

BIOPHYSICS AND BIOCHEMISTRY

Identification of Peptide AEDG in the Polypeptide Complex of the Pineal Gland

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The polypeptide complex of the epiphysis and the peptide AEDG, constructed on the basis of its amino acid analysis, exert similar biological effects. Both bioregulators normalize melatonin synthesis in the pineal gland, functioning of the brain, eye retina, cardiovascular, endocrine, and immune systems; they also act as antioxidants, stress-protectors, and geroprotectors. Within the epiphysis polypeptide complex, free amino acids (3.26%), dipeptides (23.19%), tripeptides (50.72%), tetrapeptides (22.10%), and pentapeptides (0.72%) were revealed by mass spectrometry and HPLC. Peptide AEDG was detected among the tetrapeptides of the epiphysis polypeptide complex by selective reaction monitoring method. The biological effects of the epiphysis polypeptide complex are determined by the effect of its component AEDG.

Key Words: *epiphysis polypeptide complex; peptide AEDG; biological effects*

Pineal gland polypeptide complex (PGPC) is a complex of peptides with molecular weight <10 kDa free from melatonin and other indole admixtures. PGPC participates in the regulation of the neuroimmunoendocrine status of the body through modulation of the hypothalamic—pituitary system: it normalizes pineal melatonin synthesis, circadian rhythms, functioning of the brain, retina, cardiovascular, endocrine, and immune systems; antioxidant, stress-protective, and geroprotective effects of PGPC were demonstrated in on cell cultures, in animal experiments, and in clinical studies in humans of different ages [1-4,6,8].

Analysis of the amino acid composition of PGPC most frequently revealed glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), and glycine (Gly). The tetrapeptide Ala-Glu-Asp-Gly (AEDG) was constructed and synthesized of these amino acids [3,9]. The peptide

AEDG had biological effects similar to those of PGPC, but in lower concentrations [1,4-6,9,10]. Administration of PGPC or AEDG restored melatonin-forming function of the pineal gland in elderly people and in older monkeys [1,2], increased superoxide dismutase (SOD) activity, and decreased in the amount of LPO products, diene conjugates, and ROS [2,4,9]. PGPC and peptide AEDG restored the reproductive function in old rats by increasing blood testosterone concentration in males and restoring estrous cycle in females [6,8]. Based on similar biological effects of these peptide bioregulators, we hypothesized that peptide AEDG is a component of PGPC. At the same time, peptide composition of PGPC has never been analyzed.

The aim of this study was to detect peptide AEDG within PGPC by mass spectrometry and HPLC.

MATERIALS AND METHODS

We used PGPC in the form of a solution containing water for injection and amino acid glycine as inactive ingredients. PGPC concentration was 2.5 mg/ml.

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Peptides and free amino acids in PGPC were detected by mass spectrometry in the frontal dependent tandem mode and directed tandem mode with preliminary precursor ion isolation on a time-of-flight quadrupole high resolution Agilent G6550A Q-TOF LC/MS mass spectrometer (Agilent). PGPC components were separated by HPLC under various elution gradient conditions for directed separation of neutral, polar, or hydrophobic components with a modular UPLC system Agilent Infinity Series 1290 (Agilent) in the microcurrent mode using a diode-matrix detector with variable wavelength (UV detector) and a cell volume of 10 μ l. Short peptides within PGPC were isolated by chromatography-mass spectrometry using the selective reaction monitoring (SRM) method on an Eclipse Plus C-18 RRHD inverted stationary phase chromatography column (2.1 \times 100 mm, particle size 1.8 μ ; Agilent). The chromatographic column had the following characteristics: operating range pH 3-8, maximum pressure 600 bar, total sample analysis time 60 min. Distribution of the isolated and identified peptides was recorded based on the relative hydrophobicity coefficient [13]. PGPC mass spectra were recorded in the mass-to-charge ratio range of (m/z) 420-1200 [7,11,12,15]. To obtain mass spectra, no more than 5 precursor ions were selected during one full scanning cycle in the frontal mode with intensity of no less than 300 units, with relative intensity of precursor ions to the base peak in the spectrum of over 0.05%. Precursor ions were selected by the charge in the following priority order: 2+>3+>1+>(3+/N+), where N is any charge, other than those indicated.

RESULTS

A total of 341 organic components were identified in PGPC. The components contained free amino acids and short peptides. The distribution of amino acids and short peptides in PGPC was as follows: 3.26% free amino acids, 23.19% dipeptides, 50.72% tripeptides, 22.10% tetrapeptides, and 0.72% pentapeptides. Among the revealed short peptides and free amino acids, 87% molecules were with amino acid residue L-isomers and 17% — with modified amino acid residues (amidation, oxidation, and lactamization). The detected modifications were induced and posttranslational.

