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Pineal peptide preparation epithalamin increases the lifespan of fruit flies, mice and rats

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Abstract

Treatment with pineal peptide preparation epithalamin® was followed by the increase of the mean lifespan of female *D. melanogaster*, SHR mice, C3H/Sn mice and LIO rats by 11–31% ($P < 0.05$). Ninety percent mortality as well as maximum lifespan were increased in fruit flies, C3H/Sn mice and rats. Mortality rate was decreased by 52% in *D. melanogaster*, by 52% in rats, by 27% in C3H/Sn mice. It did not change in SHR mice exposed to epithalamin. Treatment with the pineal peptide increased MRDT in flies, C3H/Sn mice and rats. It has been shown that epithalamin increases synthesis and secretion of melatonin in rats and inhibits free radical processes in rats and in *D. melanogaster*. It is suggested that antioxidative properties of epithalamin lead to increased lifespan of three different animal species. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Pineal peptide preparation; Lifespan; *D. melanogaster*; Mouse; Rat

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1. Introduction

During the last decade a number of reports describing the regulatory role of the pineal gland during aging has been published (Armstrong and Redman, 1991; Pierpaoli, 1991; Trentini et al., 1991; Reiter, 1995). The modulating effect of the pineal gland on neuroendocrine and immune systems was shown to be altered with aging (Maestroni et al., 1989; Reiter, 1990; Yu and Reiter, 1993; Reiter, 1995). The pinealectomy of rats was followed by the reduction of their lifespan (Malm et al., 1959). The administration of pineal hormone melatonin to old mice or grafting of pineal gland from young donors to the thymus or in situ of old mice was shown to prolong their lifespan (Pierpaoli et al., 1991; Pierpaoli and Regelson, 1994). Recently, reports on the lifespan-prolonging effects of melatonin were strongly criticized (Reppert and Weaver, 1995; Turek, 1996). The murine strains used in that studies (C57BL, NZB and BALB/c) were described to have a genetic defect in pineal melatonin biosynthesis and cannot produce melatonin (Goto et al., 1989). The melatonin treatment reduced survival in one of the mouse strains (C3H/He) which produced melatonin (Pierpaoli et al., 1991). However, recently an independent group of researchers has published data that melatonin significantly increases lifespan in the rat (Oakin-Bendahan et al., 1995).

The majority of investigators have regarded melatonin as the primary mediator of endocrine function of the pineal gland. However, there is evidence that some of the effects of the pineal gland may be a result of the pineal peptide secretion (Noteborn et al., 1988; Anisimov and Reiter, 1990). It was shown that some crude peptide extracts or purified peptides isolated from pineal gland have anti-gonadotropic and anti-tumor activity (Anisimov and Reiter, 1990; Yuwiler and Brammer, 1993; Vaskovsky et al., 1993). In 1960, the Roumanian researcher C.I. Parhon reported the life-span prolonging effect of pineal extract in old rats (Parhon, 1960). No details were given on the method of pineal extraction or on the experiment.

Two decades ago we published the first evidence that administration of the low-molecular weight pineal peptide preparation, the commercial drug form of which was named later as Epithalamin®, was followed by restoration of estrus cycle in old female rats with persistent estrus and by lowering of the threshold of sensitivity of hypothalamo-pituitary complex to feedback inhibition by estrogens in old animals (Anisimov et al., 1973). Since then the effect of epithalamin on the function of the reproductive, neuroendocrine and immune systems was systematically studied in our and our colleagues' experiments. Epithalamin showed high biological activity. Long-term treatment with the preparation prolongs the lifespan of rats, mice and fruit flies (Dilman et al., 1979; Anisimov et al., 1982, 1989, 1992, 1994, 1997b), slows down the aging of reproductive system, improves immune functions and inhibits the development of spontaneous, induced by chemicals or X-irradiation and transplanted tumors (Anisimov and Reiter, 1990; Morozov and Khavinson, 1996; Anisimov et al., 1994, 1997b).

This paper presents the calculation of the additional parameters of lifespan and reevaluation based on the data from our previous studies on the effect of epithalamin.

