Melatonin as a Geroprotector: Experiments with *Drosophila melanogaster*

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The lifespan of *D. melanogaster* treated with melatonin in a concentration of 0.08% at the stage of development is studied. The geroprotective effect of melatonin is the most obvious (up to 12-18%) in comparison with controls with a short lifespan; if the controls' lifespan was relatively long, the hormone exerts negligible or toxic effect shortening the lifespan by up to 10%. Antioxidant mechanism of geroprotective effect of melatonin is discussed in light of the concept of fluctuations in the lifespan in successive *D. melanogaster* generations.

Key Words: *melatonin; lifespan; geroprotector; Drosophila melanogaster*

Melatonin (N-acetyl-5-methoxytryptamine), first known as a pineal hormone, attracts attention due to its numerous positive effects on biological systems of various complexity [3,13]. The efficacy of melatonin *in vitro* surpasses that of glutathion, mannitol, α-tocopherol, and ascorbic acid [11,14]. Melatonin regulates circadian rhythms in live organisms [13].

The geroprotective activity of melatonin has been little studied in experiments. Melatonin added to daily rations of mice during the whole life increased the mean lifespan (LS) by 25%. However, no geroprotective effect was detected after addition of the drug at the late larval stages of *D. melanogaster* development.

All this necessitates further investigation of geroprotective properties of melatonin. We studied the LS of *D. melanogaster* treated with melatonin.

MATERIALS AND METHODS

Crystalline melatonin (Sigma) was used.

Lipophilic substance dissolved in ethanol was added to ready nutrient medium cooled to 45°C. For control groups, the same amount of ethanol was added into the nutrient medium (final ethanol concentration 0.1%/unit). Preliminary tests showed that this concentration of ethanol did not modify the rate of development and LS of *D. melanogaster,* which is in line with published data [4]. In preliminary experiments melatonin was used in concentrations 0.01-0.15%. The concentration of 0.08% per unit of the nutrient medium was chosen as the optimal.

D. melanogaster, Canton-S wild strain, was used. All experimental procedures and growth conditions have been described in detail [5]. The LS distribution in the control and experimental groups was compared by pairs using the Kolmogorov—Smirnov test. Correlations between LS in the control population and the geroprotector efficacy was analyzed using Spearman's test [15].

RESULTS

The LS in the control groups greatly varied: the extreme values differed by more than 40% in females and by almost 30% in males (Table 1). These results agree with previous reports on the natural fluctuations in LS of successive *D. melanogaster* generations [6,7].

Paired differences in LS between the control and experimental groups varied: we observed a significant geroprotective effect of melatonin prolonging the LS by 10-18% and lack of the effect or even a 10% shortening of LS. For elucidating the relationship between LS in the control and experimental groups, each mean

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TABLE 1. Mean Lifespan of Insects Grown in Control and Melatonin-Containing Medium ($M \pm m$)

Experiment No.	Control	Experiment	Relative effect, %
Males			
1	36.94±10.15	40.71±10.41***	+10.2
2	39.57±10.91	35.61±10.02*	-10.0
3	32.66±17.57	38.71±13.54**	+18.5
4	37.47±13.85	37.15±13.80	0
5	27.21±10.38	30.08±11.54**	+10.5
Females			
1	41.88±11.33	42.86±9.09	+2.3
2	36.76±11.85	38.52±9.89	+4.8
3	34.87±12.56	37.21±11.79	+6.7
4	35.77±11.90	36.67±12.83	+2.5
5	32.73±12.44	36.68±11.43***	+12.1

Note. *$p<0.01$, **$p<0.02$, ***$p<0.05$ *vs.* control (Kolmogorov—Smirnov's test).

LS value was standardized by the mean group value calculated from 5 experiments; this helped us to pool the data for both genders. The two resultant samplings, 10 values in each, were subjected to analysis of correlations. The critical value of the correlation coefficient for samplings of $n=10$ and <1% error probability was 0.79. The ranked correlation coefficient for our data was $r=-0.83$. This indicates a highly significant inverse correlation between LS in the control group and geroprotective effect of melatonin.

The results indicate a geroprotective effect of melatonin for *D. melanogaster* in comparison with the control group with relatively short LS. A relatively long LS in the control group levels the geroprotective effect of melatonin; the LS even decreases because of drug toxicity. Therefore, varying viability of the population from which the control and experimental groups are formed determines the geroprotective effect of melatonin. Previously we revealed a similar relationship for the geroprotector spin radical trap 4-hydroxy-tetramethyl-piperidine-N-oxyl [5].

The geroprotective effect of melatonin at larval stages of the insect development indirectly suggests the antioxidant mechanism of its effect. Antioxidants exert favorable aftereffects by prolonging LS of adult insects, which during the preimaginal stage were developing in a nutrient medium with bioactive substances. A probable mechanism of this phenomenon has been discussed previously [10]. Similar effects are exhibited by 3-hydroxypyridine [10], vitamins A and E [2,9], and 4-hydroxy-tempo [5].

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