BIOGERONTOLOGY

Effects of Peptides on Generation of Reactive Oxygen Species in Subcellular Fractions of *Drosophila Melanogaster*

V. Kh. Khavinson, S. V. Myl'nikov*, T. I. Oparina, and A. V. Arutyunyan****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 132, No. 7, pp. 84-87, July, 2001
Original article submitted January 31, 2001

We studied the effects of Epithalon (Ala-Glu-Asp-Gly) and Vilon (Lys-Glu) on free radical processes in highly inbred HA^+ line of *Drosophila melanogaster*. Vilon inhibited generation of reactive oxygen species in mitochondria, but stimulated this process in the cytosol. We found sex- and age-related differences in the generation of reactive oxygen species and cytosol antioxidant activity.

Key Words: *reactive oxygen species; mitochondria; peptides*

The search for efficient geroprotectors is an important problem of gerontology. This search is based Harman free radical theory [10], which postulates that geroprotectors can be found among natural and synthetic antioxidants. We previously studied antioxidant and geroprotective effects of peptides synthesized at the St. Petersburg Institute of Bioregulation and Gerontology. Epithalon (Ala-Glu-Asp-Gly) added to nutrient medium activates antioxidant enzymes in *Drosophila melanogaster* [5]. This effect can be explained by intensive generation of reactive oxygen species (ROS) in mitochondria. Under these conditions activation of antioxidant enzymes is an inducible compensatory reaction. Here we studied the effects of Epithalon and dipeptide Vilon (Lys-Glu) on generation of ROS in the cytosol and mitochondria and total antiradical activity of the cytosol in *Drosophila melanogaster*.

St. Petersburg Institute of Bioregulation and Gerontology, Northwestern Division of the Russian Academy of Medical Sciences; *Department of Genetics and Selection, St. Petersburg State University; **D. O. Ott Institute of Obstetrics and Gynecology, Russian Academy of Medical Sciences, St. Petersburg. ***Address for correspondence:*** vvm@medport.ru. Khavinson V. Kh.

MATERIALS AND METHODS

Experiments were performed on highly inbred strain *D. melanogaster* HA^+ selected for high sexual activity of males [3] and maintained for several hundreds generations and selections.

Epithalon and Vilon dissolved in physiological saline were added into the nutrient medium in a concentration of 0.00001 wt%. These substances affected the second and third larval stages; the total exposure did not exceed 2 days. In the control, physiological saline was added to the medium. Imagoes were maintained in a medium containing 100 g yeasts, 10 g agar, 30 g sugar, 30 g plum, and 30 g farina per 1 liter water. The medium was changed every other day. For isolation of subcellular fractions, 50 mg flies aging 10 (young) and 25 days (adult) were homogenized in 0.25 M sucrose in 10 mM Tris-HCl buffer (pH 7.4). The mitochondrial fraction was isolated routinely [14]. The homogenate was centrifuged at 500*g* for 3 min to precipitate the nuclear fraction. Mitochondria were precipitated by centrifugation at 7000*g* for 10 min. The supernatant containing microsomes and soluble

0007-4888/01/1321-0682$25.00

cell fractions was considered as the cytosol. Subcellular fractions were frozen and defrosted to obtain the mitochondrial matrix. The mitochondria were suspended in 0.5 ml K^+-phosphate buffer containing 60 mM KH_2PO_4 and 105 mM KCl (pH 7.45, protein concentration 0.6-1.0 mg/ml). The purity of the mitochondrial fraction was evaluated by succinate dehydrogenase activity. In our experiments succinate dehydrogenase activity in mitochondrial fraction 200-500-fold surpassed that in the cytosol.

Generation of ROS in the mitochondrial fraction was measured by luminol-dependent chemilu-

