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Effect of melatonin and pineal peptide preparation epithalamin on life span and free radical oxidation in *Drosophila melanogaster*

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Abstract

It was shown previously that epithalamin delays age-related changes in reproductive and immune systems and increases the life span of mice and rats. These effects could be mediated by stimulating influences of epithalamin on synthesis and secretion of melatonin and on free radical processes. A comparative study on the effect of epithalamin and melatonin on both the life span of *Drosophila melanogaster* (strain HEM) and on the intensity of lipid peroxidation and activity of antioxidative enzymes in their tissues was the main aim of this work. Melatonin and epithalamin was added to the nutrition medium (100 µg/ml) during 2–3rd age of larvas. For survival analysis the flies were passed (five couples per vessel) each 3–7 days. Lipid peroxidation was evaluated as the level of ketodienes (KD) and conjugated hydroperoxides (CHP) in fly tissues at the age of 11 days. Activity of Cu, Zn-superoxide dismutase (SOD) and catalase was evaluated as well. The mean, median and maximum life span (MLS) were estimated. Mortality rate (MR) was calculated as α in the Gompertz equation ($R = R_0 \cdot (\exp \alpha t)$) and mortality rate doubling time (MRDT) as $\ln 2/\alpha$. These parameters in groups of male and female flies exposed to melatonin and in male flies exposed

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to epithalamin were no different from the parameters for controls. However, exposure to epithalamin was followed in females by a significant increase in mean life span (by 17%, $P < 0.02$), of median (by 26%), of MLS by 14% and by a 2.12 times decrease of MR ($P < 0.01$) and MRDT (by 32%) compared with female controls. The level of CHP and KD in the tissues of male control flies was 40 and 49% less than that in females and indirectly correlates with male life span. Exposure to melatonin was followed by a decrease in the level of CHP and KD in females and the deletion of sex differences in them. Exposure to epithalamin significantly decreased the level of CHP and KD in female flies compared to controls (2.3 and 3.4 times, respectively, $P < 0.001$). Exposure to melatonin failed to influence the activity of catalase in males but increased it in females by 24% ($P < 0.02$) and failed to influence SOD activity both in males and females. Exposure to epithalamin was followed by a significant increase in activity of catalase, 20% in males and 7% in females and by an increase in SOD activity in males (41%). Thus, it was shown that exposure to epithalamin significantly increases the mean life span and MLS of female *D.melanogaster* and slowed down their aging rate by 2.12 times. This effect is in good agreement with the inhibiting effect of epithalamin in lipid peroxidation processes in fly tissues. © 1997 Elsevier Science Ireland Ltd.

Keywords: Melatonin; Epithalamin; Life span; Free radical oxidation; *Drosophila melanogaster*

1. Introduction

There is significant evidence to support the proposition that oxidative damage may play a significant role in aging [1–3]. According to the free radical theory of aging some active molecules of oxygen, superoxide (O_2^-), H_2O_2 , hydroxyl radical (HO^\bullet) and possibly singlet oxygen (1O_2) damage cellular macromolecules that lead to mutations, genome instability followed by aging and age-related pathology, including atherosclerosis, immunodepression, brain disfunction, cataracts, cancer and others [1–3]. Some natural endogenous factors (Cu, Zn-superoxide dismutase (SOD), glutathione peroxidase, catalase, coeruloplasmin, β -carotene, α -tocopherol (vitamin E), ascorbic and uric acids) defend macromolecules from oxidative damage [1,2]. Exposure to some natural or synthetic antioxidants increases the life span of laboratory rodents and flies [1,4,5]. It was shown that transgenic flies which overexpressed both catalase and SOD have greater mean and maximum longevity and slowing of aging [6].

In recent years it has been reported that the pineal indole hormone melatonin is a highly potent hydroxyl radical and peroxy radical scavenger both in vitro and in vivo [7–10]. Like some other antioxidants, melatonin increased the life span of mice and rats [11,12], however some serious criticism of the results emerged [13–15].

