BIOGERONTOLOGY

Short Peptides Stimulate Serotonin Expression in Cells of Brain Cortex

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 157, No. 1, pp. 89-93, January, 2014
Original article submitted May 15, 2013

Peptides Glu-Asp-Arg and Lys-Glu-Asp stimulate serotonin expression in aging cultures of brain cortex cells. Peptide regulation of 5-tryptophan hydroxylase gene encoding the enzyme involved in serotonin synthesis was demonstrated by the molecular docking method. The CCTGCC nucleotide sequence in 5-tryptophan hydroxylase gene was found to be complementary to these peptides. Hence, Glu-Asp-Arg and Lys-Glu-Asp peptides epigenetically regulate serotonin synthesis in the brain cortex, which indicates their neuro- and geroprotective activities.

Key Words: *tripeptides; molecular simulation; serotonin; cell aging; cerebral cortex*

Signal molecules involved in homeostasis maintenance are the main objects of research in modern biology and pharmacology [10]. Studies of the processes underlying regulation of homeostasis by endo- and exogenous bioactive substances open new approaches to the diagnosis, treatment, and prevention of various diseases, including those associated with aging [12]. Serotonin is one of the most important signal molecules regulating brain functions and affecting lifespan. This neurotransmitter is formed from tryptophan in the reaction catalyzed by 5-tryptophan hydroxylase (TPH) [13]. N-acetylserotonin (NAS) is synthesized from serotonin; NAS is a melatonin precursor with pronounced geroprotective effect on the brain cells, several-fold higher than the effect of melatonin [5]. NAS regulates the cognitive functions of the brain, produces antidepressant and antihypertensive effects, prolongs lifespan, and abolishes the effects of β-amyloid toxins on neurons [12]. Hence, stimulation of serotonin synthesis contributes to prevention of neurodegenerative diseases.

Peptides play an important role in signal regulation of cell development and functioning. Along with the systems of classical polypeptide hormones and cytokines, studied in detail, there are less studied systems of peptide regulators. These are short peptides discovered and studied by Russian scientists [5-7]. However, many problems concerning the mechanism of biological activity of short peptides in the realization of some important functions, primarily the peptidergic regulation of serotonin synthesis in neurons, are little studied.

We analyzed the effects of peptides on serotonin synthesis in aging brain cells.

MATERIALS AND METHODS

The study was carried out on primary dissociated cultures of brain cortex cells from young (3 months)

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Wistar rats. Culture medium contained 15% fetal calf serum, 82.5% DMEM, 1.5% HEPES, L-glutamine, and gentamicin. Primary culture was isolated in Petri dishes treated with gelatin (BioloT). Subsequent culturing was carried out in 50-ml flasks with pre-treated surface (JetBiofil; 25 cm^2; BioloT). The cells were cultured in 5-ml culture medium per flask and in 3 ml culture medium per 3.5-cm Petri dish. Passage 1 was regarded as "young" and passage 14 as "old" cultures in accordance with the recommendation of International Association of Cell Culture Research (USA, San Francisco, 2007). Cell cultures were divided into 3 groups. Saline was added to group 1 (control) cultures, Glu-Asp-Arg peptide was added to group 2, and Lys-Glu-Asp peptide was added to group 3 cultures [1,4,8,11]. The peptides were added in a concentration of 20 ng/ml during each passage. For immunocytochemical study, monoclonal antibodies to serotonin (1:50; Dako) and biotinylated mouse Ig (Novocastra) were used as first and second antibodies, respectively. The reaction was visualized with horseradish peroxidase and diaminobenzidene (EnVision Detection System, Peroxidase/DAB, Rabbit, Mouse). The results of immunocytochemical staining were evaluated morphometrically using Nikon Eclipse E400 microscope with Nikon DXM1200 digital camera and VideoTest Morphology 5.2 software. Five visual fields were analyzed at ×200 in each case. The area of expression was estimated as the proportion of area occupied by immunopositive cells to total area of cells in a visual field and expressed in percent.

