant effects were shown [1, 2, 8]. In the structures of the mammalian brain, the contents of the PA is different. The uneven distribution of PA between glia and neurons in large concentrations they was found in glial cells. ODC expression in glia is observed in the functional activation caused by neuronal transplantation and chemical hypoxia [17]. Adding a PA can have a toxic effect on the culture of neurons. It was shown that incubation of a pure neuronal culture with 50 μm spermine led to cell death, whereas in the holistic structure of slices of the brain, consisting of neurons and glia (astrocytes), this concentration of spermine did not cause their death [8]. The fact that PAS are synthesized in

neurons and accumulates in glia allowed Skachkova et al. [8] to suggest a hypothesis about the role of the PA as gliotransmitters that performs a regulatory function in relation to the neurons. With receipt PA glia to neurons in certain cases, is carried out through a PA system and endothelium of brain capillaries. Especially important is the study of the role of gliotransmitters-PA in the pathogenesis of ischemic stroke [8, 30].

Polyamine system in ischemia: neurotoxicity and neuroprotection. PA may participate in the development of the OS, inducing, first, the increase of reactive oxygen species (ROS), superoxide anion radical, H2O2 and hydroxyl radical (OH*) and, second, the formation of unsaturated aldehydes such as 3-AP and acrolein [25, 30]. The addition of spermine and spermidine in cell cultures containing serum causes an inhibition of proliferation of any cell types due to the formation of oxidation products of PA, H2O2 and acrolein [15]. In neuronal cell culture complete growth inhibition of the cells is achieved by incubation with 10 μm of acrolein, 100 μm 20 μm H2O2 and OH* [25]. The acrolein is more toxic to the brain tissue than the ROS. On different models of cerebral ischemia in rats it was shown that the introduction of neuroprotective agents (acetylcysteine, carnosine) results in neutralization of acrolein, 3-AP and H_2O_2 [2, 8].

Introduction to neuronal culture containing toxic doses of spermine of the aldehyde dehydrogenase enzyme prevented cell death [25]. In the model of ischemia caused by glucotoxicity deprivation, M. Nakamura et al. found that Ca2+ was a factor inducing the toxicity of PA in neurons [24], and that Ca2+ triggered the release of PA from the ribosome. One of the main pathways for Ca2+ entry into neurons are the channels of the AMPA and NMDA receptors. In animal experiments it was shown that the introduction of N1, N4, N8-tribenzylspermidine channel blocker of NMDA receptors leads to a decrease in the content of Ca2+ and PCAcrolein in neurons [21]. It is established that PA glia is able to protect neurons from ischemic death by adjusting the data receptor channels of neurons [8]. It is shown that the neuroprotective effect of the drug diazoxide, which is known as an activator of potassium channels and is able to reduce the flow of calcium ions, in experimental ischemia/reperfusion in rats is inhibited with the introduction of spermine [12], which confirms the different effects of PA in cerebral ischemia.

In models of global cerebral ischemia it was shown that in the hippocampus the activity of the enzyme of synthesis of PA is increased 16-fold, while the content of putrescine 4-fold [30]. At the same time, in these conditions there is activation of enzymes of the collapse of the PA with the formation of various cytotoxical

aldehydes 3-AP, acrolein and H2O2 [28].

The introduction of inhibitors of ODC prevents the development of ischemic brain damage, indicating a critical role of the accumulation of PA [30]. Similar results were obtained in an experimental model of ischemic stroke caused by 24 h photochemically-induced thrombosis in mice, which showed the increase in the content of PCAcrolein 28-fold in the ischemic lesion accompanied by a decrease in the content spermidine and spermine and an increase in the activity of enzymes of the collapse of the PA - SMO and AcPAO in the outbreak [25]. Later it was found a higher degree of correlation between the size of brain infarction in mice with contents PCAcrolein than with H2O2 [16].