The pineal peptide preparation epithalamin [16,17] has been shown effectively to increase the life span of mice and rats, slow down the aging of their reproductive and immune systems [16–20] and revealed significant antioxidative potential [21–

23]. Because exposure to epithalamin increased pineal synthesis and secretion of melatonin [17,20] it was suggested that some effects of epithalamin are mediated by this indole hormone.

It is worthy of note that melatonin is present in mammals as well as in many invertebrate taxa and insects [24,25]. The comparative study on effects of melatonin and epithalamin on life span, lipid peroxidation and activity of antioxidative enzymes, SOD and catalase, was the main aim of the present work.

2. Materials and methods

2.1. Animals

The HEM strain of *Drosophila melanogaster*, selected for a high rate of embryonal mortality from a wild population Lerick and then passed through about 300 generations of strict inbreeding [26], was used in the experiments.

2.2. Chemicals

Melatonin was from Sigma, stored at -4°C and officinal pineal peptide preparation epithalamin [16,17] was from the St. Petersburg Plant of Medical Preparations.

2.3. Experiment

Melatonin and epithalamin were dissolved 'ex tempore' in 0.01% ethanol and were added to nutrient medium at a concentration $100\ \mu\text{g/ml}$, being fed on by larvae of 2–3rd stages that most effectively resulted in life span modification in the adult flies [27]. Flies were kept, five couples per vessel, passing every 3–7 days. Control groups of larvae were exposed to the solvent.

2.4. Biochemical study

Some of the adult flies from each group was sacrificed at the age of 11 days. Products of lipid peroxidation were extracted with heptan–isopropanol (1:1, v:v) containing 0.1% butylated hydroxytoluene as antioxidant. Intensity of lipid peroxidation was estimated at an optical density of wave length 274 nm (ketodiene, KD) and 232 nm (conjugated hydroperoxides, CHP) [28] with a Beckman spectrophotometer DU-65. Obtained values were related to the flies weight at sampling. There were 3–5 samples from each group containing 100 flies per sample. Activity of catalase (EC 1.11.1.6) and SOD (EC 1.15.1.1) was evaluated in flies according to [29]. There were 4–7 samples from each group containing 40–50 flies per sample.

Table 1
Parameters of life span in *D.melanogaster* exposed to melatonin or to epithalamin

Exposure group	Sex	No. of flies	Life span (days)		Mortality rate ($\alpha \times 10^2$, days $^{-1}$)		MRDT (ln 2/ α)
			Mean	Median	Maximum		
Controls	Females	199	24.7 ± 1.21	23	80	7.0 ± 1.29	3.7
	Males	189	28.3 ± 1.24*	23	82	6.5 ± 1.29	4.3
Melatonin	Females	206	23.7 ± 1.19	23	75	6.2 ± 1.56	4.3
	Males	190	26.4 ± 1.24	23	75	9.0 ± 1.18	3.9
Epithalamin	Females	207	28.8 ± 1.19**	29	91	3.3 ± 0.33***	4.9
	Males	186	26.0 ± 1.25	23	86	7.2 ± 1.11**	4.2

The difference with corresponding parameter in females is significant: * $P < 0.05$; ** $P < 0.01$.

The difference with sex-matched controls is significant: *** $P < 0.02$.

2.5. Statistics

Two way analysis of variance and regression analysis were used for statistical treatment of the results [30]. The mortality rate was evaluated as a in Gompertz equation $R = R_0 (\exp \alpha t)$ [31] and as a mortality rate doubling time (MRDT) $\ln 2/\alpha$. Differences in the parameters of life span and biochemical parameters were evaluated with Mann-Whitney and Student's t criteria [30].

3. Results

3.1. Effect of melatonin and epithalamin on the life span of *D. melanogaster*

Mean life span of control male flies was 14.6% shorter than that of control females ($P < 0.05$) whereas the sex difference in both median and aging rate calculates as α in Gompertz equation were statistically insignificant (Tables 1 and 2). None from these parameters were statistically different from those for exposure to melatonin flies. Melatonin treatment abolished sex differences in the mean life span of the flies.

