BINDING OF PROSTAGLANDINS E BY PLASMA MEMBRANES OF THYROID CELLS

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The ability of the plasma membranes of human and bovine thyrocytes to bind prostaglandins of the E group (PGE) was investigated by the use of material from kits for the radioimmunological determination of PGE. Sites for specific binding of PGE with high and low affinity constants for prostaglandins were found to exist on the plasma membranes of human and bovine thyrocytes. Binding of PGE by the membrane is under the influence of thyrotrophin and cyclic nucleotides.

KEY WORDS: prostaglandins; plasma membrane; thyroid gland, cyclic nucleotides.

Prostaglandins of the E group (PGE) are known to take part in the mechanism of the stimulating action of thyrotrophin on the thyroid gland [3, 4, 12]. Meanwhile the problem of the stage of this process at which PGE participates has not yet been finally settled. It is suggested [5, 6] that PGE are intermediaries between the receptor and effector part of the adenylate cyclase system of the cell. However, investigations of prostaglandin synthesis with the use of inhibitors have yielded conflicting data on relations between thyrotrophin, PGE, and cyclic adenosine-3',5'-monophosphate (cyclic AMP) [1, 7, 11]. The possibility cannot be ruled out that stimulation of PGE synthesis in thyroid cells is mediated by cyclic AMP, and that, like the effect of thyrotrophin, the PGE exert their stimulating action through the plasma membranes, on which binding sites for PGE are located. It has been shown [8] that PGE-^{3}H are bound by the bovine thyroid membrane and that the affinity constants for this process is quite high (2.6×10^8 M^{-1}). Unfortunately, the method suggested by the authors cited cannot be used for research on the human thyroid gland, for a large quantity of thyroid tissue is needed.

The object of the present investigation was to study the possibility of specific binding of PGE by plasma membranes of human as well as bovine thyroid cells.

EXPERIMENTAL METHOD

Histologically unchanged thyroid gland tissue was taken from patients with nodular goiter after subtotal thyroidectomy and, together with bovine thyroid tissue, was used for the investigation. The tissues were kept at −20°C. The plasma membranes of the thyroid cells were obtained by Neville's method in the modification of Wolff and Jones [10]. The protein concentration in the membranes was determined by Lowry's method. To study the binding of PGE with the membranes, materials from the kit for radioimmunological determination of prostaglandins, from Clinical Assay Inc., USA, were used (PGE-^{3}H, standard PGE, normal rabbit serum, goat antirabbit serum, isogel, Tris-buffer).

Different quantities (from 40 to 7500 ng protein in 100 μl buffer) were added to test tubes with solution containing 1000 μl buffer with 5 mM Ca^{++} (pH 7.2) and 50 μl labeled PGE (specific activity 10 μCi/liter). The mixture was thoroughly shaken and incubated for 1 h at 37°C. Next, 100 μl rabbit serum and 100 μl goat antirabbit serum were added and the solution was again incubated (18-20 h, 4°C). After incubation the mixture was centrifuged (1600g, 4°C, 30 min). The supernatant was removed and the residue dissolved in 1 ml 0.1M NaOH, after which radioactivity was counted in an "Isocap-300" liquid scintillation system (Nuclear Chicago, USA).

To determine the degree of specific binding of PGE-^{3}H with the plasma membrane, different quantities of unlabeled PGE (from 8.2 to 2000 pg) or of one of the following substances – oleic acid (5 μM), thyrotrophin (0.09, 0.9, or 9 units ml), corticotrophin (2 μM), cyclic AMP (0.5 μM), cyclic GMP (0.1 μM) – were added to the solution before the first incubation.

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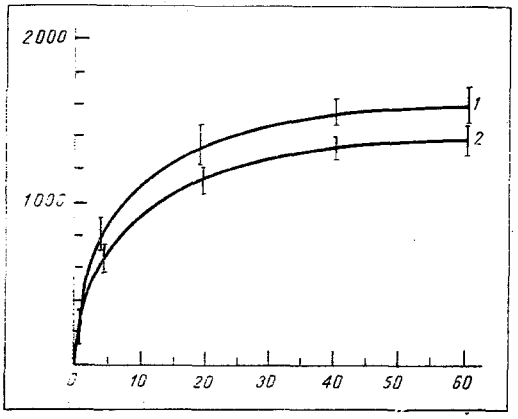

Fig. 1. Binding of PGE-^{3}H by human (1) and bovine (2) thyrocyte membranes. Abscissa, protein content (in μg); ordinate, radioactivity (in cpm).

