GERONTOLOGY

Effect of Ala-Glu-Asp-Gly Peptide on Life Span and Development of Spontaneous Tumors in Female Rats Exposed to Different Illumination Regimes

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The effects of Ala-Glu-Asp-Gly peptide (Epithalon) on the life span and development of spontaneous tumors were studied in female rats exposed to standard, natural for North-Western Russia, and constant illumination. The mean life span of animals exposed to constant or natural illumination decreased by 13.5 and 25.5%, the maximum by 9 and 7 months, respectively, and spontaneous tumors developed much more rapidly than in animals living under conditions of the standard light regimen. Epithalon (0.1 μg daily 5 times a week from the age of 4 months) did not change the life span of rats living under conditions of standard day/night regimen, while in rats exposed to the natural and constant light it promoted prolongation of the maximum life span by 95 and 24 days, respectively. Epithalon prolonged the mean life span of the last 10% of rats exposed to natural and constant illumination, treated with Epithalon, by 137 and 43 days, respectively. This peptide exhibited virtually no effect on the development of spontaneous tumors in rats exposed to standard and constant illumination, but significantly inhibited their development in rats exposed to natural light.

Key Words: *light regimen; Epithalon peptide; life span; spontaneous tumors; rats*

Numerous studies demonstrate the important role of circadian rhythms and the pineal gland in aging and tumor development. Blocking of the pineal function during exposure to constant light or its dysfunction caused by seasonal fluctuations in illumination accelerates aging, shortens the life span, and stimulates carcinogenesis in female rats [1,7, 13,14]. It is known that exposure of humans to light at night (often called photopollution), leading to suppression of the pineal function and production of the pineal hormone (melatonin), causes disorders in the homeostasis and stimulates some age-associated diseases [1,7,12-14]. Exposure of laboratory animals (fruit flies, rats, mice) to constant (24-h) illumination reduced their life span, in the rodents it resulted in higher incidence of proliferative processes and spontaneous tumors [1,7,14]. Treatment with Epithalon (peptide synthesized on the basis of the analysis of polypeptide preparation from the pineal gland, epithalamine) stimulated nocturnal production of melatonin, normalized many hormonal and metabolic values, prevented early aging,

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and did not stimulate tumor development in animals [5].

Since the function of the pineal gland depends on the photoregimen, evaluation of effects of Epithalon on life span and development of spontaneous tumors in animals exposed to different photoregimens is an important task. The data indicating that newcomers to high latitudes characterized by long periods of "white nights" in spring and summer and long polar night in autumn and winter exhibit signs of rapid aging and higher incidence of age-associated diseases [1,6,7] need experimental validation. However, there are virtually no scientifically-based recommendations on the prevention of these diseases.

We compared the effects of Ala-Glu-Asp-Gly peptide (Epithalon) [5] on the life span and development of spontaneous tumors in female rats exposed to the natural light regimen of North-Western Russia (Petrozavodsk), constant light, and standard illumination (12:12 h light:darkness regimen).

MATERIALS AND METHODS

Experiments were carried out on 292 female LIO rats born at the beginning of May, 2003, in vivarium of Petrozavodsk State University. At the age of 25 days the animals were divided at random into 3 group. Group 1 animals were kept under conditions of fixed standard illumination with fluorescent lamps (12:12 h light:darkness; LD), 750 Lux at the level of cages. Group 2 animals were kept under conditions of natural light (NL) [2]. The illumination in this regimen was determined by the season: in winter, minimum duration of daylight was 4.5 h, in summer 24 h ("white nights"). Illumination of the room changed 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 cloudy day, and 150-500 Lux in the evening. Group 3 rats were kept under condition of constant light (24 h - day and night; LL) with fluorescent lamps (750 Lux at the level of cages).

The animals were kept in standard plastic cages at 21-23°C and received standard balanced granulated fodder and water *ad libitum*. At the age of 4 months, the rats of all groups were divided at random into 2 subgroups. One subgroup received subcutaneous injections of Epithalon (0.1 μg in 0.1 ml saline) from this age and throughout their life (Epithalon was synthesized at St. Petersburg Institute of Bioregulation and Gerontology by E. I. Grigoryev, Cand. Chem. Sci.); another subgroup received an equivalent volume of the solvent.

