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STRUCTURAL-FUNCTIONAL ANALYSIS OF BIOPOLYMERS AND THEIR COMPLEXES

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Epigenetic Mechanisms of Peptide-Driven Regulation and Neuroprotective Protein FKBP1b

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Abstract—Cortexin is a clinically approved cerebral cortex polypeptide complex in calves. The mechanism of cortexin action is not understood well. Two cortexin derivatives, short peptides EDR and DS with neuroprotective activity, were synthesized. According to the data of molecular modeling, these peptides are able to bind to the histone H1.3 protein. This can affect the conformation of histone H1.3, which leads to a change in the chromatin structure in the loci of some genes, in particular *Fkbp1b* encoding the FK506-binding protein. Electrophysiological processes associated with the Ca^{2+} exchange are disturbed in the pyramidal neurons of the hippocampus during aging of the brain. The *Fkbp1b* gene encodes peptidyl-prolyl *cis-trans* isomerase, regulating the release of calcium ions from the sarcoplasmic and endoplasmic reticulum of neurons. The activation of the *Fkbp1b* gene transcription under treatment with short peptides can promote the synthesis of its protein product and the activation of the Ca^{2+} release from organelles of the sarcoplasmic and endoplasmic reticulum of neurons, which, in turn, can lead to an increase in the functional activity of neurons.

Keywords: short peptides, neuroprotection, aging, molecular modeling, FKBP1b
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INTRODUCTION

During 1973–2017, more than 20 physiologically active polypeptide complexes were isolated in calves, then 17 short peptides patented in many countries (United States, Canada, Australia, Europe, Japan, Korea, Israel, etc.) were designed based on that data in [1, 2]. Clinical trials of thymus polypeptide complexes, the pineal gland, the brain (cortexin), the prostate gland, and the retina were performed in [3, 4]. The EW dipeptide, an immunity regulator, which also successfully passed clinical trials, was synthesized based on the polypeptide complex of the thymus in [1]. Amino acid analysis of cortexin revealed several short peptides with neuroprotective properties, in particular, pinealon (EDR) and DS. The neuroprotective properties of peptides EDR and DS were studied mainly in animals and cell cultures, however, the molecular mechanism of their action is not fully understood. We show via molecular modeling that peptides EDR and DS are capable of binding to the histone H1.3 protein. This interaction can lead to a change in the conformation of the histone, which results in a change in chromatin in the loci of certain genes, in particular *Fkbp1b* encoding the FK506-binding protein.

Abbreviations: CNS, central nervous system; Brn3, brain-specific homeobox-containing transcription factor; CXCL12, chemokine of the CXC subfamily; EDR, pinealon; FK506, rapamycin; FKBP1b, FK506-binding protein 1b; IL-2, interleukin-2; mTORC1, mTOR1 complex; Pax6, aniridia type II protein or oculorhombin; PLB, propensity for ligand binding; Prox1, homeobox-containing protein 1 with Prospero domain; RyR, ryanodine receptor; sAHP, slow K^+-dependent hyperpolarization.

PEPTIDE NEUROPROTECTORS

Pinealon is a neuroprotective Glu–Asp–Arg peptide with a molecular weight of about 418 Da in [5]. In the clinical study, 72 patients (age range at 30–74 years) with traumatic brain injury and encephalasthenia orally received the EDR peptide, daily, for 20–30 days in addition to standard therapy. An improvement in memory, a decrease in the duration and intensity of headaches, and the emerging emotional balance were noted. In patients with the traumatic brain injury, there was a regression of focal symptoms and an improvement in speech function under motor and sensory aphasia. In

patients with encephalasthenia, the number of errors while performing correction work decreased, the integral indicator of working capacity increased under the influence of the EDR peptide in [6].

The oral administration of the EDR peptide for the correction of the psychoemotional and functional state of CNS in 75 elderly individuals led to an increase in short-term and long-term memory, as well as a decrease in the severity index of the state in [7].

The effect of the EDR peptide on the functional activity of CNS was also studied in rats with experimental prenatal hyperhomocysteinemia. The induction of oxidative stress is associated with increasing homocysteine in the blood of animals, and with a decrease in cognitive abilities and an impaired glutamatergic system of the brain. The intramuscular injection of the EDR peptide contributed to improvement of the spatial orientation and learning ability of rat offspring in the Morris water maze test. We could assume that the protective effect of tripeptide is associated with its ability to suppress the ROS accumulation in neurons, increasing their resistance to oxidative stress and preventing the interaction of homocysteine and its derivatives with the glutamate receptors in [8]. The effect of the EDR peptide on the MAP kinase activation in cerebellar granule cell cultures was also evaluated. The MAP kinase activation profile determines which genes associated with adaptation or apoptosis will be expressed in a given period of time. The addition of the tripeptide to cell cultures led to a prolongation of the lag period of MAP kinase activation, which can be considered a defense against the toxic action of homocysteine in [9, 10].