Neuroprotective effects of PA in cerebral ischemia is indicated for prophylactic introduction of the PAS (putrescine, spermine and spermidine) at a dose of 10 mg/ kg body weight of the experimental animals [11, 18, 30]. The mechanism of the protective effect of PA is explained by the fact that they function as traps of free radicals upon the induction of lipid peroxidation in homogenates of the brain by different Pro-oxidants [10]. Enzyme inhibition the collapse of the PA polyaminoacids as a specific inhibitor 72527 and nonspecific provides neuroprotection in models of occlusion of the middle cerebral artery in rats [24].

On the model of focal cerebral ischemia in rats with spontaneous hypertension by MRI it was shown that administration of spermine contributes to the reduction of neurological deficit and the size of the zone of the hearth ischemic stroke [8, 26].

Uemura et al. showed that young animals are more resistant to cerebral ischemia than the old ones, that correlated with PA levels in the brain [29]. These data are consistent with the work performed in our laboratory on SAM (senescence accelerated mice), with two sublineage mice: susceptible to accelerated rate of aging SAMP (prone) and stable – SAMR (resistant). In the brain rapidly aging mice characterized by increased sensitivity to hypoxia, the content of PA was significantly lower than in the controls [6]. Hypoxic episode caused more significant changes in the content of PA in rapidly aging mice than in the brain of the control group animals. So, in the brain of SAMP mice, the content of putrescine was increased by 126% compared to control, with a simultaneous decrease in the content spermidine and spermine by 42 and 45 %; in the brain of the control line SAMR, hypoxia causes an increase in the putrescine content of 35% and a reduction spermidine and spermine for 30 and 20 % compared to normoxia (Fig.3)

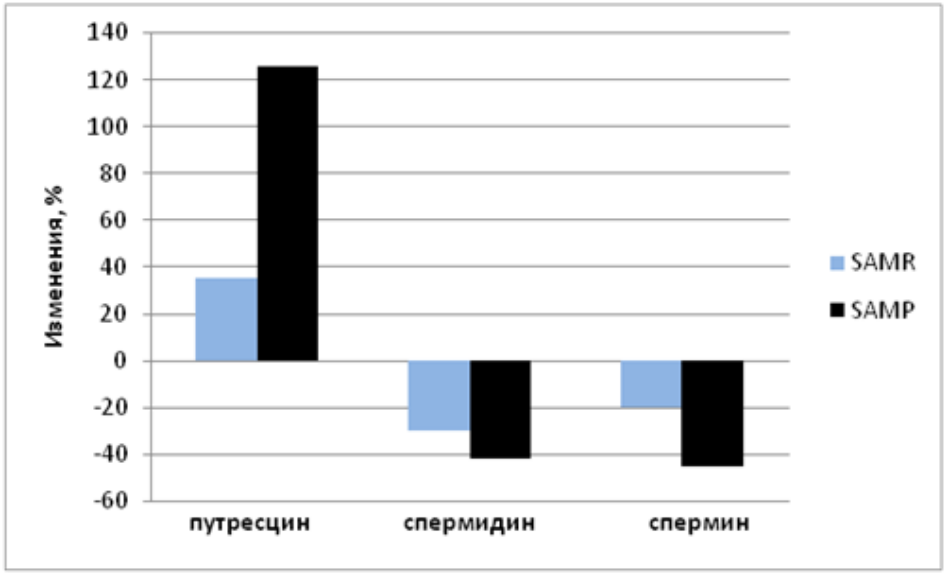

Fig. 3 Changes of polyamines in the brain tissue of the mice of line SAMP with acute hypoxic hypoxia, compared with SAMR mice (p≤0, 05 compared with normoxia %)