2. Materials and methods

Our previous studies reported data on the effect of epithalamin on the lifespan of female rats (Dilman et al., 1979), female mice of two strains, C3H/Sn (Anisimov et al., 1989) and Swiss-derived SHR (Anisimov et al., 1992), and of female *D. melanogaster* (Anisimov et al., 1997b). These data were reevaluated and we calculated the following parameters: mean lifespan; median of lifespan, in days; days of 90 and 100% mortality; MR (mortality rate) calculated as α (slope) in the Gompertz equation: $R = R_o \exp(\alpha t)$; MRDT (mortality rate doubling time) calculated as $\ln 2/\alpha$. Survival curves and Gompertz plot for mortality were drawn using a computer program. Statistical treatment was done using Student's t criterion (Gubler, 1978). Regression and multifactorial analysis of variance were used as well (Gubler, 1978).

3. Results

The results of the calculation of lifespan of various animals exposed or not exposed to epithalamin are presented in Table 1. Survival curves for controls and treated with epithalamin groups are shown in Fig. 1, and their Gompertz plots are shown in Fig. 2. Treatment with epithalamin increases the mean lifespan of all studied species from 11 to 31%, $P < 0.05$. Ninety percent of mortality, as well as the maximum lifespan (100% mortality) were increased in *D. melanogaster*, C3H/Sn mice and rats. However, these parameters did not change in SHR mice. Mortality rate after exposure to epithalamin was decreased by 52% in *D. melanogaster*, by 52% in rats, by 27% in C3H/Sn mice and did not change in SHR mice. Treatment with the pineal peptide increased MRDT in flies, C3H/Sn mice and rats. Thus, exposure to epithalamin was followed by a positive effect on the parameters of lifespan in three animal species.

4. Discussion

Our results have shown that the peptide pineal preparation epithalamin prolongs lifespan of fruit flies, mice and rats. Epithalamin increases mean lifespan of all species tested, and slows down the rate of aging rate in *D. melanogaster*, C3H/Sn mice and rats. The effect of the drug was less expressed in SHR mice. This observation could be related to smaller dosage of epithalamin in SHR mice as compared to C3H/Sn (Anisimov et al., 1982, 1989, 1994).

Table 1
Effect of epithalamin on parameters of life span in females of various species

Treatment	Number of animals	Mean life span (days)	Mortality (days)			Mortality rate	MRDT
			Median	90%	100%	$\alpha \times 10^3$ (days^{-1})	$\ln 2/\alpha$
Drosophila melanogaster							
Controls	199	25 ± 1.21	23	54	80	70 ± 12.9	3.7
Epithalamin	207	29 ± 1.19**	29	60	91	33 ± 3.3**	4.9
Changes (%)		+16	+26	+11	+14	−52	+32
SHR mice							
Control	31	564 ± 22.3	558	750	843	6.8 ± 0.36	5.7
Epithalamin	32	627 ± 20.9*	634	750	827	6.9 ± 0.18	5.7
Changes (%)		+11	+14	0	−2	+2	0
C3H/Sn mice							
Control	21	487 ± 29.4	511	691	776	7.0 ± 1.50	5.7
Epithalamin	32	640 ± 33.1**	679	757	885	5.1 ± 1.28	6.0
Changes (%)		+31	+32	+20	+14	−27	+5
Outbred rats							
Control	75	681 ± 14.5	705	825	1054	7.9 ± 0.45	5.5
Epithalamin	33	852 ± 33.8***	873	1050	1112	3.8 ± 0.77	6.3
Changes (%)		+25	+24	+27	+6	−52	+15

MRDT, mortality rate doubling time.
The difference compared to controls is significant: *$P<0.05$; **$P<0.01$; ***$P<0.001$.

The mechanisms of the effect of epithalamin on the lifespan remain unknown. However, certain effects of the preparation could be taken into consideration. It was shown that exposure to epithalamin decreases level of luteinizing hormone and prolactin in adult male rats, decreases the threshold of hypothalamo-pituitary complex to feedback inhibition by estrogens in old female rats, inhibits compensatory ovarian hypertrophy in hemiovariectomized rats, inhibits chorionic gonadotropin-induced uterine weight increase in infantile mice and rats, induces

