BIOGERONTOLOGY

Geroprotective Effect of Ala-Glu-Asp-Gly Peptide in Male Rats Exposed to Different Illumination Regimens

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Exposure of male rats to permanent or natural illumination of North-Western Russia accelerated their death in comparison with animals exposed to standard (12 h) light. Permanent illumination promoted the development of spontaneous tumors in comparison with the standard photoregimen. Injection of epithalone (synthetic Ala-Glu-Asp-Gly peptide; subcutaneously 0.1 μg/rat 5 times a week from the age of 4 months until natural death) virtually did not change the mean lifespan of male rats, but was associated with a significant ($p<0.05$) normalization of population aging rate and hence, time of mortality rate doubling in groups exposed to natural or constant illumination. Epithalone injected to rats exposed to any photoregimen significantly inhibited the development of spontaneous tumors, primarily testicular leydigomas and leukemias.

Key Words: *photoregimen; epithalone peptide; lifespan; spontaneous tumors; male rats*

Exposure of humans to light during the night hours (often called photopollution), leading to suppression of the pineal function and production of melatonin (pineal hormone), disorders the circadian rhythms, homeostasis in general, and stimulates the development of some age-associated diseases [1,7,11-13]. Exposure of laboratory animals (flies, rats, mice) to permanent (24-h) illumination is associated with shortening of the lifespan, and in rodents it is also linked with increased incidence of proliferative processes and spontaneous tumors [1,3,7,13]. On the other hand, treatment with epithalone peptide (Ala-Glu-Asp-Gly), synthesized on the base of epithalamine (pineal polypeptide preparation), stimulates the nocturnal production of melatonin, normalizes the effects on many hormonal and metabolic parameters, prevents early aging and development of tumors in animals [3,5].

Since the function of the pineal gland depends on the photoregimen, it is interesting to investigate the effects of epithalone on the lifespan and development of spontaneous tumors in animals exposed to different photoregimens. Experimental validation of observations indicating that newcomers to high latitudes characterized by long "white nights" period and long polar night during autumn and winter develop signs of rapid aging and high incidence of age-associated diseases [1,6,7] is an important task. However, there are virtually no scientifically based recommendations on prevention of these abnormalities.

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We previously studied the effects of different photoregimens and epithalone on female rats [3]. Here we compared the effects of this peptide on the lifespan and development of spontaneous tumors in male rats exposed to natural photoregimen of North-Western Russia (Petrozavodsk) and to permanent illumination and in animals kept under conditions of standard (12-h) illumination.

MATERIALS AND METHODS

Experiments were carried out on 307 male LIO rats from a vivarium of Petrozavodsk State University, born at the beginning of May. At the age of 25 days the animals were randomly divided into 3 groups. Group 1 rats (LD) were exposed to standard fixed illumination by fluorescent lamps (12:12 h light: darkness; 750 lux illumination at the level of cages). Group 2 animals (NL) were exposed to natural illumination [2]. This photoregimen is determined by the season: the minimum duration of daylight period in winter is 4.5 h, while in summer it reaches 24 h during the white nights. Illumination of the rooms varied during the day: 50-200 lux at the level of cages in the morning hours, up to 1000 lux on a bright day and 500 lux on a gloomy day, and 150-500 lux in the evening. Group 3 rats (LL) were exposed to permanent illumination by fluorescent lamps (750 lux at the level of cages).

The animals were kept in standard plastic cages at 21-23°C on balanced granulated fodder and water *ad libitum*. At the age of 4 months, the rats of each group were divided into 2 subgroups; animals of one subgroup starting from this age and until death were 5 times weekly subcutaneously injected with epithalone (synthesized by E. I. Grigoryev, Cand. Chem. Sci., at St. Petersburg Institute of Bioregulation and Gerontology) in a dose of 0.1 μg/rat dissolved in 0.1 ml NaCl, and the other subgroup (control) were injected with an equivalent volume of the solvent.

Some animals in each group were sacrificed for biochemical analysis; the results were presented previously [2,4]. The remaining rats were observed until their natural death. All rats dead during the experiment were autopsied. The viscera and tissues with suspected neoplastic changes were studied by histological methods. The detected tumors were classified in accordance with IARC recommendations [14].

The results were processed by parametrical and nonparametrical methods of variation statistics using Statgraph, Statistica 5.5, and Stadia software [3]. The kinetic parameters of population aging were calculated using Gompertz' model for survival function:

$$S(x)=\exp\{-\frac{\beta}{\alpha}[\exp(\alpha x)-1]\}$$

where α and β are parameters of population aging rate and initial mortality rate, respectively [10]. Parameter a is often characterized also by the mortality rate doubling time (MRDT), calculated as ln(2)/a. Confidence intervals for aging rate values were calculated using log-like functions [9].