Participation of PA in ischemic pathochemical cascade is shown in the very early stages of cerebral ischemia, while an increase in the metabolism of PA with induction of both enzymes for their synthesis and decay [30]. Putrescine and 3-AP accumulated in the zone of ischemic penumbra within 2 h after cerebral ischemia in rats, thus supporting a sharp increase in the activity of metabolic pathways for the interconversion of PA [25, 30]. The accumulation of 3-AP and acrolein in the lysosomes within 3-4 h after ischemia causes the release of proteolytic enzymes, leading to disruption of mitochondrial integrity [30]. There is a time dependence in the quantitative change of PA in the brain during ischemia: the contents of spermine and spermidine reduced after 6 h and 24 h after occlusion of the middle cerebral artery in ishemizirovanna cerebral hemisphere [30]. However, 5 days after occlusion of the middle cerebral artery in rats, the content of acetyl derivatives of spermine and spermidine in the brain tissue increases [27]. The tests on animals made it possible to develop markers of ischemic stroke for clinical trials. The diagnostical significance of the determination of the content of the PA, their degradation products, and enzymes of the metabolism was confirmed during examination of patients with lacunar stroke [25, 31].

In clinical and biochemical studies patients with stroke it was shown the increase of the content of degradation products of PA 3-AP, acrolein, PCAcrolein in the blood and in the CSF [15, 16, 28, 31], and activation of the enzyme of their degradation [25].

In our laboratory, it was shown that in the first 48 hours after stroke the content of free polyamines increased, and associated PA forms decreased in blood plasma and in erythrocytes of patients, which indicates the transition of polyamine from the associated with nucleic acids form into the form of polyamines. In the blood plasma of patients, there were an increase in the content of free polyamines and the decrease of the content related in comparison with donors. So, a free form of putrescine was increased by 96%, spermidine by 131%, and spermine by 32%, while the bound forms were decreased (Fig.4). In the early stages of ischemic stroke in plasma and erythrocytes of patients there was an increased content of free-polyamines compared to control. The content of bound forms of polyamines in plasma and erythrocytes of the patients ia shown on Fig. 4.

Therefore, cerebral ischemia triggered the pathogenetic mechanisms of formation of toxic breakdown products of the PA in neurons, glia and overall body. Treatment of stroke at different stages should be aimed at breaking the links of the ischemic cascade [3, 4, 7]. Experimental and clinical-biochemical studies show that cerebral ischemia PA plays a dual role: it participates in the launch of the ischemic cascade and production of toxic aldehydes and ROS and, on the other hand, has neuroprotective effect.

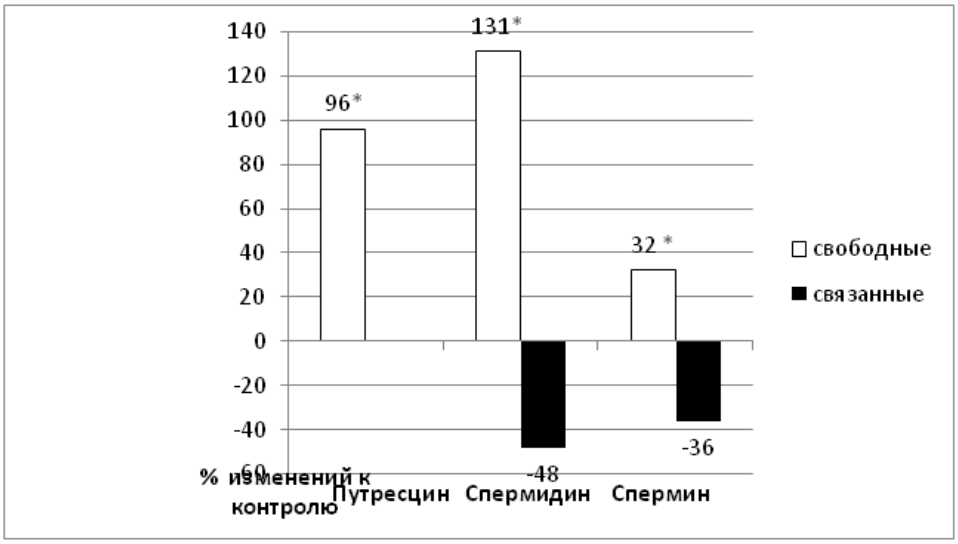

Fig. 4. The contents of polyamines in plasma of patients with stroke (percentage of control taken as 100%).

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