The ability of the EDR peptide to restore the morphology of spines in the striatum neurons of mice, in which Huntington's disease was simulated, was found in [11]. In the primary culture of mouse hippocampal neurons under amyloid synaptotoxicity (an Alzheimer's disease model), the EDR peptide at a concentration of 200 ng/mL increased the number of mushroom-shaped spines of neurons by 71%, bringing them to a normal level in [12].

The DS peptide (Asp-Ser) normalizes medium-term memory and the ability of agnts3 line *Drosophila melanogaster* flies to learn. In flies of this line, the *limk1* gene, which encodes the key enzyme of actin remodeling (LIMK1), is mutated; the disruption of its synthesis leads to the development of Parkinson's disease. The DS peptide can be studied in models of Parkinson's disease and other neurodegenerative diseases.

The Brn3 proteins (differentiation of retinal ganglion cells), Pax6 (initial stages of retinal cell differentiation), Math1 (differentiation of retinal amacrine neurons), TTR (transthyretin, a marker of retinal pigment epithelium), Prox1 (differentiation of retinal amacrine and ganglion neurons), Ki67 (proliferative protein) are DS peptide targets in the organotypic culture of chicken and rat retinal tissuets. The DS peptide stimulates the synthesis of proteins Brn3, Pax6, Math1, TTR, Prox1, CXCL12, and Ki67, and reduces the synthesis of proapoptotic p53 protein in retinal tissue. The DS peptide stimulates proliferation, differentiation, and functional activity, and reduces the level of apoptosis of pigment epithelial cells and neurons in the organotypic culture of the retina tissue.

This work is aimed at verifying the possible association of the neuroprotective effect of these peptides with their effect on the expression of the *Fkbp1b* gene, the product of which contributes to the restoration of the function of calcium channels in neurons of the brain.

We suggested that peptides EDR and DS affect expression of the *Fkbp1b* gene. Short biologically active peptides, including EDR, were shown to bind to FITC-labeled wheat histones. For histone H1, the peptides bind to the N-terminal regions of histones, which contain homologous peptide-binding motifs. The binding of peptides to histones and histone–deoxyribooligonucleotide complexes depends on the nature of the histone, the primary structure of the peptide and the oligonucleotide, i.e., there is a site-specific interaction of short peptides with histones and histone–oligonucleotide complexes. Histone H1 preferably binds to single-stranded oligonucleotides through homologous regions in the C-terminal region of the protein. In contrast to histone H1, cortical histones bind predominantly to double-stranded methylated oligonucleotides and methylated DNA. The values of the Stern–Volmer constant of histones H1 and core histones interaction with double-stranded semimethylated oligonucleotides are higher than with the unmethylated. DNA or deoxyribooligonucleotides in the complex with histones can enhance or inhibit peptide binding. It is assumed that site-specific interactions of short biologically active peptides with histones within chromatin can serve as a control mechanism for the regulation of gene activity and cell differentiation in [13].

To confirm this assumption via the methods of molecular docking and geometry minimization using Molecular Operating Environment 2016.08, we determined site-specific histone binding, with which short peptides EDR and DS interact.

METHODOLOGY FOR HISTONE–PEPTIDE INTERACTION ANALYSIS

The calculation of the spatial conformation of di-, tri-, and tetrapeptides was performed via the method of molecular mechanics. This approach allows calculating the potential energy of a given system according to Hooke's law: atoms in the molecule were considered elastic balls of various sizes (according to the atom type) connected via springs of various lengths. The peptides were constructed in the levorotatory conformation. In the calculations, the total energy was minimized relative to the origin of coordinates:

$$E_{tot} = E_{str} + E_{bend} + E_{tors} + E_{vdw} + E_{elec}+,$$

where E_{tot} is the total potential energy of the macromolecule, E_{str} is the bond strain energy, E_{bend} is the valence angle strain energy, E_{tors} is the torsion angle strain energy, E_{vdw} is the van der Waals interaction energy, and E_{elec} is the electrostatic interaction energy. The total steric energy of the system was calculated taking into account the force field, which contains a set of adjustable empirical parameters (force constants) and standard values of bond lengths, torsion, and valence angles. The van der Waals interactions in the molecule were also considered. The first term of the equation describes the change in energy when the bond is stretched or compressed relative to its standard length:

$$E_{str} = \frac{1}{2} k_b (b - b_0)^2,$$

where k_b is the constant force of bond stretching, b_0 is the standard bond length, and b is the current bond length.

Angular deformations were described via the following equation:

$$E_{bend} = \frac{1}{2} k_0 (\theta - \theta_0)^2,$$

where k_θ is the constant force of valence angle deformation, θ_0 is the equilibrium value of the valence angle, and θ is the current value of the valence angle.

The contribution of the rotation around dihedral angles to the potential energy was calculated using the following equation:

$$E_{tors} = \frac{1}{2} k_\phi (1 + \cos(n\varphi - \phi_0),$$

where k_φ is the torsion barrier (rotation barrier), φ is the current value of the torsion angle, n is the period (the number of energy minima per one full cycle), and φ_0 is the standard value of the torsion angle.