Some animals in each group were sacrificed for biochemical studies; the results were presented previously [2,4]. The rest rats were observed until natural death. All rats dead during the experiment were autopsied. All viscera and tissues with presumable neoplastic changes were studied by histological methods. The detected tumors were classified in accordance with recommendations of the International Agency for Cancer Research [10,15].

The results were processed using parametric and nonparametric methods of variation statistics, using Statgraph, Statistica 5.5, and STADIA software [3,10]. Kinetic parameters of population aging were calculated using the predictive Gompertz model:

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

where α and β are related to the population aging rate and initial mortality rate, respectively [11]. The α parameter is often characterized by the mortality rate doubling time (MRDT), calculated as $\ln^2/\alpha$. Confidence intervals for aging rate values were calculated using log-like functions [9].

RESULTS

The mean life span of rats kept under conditions of NL and LL decreased by 13.5 and 25.5%, respectively, the maximum life span by 9 and 7 months, respectively, in comparison with rats kept under LD conditions. The population aging of rats kept under NL conditions (coefficient α) increased by 2.1 times in comparison with the control, the MRDT decreased accordingly. Constant illumination had virtually no effect on kinetic parameters of population aging (Table 1).

The survival curves for NL and LL groups were shifted significantly to the left in comparison with survival curve for LD rats (Fig. 1, *a*). The differences between the survival curves were significant for LD and NL groups (p=0.0000243, χ^2=22.2) and LD and LL groups (p=0.0000162, α^2=23.0). Multifactorial analysis of dispersions (ANOVA test) detected a relationship between the life span and light regimens (15.45%, F=15.32, p<0.001).

Epithalon injected to rats virtually did not change the mean life span, but prolonged the maximum life span of LD animals by 68 days (Table 1). Epithalon prolonged the maximum life span of rats kept under NL and LL conditions by 95 and 24 days, respectively, and by 137 and 43 days the mean life spans of 10% rats of these groups in comparison with animals receiving no peptide. The protective effect of Epithalon manifested also by significant inhibition of the population aging rate and increase of MRDT in the NL group. The survival curve of LD rats treated with Epithalon was shifted to the left, while the survival curves of NL

TABLE 1. Effects of Epithalon on Life Span of Female Rats

Parameters	Light regimen (rat group)					
	LD		NL		LL	
	control	epithalon	control	epithalon	control	epithalon
Number of rats	40	55	48	50	54	45
Mean life span, days	706.0±46.2	644.0±29.1	611.0±29.5	619.0±34.3	526.0±30.4	580.0±34.6
Maximum life span, days	1167	1095	897	992	956	980
Mean life span of the last 10% rats, days	1119.0±16.7	973.0±33.4^{+}	830.0±18.9*	967.0±13.5^{+}	909.0±19.1*	952.0±7.1^{+}
$\alpha\times10^3$, day^{-1}	5.00 (4.73-5.30)	6.30^{+} (6.10-6.42)	10.5* (10.3-11.2)	6.73^{+} (6.53-6.94)	5.21 (5.13-5.35)	5.00 (4.33-5.18)
MRDT, days	138.6 (130.8-146.6)	110.0^{+} (108.0-113.6)	65.8* (61.7-67.0)	103.1^{+} (100.0-106.1)	133.1 (129.6-135.1)	138.7 (133.9-160.2)

Note. *$p<0.95$ compared to the control (LD); $^{+}p<0.05$ compared to the rats kept under conditions of the same light regimen without Epithalon (95% confidence intervals are shown in parentheses).

