The observed rise in the blood CO level during repeated immobilizations may thus be included among the list of tests providing information about lasting disturbances of metabolism of connective-tissue biopolymers during stress.

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INTERACTION OF PROSTAGLANDINS E_2 WITH RECEPTORS AND THEIR EFFECT ON ADENYLATE CYCLASE ACTIVITY IN HUMAN THYROID GLAND TISSUE

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Prostaglandins of the E_2 group (PGE_2) have an action on thyroid gland cells similar to the stimulating effect of thyrotrophic hormones (TSH) [5, 13]. The writers previously determined the kinetic characteristics of specific binding of TSH and PGE_2 with isolated cells of euthyroid tissue of the human thyroid gland (TG) [1] and studied the dynamics of changes in intracellular PGE_2 and cyclic nucleotide levels at the initiating stage of transmission of the thyrotrophic signal [2]. It could be concluded from the results that PGE_2 play a modulating role in the realization of the effect of TSH on target cells in the normal human TG. However, the role of these compounds under pathological conditions and, in particular, in thyrotoxicosis, has been studied extremely inadequately. We know that TG may be to some degree outside the control of TSH. Whether under these circumstances PGE_2 retain their stimulating effect on thyroid function is not known, but some workers regard these compounds as directly connected with the development of thyrotoxicosis [10, 15].

The aim of this paper was to compare parameters of ligand-binding capacity and affinity of receptors for PGE_2, the basal level of PGE_2, and the effect of PGE_2 on adenylate cyclase activity in euthyroid and thyrotoxic tissue of the human TG.

EXPERIMENTAL METHOD

Histologically unchanged paranodal TG tissue, obtained at operations on patients with modular euthyroid goiter, and thyroid tissue from patients with diffuse toxic goiter (thyrotoxicosis) were used. Isolated thyrocytes were obtained by the method in [7]. PG-receptor interaction was studied by the use of ^3H-PGE_2, with specific activity of 150 Ci/mmole (England), by the method described previously [1]. Affinity constants and ligand-binding capacities of the receptors were determined by the method in [6]. Preliminary chromatographic

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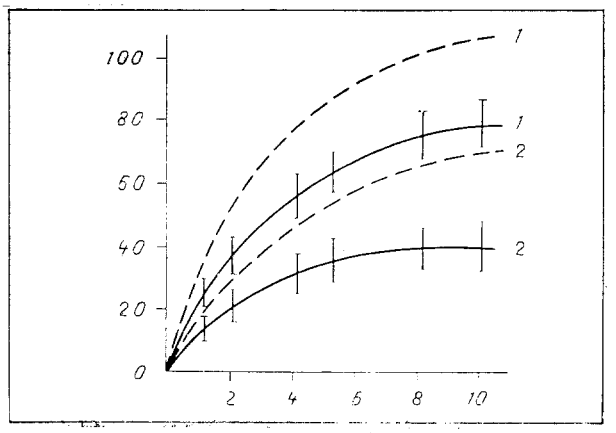

Fig. 1. Binding of ^{3}H-PGE$_2$ by isolated human thyrocytes. Abscissa, concentration of ^{3}H-PGE$_2$ (ub nm/10^9 cells); ordinate, binding of ^{3}H-PGE$_2$ (in %). Broken line — total binding of ^{3}H-PGE$_2$; contiuous line — specific binding of ^{3}H-PGE$_2$. 1) TG tissue from patient with nodular euthyroid goiter; 2) TG tissue from patient with thyrotoxicosis.

