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 Thyrotoxic Thyroid and Its Secretory Activity

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In our preceding study we confirmed the inhibitory effect of pineal peptides (the drugs epithalamine, epiphisan) on the functional activity of suboperatively obtained intact tissue of human thyroid gland (TG) *in vitro*. Since the development of diffuse toxic goiter (DTG) with signs of thyrotoxicosis changes the reactivity of the TG to regulatory influences [1,2], it seemed interesting to define its reaction to pineal peptide hormones. If the inhibitory effect of the peptides were to be preserved in relation to the thyrotoxic TG, the drugs mentioned above might be useful in therapy.

The previous results [3] showed that in some patients with DTG the dependence of the TG on the specific thyroid-stimulating effect ("TSH-sensitive gland") is preserved, while in other cases TG "escapes" the thyroid-stimulating effects ("TSH-refractory gland"). The difference in reaction to the basic regulator of thyroid function requires separate determination of the nature of the effect of pineal peptides on both types of thyroid gland.

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MATERIALS AND METHODS

Suboperatively obtained tissue of human thyrotoxic TG was taken for *in vitro* study. The sensitivity of the TG tissue to the addition of 100 IU/ml TSH to the incubation medium was examined before determination of its reaction to the administration of epithalamine (Ep), a water extract of cattle pineal glands. Samples of the gland weighing 50 mg were washed with saline and preincubated without effector in medium 199 at 37°C for 1 h. Incubation with effector was performed for 20 min to determine the level of cAMP and cGMP accumulation in the gland using Czechoslovakian test kits and for 1 h to determine the content of free T_3 and T_4 in the incubate with Amerlex M-Free T_3 and T_4 kits (Amersham, UK). Paranodal intact tissue of TG from patients with euthyroid nodal goiter was used as a control. Epithalamine was added to the incubation medium alone and with TSH in a dose of 100 μg/ml.

RESULTS

The addition of Ep to medium containing the samples of intact human TG led to a marked

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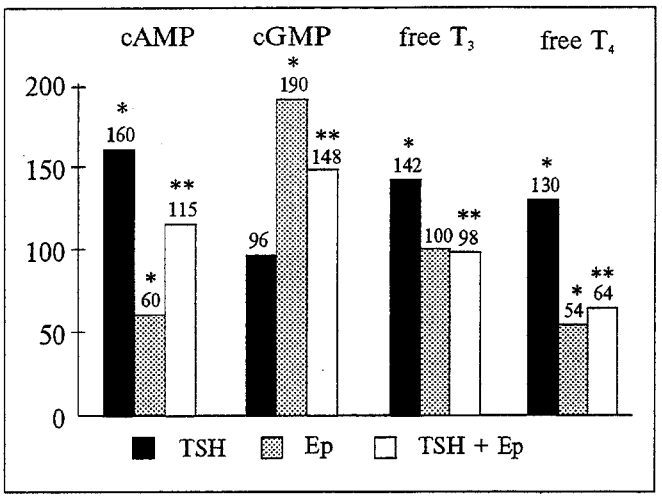

Fig. 1. Effect of separate and combined addition of TSH and Ep to the incubation medium on the level of cyclic nucleotides and thyroid hormones in incubate containing samples of intact tissue of human TG (in % of indexes). Here and in Fig. 2. Asterisks signify the reliability of the differences: * as compared with control; ** as compared with the results of TSH action.

decrease of cAMP accumulation and to a synchronous rise of cGMP accretion in the gland, with the result that the cAMP/cGMP index dropped from 11.0 to 3.5 (Table 1, Figs. 1 and 2). The level of T_3 release in the incubate did not change, but the secretion of T_4 decreased significantly (from 29.9±2.81 to 19.2±2.10 nmol/ml). There was a marked elevation of cAMP accretion induced by TSH with no changes of the cGMP level in the TG, while the release of T_3 and T_4 was significantly increased (more than 1.5-fold). When Ep and TSH were injected into the incubation medium synchronously, the capacity of TSH to increase cAMP accumulation was blocked, the release of T_3 normalized, and the content of T_4 corresponded to the indexes obtained with the administration of Ep alone.

