Effect of Pineal Peptide Hormones on the Functional Activity of the Human Thyroid *in Vitro*. Effect of Pineal Peptides on the Accumulation of Cyclic Nucleotides in the Normal Human Thyroid and Its Secretory Activity

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UDC 616.441-089.873-07:616.831.45-008.6

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol.116, № 11, pp. 521-523, November, 1993
Original article submitted May 25, 1993

Key Words: *pineal gland; pineal peptides; epithalamine; epiphisan; thyroid gland; thyroid-stimulating hormone; thyroid hormones*

In the context of the pineal-thyroid relationships it is mainly the pineal hormones belonging to the group of indolalkylamines which have atracted the attention of scientists. There is no documentation of the regulatory effects of pineal peptides on the thyroid gland (TG), except for the data of Romanian authors published back in the 70s [13,14]. Yet the broad spectrum of biological effects of the latter group of compounds [7-10] and of epithalamine (Ep), a drug synthesized on the basis of them [1,3,5,6], calls for a study of the effect of pineal peptides on the structure and function of the TG.

This study was undertaken to assess the effect of two pineal peptides, Ep and epiphisan (Eph), on the functional activity of human TG *in vitro* and on the realization of the effect of the specific thyroid stimulator, thyroid-stimulating hormone (TSH). The study was performed in two stages: the effect of pineal peptides on the accumulation of cyclic nucleotides and secretory activity of intact human TG was examined in the first stage, after which the same parameters of thyrotoxic TG from diffuse toxic goiter (DTG) patients were analyzed. This report describes the first stage of the study.

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MATERIALS AND METHODS

For the *in vitro* study, fragments of suboperatively obtained TG were washed with saline and preincubated in medium 199 at 37°C for 1 h. Samples weighing 50 mg of structurally and functionally preserved paranodal zone of TG from nodal euthyroid goiter were studied. The tissue was incubated with effector for 20 min for determination of the level of cAMP and cGMP accumulation in the gland and for 1 h for determination of the content of free T_3 and T_4 in the incubate. The content of TG cyclic nucleotides was determined using Czechoslovakian test kits; the content of free T_3 and T_4 in the incubation medium was determined using Amerlex M-Free T_3 and Amerlex M-Free T_4 RIA Kits (Amersham, UK). Test preparations of pineal gland were Ep and Eph. The first is an extract from the pineal gland of cattle not containing substances of indole structure (melatonin, etc.); the second is one of the active peptide components of the pineal gland, exhibiting a pro-

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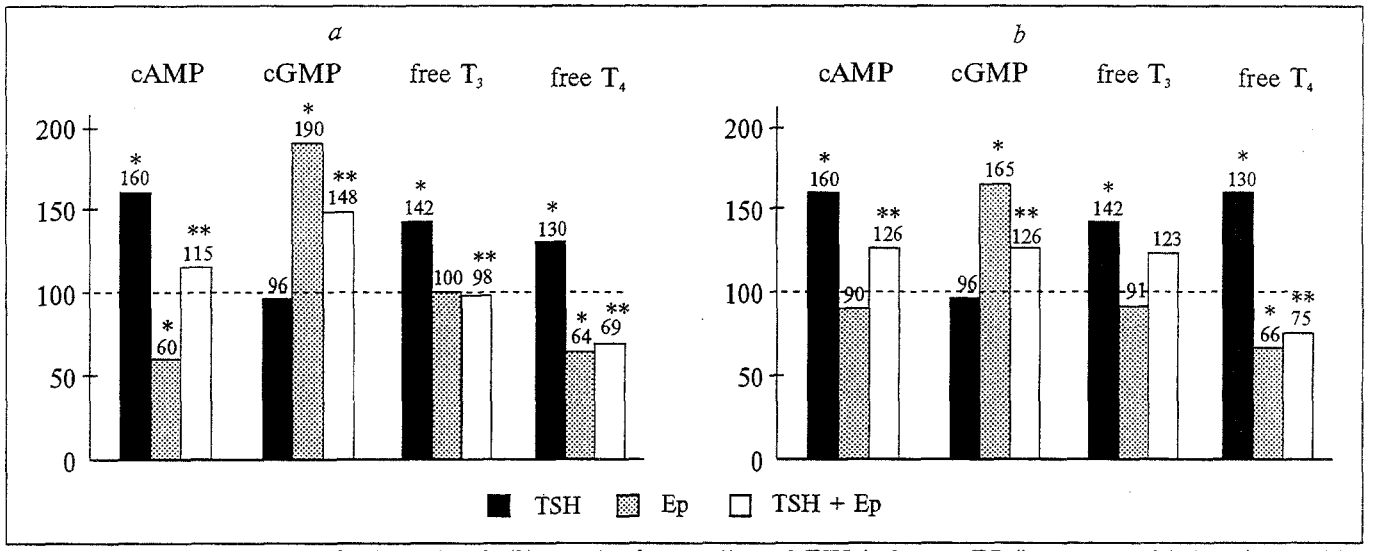

Fig. 1. Modulatory effect of Ep (*a*) and Eph (*b*) on stimulatory effect of TSH in human TG (in percent of indexes). Asterisks mean the reliability of the differences of indexes: * as compared to the control, ** as compared to the results of TSH action.

nounced antigonadal effect [4] (Ep was made available by V. Kh. Khavinson, Military-Medical Academy, St-Petersburg; Eph was supplied by R. I. Kirilenko, Ukhtomskii Institute of Physiology, St. Petersburg University). Both drugs were added to the medium at the beginning of incubation in a dose of 100 μg/ml. The baseline contents of cyclic nucleotides in the TG and of thyroid hormones in the incubate, as well as the modulating capacity of the pineal peptides vis-a-vis the action of TSH added to the medium in a concentration of 100 IU/ml, were determined. The results were processed statistically using the Student *t* test. The graphic characteristics of the normalized values of the independent and modulating effects of Ep and Eph related to TSH action are depicted in Fig. 1.