The van der Waals interactions between directly bound atoms are usually expressed via the Lennard–Jones potential:

$$E_{vdw} = \sum \frac{A_{ij}}{d_{ij}^{12}} - \frac{B_{ij}}{d_{ij}^{6}},$$

where A_{ij} is the coefficient of the repulsion contribution, B_{ij} is the coefficient of the attraction contribution, and d_{ij} is the distance between atoms i and j.

Electrostatic forces are described via the function representing the expression for the Coulomb interaction:

$$E_{elec} = \frac{1}{\varepsilon} \frac{Q_1 Q_2}{d},$$

where ε is the permittivity, Q_1 and Q_2 are charges on the interacting atoms, and d is the interatomic distance.

When calculating the molecule conformations, an important task was to correctly determine the force field in [14]. There are several ways to specify the force field: MMFF94x, Amber12EHT, BIO+, and OPLS, each differing by the degree of possible approximations and assumptions. When using the MMFF94x field, potential fields formed by all atoms of the molecular system are considered. The method provides high accuracy and is suitable for small molecules. Amber12EHT is an all-atom force field developed to calculate conformations of proteins, nucleic acids, and small molecules in [15]. In this work, the conformations of short peptide molecules were calculated using the Amber12EHT force field. After construction of peptide computer models, an important stage was the geometrical optimization of their structures by minimizing energy via methods of steepest descent and conjugate gradients. The method of steepest descent is based on the gradual shift of atoms of the peptide molecule along the coordinate axes to find a new position with a lower potential energy. When the specified condition of the energy minimum is reached, the minimization stops. This method is used for structures that are far from the energy minimum. For a more accurate calculation, the conjugate gradient method is used, the main idea of which is gradual accumulation of information on the minimized function from iteration to iteration. The energy gradient is also considered at each stage of the minimization, which is further used as additional information to calculate the new vector directions for the minimization procedure. Each subsequent stage continuously clarifies direction towards the minimum.

The molecule after geometric optimization has many isomers, which correspond to different energy values. An important task is to find the most energy-efficient isomers of the molecule, which have similar values of total potential energy (E_{tot}) within certain minima.

In our work, the conformational search was carried out using methods of molecular dynamics, the purpose of which was to reproduce the motion of the molecule at a given time interval, in this case 1 fs. The basis of the method is the classical Newton equation of motion:

$$F_i(t) = m_i^* a_i(t),$$

where $F_i(t)$ is the force acting on atom i at time t, m_i is the mass of the i-th atom, and $a_i(t)$ is the acceleration of atom i at time t.

At the final stage of the conformational search, the physicochemical features of the peptide conformers were evaluated, and the length of the peptide chain of the molecule, the charges, and the mean potential energy were calculated. Since peptides consisting of four amino acid residues were investigated in the study, only three torsion angles were measured. The number of conformations found in the peptides showed to what extent the molecules are spatially stable or unstable. It was assumed that the energetically most favorable stereoisomers of the molecules possess

Table 1. Amino acid sequences of histones

Histone of wheat *Triticum aestivum*, FASTA	PDB structure and its amino acid sequence, used to construct homologous histone models, FASTA	Identity of original sequence and template, %
>H1.1 MSTDVVADVPAPEVAAAADPVVE TTAEPAAGDANAAKETKAKAAKA KKPSAPRKPRAAPA**HPTYAEMVSE** **AITALKERTGSSPYAIAKFVEDKH** **KAHLPANFRKILSVQLKKLVASG** **KLTKVKASYKLSAAAAKPK**PAAK KKPAAKKKAPAKKTATKTKAKAP AKKSAAKPKAKAPAKTKAAAKPK AAAKPKAKAPAKTKAAAKPKAAA KPKGPPAKAAKTSAKDAPGKNAG AAAPKKPAARKPPTKRSTPVKKAA PAKKAAPAKKAPAAKKAKK	>5NL0_Z **HPKYSDMILAAVQAEKSRS** **GSSRQSIQKYIKNHYKVGEN** **ADSQIKLSIKRLVTSGALKQ** **TKGVGASGSFRLAK**	34
>H1.3 MSTEVAAADIPVPQVEVAADAAVD TPAANAKAPKAAKAKKSTGPKKPR VTPA**HPSYAEMVSEAIAALKERSGS** **STIAIGKFIEDKHKAHLPANFRKI** **LLTQIKKLVAAGKLTKVKGSYKL** **AKAPAAV**KPKTATKKKPAAKPKA KAPAKKTAAKSPAKKAAAKPKAK PAKAKAVAKPKAAAKPKAAAKP KAKAAAKKAPAAATPKKPAARKPP TKRATPVKKAAPAKKPAAKKAKK	>5NL0_Z **HPKYSDMILAAVQAEKSRS** **GSSRQSIQKYIKNHYKVGEN** **ADSQIKLSIKRLVTSGALKQ** **TKGVGASGSFRLAK**	36
>H1.6 PVPQVEVAADAAVDTPAASAKAPK AAKAKKSTGPKKPRVTPA**HPSYAE** **MVSEAIAALKERSGSSTIAIAKFIE** **DKHKAHLPANFRKILLTQIKKLV** **AAGKLTKVKGSYKLAKAPAAV**K PKTATKKKPAAKPKAKAPAKKTAA KSPAKKAAAKPKAKAPAKAKAVA KPKAASKPKAAAKPKAKAAAKKA PAAATPKKPAAARKPPTKRATPVK KAAPAKKPAAKKAKK	>5NL0_Z **HPKYSDMILAAVQAEKSRS** **GSSRQSIQKYIKNHYKVGEN** **ADSQIKLSIKRLVTSGALKQ** **TKGVGASGSFRLAK**	36
>H2b MAPKAAKKPAAKKPAEEEPAAEK **AEKTPAGKKPKAERRIPAGKSAA** **KAGGDKKGKKKAKKSVETYKKY** **IFKVIKQVHPDIGISSKAMSIMNSF** **INDIFEKLAGEAAKIARYNKKPYI** **TSREIQTSVRLVLPGELAKHAVSE** **GTKAVTKFT**SAS	>1KX5_H **PEPAKSAPAPKKGSKKAVT** **KTQKKDGKKRRKTRKESY** **AIYVYKVLKQVHPDTGISSK** **AMSIMNSFVNDVFERIAGEA** **SRLAHYNKRSTITSREIQTA** **VRLLLPGELAKHAVSEGTK** **AVTKYTSAK**	65