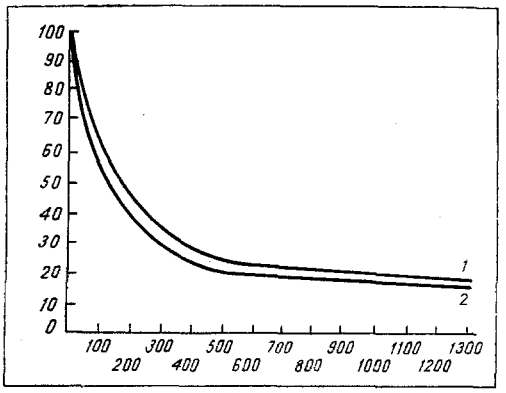

Fig. 2. Effect of various concentrations of PGE on formation of labeled PGE–membrane complex for bovine (1) and human (2) thyrocytes. Abscissa, quantity of PGE (in pg) added to membrane (40 μg protein/ml); ordinate, binding of PGE-^{3}H (in %).

TABLE 1. Effect of Thyrotrophin, Corticotrophin, Oleic Acid, and Cyclic Nucleotides on Character of Binding of PGE-^{3}H with Human and Bovine Thyrocyte Membranes (M ± m)

Substance added	Human thyrocyte membranes		Bovine thyrocyte membranes	
	radioactivity of residue, cpm (n=8)	binding, %	radioactivity of residue, cpm (n = 5)	binding, %
Control	1700±83 (8)	100	1215±108	100
Thyrotrophin in doses of:				
0,09 units/ml	1616±104	95,0	1010±84	83,1
0,9 units/ml	1000±80	59,0*	700±115	57,6*
9 units/ml	460±75	27,0*	470±56	39,0*
Corticotrophin (2 μM)	1640±116	96,5	1080±96	88,9
Oleic acid (5 μM)	1600±150	94,1	1185±173	96,0
Cyclic AMP (0.5 μM)	720±44	42,4*	590±36	48,5*
Cyclic GMP (0.1 μM)	700±100	41,2*	1040±87	

Legend. n) Number of experiments, *) values for which $P < 0.05$ compared with control.

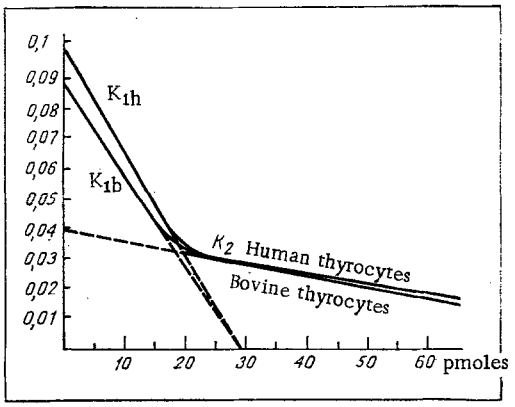

Fig. 3. Scatchard plot for determining affinity constant of PGE for thyrocyte membrane. Abscissa, quantity of PGE-^{3}H (in pmoles) bound by 1 g membrane protein; ordinate, ratio of bound to unbound PGE-^{3}H. K_{1h}) High affinity constant of PGE for human thyrocyte membrane; K_{1b}) high affinity constant of PGE for human and bovine thyrocyte membrane.

The affinity constant of PGE for the membrane was determined by calculating the regression coefficient using data of a Scatchard plot [2, 9].

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the radioactivity of the precipitate formed was clearly dependent on the quantity of membrane suspension (in μg protein) added to the incubation medium. Bovine thyroid gland membrane was found to bind PGE rather less strongly than human thyrocyte membrane. On incubation of human and animal cell membranes (with a protein concentration of 4000 ng/100 μl) with PGE this difference was significant ($P < 0.05$).

