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The Pineal Peptides: Interaction with Indoles and the Role in Aging and Cancer

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At present there has been studied the role of the pineal gland as one of the central regulators of endocrine functions. The function of the epiphysis as of an endocrine gland has been commonly accepted, however, still now the discussion is in progress on the number of hormones synthesized and secreted by it. The majority of researchers consider melatonin as a major mediator of various effects of pineal gland on the neuroendocrine system, however, some other metabolites of serotonin, e.g. 5-methoxytryptamine have been shown to be also secreted by the gland (Reiter, 1988). Finally, it has been confirmed that some effects of pineal gland occur as a result of secretion of peptide hormones by it (Anisimov, 1980; 1988; Blask et al., 1988).It should be noted that almost no data on theinteraction of these two classes of the pineal hormones are available. Nothing is known on possible influence of the pineal peptides on the production of indole metabolites in the pineal gland. The importance of studying this problem is also stressed by the data on the modulating influence of both melatonin and pineal peptides on the immune system, tumor development and life span of rodents (Anisimov, 1980, 1988; Blask et al., 1988; Maestroni et al., 1989).

In our experiments, we studied the effects of the peptide drug epithalamin obtained from the bovine pineal gland (Anisimov et al., 1982).

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

Epithalamin was administered subcutaneously a single time to Wistar male rats aged 3-4 months, at dose of 2.5 mg per kg of body weight, at 10.00. Besides, some animals were given the drug at the same dose at 10.00 during 5 days. The animals were decapitated in the period of maximal pineal activity (between midnight and 3.00).

Serotonin, 5-methoxytryptamine (5-MT), melatonin, N-acetylserotonine (NAS), 5-hydroxy- and 5-methoxyindol acetic acid (5-HIAA + 5-MIAA) were detected in the pineal gland by fluorometric method.

The results of investigation are given in Fig.1, where indole changes under the influence of epithalamin in the pineal gland are represented in per cent in comparison to

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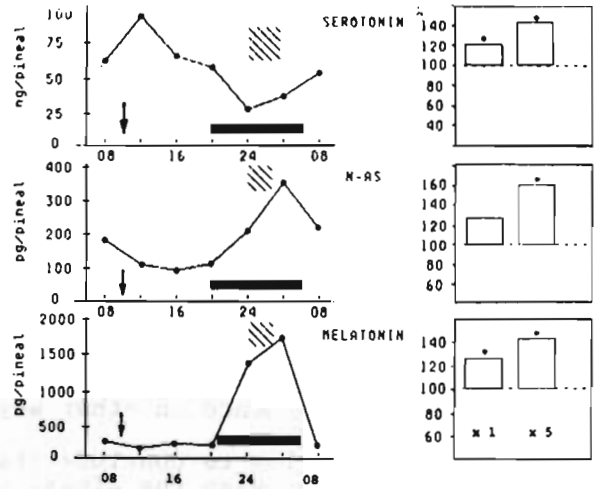

Fig.1 Effect of epithalamin on nocturnal levels of of indolamines in rat pineal gland
Arrow- injection of epithalamin. Black line - dark time; inclined strokes indicates time of indolamine estimation. xl - single injection; x5 - 5 daily injections of epithalamin. Ordinate on right part of figure - ratio to control level, in percent. *-p 0.05.

control. In the first series of experiments, epithalamin was injected in the morning (at 10.00). In 14-16 hours after administration, i.e. at night, experimental animals revealed a moderated increase of pineal concentrations of serotonin, NAS and melatonin, as well as no change in the contents of 5-MT and a total fraction of indolacetate. These data permit to suggest that morning epithalamin injection stimulate pineal gland night activity, facilitating not only transformation of tryptophan into serotonin, but also the reaction of serotonin metabolism on the way of its N-acetylation and subsequent 0-methylation, producing no influence on the process of direct 0-methylation and oxidative desamination of serotonin.