Table 1. (Contd.)

Histone of wheat *Triticum aestivum*, FASTA	PDB structure and its amino acid sequence, used to construct homologous histone models, FASTA	Identity of original sequence and template, %
>H3 **ARTKQTARKSTGGKAPRKQLAT** **KAARKSAPATGGVKKPHRERPG** **TVALREIRKYQKSTELLIRKLPFQ** **RLVREIAQDFKTDLRFQSSAVAAL** **QEAAEAYLVGLFEDTNLCAIHAK** **RVTIMPKDIQLARRIRGERA**	>1KX5_A **ARTKQTARKSTGGKAPRKQ** **LATKAARKSAPATGGVKKP** **HRYRPGTVALREIRRYQKS** **TELLIRKLPFQRLVREIAQD** **FKTDLRFQSSAVMALQEAS** **EAYLVALFEDTNLCAIHAKR** **VTIMPKDIQLARRIRGERA**	96
>H4 SGRGKGGKGLGKG**GAKRHRKVL** **RDNIQGITKPAIRRLARRGGVKRI** **SGLIYEETRGVLKIFLENVIRDAV** **TYTEHARRKTVTANDVVYALKR** **QGRTLYGFGG**	>1EQZ_D **GAKRHRKVLRDNIQGITKP** **AIRRLARRGGVKRISGLIYE** **ETRGVLKVFLENVIRDAVTY** **TEHAKRKTVTAMDVVYAL** **KRQGRTLYGFGG**	97

Amino acid sequences of histones with resolved secondary structure taken from PDB are highlighted in bold. The identity of the sequences was calculated via BLASTp (https://blast.ncbi.nlm.nih.gov).

biological activity and perform functions in precisely this conformation.

To develop a computer model of the interaction of peptides with wheat histones, we designed their homologous models, since the spatial structures of wheat histones have not previously been resolved. To develop models of histones H1, H2b, H3, and H4, we used structures from the Protein Data Bank (PDB) 5NL0, Z chain; 1KX5, chain A and chain H; and 1EQZ, chain D.

Table 1 shows amino acid sequences of wheat histones and the corresponding histone sequences from the PDB database.

To construct histone models, we used the homologous modeling method, which allows estimation of the degree of primary structure homology of the studied proteins using three-dimensional structures from the PDB database, resolved with X-ray structural analysis or nuclear magnetic resonance.

Active binding sites for peptides in histone molecules were found via the Edelsbrunner method in [16]. This method is based on the search for the most energetically preferable binding sites of peptides isolated in the histone structure by alpha-spheres. This method is used to determine regions of rigid atomic packing, but does not take into account sites that are too susceptible to the solvent (sites located on the surface of the molecule). The sites were ranked by propensity of ligand binding (PLB), based on the amino acid composition of the pocket in [16, 17].