The use of different doses of unlabeled PGE in the reaction led to the discovery that the binding of PGE-^{3}H by the membrane decreased with an increase in the concentration of prostaglandins present in the incubation medium (Fig. 2). Analysis of dependence of the ratio between bound and unbound labeled PGE and the total quantity of prostaglandins-^{3}H bound by 1 μg protein revealed two constants of affinity of PGE for combining sites on the plasma membrane of the human and bovine thyrocytes at pH 7.2: a constant of a high degree of affinity (6.0×10^9 M^{-1} for human thyrocytes and 4.4×10^9 M^{-1} for bovine thyrocytes) and a constant of a low degree of affinity (1.6×10^8 M^{-1} for human and bovine thyrocytes). The quantity of bound prostaglandins, determined by the extrapolation method on a Scatchard plot, was 27.5 pmoles/g membrane protein for the high affinity constant and 170 pmoles/g membrane protein for the low affinity constant (Fig. 3). These results do not agree with those obtained by Moore and Wolff [8], who determined one constant of affinity of prostaglandins for bovine thyrocyte plasma membrane at pH 7.0 (2.6×10^8 M^{-1}).

It will be clear from Table 1 that oleic acid (a compound of the same class as prostaglandins) and corticotrophin had no significant effect on the percentage binding of PGE with the membrane. Meanwhile, thyrotrophin considerably altered the character of binding of PGE, for the quantity of PGE–membrane complex formed was reduced depending on the dose of hormone added. Cyclic AMP also reduced binding of PGE. The addition of cyclic GMP, on the other hand, affected binding of prostaglandins with the membrane of the human, but not of the bovine gland.

Specific binding sites for PGE are thus present in the plasma membranes of human and bovine thyrocytes. Thyrotrophin and cyclic nucleotides affect the binding of prostaglandins with the membrane. These results confirm that the physiological role of PGE in the thyroid gland is not one of mediator between thyrotrophin and the effector part of the adenylate cyclase system.

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EFFECT OF $N^6O^{2'}$-DIBUTYRYL-CYCLIC ADENOSINE-3',5'-MONOPHOSPHATE ON PROTEIN SYNTHESIS IN THE SUBESOPHAGEAL GANGLIONIC COMPLEX OF Helix pomatia

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Electrophoretic fractionation of proteins of the subesophageal ganglionic complex of Helix pomatia showed that the relative electrophoretic mobility of most proteins is 0.2-0.6. On incubation of the subesophageal ganglionic complex with L-leucine-4,5-^{3}H for 3, 6, and 24 h the mean relative radioactivity of the neurospecific proteins increased. Dibutyryl-cyclic AMP ($2 \cdot 10^{-6}$ M) was shown to inhibit the processing of low-molecular-weight neurospecific proteins.

KEY WORDS: Dibutyryl-cyclic AMP; specific proteins of neurons; processing.

It has been shown in the last decade that the nerve tissue of vertebrates and invertebrates contains a number of unique proteins that are not found in the other organs of these animals [5, 9]. In particular, Peng Loh and Gainer [6, 7] found such proteins in the mollusk Aplysia californica. The specific low-molecular-weight proteins of the neurons of this animal were found to be split into fragments of lower molecular weight [7] during transport along the axon.

It was decided to study how dibutyryl-cyclic AMP affects the processing of the neurospecific low-molecular-weight proteins of the mollusk Helix pomatia.

EXPERIMENTAL METHOD

Snails active for two weeks were chosen for the experiment and killed; the subesophageal ganglionic complex was removed, its connective-tissue membrane opened, than the ganglia were kept for 1 h in physiological medium [3]. The ganglia were then transferred to 1 ml of this medium with 100 μCi L-leucine-4,5-^{3}H and $2 \cdot 10^{-6}$ M dibutyryl-cyclic AMP. Incubation continued for 4, 6 or 24 h at 20-22°C. After the end of incubation the following ganglia were excised from the complex, the right and left pleural, the right and left parietal, and the visceral [3]. All ganglia were homogenized in 0.2 ml of 0.9 M acetic acid and 10 M urea (pH 2.4). The homogenate was applied to disks of 10.5% polyacrylamide gel (PAG) and subjected to electrophoresis. Pyronine was used as the reference substance. The disks of gel were then cut into 3-mm fractions, 0.12 ml 30% H_2O_2 was added, and the samples were kept at 4-5 h at 40°C, after which 10 ml toluene-based scintillator was added. The

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