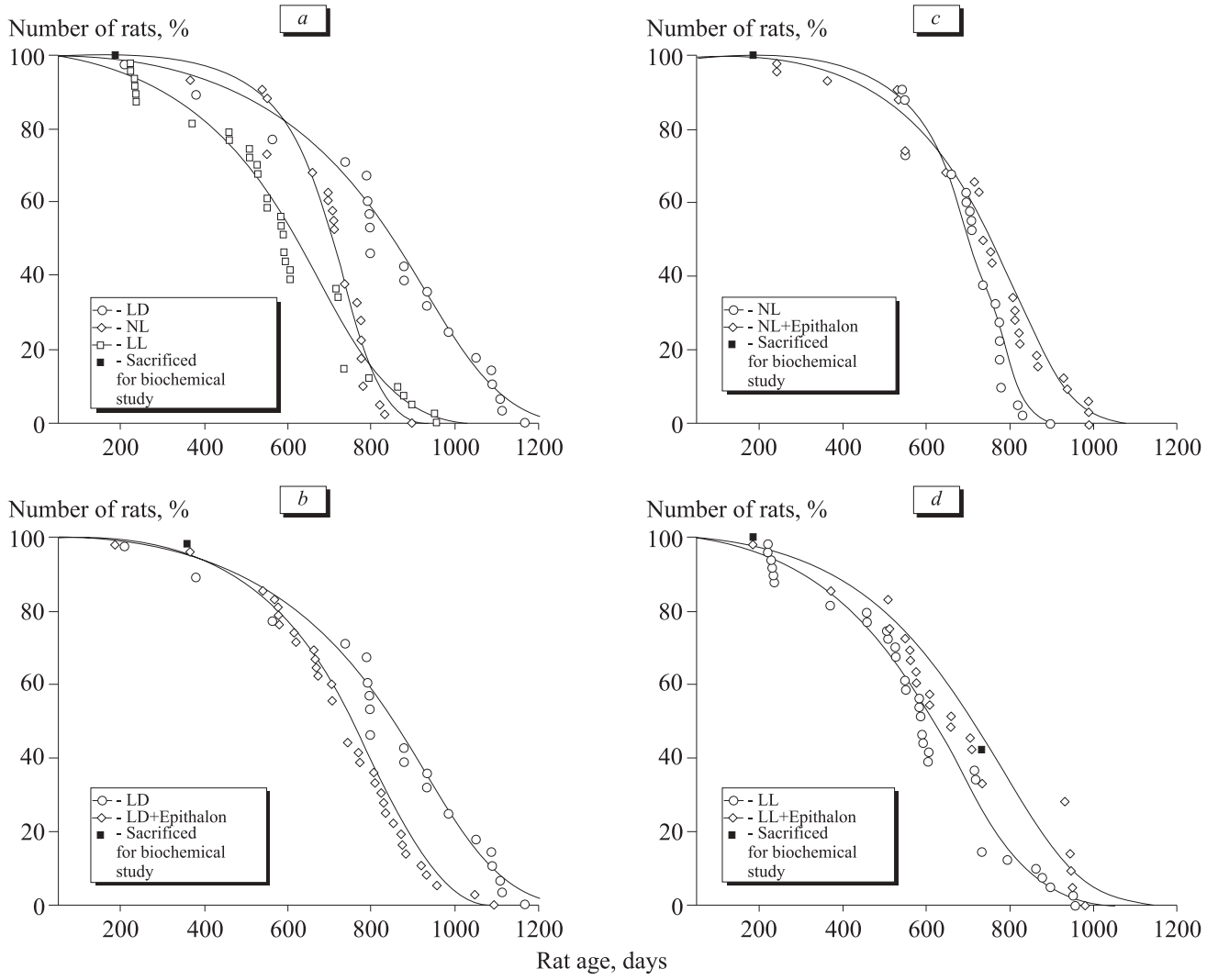

Fig. 1. Effects of light regimen and Epithalon on survival of female rats. Here and in Fig. 2: *a*) data for all groups; *b*) LD group; *c*) NL group; *d*) LL group.

TABLE 2. Effects of Epithalon on the Development of Spontaneous Tumors in Female Rats Kept under Conditions of Different Light Regimens