Docking is a computer simulation of interactions between the ligand (peptide) and the active site of the receptor (histone). The docking method includes the placement of the ligand in different conformations at the binding site, as well as the calculation of the optimal mutual orientation of the peptide and histone molecules during their binding and the binding energy (kcal/mol). The semiflexible docking, where only the peptide conformational mobility was considered, while the side groups of the histones were rigid, was used. When calculating the optimal spatial conformations of the interaction between peptides and histones, the contact area, the number of hydrogen bonds, the parameters of hydrophobic, and electrostatic interactions were considered. The Amber12EHT force field and the GBVI/WSA genetic algorithm of the search were used. Docking solutions were ranked according to the values of the fitness function (ΔG), which was calculated using the following formula:

$$\Delta G = c + \alpha(2/3(\Delta E_{coul} + \Delta E_{sol}) + \Delta E_{vdw} + \beta\Delta SA_{weighted}),$$

where c is the value of the loss of rotational and translational entropy of the complex; α, β are experimentally defined constants that depend on the force field; E_{coul} is the Coulomb energy value, which is calculated using the charge of the system with the permittivity of 1; E_{sol} is the value of the electrostatic energy of the solvent; E_{vdw} is the van der Waals contribution to the interaction energy; $SA_{weighted}$ is the contribution of molecular shells to the energy value.

Docking solutions were ranked in descending order from energetically most advantageous to least favorable. After analyzing the docking data, the energetically most favorable complex of the peptide and histone was selected.

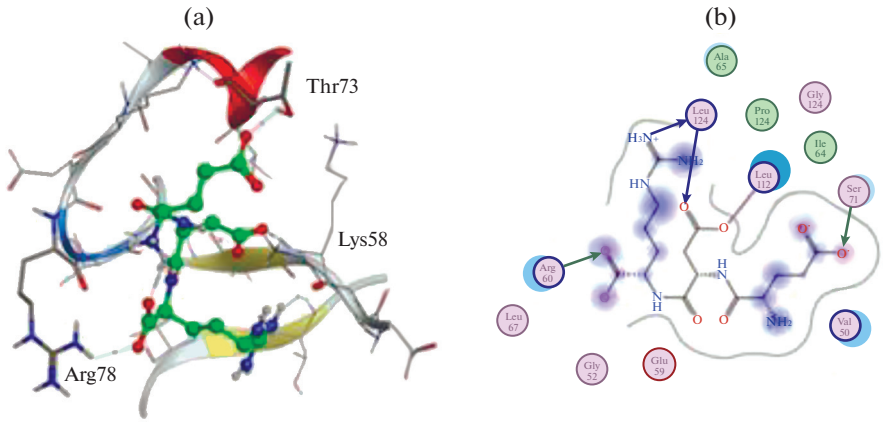

Fig. 1. Interaction of EDR peptide with histone H1.3.

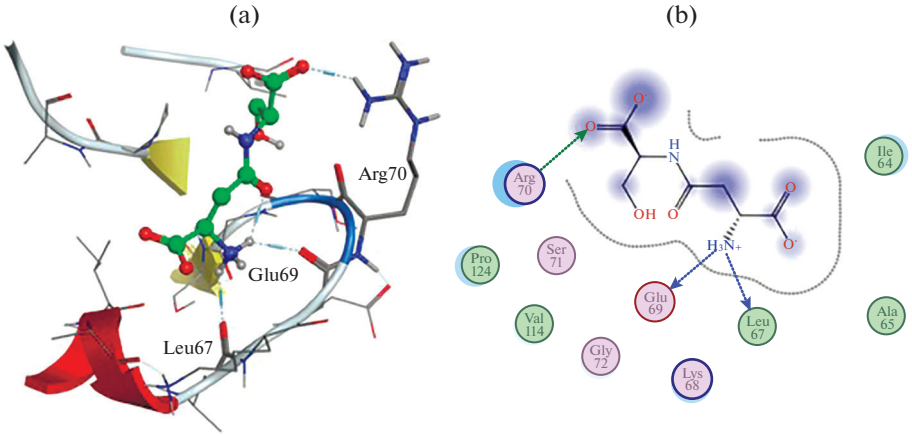

Fig. 2. Interaction of DS peptide with histone H1.3.

The Ligand Interactions module, which provides the ability to visualize the active site of the complex in the form of a diagram, is implemented in the Molecular Operating Environment 2016 program. The amino acid residues in the active site are identified in two stages: (1) receptor amino acids, ions, and solvent molecules that interact strongly with the ligand (interactions during the formation of hydrogen bonds); (2) receptor amino acids and ions that are close to the ligand, but with weak or diffuse interactions, such as collective hydrophobic or electrostatic interactions.

RESULTS HISTONE–PEPTIDE INTERACTION ANALYSIS

In our work, peptides were associated mainly with histones H1.1, H1.3, and H1.6 in the region formed by Tyr, His, Arg, and Lys residues localized in loops. Figures 1 and 2 show the energetically most favorable variants of histone–peptide interactions.

We showed that peptides preferably bind to histones at sites rich in positively charged arginine and lysine residues, found in large amounts in the N-terminal regions of histones. We assume that competitive binding of peptides, and other regulatory factors to histones, occurs at these sites. In this regard, it is assumed that the binding of the peptide to histones can serve as an additional mechanism through which the biological effect is realized.