Exposure to epithalamin failed to change significantly any life span parameter in male flies, however in females it significantly increased the mean life span (17%, $P < 0.02$), median (26%) and maximum longevity (14%), and decreased both the mortality rate of the population (2.12 times, $P < 0.01$) and MRDT (32%) as compared with controls (Tables 1 and 3). The survival curve was shifted to the right and the slope of the Gompertz plot decreased only in female flies exposed to epithalamin (Fig. 1).

Table 2
Effect of melatonin and epithalamin on sex ratio (females:males) of parameters of life span and free radical oxidation in *D. melanogaster*

Parameters	Controls	Melatonin	Epithalamin
Life span			
Mean	0.87*	0.90	1.11
Mediana	1.00	1.00	1.26
Maximum	0.98	1.00	1.13
Mortality rate (α)	1.08	0.69	0.46*
MRDT ($\ln 2/\alpha$)	0.86	1.10	1.17
Conjugated hydroperoxides	1.67*	1.08	1.37
Ketodiene	1.97*	1.18	1.10
SOD	1.01	1.04	0.78
Catalase	0.46*	0.59*	0.52*

* The sex difference was estimated as significant, $P < 0.05$.

Table 3

Comparative effect of melatonin and epithalamin on parameters of life span and free radical oxidation in *D.melanogaster*

Parameters	Change (%) to control value			
	Melatonin		Epithalamin	
	Female	Male	Female	Male
Life span				
Mean	−4.0	−6.7	+16.6**	−8.1
Mediana	0	0	+26.1	0
Maximum	6.3	−8.5	+13.8	+4.9
Mortality rate (α)	−11.4	+38.5	−52.9**	+10.8
MRDT (ln 2/ α)	+16.2	−9.3	+32.4	−2.3
Conjugated hydroperoxides	−52.7**	−27.1	−57.4**	−47.9*
Ketodienes	−61.1**	−41.7	−70.7**	−47.9
SOD	−16.1	−18.3	+8.1	+41.2*
Catalase	+24.1**	−1.7	+19.6**	+6.9*

The difference with sex-matched controls is significant, * $P < 0.05$; ** $P < 0.02$.

3.2. Effect of melatonin and epithalamin on lipid peroxidation and antioxidant enzyme activity in *D.melanogaster*

The tissue level of CHP and KD was significantly higher in control females than males (by 40 and 49%, respectively, Table 4), that inversely correlated with the mean life span of the flies. Exposure to melatonin significantly reduced lipid peroxidation in females and abolished sex differences between the groups. Most significantly the content of CHP and KD was decreased in female flies exposed to epithalamin (by 2.3 and 3.4 times, respectively, $P < 0.001$) compared to controls. Sex differences in the content of the products of lipid peroxidation were diminished as well.

The activity of catalase was 2-fold higher in control males than that in control females, whereas the activity of SOD was the same in both sexes. Exposure to melatonin failed to influence the activity of catalase in males but increased it in females (by 24%, $P < 0.02$) and failed to influence the activity of SOD both in males and females. Exposure to epithalamin was followed by a significant increase in the activity of catalase by 20% in males and by 7% in females and by the increase in SOD activity in males (41%).

4. Discussion

Our results have shown significant sex differences in some parameters studied in control flies. The mean life span of the HEM strain of *D. melanogaster* was inversely correlated with levels of conjugated hydroperoxides and ketodienes that is

in agreement with data published earlier [32]. Contrary, the mean life span directly correlated with activity of catalase. These findings are in a good agreement with the postulates of the free radical theory of aging [1–3,33].