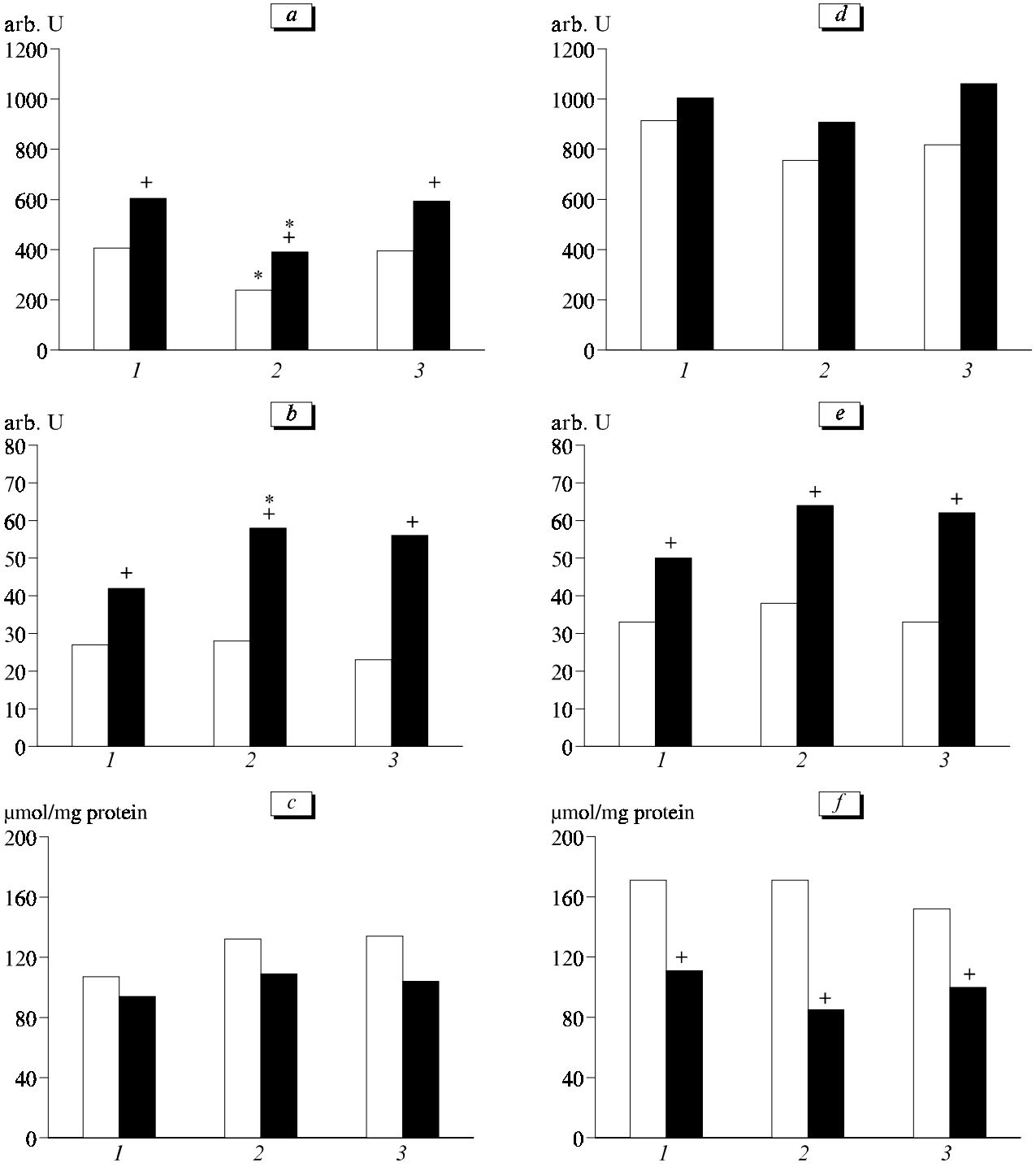

Fig. 1. Concentration of reactive oxygen species in mitochondria (*a*, *d*) and cytosol (*b*, *e*) and antioxidant activity (*c*, *f*) in subcellular fractions in female (*a*-*c*) and male (*d*-*f*). *Drosophila melanogaster* HA^+. Light bars: young flies; dark bars: adult flies. Control (*1*), Vilon (*2*), and Epithalon (*3*). $p<0.05$: *compared to the control; $^+$compared to young flies.

minescence. This method is widely used for evaluation of production of ROS and other free radicals [2,9]. Chemiluminescence was measured in a medium containing 0.7 ml K^+-phosphate buffer (pH 7.45), 0.05 ml 0.1 M luminol, and 0.05 ml mitochondrial fraction (dilution 1:4) on an Emilite-1105 chemiluminometer at 37°C for 2 min. The reaction was initiated by adding 0.2 ml 2% H_2O_2 (E_{230} 3.50) to the reaction mixture. Antiradical activity of the cytosol was measured using stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). DPPH dissolved in ethanol (E_{230} 0.70-0.75) was added to 1.5 ml protein-free extract from the cytosol fraction obtained after precipitation of cytosol proteins with phosphotungstic acid. After a 10-min incubation DPPH was extracted with 4 ml toluene. The measurements were performed on a Beckman DU-65 spectrophotometer at 517 nm [1].

Each experiment was repeated 3-4 times. The results were analyzed by dispersion analysis. The data were presented in the logarithmic form to minimize intrasample variations.

RESULTS

All studied parameters demonstrated an the age-related dynamics (Fig. 1). Generation of ROS in the mitochondrial fraction from HA^+ females increased during aging, but did not differ between 10- and 25-day-old males. However, ROS generation in the cytosol in 25-day-old males and females was higher than in 10-day-old flies. Antiradical activity of the cytosol in males decreased during aging, but in females this parameter remained unchanged. These results are consistent with previous data that the mean life-span of HA^+ males and females are 22 and 30 days, respectively. In our experiments the age of females was below, while the age of males was above the mean life-span. Therefore, HA^+ males were characterized by the age-related decrease in antiradical activity.

