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Effect of Pineal Peptide Preparation (Epithalamin) on Life Span and Pineal- and Serum Melatonin Level in Old Rats

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INTRODUCTION

During the last decade the interest in the role of the pineal gland in aging and cancer has increased.[1,2] It has been shown that with age significant morphological changes in the pineal gland of rodents and humans develop that could be interpreted as a decrease in pineal function.[1,2] These observations are supported by the data on age-related decrease in the level of the night peak of melatonin in the pineal gland, serum, and urine of animals and humans.[1,2]

At the same time there is morphological, biochemical, and endocrinological evidence of the decrease of pineal function both in rodents bearing transplanted or induced tumors and in cancer patients.[1–3] Inhibition of pineal gland function by pinealectomy or exposure to constant light stimulated tumor development, whereas administration of melatonin or pineal peptide preparations or exposure to constant darkness inhibited tumor development.[1–3]

The pinealectomy of rats reduced their life span,[4] and administration of the pineal hormone melatonin to old mice increased their longevity.[5] In experiments with mice of two strains and with rats we have shown that long-term administration of the polypeptide pineal preparation epithalamin started at the age of 3.5 months prolonged the life span of rodents and diminished the incidence of spontaneous tumor development.[2,6–8] When administration of epithalamin to female SHR mice was started at the age of one year, the increase in life span was smaller then that of young mice.[6]

Whereas there is no doubt concerning the role of melatonin as mediator of many effects of the pineal gland, available data give evidence that some of the effects of the pineal gland may be a result of pineal peptide secretion.[1,2,8] Recently it was shown that the pineal peptide preparation epithalamin administered in the morning to male rats increases the night metabolism of serotonin into melatonin and the night peak of this indolamine in the pineal gland.[6]

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TABLE 1. Effect of Epithalamin on Pineal Indolamines in Pineal Gland of Young and Old Male Rats

Pineal Level of Indolamines (ng/mg of Tissue)	Age			
	4–5 Months		18–20 Months	
	Saline (n = 8)	Epithalamin (n = 13)	Saline (n = 6)	Epithalamin (n = 7)
Serotonin	3.65 ± 0.18	5.10 ± 0.55[a]	5.20 ± 0.98	5.93 ± 1.14
N-Acetylserotonin	1.70 ± 0.26	2.77 ± 0.37[b]	3.39 ± 1.41	2.28 ± 0.19
Melatonin	2.54 ± 0.19	3.61 ± 0.31[a]	1.66 ± 0.23[c]	2.32 ± 0.25[c]
5-Methoxytryptamine	7.13 ± 0.39	7.83 ± 0.58	3.43 ± 0.30[d]	3.08 ± 0.74[d]
5-HIAA + 5-MIAA	16.96 ± 1.07	18.53 ± 1.22	14.84 ± 1.40	14.02 ± 1.57[e]

[a] Difference from control of the same age is significant, $p < 0.01$.
[b] Difference from control of the same age is significant, $p < 0.001$.
[c] Difference from corresponding young group is significant, $p < 0.02$.
[d] Difference from corresponding young group is significant, $p < 0.001$.
[e] Difference from corresponding young group is significant, $p < 0.05$.

In the present paper new data on the effect of epithalamin on melatonin synthesis and secretion in young and old rats and on the life span of old female rats are presented.

EFFECT OF EPITHALAMIN ON SEROTONIN METABOLISM IN THE PINEAL GLAND OF MALE RATS

Epithalamin (2.5 mg/kg) was administered subcutaneously at 10:00 A.M. for 5 days to male rats of the age of 4–5 months and 18–20 months. The animals were decapitated at the period of maximal pineal activity (between 2400 and 0300 h). Serotonin and its metabolites were detected in the pineal gland by the fluorometric method and in serum by radioimmunoassay (RIA) with the "DRG-Instrument" kit (USA) as described elsewhere.[6]

It was shown that in young rats an active metabolism of serotonin through *N*-acetylserotonin (N-AS) into melatonin takes place. In old rats the increased concentration of pineal serotonin level was dependent on decrease of its utilization in the pathway of *N*-acetylation and subsequent *O*-methylation (TABLE 1). Morning injections of epithalamin to young rats for 5 days resulted in an increase of the night level of serotonin, N-AS, and melatonin in the pineal gland, whereas in old rats an increase only in melatonin level tended to occur. Both in young and old rats injections of epithalamin had no influence on reactions of direct *O*-methylation in 5-MT, and on oxidative deamination and subsequent *O*-methylation into 5-HIAA and 5-MIAA (TABLE 1).

It is possible that epithalamin stimulates synthesis of serotonin from tryptophan and melatonin synthesis through N-AS. In old rats an increase in melatonin level induced by epithalamin could depend on stimulation of metabolism of N-AS into melatonin. These data are in accordance with the results of RIA of melatonin in the serum of old rats. Injections of epithalamin for 5 days was followed by a 49% increase in the night serum melatonin level in comparison to rats treated with saline: 167 ± 15.0 pg/ml and 112 ± 17.4 pg/ml, respectively ($p < 0.05$).