Molecular simulation of DNA–peptide complex was carried out using Molecular Operating Environment 2012.10 software (Chemical Computing Group Inc., 1010 Sherbrooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2012). 5'-CCTGCC-3' B-form duplex containing presumable peptide binding site was selected as double-stranded DNA molecule. The optimal mutual orientation of the peptide and DNA molecules during binding and formation of a stable complex was calculated using the molecular docking method. The calculation was made from the area of contact, number of hydrogen bonds, parameters of hydrophobic and electrostatic interactions. Amber 12EHT force field and genetic search algorithm were used, the major groove of DNA molecule was selected as the binding site. The geometry of the DNA–peptide complex was then optimized for each docking solution. The binding energy was estimated as the difference between the potential energies of the DNA–peptide complex and isolated molecules. The entropy contribution was then calculated (12 kcal/mol). The peptide–DNA interactions were evaluated by the energy of the complex, which was estimated as the difference of energies between the DNA–peptide complex and peptide or DNA alone. The interactions were stronger at more negative energy. The TPH gene sequence was taken from GenBank database (No. X52501.1) [3].

The data were statistically processed by Statistica 7.0 software. The differences between the groups were evaluated by the nonparametric Mann–Whitney *U* test. The differences were considered significant at $p<0.05$.

RESULTS

The effects of Glu-Asp-Arg and Lys-Glu-Asp peptides on serotonin synthesis were studied on dissociated cultures of rat brain cortex cells (Table 1, Fig. 1). Virtually no differences in serotonin synthesis were revealed between the control cultures of "young" and "old" cells. Morphological analysis of young cell cultures showed weak staining for serotonin marker, which indicated low level of its synthesis in the cells (Table 1). The intensity of cell staining increased in group 2 under the effect of Glu-Asp-Arg peptide, but staining was more intense in response to Lys-Glu-Asp peptide (Table 1, Fig. 1).

Addition of Glu-Asp-Arg and Lys-Glu-Asp peptides to "young" cell cultures led to significant increase of serotonin synthesis: by 1.9 and 2.4 times, respectively, in comparison with the control group (Fig. 1). Lys-Glu-Asp peptide stimulated also "old" cells and increased serotonin synthesis by 1.2 times in comparison with the corresponding control (Table 1).

The nucleotide sequence, the most likely binding site for the studied peptides, was chosen by the molecular docking method. The sequence was found by the complex energy values. The presumable binding site located in TPH gene promotor region was d(CCTGCC) sequence (Table 2). The energy of peptide Lys-Glu-Asp complex with d(CCTGCC) site was -17.2 kcal/mol, of peptide Glu-Asp-Arg complex with this site -23.0 kcal/mol. Lysine side chain in Lys-Glu-Asp peptide made the main contribution to the energy (Figs. 2 and 3).

The hydrogen bond formed between lysine lateral chain amino group and guanine N7 was -10.3 kcal/

TABLE 1. Peptide Effects on Serotonin Expression Area in Cerebrocortical Cells (*M*±*m*; %)

Cultures	Control cell cultures	Peptide	
		Glu-Asp-Arg	Lys-Glu-Asp
"Young"	1.20±0.05	2.32±0.21*	2.81±0.16*
"Old"	1.11±0.05	1.24±0.11	1.40±0.04*

Note. *$p<0.05$ in comparison with the control cell cultures.