DISCUSSION

A change in the concentration of calcium ions in mammalian neurons is considered a sign of an aging brain. Dysregulation of the functions of Ca^{2+}-dependent neurons during aging, largely associated with the type of cells and the CNS section in which they are localized, was discovered in the last century in [18, 19]. One of the most significant signs of aging of dorsal hippocampus CA1-pyramidal neurons, (the hippocampus is responsible for learning and memory) is an increase in the Ca^{2+}-dependent potential followed by slow K^+-dependent hyperpolarization (sAHP) in [20–22]. sAHP is generated by hyperpolarizing Ca^{2+}-sensitive K^+ channels, which are activated via the

Ca^{2+}-induced action potential and are characterized by the release of Ca^{2+} from ryanodine-sensitive (RYR) calcium depots in [23–25].

During aging, an increase in the amplitude and the development of the slow posthyperpolarization of electrophysiological processes associated with the Ca^{2+} exchange is observed in the pyramidal neurons of the hippocampus. Changes in the conformation of L-shaped ryanodine Ca^{2+} channels, correlating with a decrease in the plasticity of neurons, which is accompanied by a disruption in the processes of learning and memorizing, were identified in [26, 27]. An age-related decrease in the expression of the gene encoding FK506-binding protein 12.6/1b (FKBP1b, peptidyl-prolyl *cis-trans* isomerase, inhibiting the release of Ca^{2+} from RyR channels of the sarcoplasmic and endoplasmic neuronal reticulum) was also in [28]. FKBP1a/1b, or FKBP12/12.6 proteins, belong to the class of immunophilins that bind the immunosuppressant rapamycin. In muscle cells, FKBP1a/1b also interacts with RyR and interferes with Ca^{2+} release in [22]. During aging, FKBP1b protein synthesis is reduced in the hippocampal neurons, while the synthesis of RyR2 and FRAP/mTOR (rapamycin target) is activated in [22]. Impaired function of the FKBP1b protein, destabilizing Ca^{2+} homeostasis in the hippocampal neurons, leads to signs of CNS aging in young animals. Suppression of the *Fkbp1b* gene activity in the hippocampal neurons of young rats enhances the Ca^{2+} current, which disturbs the excitability of cells, causing accelerated aging of the nervous system in [29, 30].

The cytoplasmic protein FKBP1a is a receptor for the immunosuppressant rapamycin, used to suppress the rejection reaction during transplantation. Rapamycin prevents the activation of T and B cells by interleukin-2 (IL-2) and is widely used in stenting in [31]. In mouse models, the effect of rapamycin on autoimmune diseases was demonstrated in [32]. Rapamycin binds to the FKBP1a protein, after which the (FKBP1a)/rapamycin complex recognizes the FRB domain (rapamycin binding domain) of mTOR and inhibits the mTOR complex 1 (mTORC1). Rapamycin binding to the FKBP1a protein leads to the destabilization of mTORC1 in [33], which regulates autophagy, cell growth and survival. Rapamycin also suppresses the response to IL-2, thereby preventing the development of immune response.

Rapamycin displaces the FKBP1b protein from RyR in myocytes, increases sAHP, and also increases the release of Ca^{2+}. Moreover, FKBP1b knockout is associated with increased synthesis of RyR2 and mTOR, related to the translation regulation of proteins responsible for proliferation. Thus, FKBP1b dysfunction can accelerate the aging process, affecting the regulation of Ca^{2+} channel functioning in the hippocampal neurons in [29, 30, 34].

Selective dysfunction of the FKBP1b protein in the hippocampal neurons of young rats, caused by a microinjection of vectors based on adeno-associated viruses expressing small interfering RNAs to FKBP1b, or by rapamycin administration, increases sAHP and promotes Ca^{2+}-dysregulation of the hippocampus. Moreover, expression of the *Fkbp1b* gene is reduced in the hippocampus of rats at early stages of Alzheimer's disease. All this suggests that the decrease in FKBP1b expression can be considered a key factor, associated with aging and the development of neurodegenerative diseases caused by impaired calcium signaling in brain neurons, and as a new therapeutic target in [30].

It was shown that overexpression of the FKBP1b protein in the hippocampus prevents aging-induced memory loss and dysfunction of Ca^{2+}-dependent neurons. To establish this, a vector expressing *Fkbp1b* was introduced in the hippocampus of 13- and 19-month-old male rats. When the animals reached the age of 21 months, their working capacity and memory status were assessed in the Morris water maze in [35]. An increase in the FKBP1b activity is accompanied by an improvement in spatial memory in old rats. They chose a shorter way to exit the water maze and did it faster than the control rats that were injected with a green fluorescent protein gene. After that, the change in expression of 2342 genes in the hippocampal cells of old and young animals was compared. The results led to the conclusion that aging of the nervous system correlates with a decrease in the *Fkbp1b* gene expression. Genes, the expression of which decreases with aging and is restored when the *Fkbp1b* gene is activated, are associated mainly with various structures of neurons, including the cytoskeleton and membrane channels. On the contrary, the genes, whose activity is enhanced with aging and is not restored when the *Fkbp1b* gene is activated, are primarily associated with dysfunction of the ribosomes and glial lysosomes. The restoration of the structure of neuronal microtubules was confirmed via immunohistochemical methods. Apparently, in the hippocampus and other brain structures responsible for behavior, short-term and long-term memory, there is a network of genes that modulate the functions of neurons. During aging, dysregulatory processes develop in this network, leading to memory impairment, which can be restored by overexpression of *Fkbp1b* [35].

