INHIBITION OF THYROTROPIN-STIMULATED CYCLIC AMP ACCUMULATION BY HUMAN THYROGLOBULIN IN HUMAN CULTURED THYROID CELLS

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1. Introduction

Recent data show that thyroid membrane has receptors which interact with thyroglobulin. The latter can significantly inhibit thyrotropin binding to thyroid plasma membrane [1,2]. The possible correlation of this effect to a reduction of adenylate cyclase activity is controversial [3,4]. Here we describe the effect of pre-incubation with human native thyroglobulin on TSH-stimulated cyclic AMP accumulation in human cultured thyroid cells. These data show that native thyroglobulin reversibly inhibits the response to TSH.

2. Materials and methods

Thyroid cells were prepared from the counterside lobe obtained during thyroidectomy, in cases of monofocal cancer, by mechanical dispersion after trypsinization as in [5]. Dispersed cells suspended in McCoy's 5a medium containing 20% foetal calf serum (FCS) were seeded in Linbro multi-well plates and kept in culture for 7 days. Adrenocortical cells were isolated from human adrenal gland obtained during nephrectomy by mechanical dispersion after trypsinization with some modifications of the method used for thyroid cells and kept in the same culture medium containing synthetic 1–24 ACTH (3.5 μM) for 7 days. The study of adenylate cyclase–cAMP system was done in Krebs-Ringer bicarbonate (KRB) buffer, containing 0.6 mM 3-isobutyl-1-methylxanthine (IMX), 10 mM glucose and 2 g/l human serum albumin (HSA) according to [5]. Each experiment was performed with triplicate incubations and repeated 3 times.

Human thyroglobulin was purified from saline extracts of normal thyroid slices by 1.4–1.8 M ammonium sulphate precipitation [6] followed by gel filtration on Sephacryl S-300 immediately before use on cultured cells; the 19 S peak was diluted to appropriate concentrations. Protein concentration was measured by A_{280} ($\epsilon^{1\%}_{1\,cm} = 10.0$). Intracellular accumulation of cAMP was measured in triplicate in the freezed-dried 2000 × g supernatant of the ethanol-treated homogenate, by saturation analysis using a cytosol protein purified from bovine adrenal cortices [7]. The DNA content was measured in the pellet by a fluorimetric method [8] after complete extraction of lipids. Statistical analysis was performed by one-way analysis of variance.

The bovine TSH (bTSH; batch no. 53/11) was generously supplied by National Institute for Biological Standard and Control, Holly Hill, London; cAMP and IMX were purchased from Sigma (St Louis, MO); [2,8-^{3}H]adenosine 3′,5′-cyclic phosphate ammonium salt from the Radiochemical Centre (Amersham); synthetic 1–24 ACTH (Synacthen®) from Ciba-Geigy (Basel); Sephacryl S-300 from Pharmacia (Uppsala); McCoy's 5a medium and foetal calf serum from Gibco (Grand Island, NY); culture multi-well plates from Linbro, Flow Labs. (CN); porcine trypsin and all other reagents analytical grade from Merck (Darmstadt).

3. Results

Cultured thyroid cells maintained the capacity to respond to bTSH, measured as cAMP intracellular accumulation, from a dose as low as 0.1 mU/ml. A dose-dependent increase in cAMP intracellular levels was observed after 30 min incubation up to 2.5 mU/ml of bTSH (mean ± SDM: 9.27 ± 0.38 pmol cAMP/μg DNA vs basal values: 0.67 ± 0.08 pmol

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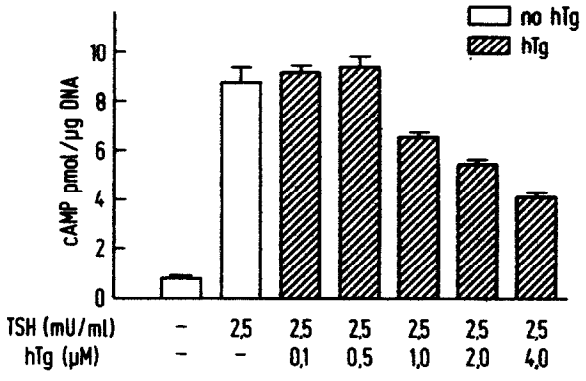

Fig.1. Effect of different preincubation times with native human thyroglobulin on cAMP accumulation induced by bTSH in human cultured thyrocytes. About 1.5 × 10^5 thyrocytes were preincubated in triplicate varying from 15–240 min at 37°C in McCoy's 5a medium supplemented with 20% FCS and 1.5 µM thyroglobulin. At the end of preincubation cells were stimulated in KRB buffer (pH 7.4) containing 2.0 g/l glucose, 2.0 g/l HSA and 0.6 mM IMX at 37°C with 2.5 mU/ml of bTSH. Results are expressed as pmol cAMP/µg DNA, vertical bars represent standard deviation of mean.