RESULTS

It is shown in Table 1 that the addition of 100 IU/ml TSH to incubating medium containing fragments of paranodal intact tissue of human TG substantially increased the accumulation of cAMP by the gland but did not affect the accretion of cGMP, so that the cAMP/cGMP ratio rose from 11.0 in the control to 16.4. Simultaneously there was a significant increase of T_3 (from 1.9±1.40 to 19.8±1.85 nmol/ml) and T_4 (from 29.9±2.81 to 36.8±2.87 nmol/ml) release.

The addition of Ep by itself in a concentration of 100 μg/kg to the incubation medium decreased the accumulation of cAMP (nearly 2-fold) and simultaneously stimulated to equal extent the accumulation of cGMP (the cAMP/cGMP ratio dropped to 3.5). The level of T_3 release was not affected by Ep, but the secretion of T_4 decreased by 46%. It may be assumed that Ep inhibits the secretion of T_4, while at the same time its intrathyroid deiodization with the production of T_3 is enhanced (probably adaptively) (Fig. 1).

Combined addition to the incubation medium of Ep and TSH in the concentrations mentioned above significantly offset the effect of TSH in relation to the accumulation of cAMP (from 60 to 15%), and under these conditions the rise of the cGMP content in the TG was also less pronounced than when Ep was administered by itself. Simultaneously Ep blocked the TSH-induced in-

TABLE 1. Effect of Ep and Eph on Functional Activity of Human TG and on Effect of Thyrotropic Stimulation

Effector (addition to incubating medium)	cAMP, pmol/g tissue	cGMP, pmol/g tissue	cAMP/cGMP	free T_3, nmol/ml	free T_4, nmol/ml
Control (n=10)	1100±160	100±17	11.0	13.9±1.40	29.9±2.81
TSH, 0.1 IU (n=11)	1760±109*	96±20	16.4	19.8±1.85*	38.8±1.90*
Ep, 100 μg/ml (n=12)	660±57*	190±16*	3.5	13.9±2.41*	19.2±2.10*
Eph, 100 μg/ml (n=11)	996±88	165±28	6.0	12.7±1.45	19.9±1.97*
Ep+TSH (n=10)	1210±156*,**	148±17*,**	8.2	13.6±1.45	20.9±3.84*,**
Eph+TSH (n=8)	1390±118	156±31*,**	8.9	17.2±1.38	22.5±2.79*,**

Note. Asterisks signify reliability of the differences, * means as compared to control, ** as compared to the results of TSH activation of TG tissue.

crease of T_3 and T_4 secretion: the secretion of T_3 dropped to the control level and of T_4 by 60% on average. So, Ep by itself produces an inhibitory effect on the TG and markedly diminishes the specific effect of TSH.

The study of the effect of Epb6 performed according the same scheme, showed that the addition of Eph to the incubation medium practically did not change the level of cAMP accretion in the TG but significantly increased the content of cGMP, so that the index cAMP/cGMP was 6.0. The content of T_3 in the incubate was not affected by Eph but the level of T_4 fell markedly (by 34%).

The stimulating effect of TSH on the accumulation of cAMP was largely offset by Eph. Against the background of TSH administration the positive effect of Eph on the accretion of cGMP was manifested less strongly and the stimulating effect of TSH on the secretion of thyroid hormones was abolished, as a result of which the content of T_3 was 86% and of T_4 61% of the level achieved with the independent administration of TSH. Comparison of the effects of Ep and Eph revealed that the effects on all studied indexes and the modulating effect on TSH were more pronounced for the first drug than for the second.

Thus, common tendencies are noted in the nature of the effects of Ep and Eph on the TG: a) an inhibitory effect on the secretory activity of the TG; b) a dissimilar effect on the level of cAMP and cGMP accumulation; c) a modulating effect of both drugs in relation to the effect of the specific stimulator of thyroid activity, TSH. It may be assumed that the effects of Ep are to a certain extent mediated by Eph peptide, which belongs to the peptide components of the pineal gland.

Concerning the pathways of realization of the inhibitory effect of pineal peptides on the TG, it is to be especially noted that the effect of TSH on thyroid hormone secretion, both according to published data [2,11,15] and the present findings, is mediated via a cAMP-dependent mechanism. In this connection the modulation of the TSH effect by pineal peptides attests to their participation in this process. However, both Ep and Eph change the level of cGMP accretion when added by themselves to the incubating medium. There is thus reason to assume that the effects of pineal hormones are mediated independently of the cAMP-dependent mechanism. In our opinion, the capacity of Ep and Eph to change the ratios of the intracellular concentrations of cyclic nucleotides may be the key to the modulating effect of these drugs on the functional activity of the TG. It has been noted by other authorities [12] that inhibition of thyroxine release from the TG may be related to hydrolysis of membrane phosphoinositides and hence to the production of inositol phosphates as secondary messengers. Although these scientists studied another group of compounds, it is not to be ruled out that this mechanism has a direct bearing on the effect of pineal peptides on the thyroid.

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