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AEDG Peptide Regulates the Expression of Circadian Genes Clock, Cry2, Csnk1e in Human Immune Cells

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Introduction

The pineal hormone melatonin (MT) is an essential regulator of circadian rhythms. MT activates circadian genes *Bmal, Clock, Per, Cry* due to its effect on membrane or nuclear receptors MT1 and MT2, or due to a direct effect on transcription factors [2, 4, 5]. It was found that MT synthesis is regulated by the polypeptide complex of epiphysis (Epithalamin) [1, 3]. AEDG peptide, which is a part of epithalamin, regulates MT synthesis and possesses a wide range of physiological properties similar to this hormone [1, 3]. This study aims to find out the effect of AEDG on the expression of circadian genes *Clock, Cry2,* and *Csnk1e* in human immune cells.

Sujects and Methods

Blood sampling was performed in middle aged women (40-59 years, n = 75), working mainly on night shifts from 21:00 to 09:00 (doctors and nurses) for a period of 1 year preceding the study. The patients with metabolic excretion level of MT 6-COMT corresponding to the age norm were included in the control group (n = 35). The patients with reduced melatonin-forming function of the pineal gland (this indicator was at the level of elderly people, n = 40) were randomly divided into a placebo group and a group with sublingual administration of AEDG peptide (dietary supplement in a form of a 20 ml bottle, Epitalon ® (Solution-Spray), Nanopep, France). The patients of the placebo group were administered with 0.9% sodium chloride solution sublingually (daily, 2 times a day, in the morning from 8:00 to 10:00s and in the afternoon from 12:00 to 14:00, before meals, 3 sprays under the tongue for 20 days). The patients of the second group were

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administered with AEDG peptide in a similar manner (1 ml of the solution corresponded to a daily intake of the peptide at a dosage of 0.5 mg).

Since the intravital study of human pinealocytes is impossible, the use of immune blood cells appears to be a promising method for assessing the level of circadian genes [1, 2, 3]. Genomic DNA was isolated from leukocytes and whole blood lymphocytes by phenol-chloroform extraction using proteinase K. The expression of *Clock, Cry2* genes in leukocytes, and *Csnk1e* in blood lymphocytes was determined by real-time polymerase chain reaction. The results were processed in the CFX Manager Software. Statistical data processing was performed in "Statistica 7.0." The differences between the groups were evaluated by Student's t-test and were considered statistically significant at $p < 0.05$.

Results and Discussion

This study shows that in patients of the placebo group with reduced melatonin-forming function of the pineal gland, expression of the *Cry2* gene in blood leukocytes was 2.3 times lower ($p < 0.05$) as compared to the control. *Cry2* expression in leukocytes of the patients with reduced melatonin-forming function of the pineal gland before treatment with AEDG peptide corresponded to this indicator in the placebo group. After AEDG peptide administration, the expression of the *Cry2* gene in blood leukocytes was 2 times higher ($p < 0.05$) compared to this parameter before treatment and 1.2 times higher ($p < 0.05$) compared to the placebo group. The mechanism of melatonin synthesis via Cry2 is shown below [6], Figure 1.

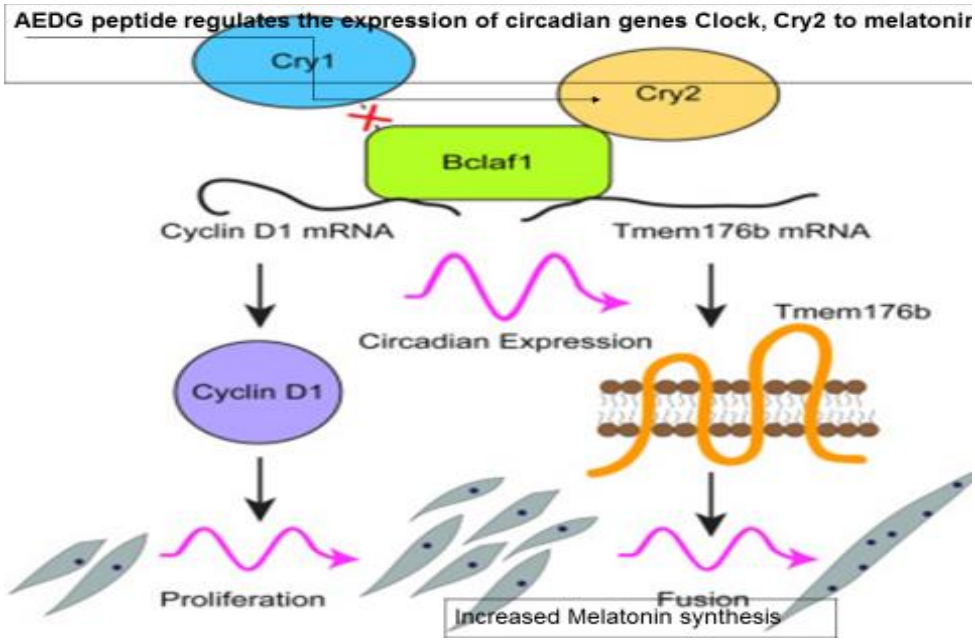

Figure 1. Effects of AEDG peptide on Cry 2 gene expression with increased melatonin synthesis (Adapted from [6]).

An experimental study reported that one of the core regulators of circadian rhythms, Cry2, but not Cry1, is critical for the circadian patterns of these two critical steps in myogenic differentiation [6]. It may be generated via the specific interaction between Cry2 and Bclaf1, which stabilizes mRNAs encoding cyclin D1, a G1/S phase transition regulator, and Tmem176b, a transmembrane regulator for myogenic cell fusion. Myoblasts lacking Cry2 display premature cell cycle exit and form short myotubes because of inefficient cell fusion. Consistently, muscle regeneration is impaired in Cry2−/− mice. Bclaf1 knockdown recapitulated the phenotypes of Cry2 knockdown: early cell cycle exit and inefficient cell fusion, which uncovers a post-transcriptional regulation of myogenic differentiation by circadian rhythms [6].

In the placebo group the expression of the Csnk1e in blood lymphocytes was 3.26 times higher ($p < 0.05$) compared to the corresponding parameter of the control group. The expression of *Csnk1e* in lymphocytes of the patients with reduced melatonin-forming function before the application of AEDG peptide was consistent with the same indicator in the placebo group. After the administration of AEDG peptide, the expression of the *Csnk1e* gene in blood lymphocytes decreased 2.1 times ($p < 0.05$) as compared to the pre-treatment indicator and 1.8 times ($p < 0.05$) as compared to the placebo group.

In brief, it should be assumed that the gero-protective effect of the AEDG peptide is based on its ability to restore melatonin-forming function of the pineal gland through regulation of the expression of circadian genes *Clock*, *Cry2* and *Csnk1e*.

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