Frontal chromatography-mass spectrometry did not provide convincing evidence of the presence of peptide AEDG in PGPC, probably because AEDG content in the PGPC was below the sensitivity threshold of quadrupole time-of-flight chromatography-mass spectrometric system. Therefore, to identify peptide AEDG in PGPC we used the highly sensitive chromatography-mass spectrometric system with triple quadrupole mass analyzer and conversion diode detector with electronic multiplier and Skyline software. Primary AEDG-specific transitions were calculated and selected, and following recording conditions were optimized: accelerating potential at the exit from the collision cell, activation energy of singly charged ions, ion accumulation time during the discrete scanning cycle, and the ion source parameters. The number of interfering ions was also minimized using the calculated data, obtained from primary results analysis with

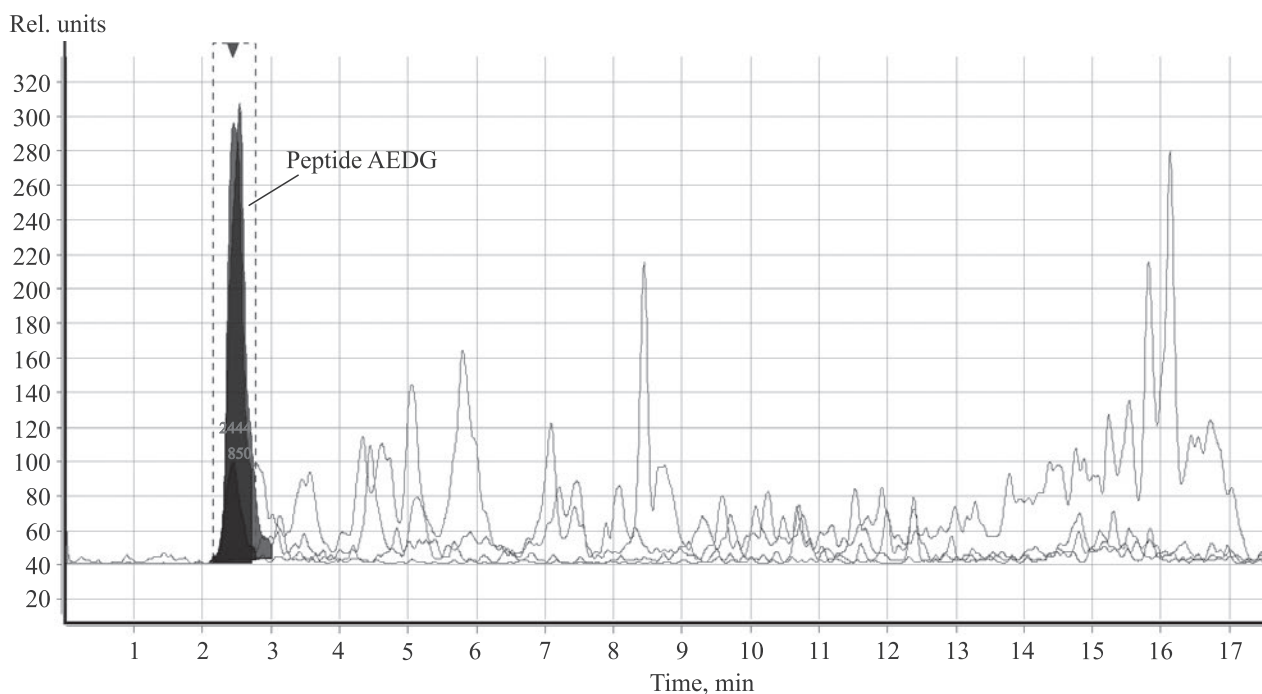


Fig. 1. Chromatogram of SRM signal of the focused ion current of isolated fragment ions of peptide AEDG as a component of PGPC.

srm2prot and SRM Collider. It was possible to record and identify the signal for short peptides (at least 75%, but not more than 90% for fragment ions and no less than 95% for precursor ions). Among them, we obtained a SRM signal (selective reaction monitoring signal) with high homoscedasticity and convergence of fragment ions from the AEDG peptide (Fig. 1).

The data indicate that 22.1% of PGPC composition is presented by tetrapeptides, among which peptide AEDG was revealed. AEDG was present in PGPC in low concentrations required for its physiological action. This was confirmed by the data on AEDG activity manifested at low (nanomolar) concentrations. For instance, in the study of AEDG effect on the synthesis of aryl-alkylamine-N-acetyltransferase (AANAT) enzyme and transcriptional protein pCREB, regulating melatonin synthesis in pinealocytes, AEDG was used in a concentration of 100 ng/ml. At the same time, the control biologically active substance norepinephrine, stimulating AANAT and pCREB synthesis in the pinealocytes, was used in a much higher concentration (1 µg/ml) [5,14].

Thus, within PGPC the peptide AEDG was revealed using high-tech physicochemical methods of analysis; it exerted biological effects of PGPC and is regarded as its active principle.

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