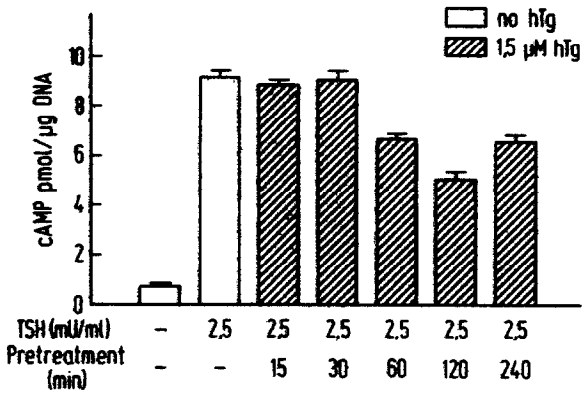

Fig.2. Effect of 120 min preincubation with native human thyroglobulin on cAMP accumulation induced by bTSH in human cultured thyrocytes. About 1.5 × 10^5 thyrocytes were preincubated in triplicate at 37°C in McCoy's 5a medium supplemented with 20% FCS, in presence of thyroglobulin at 0.1–4 µM. At the end of preincubation cells were stimulated with bTSH as in fig.1. Results are expressed as pmol cAMP/µg DNA, vertical bars represent standard deviation of mean.

cAMP/µg DNA). Human native thyroglobulin up to 4 µM had no effect on cAMP accumulation during 30–120 min incubation (mean ± SDM: 0.65 ± 0.7 pmol cAMP/µg DNA).

Pretreatment with human native thyroglobulin (1.5 µM) had an inhibitory effect on bTSH-stimulated cAMP accumulation as shown in fig.1. Inhibition appeared after 60 min incubation and reached a maximum of ~42% of the basal level after 120 min and decreased after 4 h. When cultured thyroid cells were pretreated with different doses of human native thyroglobulin (from 0.1–4.0 µM) for 120 min, a dose-dependent reduction in the subsequent bTSH stimulation of cAMP formation was observed from 1.0 µM thyroglobulin (fig.2). To verify that the inhibitory effect of native thyroglobulin was not the consequence of a permanent reduction in thyroid function, experiments were performed to determine whether the action of thyroglobulin was reversed by washing (fig.3). Thyroid cells preincubated for 120 min with thyroglobulin (1.5 µM), were washed 3 times with fresh medium and subsequently stimulated with bTSH (2.5 mU/ml) at different times after the removal of thyroglobulin (15–120 min). A normal response appeared after 60–90 min.

The inhibitory effect of thyroglobulin on bTSH response of thyroid cells appeared to be specific. In fact, pre-treatment with human native thyroglobulin of human cultured adrenocortical cells did not reduce the increase of cAMP intracellular levels evoked by 30 min exposure to 10 µM synthetic 1–24 ACTH (not shown).

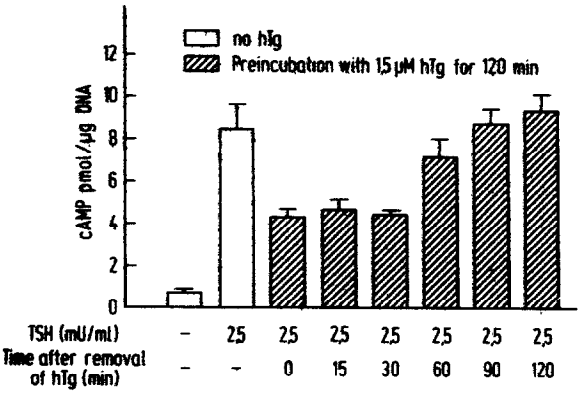

Fig.3. Effect of bTSH on cAMP accumulation in human cultured thyrocytes preincubated with human thyroglobulin for 120 min and then without thyroglobulin at different times. About 1.5 × 10^5 thyrocytes were preincubated in triplicate for 120 min at 37°C in McCoy's 5a medium supplemented with 20% FCS and 1.5 µM human thyroglobulin. Thyroglobulin was then removed by washing and cells were stimulated with bTSH, as in fig.1, 15–120 min after the removal of thyroglobulin. Results are expressed as pmol cAMP/µg DNA, vertical bars represent standard deviation of mean.

4. Discussion

These findings confirm that human native thyroglobulin, up to 4 μM, is ineffective in modifying the adenylate cyclase–cAMP system of intact thyroid cells as observed in subcellular fractions [4]. However, pretreatment of human cultured thyroid cells with homologous thyroglobulin for $\geqslant$1 h reduces the thyrotropin-induced cAMP intracellular accumulation. This inhibition seems to be specific in view of the fact that thyroglobulin pretreatment of other endocrine cells as human cultured adrenocortical ones do not modify the response to ACTH. Moreover, the inhibitory effect appears to be reversible, differently from that observed for thyroglobulin binding to thyroid plasma membrane [2].

The thyroglobulin inhibitory dose effective in thyroid cells appear $\geqslant$10-fold higher than that found effective in the plasma membrane binding [1–3]. However, it is necessary to point out that the two systems are not comparable. In fact, labelled thyroglobulin binding to thyroid cell surface can not be studied in cultured thyroid cells in view of its internalization through an endocytotic process which is thyrotropin- and cAMP-dependent (unpublished). Even if the inhibitory dose of human thyroglobulin is much higher than its circulating levels in normal subjects, our results suggest that it may play a role in modulating the in vivo response to thyrotropin when the release is increased as in Graves' disease and thyroid carcinoma.

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