RESULTS

Exposure of male rats to natural or permanent light did not modify the mean lifespan of all rats or of 10% animals with the maximum lifespan (Table 1). On the other hand, survival curves of NL and LL rats were shifted significantly to the left in com-

TABLE 1. Effect of Epithalone on Lifespan of Male Rats Exposed to Different Illumination Regimens

Parameter	LD		NL		LL	
	control	epithalone	control	epithalone	control	epithalone
Number of rats	57	51	50	50	50	49
MLF, days	664.0±34.0	650.0±41.4	613.0±32.9	644.0±37.9	580.0±35.5	631.0±35.7
MaxLF, days	1045	1185	1046	956	1005	1020
MLF for the last 10% rats, days	999.0±11.5	1065.0±34.7	972.0±22.7	1021.0±15.5	983.0±13.8	989.0±10.6
$\alpha\times10^3$, day^{-1}	6.06	6.05	6.70*	6.37	5.19*	6.49
	(5.87; 6.47)	(5.65; 6.46)	(6.50; 6.97)	(6.21; 6.75)	(4.89; 5.57)	(6.17; 6.71)
MRDT, days	112.4	114.6	103.4*	108.8°	133.6*	106.3°
	(107.1; 118.1)	(107.3; 122.7)	(99.4; 106.6)	(102.7; 111.6)	(124.4; 141.7)	(103.3; 112.3)

Note. MLF: mean lifespan; MaxLF: maximum lifespan; α: population aging rate; MRDT: mortality rate doubling time. *$p<0.05$ *vs.* LD group; °$p<0.05$ compared to animals of the same group receiving no epithalone.

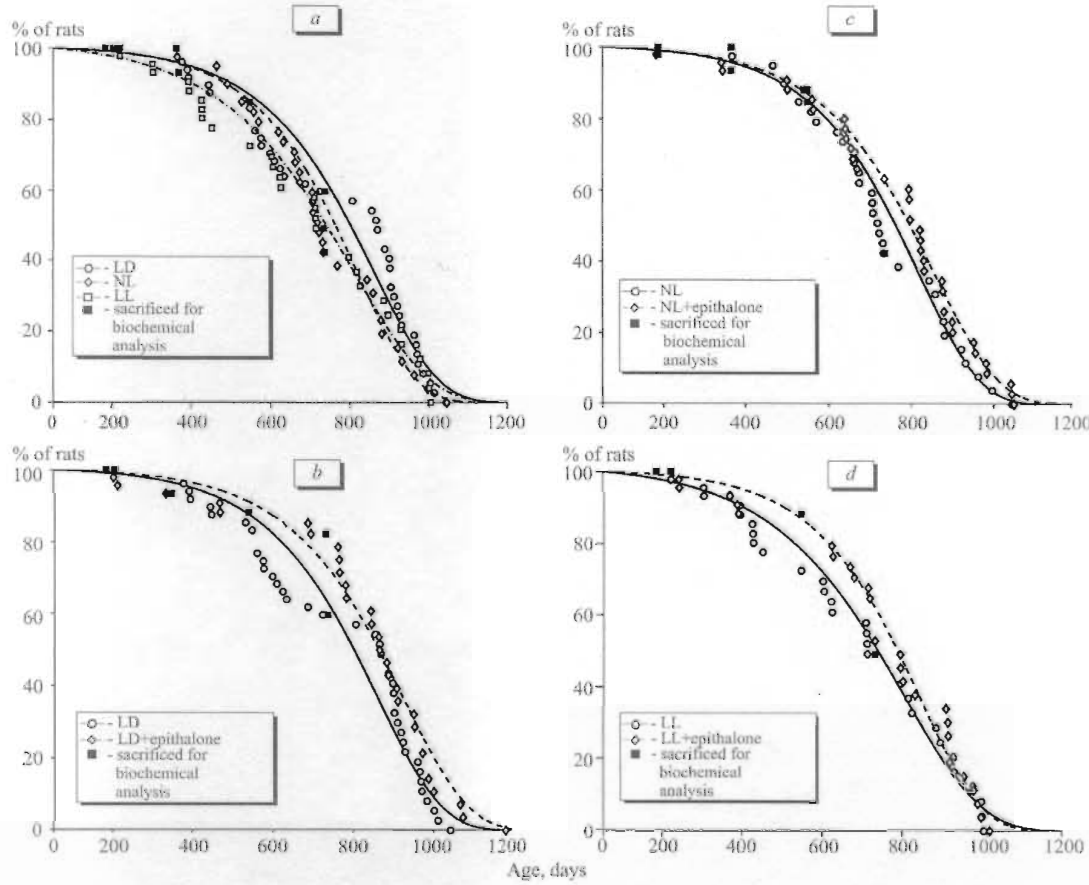

Fig. 1. Effects of illumination mode and epithalone on survival curves of male rats. Here and in Fig. 2: *a*) data for all groups; *b*) for LD group; *c*) for NL group; *d*) for LL group.