Analysis of the data on the thyrotoxic TG with a normal reaction to TSH showed that the level of cyclic nucleotides in the TG corresponded to that in the intact TG, while the level of free thyroid hormones in the incubate was even lowered (T_3 by 17% and T_4 by 37%). This seemingly paradoxical situation may be explained by the absence of a stimulating thyrotropic effect *in vitro*, as was confirmed by results of adding TSH to the incubation medium, whereupon all analyzed indexes rose, though to different degrees. This was particularly evident in relation to the release of T_4, which increased from 19.6±1.88 to 69.5±4.49 nmol/ml; the secretion of T_3 increased to a lesser degree (from 11.6±1.43 to 17.9±1.48 nmol/ml). The content of cyclic nucleotides in the TG increased due to TSH by 45% on average.

However, the drop of the level of secretion of thyroid hormones as compared to the intact TG (in the latter case there is no effect of TSH on samples of resected gland) cannot be due just to the absence of thyroid stimulation. The decisive role in the case of the thyrotoxic gland is probably played by the absence in the incubation medium of thyroid-stimulating immunoglobulins, which are the regulators of thyroid activity in DTG patients *in vivo*. Comparison of the indexes of secretory activity of the TSH-sensitive TG and of its capacity to accrete cyclic nucleotides attested to the fact that the rise of T_4 secretion in the

TABLE 1. Effect of Separate and Combined Addition of TSH and Ep to Incubation Medium on Level of Cyclic Nucleotides in TG and Thyroid Hormones in Incubate Containing Samples of Normal and Thyrotoxic Human TG

Sample of TG tissue and effector	Intact TG				Thyrotoxic TG							
					TSH-sensitive TG				TSH-refractory TG			
	cAMP, pM/g	cGMP, pM/g	Free T_3, nmol/ml	Free T_4, nmol/ml	cAMP, pM/g	cGMP, pM/g	Free T_3, nmol/ml	Free T_4, nmol/ml	cAMP, pM/g	cGMP, pM/g	Free T_3, nmol/ml	Free T_4, nmol/ml
Control (n=11)	1100 ±160	100 ±17	13.9 ±1.40	29.9 ±2.81	1080 ±113	95 ±11	11.6 ±1.43	19.1 ±1.88	2064 ±285	160 ±18	29.6 ±2.71	37.1 ±3.48
Ep, 100 mg/ml (n=10)	660 ±57*	190 ±16	13.9 ±2.41	19.2 ±2.10*	885 ±116	154 ±21*	8.5 ±0.94*	14.7 ±1.01*	2270 ±248	182 ±19	29.8 ±3.61	37.4 ±4.85
TSH, 100 IU/ml (n=12)	1760 ±109*	96 ±20	19.8 ±1.85*	38.8 ±1.90*	1564 ±121*	138 ±14*	17.9 ±1.48	69.5 ±4.49**	2167 ±270	144 ±18	27.5 ±2.85	33.4 ±3.40
TSH + Ep, 100 mg/ml (n=10)	1210 ±156	148 ±17	13.6 ±1.45 ***	20.9 ±3.84 ***	1231 ±203	119 ±42	7.7 ±0.85	14.9 ±0.95 **, ***	2477 ±370	130 ±48	29.7 ±3.58	37.3 ±3.72

Note. One asterisk signifies the reliability of differences compared with the control; two asterisks signify a high reliability of differences compared with the control; three asterisks signify the reliability of differences compared with TSH alone.

studied case is mediated not solely via a cAMP-dependent mechanism.