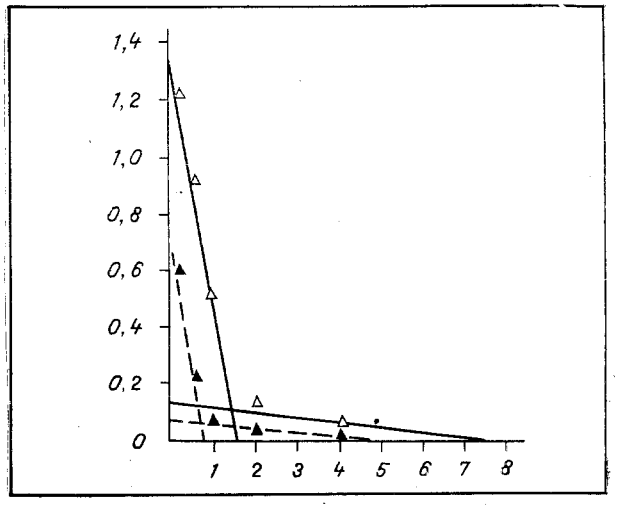

Fig. 2. Scatchard plot of specific binding of ^{3}H-PGE$_2$ with isolated human thyrocytes. Abscissa concentration of bound ^{3}H-PGE$_2$ (in $M^{-11}/10^9$ cells); ordinate, ratio of concentration of bound and free ^{3}H-PGE$_2$. Continuous line represents TG tissue from patients with nodular euthyroid goiter; broken line — TG tissue from patient with thyrotoxicosis.

TABLE 1. Number of PGE_2-Binding Sites and Their Kinetic Characteristics in Euthyroid and Thyrotoxic Human TG Tissue

Diagnosis	Number of receptors per cell		Affinity constants of receptors for PGE_2	
	with high affinity	with low affinity	with high affinity 10^{-10} M^{-1}	with low affinity 10^{-19} M^{-1}
Nodular euthyroid goiter ($n = 15$)	9500±840	46 800±9700	8,2±1,6	1,8±0,2
Diffuse toxic goiter ($n = 11$)	4800±350 <0,01	22 600±3700 <0,01	8,4±1,2 <0,05	1,9±0,3 <0,05

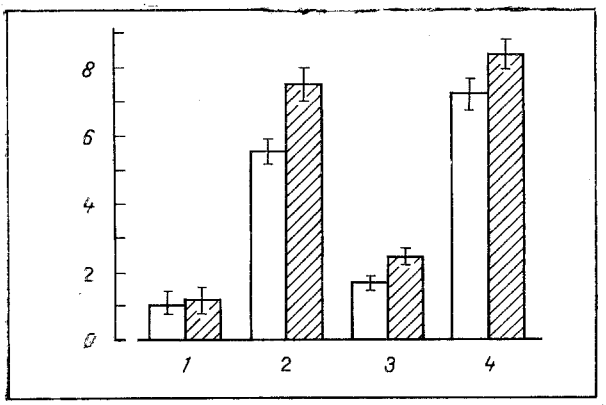

Fig. 3. Degree of activation of adenylate cyclase of euthyroid (unshaded columns) and thyrotoxic (shaded columns) human TG tissue: 1) basal adenylate cyclase activity; 2) GIDP; 3) PGE_2; 4) GIDP + PGE_2.