It should be emphasized that the intensity of ROS generation in mitochondria in males 2-fold surpassed the corresponding parameter in females. Our previous experiments showed that catalase activity in HA^+ males 1.3-fold surpassed that in females [5]. Increased catalase activity in males is probably a compensatory reactions to enhanced ROS generation. Moreover, the observed sex-related differences in ROS generation can be also explained by different activities of enzymes in the mitochondrial electron transport chain. Sex dimorphism in cytochrome *c* oxidase activity in mammalian tissues determined by different regulation of expression of genes encoding components of the electron transport chain by sex hormones was previously reported [13]. The existence of this mechanism in *D. melanogaster* cannot be excluded.

Vilon inhibited ROS generation in mitochondria in 10- and 25-day-old females, but not in males. In the cytosol, Vilon did not change ROS generation in young females but intensified this process in 25-day-old females. Thus, Vilon decreased ROS content in mitochondria in 10- and 25-day-old females by 1.7 and 1.5 times, respectively, but 1.4-fold increased ROS concentration in the cytosol of 25-day-old females.

It should be taken into account that ROS generation in mitochondria is a result of disturbances in the respiratory chain [4]. Experiments were performed on stage II larvae. Metamorphosis (transition from the larval to pupal stage) is accompanied by total lysis of tissues. Organs of imagoes are formed from small imaginal discs. In our experiments, studied parameters changed in adult flies. However, all measurements were performed 15-30 days after the exposure to peptides. Moreover, our previous studies showed that the content of lipid peroxidation products remains low for at least 45 days after treatment with the peptide [6]. It was demonstrated that geroprotectors are efficient in the larval stage of *Drosophila melanogaster*, but produce no effects on imagoes [8,11,12,15]. The mechanisms of this phenomenon remain unclear.

We hypothesize that these effects are associated with genetic and epigenetic mechanisms, which are realized at the transcriptional or posttranslational levels of regulation. In this case, inhibition of ROS generation can decelerate aging.

The hypothesis on long-term changes in the expression of genes encoding mitochondrial membrane proteins by Vilon is very interesting. Epithalon had no effect on the studied parameters, which indicates that geroprotective effects of this Epithalon and Vilon are realized via different mechanisms [7].

It remains unclear whether Vilon produces direct or indirect effects on the mitochondrial membrane. Vilon can be efficient in the therapy of diseases associated with electron transport disturbances in mitochondria, including Parkinson's disease and Huntington's chorea.

REFERENCES

1. A. V. Arutyunyan, E. E. Dubinina, and N. N. Zybina, *Methods for Studies of Free Radical Oxidation and Antioxidant Body's Protection. Methodical Recommendations* [in Russian], St. Petersburg (2000).
2. N. V. Gulyaeva, M. Yu. Stepanichev, I. A. Lazareva, and M. V. Onufriev, *Neirokhimiya*, **12**, No. 1, 37-41 (1995).
3. L. Z. Kaidanov, S. V. Myl'nikov, O. V. Iovleva, *et al*., *Genetika*, **30**, No. 8, 1085-1096 (1994).
4. V. K. Kol'tover, *Dokl. Akad. Nauk SSSR*, **256**, No. 1, 199-202 (1981).
5. V. Kh. Khavinson and S. V. Myl'nikov, *Byull. Eksp. Biol. Med*., **129**, No. 4, 420-422 (2000).

6. V. Kh. Khavinson and S. V. Myl'nikov, *Ibid*., **130**, No. 11, 585-588 (2000).
7. V. Kh. Khavinson and S. V. Myl'nikov, *Dokl. Ros. Akad. Nauk*, **373**, No. 5, 707-709 (2000).
8. V. N. Anisimov, S. V. Myl'nikov, T. I. Oparina, and V. Kh. Khavinson, *Mech. Ageing Dev*., **97**, 81-91 (1997).
9. W. H. Betts, *Handbook of Methods for Oxygen Radical Research*, Ed. R. A. Greenwald, Boca Raton (1985), pp. 197-201.
10. D. Harman, *Proc. Natl. Acad. Sci. USA*, **78**, 7124-7128 (1981).
11. D. M. Izmaylov and L. K. Obukhova, *Mech. Ageing Dev*., **91**, 155-164 (1996).
12. D. M. Izmaylov and L. K. Obukhova, *Ibid*., **106**, 233-240 (1999).
13. F. Migdadi, S. Gollant, and A. C. Brownie, *Endocrinol. Res*., **21**, 109-114 (1995).
14. R. J. Mockett, W. C. Orr, and J. J. Rahmandar, *Arch. Biochem. Biophys*., **371**, No. 2, 260-269 (1999).
15. L. K. Obukhova, N. Sh. Nakaidze, A. M. Serebryany, *et al*., *Exp. Gerontol*., **14**, 335-342 (1979).