The exposure to epithalamin significantly increased in the mean and maximum life span of female *D. melanogaster* and slowed the aging rate of the population by more than 2. These effects are in a good agreement with the strong inhibiting effect

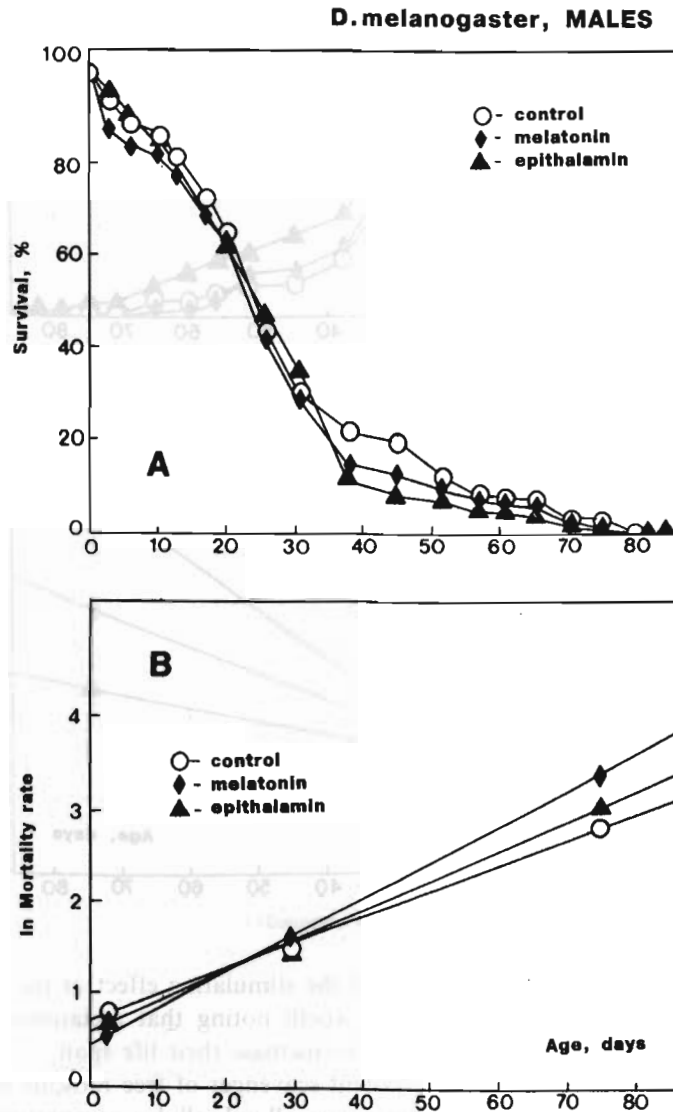


Fig. 1. Survivorship curves and mortality rates of *D. melanogaster* exposed to melatonin or epithalamin. (a) Survivorship curves; (b) mortality rates of flies are graphed on a semilogarithmic scale (Gompertz plots).