Parameters		Light regimen (rat group)					
		LD		NL		LL	
		control	epithalon	control	epithalon	control	epithalon
Number of rats		40	55	48	50	54	45
Number of rats with tumors, %		21 (52.5%)	23 (41.8%)	34 (70.8%)*	22 (44.0%)** +	24 (44.4%)	17 (37.8%)
Number of rats with malignant tumors, %		5 (12.%)	3 (5.9%)	7 (14.6%)	2 (4%)	7 (13.8%)	3 (6.7%)
Number of tumors		29	33	48	28	39	19
Number of tumors per rat with tumors		1.38	1.43	1.41	1.27	1.63	1.12
Mean life span of rats with tumors, days		769.0±63.0	725.0±41.8	683.0±22.9**	726.0±36.7	665.0±40.3	578.0±54.6
Tumor location and type							
Mammary gland	fibroma	4	4	9	3	1	3
	fibroadenoma	11	18	21	15	20	9
	adenocarcinoma	—	1	—	—	1	—
Uterus	polyp	4	5	1	4	4	2
	fibroma	1	—	—	1	1	—
	fibromyoma	—	—	2	—	1	—
	adenocarcinoma	—	—	3	1	1	—
Uterine tubes	fibroma	—	—	3	—	—	—
Adrenal	adenoma	1	1	1	2	3	—
	pheochromocytoma	2	—	—	—	—	—
	cancer	—	—	1	—	—	—
Ovary	fibroma	1	—	—	—	—	—
	luteoma	—	—	1	—	—	—
	hemangioma		—	1	—	1	— —
	androblastoma	—	—	—	—	—	1
Hemopoietic system							
	leukemia	2	1	1	1	1	1
	malignant lymphoma	1	—	1	—	3	1
Soft tissues	fibroma	—	—	1	—	1	—
	sarcoma	2	1	2	—	—	—
Kidney	liposarcoma	—	—	—	—	1	—
Ureter	fibroma	—	1	—	—	—	—
Pituitary	adenoma	—	—	—	—	1	—
Small intestine	adenocarcinoma	—	—	—	—	—	1
Trigeminal nerve	neuroblastoma	—	—	—	—	—	1
Total	benign	24	30	39	26	32	15
	malignant	5	3	9	2	7	4

Note. *$p<0.95$, **$p<0.01$ compared to the control (LD); +$p<0.05$ compared to rats kept under the same light conditions receiving no Epithalon.

and LL animals treated with Epithalon were shifted to the right in comparison with the curve for LD subgroup receiving no peptide injections (Fig. 1, *b-d*).

Violation of the light regimen had a statistically significant modifying impact for the development of spontaneous tumors in female rats (Table 2). The incidence of tumor development in rats kept under