This suggestion is confirmed by the results of the second series of experimets in which epithalamin was administered in the morning hours (at 10.00) during 5 days. As in the previous series of experiments, the drug activated a night activity of the pineal gland which is confirmed by the increased pineal concentration of serotonin, N-acetylserotonin and melatonin, however, in 5-day administration of the drug, these changes were more pronounced. The contents of 5-MT, 5-HIAA + 5-MIAA did not differ from that in control animals. Thus a prolonged administration of epithalamin stimulates the process of serotonin formation from tryptophane, as well as the process of serotonin metabolism on the way of its N-acetylation and subsequent 0-methylation, pro-

ducing no significant influence on other ways of metabolism.

The data presented allow to conclude that, firstly, an ultra-short loop exists between the pineal peptides and indoles, secondly, tha target of the pineal peptides, is first of all, the reaction of tryptophan transformation into serotonin when its stimulating effect on subsequent metabolism in N-acetylserotonin and melatonin cannot be excluded, which requires further investigation.

The data obtained correspond earlier observations on a pronounced biological effect of a long-term administration of epithalamin as compared to a single one, and suggest that some but not all properties of epithalamin admininstered in the morning hours my by mediated by the increased night peak of melatonin in the pineal gland, and, respectively, in the blood of animals.

THE EFFECT OF EPITHALAMIN ON THE LIFE SPAN AND SPONTANEOUS TUMOR DEVELOPMENT IN FEMALE MICE OF DIFFERENT AGE

It was shown in our previous experiments, that a long-term administration of epithalamin to mice and rats increased their life span and diminished the incidence of spontaneous tumors (Dilman et al., 1979; Anisimov et al.,1982, 1989; Anisimov, 1988). However, there comes forward the problem on the optimal onset of the drug administration: in the young, mature or old age.

We studied the influence of epithalamin on the life span and tumor development in virgin female Swiss-derived SHR mice. The drug was administered subcutaneously at 10.00, monthly, 5 days running, at a dose of 0.1 mg of epithalamin in 0.1 ml of saline beginning at the age of 3 (young group) or 12 (middle-aged group) months. Control animals were treated with saline.

Long-term treatment of young and middle-aged female mice with epithalamin resulted in 20% and 17% increase of mean life span in respective age groups (taking into account only time from the commencement of treatment). At the same time, the maximal life span of middle-aged epithalamin treated mice was shorter than in the young ones (Table 1).

Fig.2 shows that treatment with epithalamin markedly shifted survival curves of both age groups to the right as compared to corresponding controls. The aging rates calculation in young and middle-aged groups treated with epithalamin revealed the slow-down of the aging in young animals as compared to middle-aged ones: $\varkappa$(epithalamin, young) = 0.0052 day^{-1} vs $\varkappa$(epithalamin, middle-aged)= 0.0077 day^{-1}.

It is noteworthy that administration of epithalamin increased life span of tumor-free young mice by 20%, $p < 0.05$, i.e. exerted true geroprotective effect. In middle-aged animals this increase was respectively 36% , $p < 0.05$.

Table 1. Life span and tumor incidence in female SHR mice treated with epithalamin in different age

Parameters	Young mice		Middle-aged mice	
	Saline	Epitha-lamin	Saline	Epitha-lamin
Number of mice	31	32	41	33
Life span (days): all mice	564± 22	627±21	576±18	612±14
tumor-free mice	553± 38	643±26	557±30	626±20
Number of tumor-bearing mice: absolute	17	12	23	15
relative,%	54.6	37.5	56.1	45.5
cumulative,%	83.8	55.2	87.9	71.8
Number of tumors: total	27	14	32	18
per mouse	1.59	1.17	1.39	1.20
Number of mice with tumors of : mammary gland (adenocarcinoma)	13	5	10	6
lung (adenocarcinoma)	1	2	2	2
leukemia	4	3	2	2
other localization	3	3	9	4

Administration of epithalamin to young mice caused significant reduction in spontaneous tumor incidence, first of all because of decrease of mammary tumor incidence - 2.6 fold, $p < 0.05$. (Table 1).

As it is shown in Fig.3, administration of epithalamin shifted curves of tumor-free survival to the right in young mice, whereas in middle-aged group only feebly marked effect of epithalamin treatment was estimated.