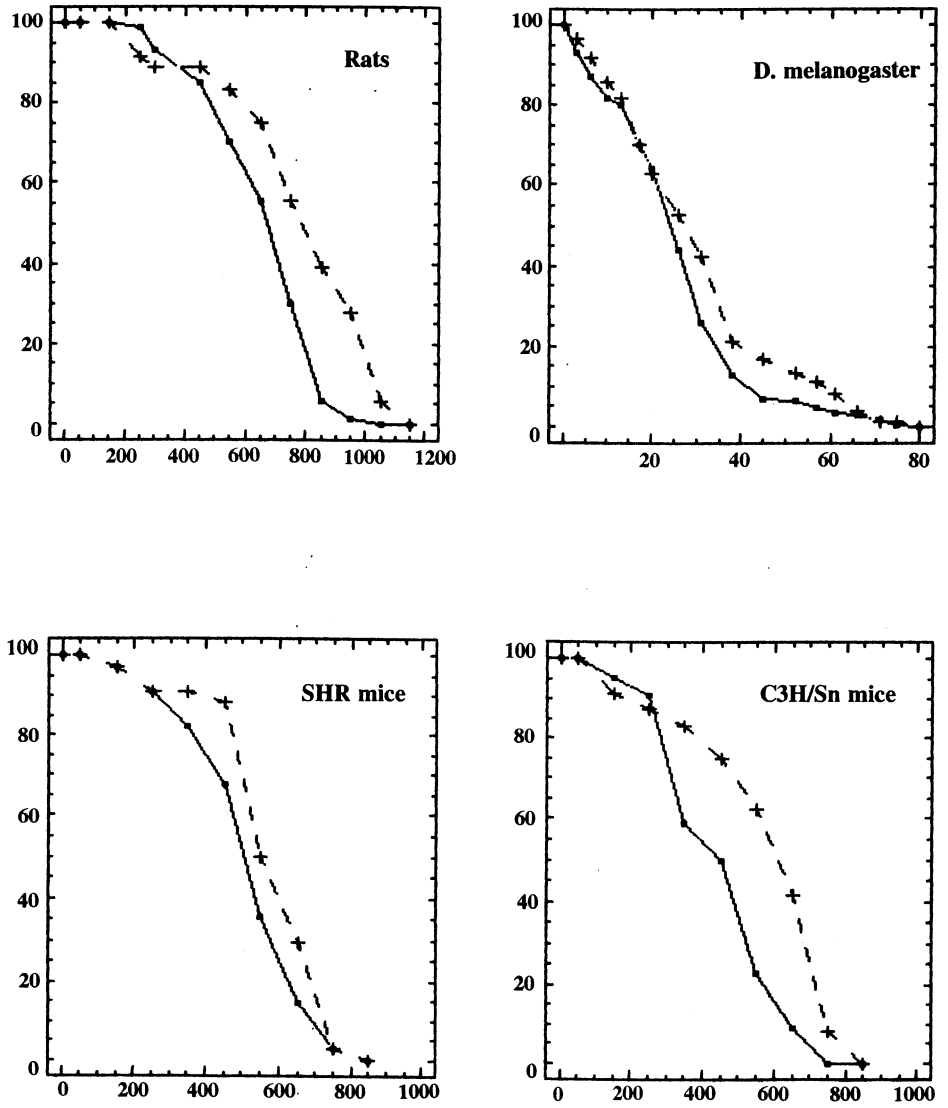

Fig. 1. Effect of epithalamin on survival curves of adult female fruit flies, mice and rats. Ordinate, number of animals (%); abscissa, age (days). (□) Controls; (+) exposure to epithalamin.