Thus, the data presented allow the suggestions that an ultrashort loop exists between the pineal peptides and indoles, and that the target of pineal peptides is first of all the metabolism of tryptophan into serotonin and its subsequent transformation into melatonin. In old rats the intensity of these reactions is decreased.

EFFECT OF EPITHALAMIN ON LIFE SPAN AND SPONTANEOUS TUMOR DEVELOPMENT IN OLD FEMALE RATS

Outbred female rats starting at the age of 15 months were injected during the morning with saline or 0.5 mg of epithalamin dissolved in saline on 5 consecutive days monthly until natural death. It was shown that administration of epithalamin to old female rats insignificantly increased their life span: by 6.2% when calculated from birth, and by 18% when calculated from the start of the experiment ($p > 0.05$). Exposure of old rats to epithalamin was followed by a slowing down of the aging rate: 23.4% of these rats treated with epithalamin survived longer than the maximum life span in rats in the control group (TABLE 2).

Cytological study of vaginal smears revealed a delaying of the age-related switching off of estrous function in rats treated with epithalamin in comparison to control rats. Thus, if at the age of 18 months the relative number of rats with persistent estrus or anestrus was equal in both groups, at the age of 27 months

TABLE 2. Life Span and Spontaneous Tumor Incidence in Female Rats Treated with Epithalamin Started at the Age of 15 Months

Parameters	Saline	Epithalamin
Effective number of rats	44	47
Life span (from birth), days		
Mean	695 ± 17.7	738 ± 22.1
Median	670	714
Maximum	888	972
Aging rate (α)[a], $days^{-1}$	0.00641	0.00153
Number of tumor-bearing rats	19 (43.2%)	13 (27.7%)
Number of rats bearing malignant tumors	5	3
Total numbers of tumors	38	19
Number of tumors per tumor-bearing rat	2.00	1.46
Tumor localization and type		
Pituitary: adenoma	8	4
Thyroid gland: adenoma	1	—
Mammary gland: fibroadenoma	20 (12)[b]	11 (9)[b]
Uterus		
Polyp	4 (3)[b]	1
Stromogenic sarcoma	1	—
Hemopoietic system		
Leukemia	4	1
Malignant lymphoma	—	2

[a] Aging rate was calculated as α from Gompertz equation: $R = R_0 (\exp) \alpha t$, where R = mortality, R_0 = mortality at t (age) = 0, α = constant.

[b] Number in parenthesis is the number of rats with tumor(s) at this site.

these disturbances were found in 50% of control rats and in 30% of rats treated with epithalamin.

These data are in accordance with our previous experiments, in which prolongation of regular estrous function in old rats treated with epithalamin from the age of 3.5 months was shown.[7] When epithalamin was injected into 16–18-month-old female rats with spontaneous persistent estrus, the restoration of regular estrous cycles and, moreover, the restoration of fertility were observed.[7] The mechanism of these effects could include the capacity of epithalamin to increase the threshold of sensitivity of the hypothalamo-pituitary complex to the homeostatic feedback action of estrogen.[7]

Administration of epithalamin to old rats was followed by a significant ($p < 0.004$) decrease of total tumor incidence (by 1.6 times) and a decrease in malignant tumor incidence by 1.8 times ($p < 0.04$). Also shown was a decrease in the incidence of pituitary adenomas and mammary tumors (TABLE 2). In our previous experiments with rats treated with epithalamin from the age of 3.5 months the mean life span was increased by 25% when calculated on date of birth.[7] This is more significant than in the present study (6%). But this difference in significance may be due to the fact that in this experiment increase in life span was calculated from the start of experiment. When epithalamin was administered to female SHR mice from the age of 3.5 and 12 months, the mean life span calculated from the start of treatment increased by 20% and 17%, respectively.[6]

In this set of experiments as well as in previous ones on mice and rats the inhibition of spontaneous tumor development under the influence of epithalamin occurred.[2,6,8] We have studied also the effect of epithalamin on the carcinogenesis induced by 7,12-dimethylbenz(a)anthracene, N-nitrosoethylurea, and total-body X-ray irradiation; and in all of the studies the inhibitory effect of this peptide pineal preparation was observed.[2,6,8] This drug also inhibited the growth of some transplanted tumors[2,8] and improved some parameters of immunity and health conditions in cancer patients.[8] The mechanism of the antitumor and anticarcinogenic effects of epithalamin is thought to involve normalization of a number of hormonal and metabolic disturbances promoting tumor development as well as stimulation of antitumor immunity,[2,8] mediated both by increasing melatonin synthesis and secretion and by direct effects of pineal peptides. The same mechanisms are probably responsible for the aging-preventing effect of the pineal peptide preparation.

Our observations provide arguments in support of the practical application of epithalamin in patients. At the same time, the problem of further isolation and characterization of individual active peptides and the study of their effects on life span and tumor development is a task of our current experiments.

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