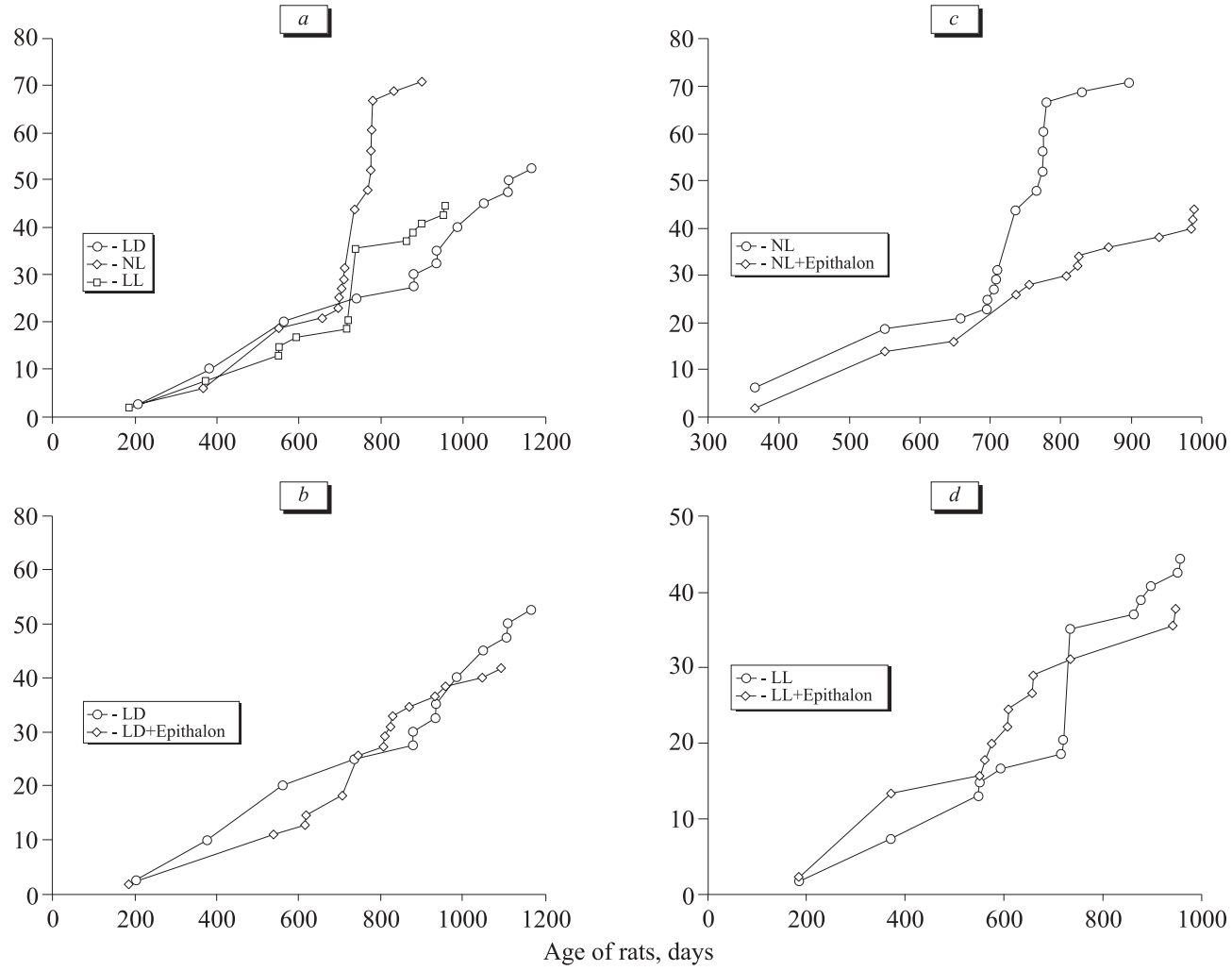

Fig. 2. Effects of light regimen and Epithalon on the dynamics of spontaneous tumor development in female rats. Ordinate: number of rats with tumors (%).

NL conditions increased significantly ($p<0.05$) in comparison with LD group mainly at the expense of 2-fold higher incidence of benign tumors of the mammary gland. Uterine adenocarcinomas ($n=3$) were detected in NL group, but not a single one in the LD group. In addition, the mean life span of NL rats with tumors was significantly lower than in LD group.

Exposure to LL virtually had no effect on the total incidence of spontaneous tumors in female rats. However, the multiplicity of tumors increased in this group, the mean life span of rats with tumors was lower, and mammary tumors were more incident (Table 2). The NL and LL exposure stimulated the development of all tumors. The long rank test demonstrated highly significant differences ($p<0.001$; Fig. 2) between the curves representing the time course of tumor development in the control and other groups.

Epithalon had virtually no effect on the development of spontaneous tumors in rats exposed to LD. Under LL conditions Epithalon suppressed tumor multiplicity and development of mammary fibroadenomas. In the NL group Epithalon treatment reduced the total incidence of spontaneous tumors ($p<0.05$; Table 2). Epithalon virtually normalized the time course of tumor development in the NL group (Fig. 2).

These data indicate unfavorable effects of LL and NL exposure on the life span of female rats, which is in good agreement with numerous data indicating a relationship between the pineal gland and endocrine regulation of metabolic processes [1,7,13,14].

A peculiar photoperiodism is characteristic of North-Western Russia; it presents as a long day in spring and summer (particularly from the middle of May to the middle of July, the “white nights” season) and just 4-5 daylight hours in autumn and winter. The natural disturbances of circadian rhythm resultant from seasonal fluctuations of na-

tural light are little studied. Suppression of the pineal function during the white nights period leads to dysfunction of the biological "clock" of the organism, responsible for adaptation of the biological rhythms of functioning to the light/darkness cycle [1,2,6,7]. Hence, we can say that aging rate is higher in female rats exposed to constant light or to naturally disturbed light regimen in comparison with animals kept under conditions of fixed alternation of light and darkness.

Many experimental studies showed that the LL mode promotes the development of spontaneous tumors of different location and chemical carcinogenesis in mice and rats [1,7,14]. Our data indicate that Epithalon, a synthetic peptide stimulating nocturnal production of melatonin and exhibiting antioxidant, geroprotective, and anticarcinogenic effects of this hormone [5,8], prevents early aging and stimulation of tumor development in female rats exposed to the NL regimen of North-Western Russia. Moreover, Epithalon somewhat inhibits the development of spontaneous tumors in rats kept under LL conditions.

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