In mice treated with epithalamin from the age of 3 months, mammary tumor development was significantly inhibited. This result is also supported by the fact of decrease of number of mammary adenocarcinomas per tumor-bearing

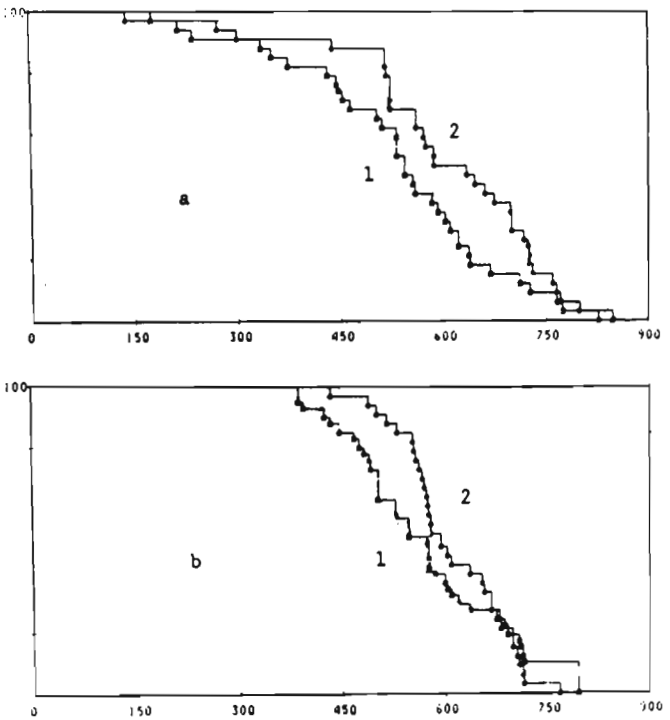

Fig.2. Survival estimation in young (a) and middle-aged (b) SHR mice treated with epithalamin (Kaplan-Meyer method).
OX - age, days: OY - % survived.
1 - saline, 2 - epithalamin.

animal: 1.46 - in control group; 1.20 - in epithalamin treated mice. In middle-aged group, this effect was absent, at the same time the response to epithalamin administration was reduced as compared to young mice (Fig. 3b).

Treatment with epithalamin did not significantly influence any other than mammary gland tumor development. It must be mentioned, however, that a tendency towards the decrease of incidence of leukemias and other tumors in middle-aged animals, treated with epithalamin was observed (Table 1).

Present experiments have shown that a geroprotective effect of epithalamin in SHR mice was less pronounced than in previous experiments with C3H/Sn mice (Anisimov et al., 1982). This may result from both strain peculiarities of the animals used and preparation dosage differences. In the experiments with C3H/Sn mice, epithalamin was administered according to tha same schedule, but a daily dose was 0.5 mg

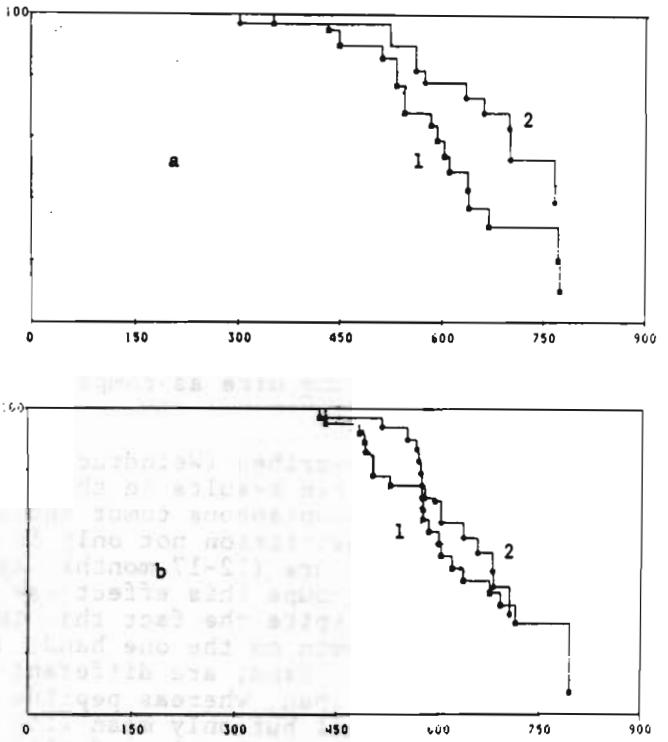

Fig. 3. Time-to-tumor estimation in young (a) and middle-aged (b) SHR mice treated with epithalamin (Kaplan-Meyer method).
OX - age, days; OY - % survived without any tumor. 1 - saline; 2 - epithalamin.