According to our hypothesis, peptides EDR and DS, penetrating into the nucleus and the nucleolus in [36], are able to bind to histone H1.3, which should lead to a change in the conformation of the histone and result in a change in the availability of genes, in particular *Fkbp1b*, for transcription. In turn, the activation of *Fkbp1b* transcription should promote the synthesis of the FKBP1b protein and the activation of the Ca^{2+} release from the sarcoplasmic and endoplasmic reticulum of neurons, which will lead to the normalization of the functional activity of neurons. The neuroprotective effects of peptides EDR and DS are a

testimony of this hypothesis in [1–3, 5–13, 36]. This hypothesis needs experimental confirmation, which we propose for further studies.

The authors declare no potential conflict of interest.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

REFERENCES

1. Anisimov V.N., Khavinson V.Kh. 2010. Peptide bioregulation of aging: Results and prospects. *Biogerontology.* **11** (2), 139–149.
2. Khavinson V.Kh. 2014. Peptides, genome, aging. *Adv. Gerontol.* **4** (4), 337–345.
3. Khavinson V.Kh., Kunik B.I., Ryzhak G.A. 2012. Peptide bioregulators, a new class of geroprotectors: 1. Results of experimental studies. *Usp. Gerontol.* **25** (4), 696–708.
4. Khavinson V.Kh., Korkushko O.V., Shatilo B.V., Antonyuk-Shcheglova I.A. 2011. A peptide geroprotector from the epiphysis retards accelerated aging in elderly persons. *Byull. Eksp. Biol. Med.* **151** (3), 366–369.
5. Khavinson V.Kh., Grigoriev E.I., Malinin V.V., Ryzhak G.A. 2008. Eurasian Patent EA 010157.
6. Morozov V.G., Ryzhak G.A., Malinin V.V., Rutkovskaya V.N. 2011. *Tsitogeny. Biologicheski aktivnye dobavki k pishche: Metod. rekomendatsii* (Cytogens: Biologically Active Dietary Supplements. Methodological Guidelines). St. Petersburg: Kosta.
7. Balashova S.N., Zhernakov G.L., Dudkov A.V. 2008. Applications of peptide bioregulators in elderly persons with psychoemotional disturbances. *Usp Gerontol.* **3** (21), 448–452.
8. Arutjunyan A., Kozina L., Stvolinskiy S. 2012. Pinealon protects the rat offspring from prenatal hyperhomocysteinemia. *Int. J. Clin. Exper. Med.* **2** (5), 179–185.
9. Khavinson V., Ribakova Y., Kulebiakin K. 2011. Pinealon increases cell viability by suppression of free radical levels and activating proliferative processes. *Rejuvenation Res.* **5**, 535–541.
10. Umnov R.S., Lin'kova N.S., Khavinson V.Kh. 2013. Neuroprotective effects of peptide bioregulators in persons of different ages: A review. *Usp. Gerontol.* **4** (26), 671–678.
11. Khavinson V., Linkova N., Kukanova E., Bolshakova A., Gainullina A., Tendler S., Morozova E., Tarnovskaya S., Vinski D.S.P., Bakulev V., Kasyanenko N. 2017. Neuroprotective effect of EDR peptide in mouse model of Huntington's disease. *J. Neurol. Neurosci.* **1(8)**, 1–11.
12. @@Красковская Н.А., Куканова Е.О., Линькова Н.С., Попугаева Е.А., Khavinson V.Kh. 2017. Tripeptides restore the numbers of neuronal spines in an in vitro model of Alzheimer's disease. *Klet. Technol. Biol. Med.* **2**, 101–104.
13. Fedoreyeva L.I., Smirnova T.A., Kolomiitseva G.Ya., Khavinson V.Kh., Vanyushin B.F. 2013. Interaction of short peptides with FITC-labeled wheat histones and their complexes with deoxyribooligonucleotides. *Biochemistry* (Moscow). **78** (2), 166–175.
14. Labute P. 2010. LowModeMD: Implicit low-mode velocity filtering applied to conformational search of macrocycles and protein loops. *J. Chem. Inf. Model.* **50**, 792–800.
15. Case D.A., Darden T.A., Cheatham T.E. 2012. *AMBER 12.* San Francisco: Univ. of California Press.
16. Edelsbrunner H., Facello M., Fu R., Liang J. 1995. Measuring proteins and voids in proteins. In: *Proc. 28th Hawaii Int. Conf. on Systems Science,* pp. 256–264.
17. Soga S., Shirai H., Kobori M., Hirayama N. 2007. Use of amino acid composition to predict ligand-binding sites. *J. Chem. Inf. Model.* **47**, 400–406.
18. Khachaturian Z.S. 1989. The role of calcium regulation in brain aging: Reexamination of a hypothesis. *Aging* (Milan). **1**, 17–34.
19. Disterhoft J.F., Thompson L.T., Moyer J.R., Mogul D.J. 1996. Calcium-dependent afterhyperpolarization and learning in young and aging hippocampus. *Life Sci.* **59**, 413–420.
20. Landfield P.W., Pitler T.A. 1984. Prolonged Ca^{2+}-dependent after hyperpolarization in hippocampal neurons of aged rats. *Science.* **226**, 1089–1092.
21. Moyer J.R., Thompson L.T., Black J.P., Disterhoft J.F. 1992. Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age- and concentration-dependent manner. *J. Neurophysiol.* **68**, 2100–2109.
22. Gant J.C., Sama M.M., Landfield P.W., Thibault O. 2006. Early and simultaneous emergence of multiple hippocampal biomarkers of aging is mediated by Ca^{2+}-induced Ca^{2+} release. *J. Neurosci.* **26**, 3482–3490.
23. Lancaster B., Hu H., Ramakers G.M., Storm J.F. 2001. Interaction between synaptic excitation and slow afterhyperpolarization current in rat hippocampal pyramidal cells. *J. Physiol.* **536**, 809–823.
24. Andrade R., Foehring R.C., Tzingounis A.V. 2012. The calcium-activated slow AHP: Cutting through the Gordian knot. *Front. Cell Neurosci.* **6**, 47.
25. Voglis G., Tavernarakis N. 2006. The role of synaptic ion channels in synaptic plasticity. *EMBO Rep.* **7**, 1104–1110
26. Tombaugh G.C., Rowe W.B., Rose G.M. 2005. The slow afterhyperpolarization in hippocampal CA1 neurons covaries with spatial learning ability in aged Fisher 344 rats. *J. Neurosci.* **25**, 2609–2616.