Addition of Ep alone to the incubation medium insignificantly lowered the accumulation of cAMP but increased 1.5-fold the accretion of cGMP by the gland, resulting in a drop of the cAMP/cGMP index to 5.7. The release of thyroid hormones into the incubation medium was markedly lowered (T_3 to 8.5±0.94 nmol/ml and T_4 to 14.7±1.0 nmol/ml). Combined administration of Ep and TSH decreased the accretion of cyclic nucleotides in the TG (cAMP by 31% and cGMP by 20%), but these results were statistically insignificant as compared to those obtained when TSH was administered alone due to the large scatter of individual values. On the other hand, the level of T_3 secretion was 3.5-fold lower than under the effect of TSH and the level of T_4 dropped more than 4-fold.

Thus, the mechanisms providing for the secretory activity of the thyrotoxic TG reacting to TSH were more sensitive than in the normal case both to stimulating and to inhibiting effects, and the modulating effect of Ep was strongly marked.

The baseline values of the functional activity of the thyrotoxic TG refractory to the action of TSH were significantly increased as compared both to the control data (intact TG) and to the results of the study of the TSH-sensitive TG, namely, the level of cAMP in the TSH-refractory gland was twice as high as in other cases, the content of cGMP was 1.5 times higher, the level of T_3 in the incubate rose to 29.6±2.71, and the level of T_4 rose to 37.1±3.48 nmol/ml (in the incubation medium containing the samples of TSH-sensitive TG these indexes were 11.6±1.43 and 19.1±1.88 nmol/ml, respectively). The data presented pointed to marked hyperfunction of the TG.

We have already noted that in patients with DTG thyroid-stimulating immunoglobulins begin to play the leading role in TG regulation [4,6], as is particularly obvious under conditions of independence of the gland of the TSH effect. Indeed, there was no change of the studied indexes in response to the addition of TSH to the incubation medium, and the fact that the TG samples preserved a high level of activity may testify to a certain autonomy of its functioning based on autoregulation and, perhaps, the effect of other stimulators, notably intrathyroid biogenic amines deposited in the sympathetic terminals, prostaglandins, etc., which seem to preserve their effect on the TSH-refractory gland as well [1,5].

The separate administration of Ep, like the combined administration of Ep and TSH, did not cause reactions of the TSH-refractory gland (the variations of all analyzed indexes did not exceed 10% and were insignificant). Hence, the thyrotoxic gland refractory to the effect of TSH appears not to be sensitive to the action of Ep either.

