DEPRESSIVE EFFECT OF THYROTROPIN IN THE PHYSIOLOGICAL DOSE RANGE ON PROSTAGLANDIN E_2 SYNTHESIS AND ACCUMULATION IN CULTURED HOG THYROID CELLS

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1. Introduction

Prostaglandins of the E type (PGE) are able to reproduce in vitro the effects of several hormones acting via the stimulation of cyclic AMP synthesis [1,2]. Moreover, as PGE stimulate the adenylate cyclase activity in membrane preparations of various origins [3–5], it seemed likely that both types of effectors, e.g., hormones and prostaglandins, act on cellular metabolism by similar mechanisms involving primarily adenylate cyclase activation. The acute stimulation of endogenous prostaglandin synthesis after cell–hormone interaction may indicate that in some systems one part at least of the hormone response can be prostaglandin mediated. This seems to occur in the thyroid gland where the rapid increase, after thyrotropin (TSH) addition, of arachidonic acid release from phospholipids allows increased PGE_2 synthesis [7]. The cyclic AMP response of cultured porcine thyroid cells to thyrotropin stimulation was shown to be regulated by the hormone itself. Both positive and negative regulation have been demonstrated [6]. In order to evaluate the role of endogenous PGE_2 synthesis in the regulation of cultured thyroid cell metabolism, we have estimated the PGE_2 content of cells and culture media and its relation to chronic TSH stimulation. Our results suggest that PGE_2 and TSH can be considered as complementary stimulators of the thyroid cell.

2. Materials and methods

2.1. *Cell culture*

Thyroid cells were obtained from porcine thyroid gland by a discontinuous trypsinization procedure [8]. Freshly isolated cells were suspended (3×10^6 cells/ml) in Eagle minimum essential medium supplemented with 10% newborn calf serum (Flow Labs) and antibiotics (penicillin, 200 units/ml, streptomycin, 50 μg/ml). They were incubated as unstirred suspensions in polystyrene Petri dishes not treated for tissue culture at 35°C in a 5% CO_2–95% air, water-saturated atmosphere.

2.2. *PGE_2 assay*

At the end of the culture period, cells were collected by centrifugation at $200 \times g$ for 5 min at 4°C. The resulting pellet was suspended in 2 ml distilled water. Medium and cell suspension were processed as follows. Tracer amounts of tritiated PGE_2 were added in order to evaluate the yield of the extraction procedure. After acidification (pH 3.0) with 1 M citric acid, PG were extracted twice with diethylether (8 vol.) and the extracts were dried under a stream of nitrogen. The dried extracts were solubilized with about 2 μl acetic acid in 1 ml benzene–ethyl acetate mixture (6:4, v/v) and submitted to silicic acid column chromatography in small columns containing 0.5 g activated silicic acid (Mallinckrodt, 100 mesh) [9].

After a 5 ml wash with benzene–ethyl acetate, PGE were eluted with 4 ml benzene–ethyl acetate–methanol (6:4:0.3, v/v). Radioimmunoassay of PGE_2 was performed on this fraction as in [10]. Anti PGE_2 antiserum was from the Institut Pasteur (Paris).

2.3. *Assay of PG synthetase activity*

After given times in culture, cells (5×10^6) were collected by centrifugation and washed twice in serum-free medium. The pellet was homogenized in 1.5 ml incubation buffer (100 mM Tris–Cl (pH 8.0), 20 mM EDTA, 1 mM GSH, 1 mM epinephrine), with a tight fitting Dounce homogenizer [11]. The homogenate was centrifuged for 10 min at $600 \times g$. The supernatant, 100 μl, preheated at 37°C, were added with stirring in a tube containing [^{3}H]arachidonic acid (5×10^5 cpm, 10^{-6} M final conc.). After 5 min incubation, 5–20 μl of the homogenate was spotted on thin-layer silica gel plates (Merck) together with 10 μg carrier PGE_2. The plates were developed in chloroform–methanol–acetic acid (180:12:10, v/v/v) at room temperature for 45 min. After drying, the plate was sprayed with iodine in methanol (1%). Spots corresponding to PGE_2 and arachidonic acid were scrapped and counted in toluene scintillator after elution of radioactivity with 0.5 ml methanol. Results are given in pmol/15 min/mg protein. Porcine TSH (2.3 U/mg) was a gift of Dr G. Hennen and PGE_2 were supplied by Dr J. Pike. [^{3}H]Arachidonic acid (80 Ci/mmol) was from New England Nuclear. Experiments were performed twice or more and values are given as means ± SE of triplicates.