per mouse, providing as a result life span prolongation 31%, whereas in the present experiment in SHR mice, a daily dose was 0.1 mg per animal. In rats, we have previously shown a geroprotective and antitumor effect of epithalamin to be dose dependent (Dilman et al., 1979). It is interesting that epithalamin possesses a geroprotective and antitumor effects when administered both in young and middle age. It is also noteworthy that the mean life span in young and middle-aged treated groups was about the same but the maximal life span was reduced in middle-aged mice. The reasons of this phenomenon are obscure now. One suggestion is the difference in the slope of survival curves (aging rate) in epithalamin treated young and middle-aged mice. Similar regularities of the same relationship between aging rate, mean and maximal life span were summarized elsewhere (Anisimov, 1987). At the same time there is a high correlation between aging rate and tumor incidence in animals untreated or treated with geroprotectors (Anisimov, 1987). These findings seem to serve a good explanation for more pronounced antitumor

effect of epithalamin in young mice as compared to middle-aged ones.

As it was previously described (Weindruch et al., 1982), restriction of dietary caloric results in the increase of life span and decrease of spontaneous tumor incidence after beginning of caloric restriction not only in young (2-3 months) but also in mature (12-17 months) age. However, in mice of older age groups this effect was less evident than in young ones. Despite the fact that the geroprotective effects of epithalamin on the one hand, and caloric restriction on the other hand, are different (the latter increases maximal life span, whereas peptide preparation did not increase maximal but only mean life span) , some mechanisms of geroprotective action of all these influences could be similar.

The mechanisms of geroprotective action of caloric restriction appear to be complex and dependent on slow-down of aging of immune system and neuroendocrine system, in particular on slowering the age-related decrease of functional activity of the pineal gland (Walker et al., 1978) exerting multifactorial modulating influences upon both systems mentioned (Anisimov, 1987, 1988; Reiter, 1988; Gupta, 1988). Previously, it was shown that epithalamin application delays age-associated immunity alterations in mice (Anisimov et al.,1982). This phenomenon may be of importance in the mechanisms of geroprotective and antitumor action of epithalamin. On the one hand, it is noteworthy that epithalamin alongside with immunomodulating effect provides normalizing influence on a series of naturally occuring age-related hormone-metabolic shifts facilitating the development of neoplasia (Anisimov, 1987, 1988).

Besides, it has been shown that a long-term administration of epithalamin delayed the age-related loss of estral function in female rats, restored fertility in old female rats with persistent estrus, and decreases a threshold of sensitivity of hypothalamo-pituitary system to estrogens by feedback inhibition (Dilman et al.,1979; Anisimov,1988).

It is quite probable that this rpoperty of epithalamin causes its multiple effects at the level of different homeostatic systems of the organism and, eventually, defines its geroprotective and antitumor effects, because the increase of threshold of sensitivity of hypothalamus to inhibition seems to play a leading role in the elevation mechanism of aging and formation of age-related pathology including cancer (Dilman, 1981).

We have studied also the influence of epithalamin on the development of tumors induced by different chemical carcinogens (7,12-dimethylbenz(a)anthracene and N-nitroso-ethylurea) and total-body X-ray irradiation. In all these cases carcinogenesis-inhibiting action of the preparation tested was observed (Anisimov, 1987, 1988).

Thus, there is a growing body of evidence indicating on the capacity of low molecular weight peptide factors extracted from the pineal gland to prolong animal's life span, simultaneously inhibiting spontaneous tumor development. These observations provide an evidence supporting a concept on the possibilities of practical application of epithalamin in clinical situations for the prolongation of the period of active life and primary cancer prevention not only in young, but also in mature age. At the same time, the problem of further isolation and characterization of individual active peptides and investigation of their effects on life span and tumor development is an actual task of our current experiments.

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