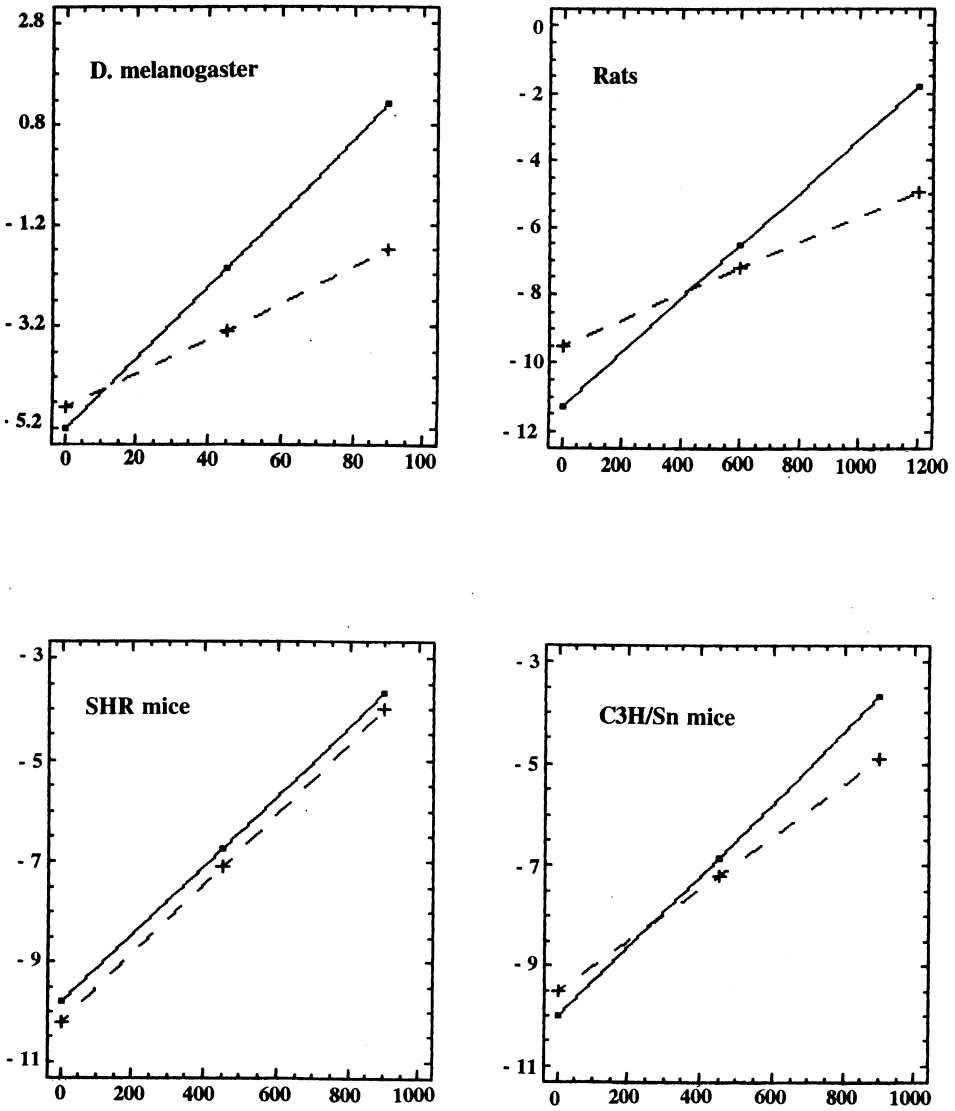

Fig. 2. Effect of epithalamin on mortality rates of adult female fruit flies, mice and rats (Gompertz plots, semilogarithmic scale). Ordinate, ln mortality rate; abscissa, age (days). (□) Controls; (+) exposure to epithalamin.

recurrence of estrus cycles and fertility in old persistent-estrus rats, slows down the age-related switching-off of estrus function in rats, increases the level of triiodothyronine and decreases level of thyroxine in serum of adult rats, decreases the level of corticosterone in serum of mice, increases the susceptibility of hypothalamo-pituitary complex to homeostatic inhibition of adrenocorticotropic function by glucocorticoids in old rats, decreases serum insulin and triglyceride levels in rabbits,

increases tolerance to glucose (Anisimov et al., 1994; Morozov and Khavinson, 1996). Treatment with epithalamin led to increase of learning capacity of rats, to increase of T- and B-cell-mediated immunity in rodents and to increase of serum titer of thymic serum factor and titer of thymosin-like compounds in old mice. It also stimulates colony-forming activity of splenocytes in pinealectomized rats (Labunets and Butenko, 1992; Anisimov et al., 1994; Morozov and Khavinson, 1996).

As exposure to epithalamin increased pineal synthesis and secretion of melatonin (Anisimov et al., 1992, 1997b) it was suggested that some effects of epithalamin are mediated by this indole hormone. During the last few years it has been reported that pineal indole hormone melatonin is a highly potent scavenger of hydroxyl and peroxyl radicals both in vitro and in vivo (Pierrefiche and Laborit, 1995; Reiter, 1995; Reiter et al., 1995).