3. Results

In a first series of experiments, we have studied the evolution of PGE_2 content of cells and media over 5 days after the onset of culturing in the presence or absence of TSH (1 mU/ml). As shown in fig.1, an accumulation of PGE_2 was observed in cells cultured in the absence of TSH. The increase was already significant after 1 day and the PGE_2 content of cells was still increasing between day 3 and day 5. PGE_2 was released into the culture medium. A plateau was observed after 3 days of culture at which time 2 nM PGE_2 was measured. If TSH (1 mU/ml) was present, cell and medium PGE_2 concentrations remained at a low level without appreciable change during the 5 day period. Since TSH inhibited the spontaneous increase in PGE_2 accumulation in cultured thyroid cells and its release in the culture medium, the influence of

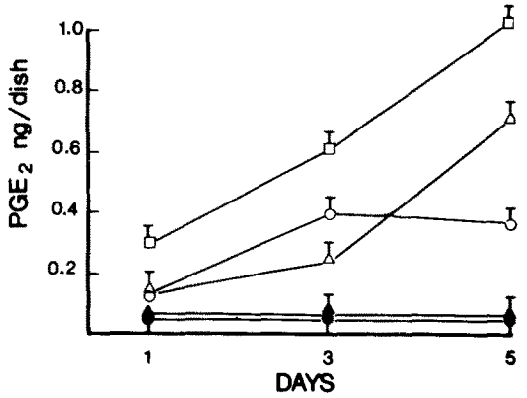

Fig.1. Prostaglandin E_2 accumulation in cultured hog thyroid cells and release into the culture medium. Cells, 4.5×10^6, were cultured as unstirred suspensions for 1, 3 or 5 days in the presence (closed symbols) or absence (open symbols) of TSH (1 mU/ml). PGE_2 content of cells and culture media were measured by radioimmunoassay. (□) Total content of the dish. (△) PGE_2 in the cells. (○) PGE_2 in the medium. (Mean ± SE.)

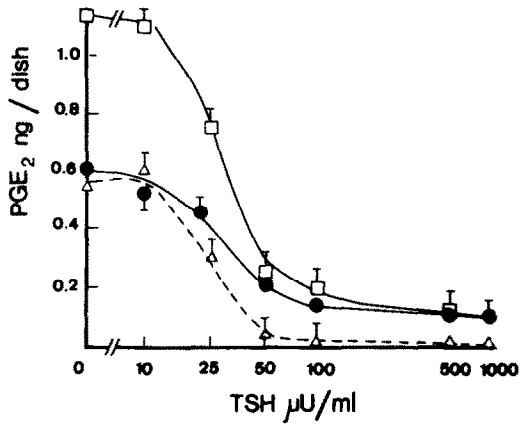

Fig.2. Dose dependence on TSH of PGE_2 accumulation in cells and its release into the culture medium. Hog thyroid cells were cultured for 4 days in the presence of increasing concentrations of thyrotropin. PGE_2 content of cells and media were measured by radioimmunoassay. (□) Total content of the dish; (○) PGE_2 in the culture medium; (△) PGE_2 in the cells. (Mean ± SE.)

various concentrations of hormone after 4 days in culture was investigated. Cells were cultured in the presence of graded concentrations of TSH from 0–5 mU/ml with special emphasis in the zone ranging from 5–100 μU/ml. Figure 2 shows that the depressive effect of TSH on PGE_2 accumulation in the cells is dose dependent. An almost complete inhibition was obtained at ~50–100 μU/ml. The inhibiting effect of TSH on PGE_2 accumulation in cultured hog thyroid cells takes place in the concentration range where positive regulation by TSH of TSH-sensitive adenylate cyclase occurs in the same system [6].

The evolution of prostaglandin synthetase activity in homogenates of cultured thyroid cells is described in fig.3. The synthesis of PGE_2 from labeled arachidonic acid was low in cells cultured in the presence of TSH whereas the conversion to PGE_2 of the precursor fatty acid increased gradually in cells cultured in the absence of hormone. This increase was similar to what had been observed for PGE_2 accumulation and release. The effect of TSH was dose dependent and took place in the dose range where PGE_2 accumulation was depressed. Inhibition of 80% was already obtained for TSH at 50 μU/ml (fig.4a). As most effects of TSH appear to be mediated by increased intracellular cAMP levels, we have tested the effects of dibutyryl-cAMP and PGE_2 on the above parameters.

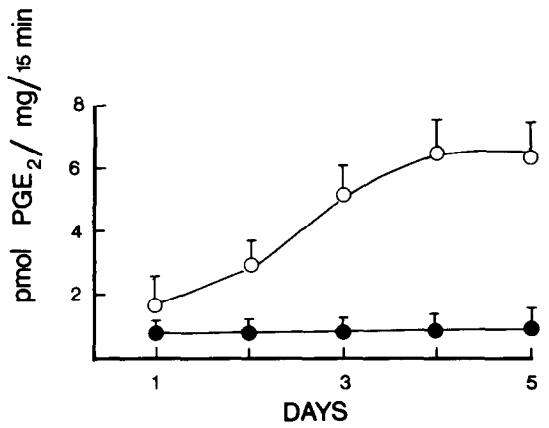

Fig.3. Prostaglandin synthetase activity in homogenates of hog thyroid cells as a function of time in culture. Cells were cultured in the presence (•) or absence (○) of TSH (1 mU/ml). After given times in culture, PG synthetase activity was measured in cell homogenates.