27. Luebke J.I., Amatrudo J.M. 2012. Age-related increase of sI(AHP) in prefrontal pyramidal cells of monkeys: Relationship to cognition. *Neurobiol. Aging.* **33**, 1085–1095.

28. Zalk R., Lehnart S.E., Marks A.R. 2007. Modulation of the ryanodine receptor and intracellular calcium. *Annu. Rev. Biochem.* **76**, 367–385.

29. Gant J.C., Chen K.C., Norris C.M., Kadish I., Thibault O., Blalock E.M., Porter N.M., Landfield P.W. 2011. Disrupting function of FK506-binding protein 1b/12.6 induces the Ca^{2+}-dysregulation aging phenotype in hippocampal neurons. *J. Neurosci.* **5**, 1693–1703.

30. Gant J.C., Blalock E.M., Chen K.C., Kadish I., Porter N.M., Norris C.M., Thibault O., Landfield P.W. 2014. FK506-binding protein 1b/12.6: A key to aging-related hippocampal Ca^{2+} dysregulation? *Eur. J. Pharmacol.* **739**, 74–82.

31. Anwer H., Vinit K., Rohini P., Hirokuni A., Qi C. 2013. Sirolimus-FKBP12.6 impairs endothelial barrier function through protein kinase Cα activation and disruption of the p120-vascular endothelial cadherin interaction. *Arteriosclerosis, Thromb. Vasc. Biol.* **10**, 2425–2431.

32. Prevel N., Allenbach Y., Klatzmann D., Salomon B., Benveniste O. 2013. Beneficial role of rapamycin in experimental autoimmune myositis. *PloS One.* **8** (11), e74450.

33. Oshiro N., Yoshino K., Hidayat S., Tokunaga C., Hara K. 2004. Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes Cells.* **4**, 359–366.

34. Gant J.C., Chen K.C., Kadish I., Blalock E.M., Thibault O., Porter N.M., Landfield P.W. 2015. Reversal of aging-related neuronal Ca^{2+} dysregulation and cognitive impairment by delivery of a transgene encoding FK506-binding protein 12.6/1b to the hippocampus. *J. Neurosci.* **35**, 30.

35. Gant J.C., Blalock E.M., Chen K.C., Kadish I., Thibault O., Porter N.M., Landfield P.W. 2017. FK506-binding protein 12.6/1b, a negative regulator of $[Ca^{2+}]$, rescues memory and restores genomic regulation in the hippocampus of aging rats. *J. Neurosci.* **38** (4), 1030–1041.

36. Fedoreyeva L.I., Kireev I.I., Khavinson V.Kh., Vanyushin B.F. 2011. Penetration of short fluorescence-labeled peptides into the nucleus in HeLa cells and in vitro specific interaction of the peptides with deoxyribooligonucleotides and DNA. *Biochemistry* (Moscow). **76** (11), 1210–1219.

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