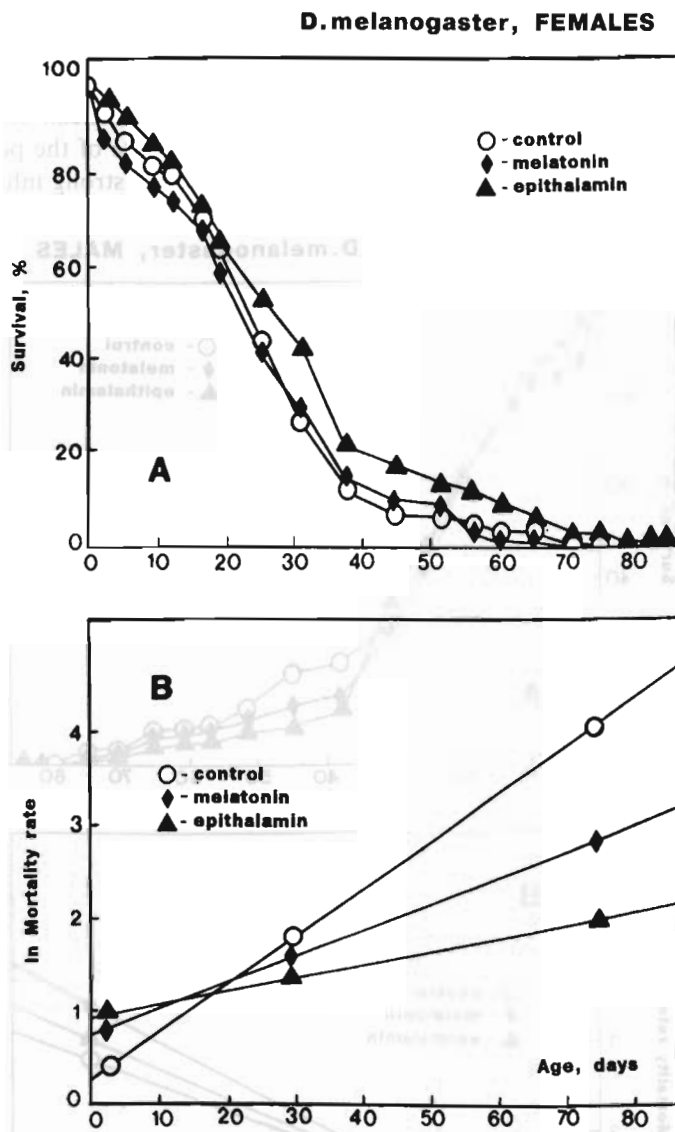


Fig. 1. (Continued)

of epithalamin on lipid peroxidation and the stimulating effect of the drug on the activity of antioxidative enzymes. It is worth noting that melatonin which also inhibits lipid peroxidation in flies failed to increase their life span.

It was shown that melatonin is a potent scavenger of free radicals both in vivo and in vitro [8–10,21–23]. Melatonin enters all subcellular compartments, stimulates one of the potent antioxidant enzymes, glutathione peroxidase, in rat brain and liver [8–10] but, however, failed to influence the activity of serum SOD and

Table 4
Parameters of free radical processes in *D. melanogaster* exposed to melatonin or epithalamin

Parameters	Controls	Melatonin	Epithalamin
Females			
Catalase ^a	41.3 ± 1.46	51.4 ± 1.58**	49.4 ± 1.76**
SOD ^b	135.8 ± 11.24	114.0 ± 12.14	146.8 ± 13.30
Conjugated HP ^c	0.976 ± 0.079	0.462 ± 0.137**	0.416 ± 0.079**
Ketodiene ^c	0.519 ± 0.044	0.181 ± 0.075**	0.152 ± 0.044*
Males			
Catalase ^a	89.3 ± 1.94*	87.8 ± 1.94*	95.5 ± 1.94*, ****
SOD ^b	134.1 ± 13.30	109.6 ± 14.87	189.4 ± 14.87***
Conjugated HP ^c	0.584 ± 0.097*	0.426 ± 0.097	0.304 ± 0.079***
Ketodiene ^c	0.26 ± 0.053*	0.154 ± 0.053	0.138 ± 0.044

^a mM H₂O₂, min.mg of protein;

^b Units/mg of protein;

^c nM/g of tissue.

The difference with corresponding parameter in females is significant, * $P < 0.001$.

The difference with sex-matched controls is significant, ** $P < 0.01$; *** $P < 0.05$.

coeruloplasmin in rats [23]. Long-term treatment with melatonin increases the life span of mice and rats [11,12]. Exposure to a constant light regimen inhibiting melatonin production was followed by a decrease in the survival time of fruit flies [24] and pinealectomy reduced the life span of rats [34]. The pineal peptide drug epithalamin stimulates synthesis of melatonin in the rat pineal gland in vitro and in vivo and increased its secretion in the blood of adult and old rats [17,20]. Like melatonin, epithalamin inhibits lipid peroxidation and increases catalase activity in rats and in flies, but unlike melatonin, epithalamin increased serum activity of SOD and caeruloplasmin in rats and SOD in flies [21–23]. Only overexpression of both SOD and catalase was followed by an increase in life span of transgenic flies, whereas the life span of flies with overexpression of SOD or catalase alone had no difference to controls [6]. It was shown that long-term exposure to epithalamin increased the mean life span and slowed the aging rate of mice and rats and inhibited the development of spontaneous and chemically induced or ionizing radiation carcinogenesis [16,17,19]. The results of our experiments with *D. melanogaster* are in agreement with the above mentioned data and suggest that both melatonin-mediated antioxidative and SOD and other antioxidative enzyme-mediated effects of epithalamin play a significant role in the mechanism of its geroprotective potential.

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