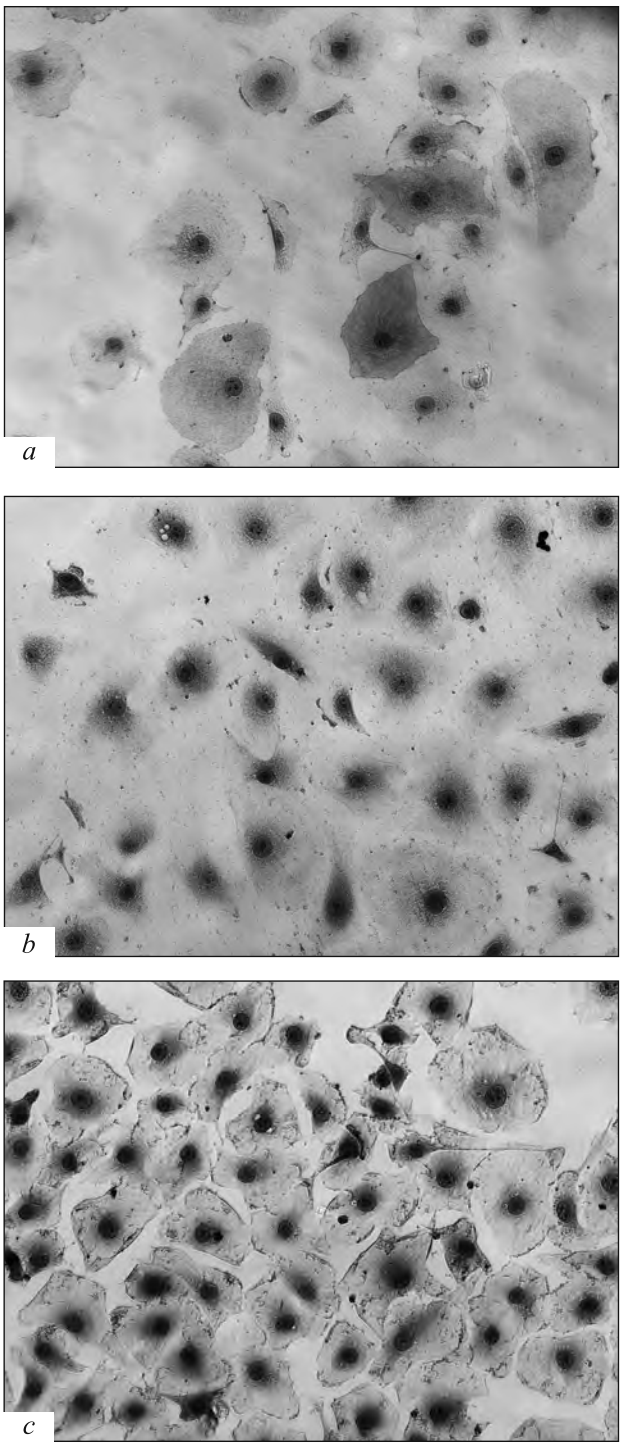

Fig. 1. Serotonin expression in "young" cultures of brain cortex cells, immunocytochemistry with hematoxylin poststaining (×200). *a*) Control; *b*) Lys-Glu-Asp peptide; *c*) Glu-Asp-Arg peptide.

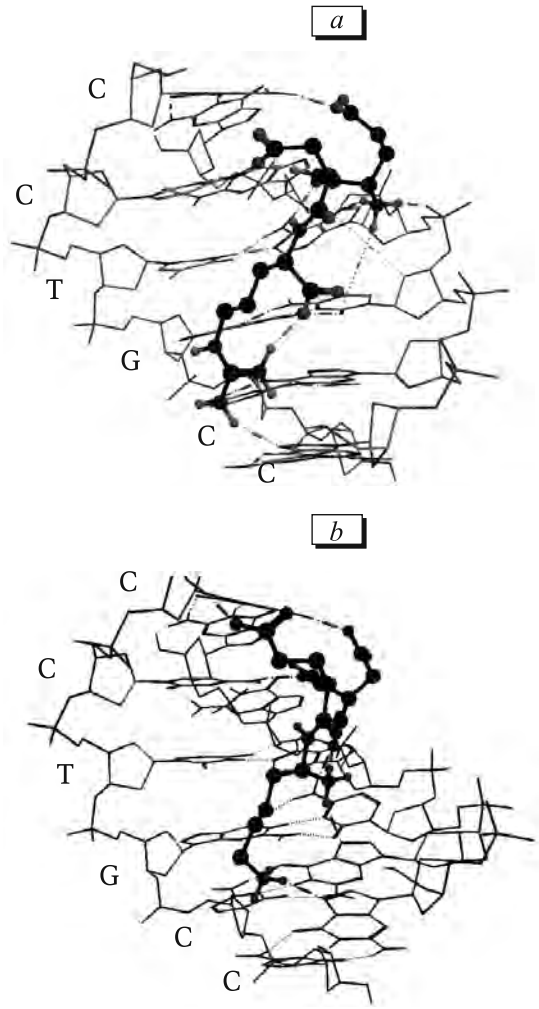

Fig. 2. Schematic presentation of interactions between Glu-Asp-Arg (*a*) and Lys-Glu-Asp (*b*) peptides and d(CCTGCC) site. Peptide molecules shown as balls, cylinders, and rods, DNA molecule is presented by cylinders and rods. Dotted line: hydrogen bonds between atoms.

mol, between arginine amino group and guanine N7 just -3.4 kcal/mol. The Glu-Asp-Arg binding energy was higher than that of Lys-Glu-Asp peptide, which correlated with experimental data (Table 1, Fig. 1). The following regularity was found: the higher the energy of the bond, the more probable the peptide interactions with the selected DNA nucleotide sequence.

