Effect of Pineal Tetrapeptide on Antioxidant Defense in *Drosophila melanogaster*

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Effects of synthetic pineal tetrapeptide L-Ala-L-Glu-L-Asp-L-Glu (Epithalon) on specific catalase activity and the content of conjugated hydroperoxides in highly inbred *Drosophila melanogaster* lines differing in reproductive functions were studied. It was shown that Epithalon is a potent modulator of the antioxidant defense, whose biological activity 1000-fold surpasses that of the complex pineal peptide preparation Epithalamin.

Key Words: *antioxidant systems; Epithalamin; Epithalon; peptides*

The free radical theory of aging is most widely accepted in gerontology. According to this theory, free radicals formed in enzymatic metabolic reactions or during respiration damage macromolecules, which leads to genome instability responsible for aging and age-related diseases [11]. It was shown that pineal hormone melatonin is a very potent natural inhibitor of free radical reactions in the body. *In vitro* experiments showed that melatonin by 5-14-fold more effectively inhibits production of hydroxyl radicals than antioxidants glutathione and mannitol [13]. Exogenous melatonin reduced the content of free radicals in mammals and drosophila [3,8,12]. Studies conducted from 1973 to 1999 demonstrated that the pineal peptide preparation Epithalamin prolongs lifespan in animals [6], decelerates aging of the reproductive and immune systems, and suppresses the growth of transplanted tumors and neoplasms developed spontaneously or induced by radiation or various chemical carcinogens [9]. Epithalamin inhibits lipid peroxidation (LPO) and increases total antioxidant activity, activity of Cu,Zn-superoxide dismutase, and the content of ceruloplasmin in rat serum [1,2]. Our previous experiments revealed inhibitory effects of Epithalamin on LPO in drosophila [3].

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Here we compared the effects of Epithalamin and synthetic tetrapeptide L-Ala-L-Glu-L-Asp-L-Glu (Epithalon, St. Petersburg Institute of Biological Regulation and Gerontology) synthesized on the basis of the amino acid sequence of Epithalamin [6] on LPO intensity and catalase activity (CA) in drosophila.

MATERIALS AND METHODS

Experiments were performed on highly inbred lines of *D. melanogaster* selected for low or high sexual activity of males LA^-, HA^-, and LA^+ [5] and maintained for several hundreds generations.

Epithalon was added into the nutrient medium in a concentration of 0.00001 wt% and, therefore, affected the second and third larval stages (L2-L3). The exposure to Epithalon did not exceed 2 days. The concentration of Epithalamin in the medium 1000-fold surpassed that of Epithalon. In the control series, physiological saline was added into the medium. LPO products in 14-day-old flies were extracted with heptane-isopropanol (1:1) mixture; hydroxytoluene was added as the antioxidant. The intensity of LPO was evaluated from the content of conjugated hydroperoxides (CHP) by measuring optical density of the extract on a SPG-55 spectrophotometer at 233 nm [7]. The obtained values were calculated per weight of flies in the sample. CA in tissue homogenates was measured spectrophotometrically [10]. Each analysis included 3-5

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TABLE 1. Content of CHP and CA in Homogenates of Flies under Various Experimental Conditions (*M*±*m*)

Line		Specific CA, μmol H_2O_2/mg protein/min		CHP, nmol/g tissue	
		females	males	females	males
LA^-	control	43.31±1.09	49.27±3.91	1.47±0.01	1.25±0.02
	Epithalon	42.57±1.83	57.44±2.89***	1.29±0.01*	1.20±0.05
	Epithalamin	46.08±1.22	57.95±0.87***	1.34±0.03***	1.23±0.01
LA^+	control	82.18±1.62	101.05±4.75	2.04±0.40	1.31±0.06
	Epithalon	98.97±5.78***	120.75±12.55	1.02±0.01*	1.04±0.02**
	Epithalamin	93.8±2.3**	99.25±2.75	1.21±0.04*	0.97±0.05**
HA^-	control	83.65±1.36	102.10±2.07	2.08±0.03	1.66±0.02
	Epithalon	82.78±2.96	100.39±3.81	1.74±0.03*	1.60±0.01
	Epithalamin	N. d.	N. d.	1.86±0.06***	1.61±0.06

Note. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to the control. N. d.: not determined.

repeated measurements on a group of 40-50 flies. The results were analyzed by one-way ANOVA.