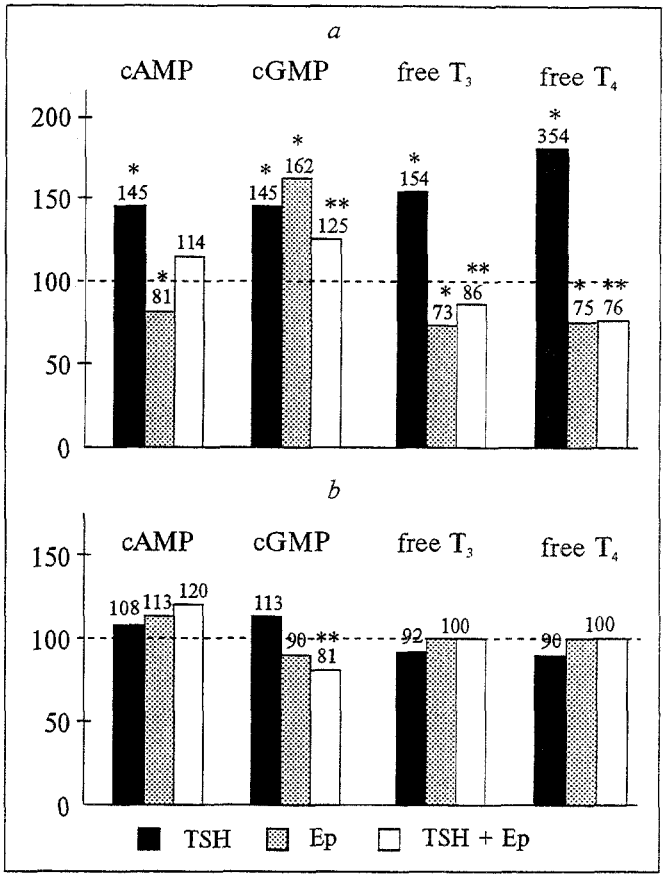

Fig. 2. Effect of separate and combined addition of TSH and Ep to incubation medium on the level of cyclic nucleotides and thyroid hormones in incubate containing samples of TSH–sensitive (*a*) and TSH–refractory (*b*) human TG in % of indexes (thyrotoxic gland).

We showed previously that the TSH-refractory TG does not react to the introduction of another pineal hormone, melatonin, which produced a strongly marked inhibitory effect on the intact and TSH-sensitive thyrotoxic gland [7]. It may thus be logically assumed that the antithyroid effect of pineal hormones is mainly modulatory, directed toward correcting the effect of thyroid stimulators.

The use of Ep in the treatment of the patients with DTG as an inhibitor of thyroid function may be promising only in a case where the dependence of the TG on thyroid-stimulating effects is preserved.

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Effect of 3-Mercaptopropionic Acid on the Toxicity of GABA-Lytics in Mice

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The substances which produce an effect on the GABA level in the brain are known to alter the toxicity of GABA-lytics [3,9,13]. An increase in the level of amino acid due to inhibition of its biodegradation goes along with a decrease in the toxicity of poisons [4,12]. On the other hand, the effect of inhibitors of GABA synthesis on the sensitivity of animals to GABA-lytics has been insufficiently studied.

In this study we assessed the effects of 3-mercaptopropionic acid (3-MPA), a catalyst of reversible inhibition of glutamate decarboxylase (GD), on the toxicity of bicuculline (BC) and picrotoxin (PT) in albino mice.

MATERIALS AND METHODS

The experiments were carried out on male albino mice weighing 18-20 g. PT and BC were suspended in physiological saline using TWEEN-80. 3-MPA was dissolved in saline and injected 20, 10, and 5 min ahead of the GABA-lytics or simultaneously with them, in a dose of 14 mg/kg (0.5 LD_{50}), which does not provoke seizures in the animals. All the preparations used in the study were purchased from Sigma (USA). The substances were intraperitoneally administered in a volume of 0.2 ml of solution per 10 g animal weight. For assessment of the toxicity no less than 5 doses were used and no less than 6 animals for each dose. The LD_{50} values were calculated using regression analysis by the method of least squares.

We studied the effect of 3-MPA (10^{-9}-10^{-4} M) on the specific binding of ^{3}H-tertbutylbicycloorthobenzoate (TBOB; Amersham, England; 1.1

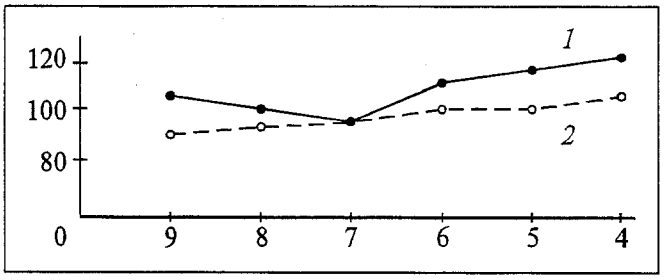

Fig. 1. Effect of 3-MPA on ^{3}H-TBOB (5 nM) and ^{3}H-GABA (100 nM) binding by synaptic membranes of the brain in intact mice. Abscissa: 3-MPA concentration, log (M); ordinate: binding of ^{3}H-GABA (*1*) and ^{3}H-TBOB (*2*), % of control. Binding of ligands in the control: 145±18 fmol/mg protein for ^{3}H-TBOB and 750±64 fmol/mg protein for ^{3}H-GABA.

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