parison with the curve the LD animals (Fig. 1, *a*). Differences in the survival curves of LD and NL animals were significant: $p=0.001$; $\chi^2=10.3$ in the log-rank test; between LD and LL groups: $p=0.01$, $\chi^2=6.7$. Multifactorial analysis of dispersions (ANOVA test) revealed a relationship between the lifespan and illumination regimen (15.45%, F=15.32, $p<0.001$). Hence, permanent and natural illumina-

TABLE 2. Effect of Epithalone on the Incidence of Tumors in Male Rats Exposed to Different Illumination Regimens

Parameter	LD		NL		LL	
	control	epithalone	control	epithalone	control	epithalone
Number of rats	57	51	50	50	50	49
Number of rats with tumors	17 (29.8)	5 (9.8)*	11 (22)	4 (8)*	13 (26)	6 (12.2)*
Number of rats with malignant tumors	7 (12.3)	1 (2%)*	6 (12)	0*	10 (26)	2 (4.1%)*
Total number of tumors	23	6	13	4	14	7
Number of tumors per rat with tumors	1.35	1.20	1.27	1.0	1.08	1.17
Day of 1st tumor detection	379	834	367	795	223	739
MLF of rats with tumors, days	824.0±49.0	1008.0±48.4	782.0±57.6	899.0±52.4	688.0±73.2	782.0±36.4

Note. MLF: mean lifespan. The percentage is shown in parentheses. Here and in Table 3: *$p<0.05$, *$p<0.02$ compared to rats kept under conditions of the same light regimen, but receiving no epithalone.