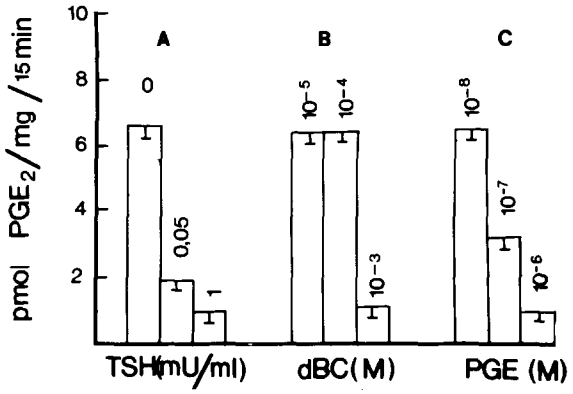

Fig.4. Prostaglandin synthetase activity in homogenates of thyroid cells cultured for 4 days in the presence of TSH (A), dBC (B) or PGE_2 (C).

Dibutyryl-cAMP added to the culture medium at concentrations of 1 mM does not mimic the depressive effect of TSH on PGE_2 accumulation. Lower concentrations (0.1 mM) increased prostaglandin accumulation and release above control values (table 1). In contrast, 1 mM dibutyryl-cAMP or 1 μM PGE_2 mimic the depressive effect of TSH on the activity of prostaglandin synthetase which remained low when these substances were added to the culture medium (fig.4b,c).

Table 1
Effect of dibutyryl cAMP on PGE_2 accumulation and release by cultured thyroid cells

Addition to culture medium	PGE_2 (pg/dish) ± SE		
	Medium	Cells	Total
Dibutyryl cAMP			
10^{-4} M	exp. 1 945 ± 78	2480 ± 156	3425
	exp. 2 516 ± 71	1835 ± 127	2351
10^{-3} M	exp. 1 340 ± 62	600 ± 156	940
	exp. 2 130 ± 12	716 ± 104	846
TSH			
1 mU/ml	33 ± 8	45 ± 9	78
None	535 ± 35	620 ± 30	1155

Cells were cultured for 4 days in the presence of added compounds. PGE_2 were measured in cells and medium by radioimmunoassay

4. Discussion

When cultured in the absence of TSH, hog thyroid cells accumulate PGE_2. After 5 day incubation, intracellular PGE_2 concentration is increased 6–10-fold. A concomitant release of PGE_2 in the culture medium is observed. When present in the culture medium, TSH inhibits this spontaneous rise. Concentrations as low as 50–100 μU/ml are sufficient to produce maximum inhibitory effects.

Control experiments using labeled PGE_2 added to the culture medium showed that no selective degradation occurred in TSH-stimulated cells (unpublished). Therefore, the observed increase in PGE_2 content in both cells and culture medium appears to be a consequence of enhanced PGE_2 synthesis. Changes in PG synthetase activity of cell homogenates are consistent with this assumption. TSH acutely stimulates PGE synthesis in thyroid tissue [7]. This acute stimulation, which may participate to adenylate cyclase activation, is dependent on the release of arachidonic acid from phospholipids [7]. This is apparently in contradiction with our observation that TSH chronically depressed PGE_2 accumulation and release in cultured hog thyroid cells. The possible relation between these two effects of TSH has to be investigated.

The spontaneous increase in PGE_2 accumulation and release in unstimulated cells deserves further attention. Various factors are known to influence PGE content and release in various cell types which are in general established lines of normal or malignant origin [12–14]. Transformation of normal lines is often followed by an increase in PGE synthesis by a mechanism which might be linked to alteration of membrane properties. The comparison between these systems and cultured thyroid cells is difficult at the present time since information on the long-term regulation of PG synthesis is scarce. PGE are known to stimulate cAMP production in thyroid slices and isolated thyroid cells [15,16]. When thyroid cells are cultured in the absence of TSH it was noticed [17] and confirmed by Takasu (unpublished) that a small but significant increase in the intracellular level of cAMP occurred after 3 days in culture. This might be a consequence of increased PG synthesis within the cells as reported for cultured fibroblasts [18]. It is therefore tempting to suggest that endogenous PGE act as a local stimulator of cAMP production, which would become efficient only when the exogenous stimulator, i.e., TSH is absent. The dose-dependence of the inhibitory effect of TSH on PGE_2 accumulation in cultured thyroid cells is especially relevant to this possibility since it occurs in the concentration range where the positive regulation of the cAMP response to TSH was recently described [6]. Under physiological conditions, when blood TSH level is around 1–10 μU/ml, a balance might occur between the thyroid stimulating effect of circulating TSH and locally produced PGE_2. This interpretation of our results gives to prostaglandins produced within the tissue a significance in the maintenance of the physiological steady state. Correlations with in vivo experiments are needed to support this interpretation.

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