RESULTS

Epithalon added to the nutrient medium elevated specific CA in LA^- males and LA^+ females, and decreased the content of CHP in females of all lines and LA^+ males (Table 1).

Comparative analysis revealed that Epithalon is a more potent antioxidant than Epithalamin. Epithalon reduced the content of CHP in LA^-, LA^+, and HA^- females by 1.2, 2.0, and 1.2 times, respectively, compared with the control. Epithalamin decreased these parameters by 1.1, 1.7, and 1.1 times, respectively. Epithalon and Epithalamin elevated CA in LA^+ females by 1.2 and 1.1 times, respectively, and in LA^- males by 1.2 times. It should be emphasized that the dose of Epithalamin 1000-fold surpassed that of Epithalon.

It was assumed that various tissues contain regulatory peptides (RP) transferring specific information encoded by amino acid sequence from one cell to another [4]. Some RP restored homeostasis in those tissues and organs, where they were isolated from [6]. Epithalamin, probably, contains short RP (including tetrapeptide L-Ala-L-Glu-L-Asp-L-Glu) with high biological activity. Taking into account that Epithalon has relatively simple structure and exerts antioxidant effects in extremely low concentrations, it can be suggested that this compound is a signal molecule, which triggers regulatory cascade processes activating the antioxidant system. This is manifested in suppression of LPO and the increase in specific CA.

Taking into account high biological activity of Epithalon, we anticipate that Epithalon-based drug will posses considerable antioxidant activity.

REFERENCES

1. V. N. Anisimov, V. M. Prokopenko, and V. Kh. Khavinson, *Dokl. Ros. Akad. Nauk*, **343**, No. 4. 557-559 (1995).
2. V. N. Anisimov, A. V. Arutyunyan, and V. Kh. Khavinson, *Ibid.*, **352**, No. 6, 831-833 (1997).
3. V. N. Anisimov, S. V. Myl'nikov, T. I. Oparina, and V. Kh. Khavinson, *Ibid.*, **352**, No. 5, 704-707 (1997).
4. I. P. Ashmarin, *Vopr. Med. Khimii*, **30**, No. 3, 2-7 (1984).
5. L. Z. Kaidanov, S. V. Myl'nikov, O. V. Iovleva, *et al.*, *Genetika*, **30**, No. 8, 1085-1096 (1994).
6. V. G. Morozov and V. Kh. Khavinson, *Usp. Sovr. Biol.*, **96**, Vyp. 3, No. 6, 339-352 (1983).
7. I. D. Stal'naya, *Current Biochemical Methods* [in Russian], Ed. V. N. Orekhovich, Moscow (1977), pp. 63-66.
8. G. K. Akbulut, B. Gonu, and H. Akbulut, *Gerontology*, **45**, No. 2, 67-71 (1999).
9. V. N. Anisimov, V. Kh. Khavinson, and V. G. Morozov, *Ann. N. Y. Acad. Sci.*, **719**, 483-493 (1994).
10. G. Cohen, D. Dembiec, and T. Marcus, *Anal. Biochem.*, **34**, 30-38 (1970).
11. D. Harman, *Proc. Natl. Acad. Sci. USA*, **78**, 7124-7128 (1981).
12. V. Rapozzi, M. Comelli, I. Mavelli, *et al.*, *In Vivo*, **13**, No. 1, 45-50 (1999).
13. R. J. Reiter, D. Melchiorri, and E. Sewerynek, *J. Pineal Res.*, **18**, 1-11 (1995).