fractionation of PG was carried out by the method in [4]. The PGE_2 concentration was determined by radioimmunoassay, using kits from Clinical Assays Inc. (USA). Adenylate cyclase activity was determined in unpurified preparations of membrane from human TG tissue, isolated by the method in [11]. To measure adenylate cyclase activity, samples were incubated at 37°C in medium containing 50 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 0.2 mM EDTA, 1 mM 3'5'-AMP, 0.1 mM ATP, (α^{32}P)-ATP (0.2-0.4) × 10^6 cmp, 20 mM creatine phosphate, and 0.5 mg/ml creatine kinase. The volume of incubation medium was 50 µl. The reaction was started by addition of the membrane enzyme preparation (30-40 µg) and it was stopped after 20 min by addition of 200 µl of 0.5 M HCl. The quantity of (α^{32}P-3'5')-AMP formed was determined by the method in [14]. For this purpose the samples were placed in a boiling water bath for 6 min and then neutralized with 200 µl of 1.5 M imidazole and applied on columns filled with neutral alumina. The 3'5'-AMP was eluted with 5 ml of water into scintillation flasks. Radioactivity was measured in a scintillation counter, using the Cherenkov effect. The yield of (α^{32}P-3'5')-AMP was determined with the aid of (^{3}H-3'5')-AMP. For this purpose, instead of incubation medium, 50 µl of a solution containing (^{3}H-3'5')-AMP was added to each tube (0.02-0.03 µCi per sample). After boiling in hydrochloric acid followed by neutralization with imidazole, the contents of the tube were applied to columns with alumina and eluted with water. The volume of eluate was adjusted to 5 ml, and aliquots of 0.5 ml were taken and introduced into flasks each containing 10 ml of ZhS-8 scintillation fluid. When adenylate cyclase activity was calculated a correction was introduced for the yield of 3'5'-AMP, which was constant for each batch of alumina, and lay between 60 and 95%. The protein concentration in the membrane adenylate cyclase preparation was determined by Lowry's method [9], after preliminary destruction of the membranes with a 1% solution of sodium deoxycholate. The following reagents were used: (α^{32}P)-ATP and ^{3}H-3'5'-AMP were from Amersham Corporation (England), guanyl-5'-imidodiphosphate (GIDP) was from Boehringer (West Germany), GTP, creatine phosphate, alumina, and imidazole were from Reanal (Hungary), ATP, 3', 5'-AMP, and Tris were from Sigma (USA). The remaining reagents were of Soviet origin (Soyuzreaktiv and Reakhim) and were of the chemically pure and high purity grades.

EXPERIMENTAL RESULTS

The results of investigation of specific binding of ^{3}H-PGE_2 by isolated cells of euthyroid (n = 15) and thyrotoxic (n = 11) tissue are given in Fig. 1. In both cases, incidentally, the formation of a PG-receptor complex was a saturable process and depended on the concentration of hormone present in the incubation mixture, but in thyrotoxic tissue binding of ^{3}H-PGE_2 was significantly depressed and amounted to 52% of the quantity of PGE_2 bonnd with isolated thyrocytes of euthyroid tissue.

For a more detailed comparative analysis we determined the principal kinetic characteristics of the processes under investigation (Fig. 2). The descending nonlinear character of the Scatchard plot suggested the presence of binding sites in both euthyroid and thyrotoxic tissue with high affinity and with low receptor capacity, as well as binding sites which evidently had lower affinity, but high receptor capacity, and which were difficult to saturate. Assuming that one molecule of ligand binds with one receptor, the number of type 1 receptors for euthyroid tissue was on average 9500 per cell and for thyrotoxic tissue 4800 per cell, and the number of type 2 receptors was 46,800 and 22,600 respectively. Affinity constants of the receptors for PGE_2 were virtually identical in both cases (Table 1). It could be concluded from these results that the number of free receptors for PGE_2 in thyrotoxic tissue is reduced; the decrease in the pool of free receptors for ^{3}H-PGE_2 under these circumstances could be the result of increasing formation of PGE_2-receptor complexes as a result of an increase in the concentration of endogenous PGE_2 in the thyrotoxic tissue.

This hypothesis required testing. For this purpose, the basal PGE_2 level in euthyroid (n = 14) and thyrotoxic (n = 15) tissue was determined by radioimmunoassay. In euthyroid tissue it was 7.7 ± 0.8 ng/g tissue, and it was almost 1.5 times lower than the basal PGE_2 level in thyrotoxic tissue (11.4 ± 1.3 mg/g tissue). Consequently, in thyrotoxic tissue the endogenous PGE_2 concentration was in fact significantly increased. Incidentally, a considerable rise in the PGE_2 level was discovered previously in [15], but in that case the venous-arterial concentration gradient of these compounds was determined, and not their concentration in the thyroid tissue of the gland.