There is significant evidence to support the supposition that oxidative damage may play an important role in aging (Ames et al., 1993; Harman, 1994; Martin et al., 1996). According to the free radical theory of aging, active molecules of oxygen, superoxide ($O_2^{\cdot -}$), H_2O_2, hydroxyl radical (HO•) and, possibly, singlet oxygen ($\uparrow O_2$) damage cellular macromolecules. It leads to mutations, genome instability followed by aging and age-related pathology, including atherosclerosis, immunosuppression, brain dysfunction, cataract and cancer (Ames et al., 1993; Harman, 1994; Martin et al., 1996). Natural endogenous factors (Cu, Zn-superoxide dismutase (SOD), glutathione peroxidase, catalase, ceruloplasmin, β-carotene, α-tocopherol (vitamin E), ascorbic and uric acids) defend macromolecules from oxidative damage (Ames et al., 1993; Harman, 1994). Exposure to certain natural or synthetic antioxidants increases the mean lifespan of laboratory rodents and flies (Emanuel and Obukhova, 1978; Harman, 1994). It was shown that transgenic flies which overexpress both catalase and SOD have greater mean and maximum longevity and slower aging rate (Orr and Sohal, 1994; Sohal et al., 1996). It is worthy to note that melatonin is present in mammals as well as in many invertebrate species, including insects (Vivien-Roels and Pevet, 1993; Arendt, 1995).

It was shown that melatonin is a potent scavenger of free radicals both in vivo and in vitro (Pierrefiche and Laborit, 1995; Reiter, 1995; Reiter et al., 1995; Anisimov et al., 1996). Melatonin enters all subcellular compartments and stimulates production of glutathione peroxidase, one of the potent antioxidant enzymes,

Table 2
Effect of epithalamin on lipid peroxidation and activity of SOD and catalase in female *D. melanogaster* (Anisimov et al., 1997b)

Parameters	Changes, in % to control value
Conjugated hydroperoxides	−57.4**
Ketodiene	−70.7**
SOD	+8.1
Catalase	+52.7**

The difference compared to controls is significant, *$P<0.05$; **$P<0.02$.

Table 3
Effect of epithalamin on lipid peroxidation and activity of antioxidation defence system in serum of rats (Anisimov et al., 1996, 1997a)

Parameters	Changes, in % to control value
H_2O_2-induced luminol-dependent chemiluminescence	−64.3**
Conjugated hydroperoxides	−75.8**
Schiff's bases	−14.4
SOD	+19.7*
Coeruloplasmin	+6.1
Total antioxidase activity (vitamins C, E, glutathione-SH, etc.)	+36.6*

The difference compared to controls is significant, $*P<0.05$; $**P<0.02$.

in rat brain and liver (Pierrefiche and Laborit, 1995; Reiter et al., 1995). However, melatonin failed to influence the activity of serum SOD and ceruloplasmin in rats (Anisimov et al., 1997a). Similar to melatonin, epithalamin inhibits lipid peroxidation and increases catalase activity in rats and in flies, but, unlike melatonin, epithalamin increases serum activity of SOD and ceruloplasmin in rats and SOD in flies (Anisimov et al., 1994, 1996, 1997b) (Tables 2 and 3). Only overexpression of both SOD and catalase led to the increase of lifespan of transgenic flies. The lifespan of flies with the overexpression of either SOD or catalase had not shown any difference from the controls (Orr and Sohal, 1994; Sohal et al., 1996).

The results of our experiments with various animals suggest that both melatonin-mediated antioxidative effect, and SOD and other antioxidative enzyme-mediated effects of epithalamin may play a significant role in mechanism of its geroprotective potential. Our observations provide evidence supporting a concept of the possibilities of practical application of epithalamin in clinics for prevention of premature aging and of age-related pathology, including cancer. Epithalamin is produced at the Factory of Medical Preparation 'Samson' (St. Petersburg) and included in the Russian Drug Directory (Moscow: INPHARMCHEM, 1993, p. 965). Its approved clinical indications are: climacteric dysfunctions, anovulatory infertility and hormone-dependent tumors. The results of clinical trials of epithalamin were reported elsewhere (Karpov et al., 1985; Slepushkin et al., 1990; Morozov and Khavinson, 1996).

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