Peptides Glu-Asp-Arg and Lys-Glu-Asp stimulated serotonin synthesis in cultures of brain cortex cells, especially in "young" cell cultures, presumably due to higher functional activity reserve of these cells and their higher sensitivity to epigenetic regulation in comparison with the "old" cells. The presumable mechanism of these peptides' action was their penetration in the cell nucleus and binding to the TPH enzyme gene promotor site. The increase of TPH content promoted an increase of serotonin synthesis from tryptophan in cerebrocortical cells, which was observed in response to peptide addition. According to a previous hypothesis, peptides interacted only with the major groove of DNA molecule, because this location offered greater possibilities for binding to the DNA nitrous bases [2,9]. The primary structure of the peptide and DNA

TABLE 2. TPH Gene Promotor Region in *Rattus Norvegicus*

Gene	Gene promoter region, range 120→100 n.p. (cDNA 5'→3')	GenBank, No.
TPH	GCTTCTCCTATAAGAGGCGGCAGCTCCCGTCCGCAGGTGACCCTC TGAACTCCAGTGGCTTTGAGGTCCTCTTTCCAGTGCCGGAT***CCTGCC***CACTG GGTCATCTTCATTCAGATTCACCATGATTGAAGACAACAAGGAGAACAAA GACCATTCCTCAGAAAGGGGGAGAGTGACTCTCATCTTTTCCT TGAAGAATGAAGTTGGAGGACTCATAAAA	X53501.1

Note. Presumable binding site for Glu-Asp-Arg and Lys-Glu-Asp peptides is types by bold underlined italics letters.

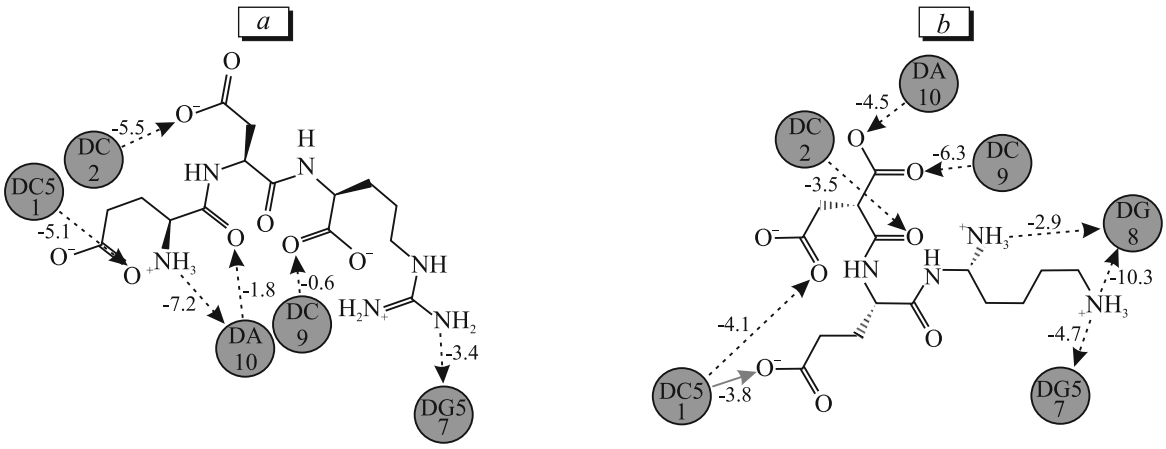

Fig. 3. Map of interactions between Glu-Asp-Arg (*a*) and Lys-Glu-Asp (*b*) peptides and d(CCTGCC) site. Dotted line: direction of proton transition from or to peptide lateral chain atom; continuous line: direction of proton transition from peptide main chain atom or to atom; circles: DNA nucleotides (their ordinal number in the chain is shown). Bond energy (in kcal/mol) specified near hydrogen bonds.

was essential for the interactions with nitrous bases. Protonated amino groups of amino acid residues were more likely to bind to guanine N7, while negatively charged amino acid residues of asparaginic and glutamic acids "preferred" cytosine N4. Hence, by their conformation characteristics Glu-Asp-Arg and Lys-Glu-Asp peptides were located in the major groove of DNA molecule with CCTCGG sequence and formed a hydrogen bond network with it.

Hence, a sequence in TPH gene promotor region served as the target for Glu-Asp-Arg and Lys-Glu-Asp peptides. Binding to this sequence, these peptides stimulated serotonin synthesis in the cerebrocortical neurons, this explaining their neuroprotective and geroprotective effects.

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