Binding of hormone with receptor is known to be an essential but insufficient condition for manifestation of the hormonal effect. For instance, in the case of binding of PG with cytoplasmic receptors an essential condition is activation of adenylate cyclase. It has recently been shown that hormonal stimulation of adenylate cyclase depends on binding of GTP to the regulatory site of the enzyme, and unhydrolyzable analogs of GTP, such as GIDP, are powerful and stable activators of adenylate cyclase [3, 8].

In the course of these investigations we determined the basal and GIDP- and PGE_2-stimulated adenylate cyclase activity in euthyroid and thyrotoxic human TG tissue. Basal adenylate cyclase activity in the tissues studied was virtually identical, and averaged 0.93 pmole of 3',5'-AMP (euthyroid tissue) and 0.99 pmole of 3',5'-AMP/mg protein (thyrotoxic tissue). However, the degree of activation of the enzyme by GIDP was significantly greater in thyrotoxic tissue than in euthyroid (Fig. 3). In the first case, activity of the enzyme increased 7.5-fold under the influence of 10^{-4} M GIDP, but in the second case the increase was 5.6-fold. Consequently, thyrotoxic tissue was much more sensitive to the stimulating action of GIDP than euthyroid tissue. PGE_2 (10^{-6} M) had an independent action on adenylate cyclase activity both in preparations of unchanged TG tissue and in thyrotoxic tissue. After simultaneous addition of GIDP and PGE_2 to the incubation mixture, their combined effect was significantly stronger than the independent effect of GIDP and much stronger than the stimulating action of PGE_2 alone. In thyrotoxic tissue, the sensitivity of adenylate cyclase to the simultaneous stimulating effect of GIDP and PGE_2 was enhanced.

In thyrotoxic tissue of the human TG a decrease in the pool of free receptors for ^{3}H-PGE_2 was thus observed, and it was due to increased formation of receptor complexes with endogenous PGE_2, the number of which in thyrotoxic tissue was significantly greater than in euthyroid tissue.

PGE_2 and GIDP have a marked stimulating effect on adenylate cyclase activity in both euthyroid and thyrotoxic tissue; in the latter case, moreover, the sensitivity of the enzyme to these stimulating effects is increased.

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EFFECT OF ETHANOL ON DNA-POLYMERASE ACTIVITY IN SUBCELLULAR FRACTIONS OF THE LIVER OF ADULT AND OLD RATS

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KEY WORDS: DNA-polymerase activity; aging; liver; subcellular fractions; ethanol

One possible cause of the slowing of replication of nuclear and mitochondrial DNA during aging [7, 8, 10] may be an age-linked change in the multienzymic DNA replication complex. The enzymes of this complex which have been characterized most completely are the DNA-polymerases. Investigations have shown changes in the activity of these enzymes during aging [6], a decrease in the accuracy of the synthesis performed by them [9], and also an increase in the thermolability of their molecules [2].

However, the question whether sensitivity of DNA-polymerases to the specific inhibitors of their activity changes in old age and, if so, whether the age change in this sensitivity depends on the subcellular localization of the enzymes, has not yet been adequately studied.

In the present investigation ethanol was used as the inhibitor. In certain concentrations ethanol is known to inhibit activity of α- and β-DNA-polymerases (above 5% for α-DNA-polymerase [4] and above 20% for β-DNA-polymerase [3]). In this case, the concentration of ethanol chosen was 5%, in which it has no appreciable inhibitory action on DNA-polymerase activity in adult animals (according to data in the literature), but can inhibit (threshold concentration) α-DNA-polymerase. It was assumed that enzyme activity in old animals will be modified by ethanol in a concentration lower than the threshold level for adult animals.

The effect of 5% ethanol on DNA-polymerase activity of subcellular fractions (nuclei, mitochondria, microsomes, cytosol) of the intact and regenerating liver was studied in adult and old rats.

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