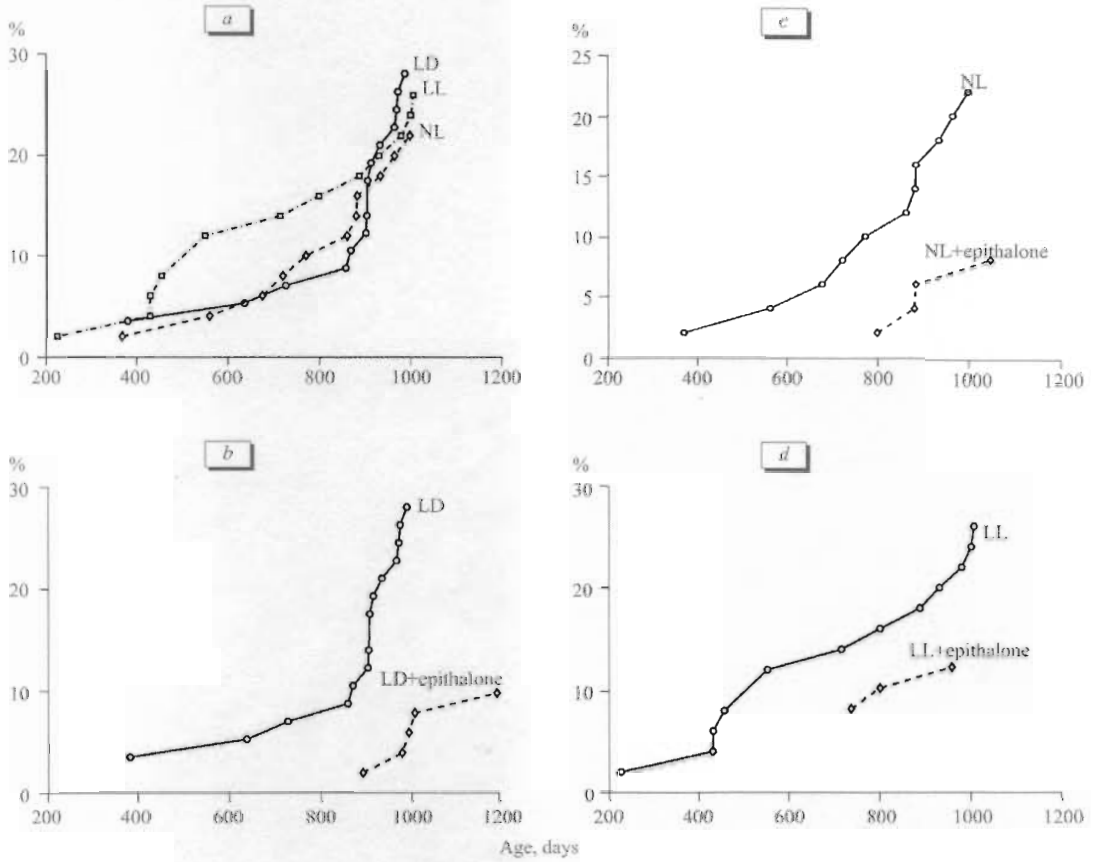

Fig. 2. Effects of illumination mode and epithalone on the dynamics of spontaneous tumor development in male rats.

tion, characteristic of North-Western Russia, accelerated animal death in comparison with the standard illumination mode. This was paralleled by shifts in the population aging rate (a) and MRDT.

Epithalone treatment virtually did not change the mean lifespan of LD animals, but increased their maximum lifespan on day 140 (Table 1). In NL or LL groups epithalone did not modify the maximum lifespan. The treatment was associated with normalization of a constant and hence, of MRDT in NL or LL groups. Analysis of the dynamics of rat mortality showed that survival curves of epithalone-treated animals from groups exposed to all illumination modes were shifted significantly to the right in comparison with the curves for rats receiving no epithalone (Fig. 1, *b-d*). This indicates prolongation of rat lifespan under the effect of the peptide.

Exposure of control animals to LL accelerated the development of spontaneous tumors in comparison with LD (reduced the mean lifespan with tumors by on average 4.5 months), without appreciably modifying tumor incidence (Table 2, Fig. 2, *a*). The first tumor in control LL rats was detected 5 months earlier than in LD group. No appreciable differences in tumor development in control and experimental NL rats were detected.

Epithalone inhibited the development of all tumors and spontaneous malignant tumors significantly at all illumination modes. This is seen from delayed appearance of tumors (Fig. 2, *b-d*) and reduced the incidence of tumors and their lower total number (Table 2). The incidence of testicular leydigomas decreased significantly under the effect of epithalone in the LL and NL groups. Tumors of the hemopoietic system (malignant lymphomas and leukemias) were detected in control subgroups of rats exposed to all illumination modes, but not a single case in animals treated by epithalone (Table 3).

Our results indicate unfavorable effect of permanent illumination on the development of spontaneous tumors in male rats, while treatment with epithalone inhibited tumor development, primarily testicular leydigomas and leukemia, under all illu-

TABLE 3. Effect of Epithalone on Tumor Type and Location in Male Rats Exposed to Different Illumination Regimens

Parameter	LD		NL		LL	
	control	epithalone	control	epithalone	control	epithalone
Testicles						
leydigoma	7	2*	6	1*	4	5
cavernous hemangioma	1	—	—	—	—	—
Malignant lymphoma/leukemia	3	—	4	—	6	—
Liver						
hepatocarcinoma	2	—	—	—	2	—
Skin						
papilloma	1	—	—	—	—	—
Soft tissues						
sarcoma	1	1	2	—	—	1
malignant fibrous histiocytoma	2	—	—	—	—	—
Lung						
adenocarcinoma	—	—	—	—	1	—
clear-cell carcinoma	1		—	—	—	—
Small intestine						
adenocarcinoma	—	—	—	—	1	—
Adrenal						
cortical adenoma	3	2	1	2	—	—
pheochromocytoma	1	—	—	—	—	—
Kidney						
liposarcoma	—	—	—	—	1	—
Ureter						
fibroma	1	—	—	—	—	—
Pituitary						
adenoma	—	1	—	1	—	—
Total						
benign	14	5	7	4	4	5
malignant	9	1*	6	0*	10	2*

mination regimen. Experimental studies showed that permanent illumination promoted the development of tumors of different location and chemical carcinogenesis in mice and rats [1,7,13]. Our previous experiments on female rats showed that permanent and natural illumination under conditions of North-Western Russia accelerated aging and promoted tumor development, while epithalone exhibited a normalizing effect [3]. The results of the present study indicate that treatment with epithalone, a synthetic peptide stimulating nocturnal production of melatonin, characterized by antioxidant, geroprotective, and anticarcinogenic effects [5,8], prevented rapid aging and abolished stimulation of tumor development not only in females, but also in male rats exposed to permanent light.

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