Studies of catecholamine effect on cyclic AMP in human cultured thyroid cells: their interaction with thyrotrophin receptor

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Abstract. Even though adrenergic nerve terminals between and around thyroid follicles and catecholamine stimulation of thyroid adenylate cyclase have been reported, there is no uniform concept on catecholamine interaction with thyrotrophin (TSH) receptors. Therefore, the effect of catecholamines on TSH-stimulated cyclic AMP (cAMP) accumulation in human follicular thyroid cells has been investigated, to thus eliminating the extrathyroidal actions of catecholamines.

Epinephrine, norepinephrine and isoproterenol appeared to be rapid and potent stimulators of intracellular cAMP accumulation, the half maximum increase doses being $4 \times 10^{-7} \text{M}$, $1 \times 10^{-5} \text{M}$ and $5 \times 10^{-7} \text{M}$, respectively. While propranolol ($1 \times 10^{-5} \text{M}$) prevented the stimulatory effect of catecholamines and failed to inhibit the effect of bovine TSH, phenolamine ($1 \times 10^{-5} \text{M}$) enhanced the potency of norepinephrine and bovine TSH, leaving that of epinephrine unchanged. The effects of epinephrine ($2 \times 10^{-8} \text{M}$) and isoproterenol ($2 \times 10^{-9} \text{M}$) were additive to that of bovine TSH ($0.5 \text{ mU/ml}$), but the effect of simultaneous stimulation with norepinephrine ($5 \times 10^{-7} \text{M}$) and bovine TSH ($0.5 \text{ mU/ml}$) was lower than expected. Prenalterol, a selective $\beta_1$-agonist, did not stimulate cAMP accumulation, while terbutaline, a selective $\beta_2$-agonist, exerted a potent stimulation. Metoprolol, a selective $\beta_1$-adrenergic blocker, did not affect the response of thyroid follicular cells to isoproterenol. These results demonstrate the existence of $\beta$-adrenergic receptors in human thyroid follicular cells, mainly of the type $\beta_2$, apparently not correlated with TSH receptor. The existence of $\alpha$-adrenergic receptors which counter-regulate TSH functional responses in human thyroid follicular cells is suggested.

Sympathetic adrenergic nerve fibres between and around human thyroid follicles have been demonstrated (Melander et al. 1974), suggesting an influence of the sympathetic nervous system directly on follicular cells in humans too. It has also been demonstrated that catecholamines stimulate the adenylate cyclase-cyclic AMP system of isolated calf thyroid cells (Maayan & Ingbar 1970), bovine thyroid plasma membranes (Marshall et al. 1975) and slices (Spaulding & Burrow 1975), but it has not been evaluated whether catecholamines may interact with thyrotrophin (TSH) receptors or not.

In the present study we have used human primary cultures as a model to eliminate the action of catecholamines on cells different from follicular ones, such as endothelial and connective tissue cells, which may modify the responsiveness to TSH. We have studied: a) the direct effect of catecholamines on cyclic AMP (cAMP) levels of thyroid cultures; b) their interaction with the response to TSH; c) the nature of involved adrenergic receptors.

The results indicate the existence of $\beta$-adrenergic receptors in human thyroid cells and suggest that their role is independent of the action of TSH. In addition $\alpha$-adrenergic receptors seem to be involved in the counter-regulation of TSH action.

Material and Methods

Cell cultures

Thyroid tissue was obtained from surgical specimens of normal tissue from the counterside lobe of patients undergoing total thyroidectomy for monofocal cancer.
Isolated human thyroid cells were prepared as previously described (Toccafondi et al. 1980), using collagenase instead of trypsin. In brief, thyroid tissue fragments were suspended in 10 ml x g tissue of McCoy's-Hepes medium, pH 7.4, containing 0.5 mg/ml collagenase and 5% foetal calf serum (FCS). Enzymatic digestion was carried out for 18 h in 60 mm plastic Petri dishes at 37°C, in humidified air containing 10% CO₂. Mechanically dispersed thyroid cells were seeded at 1 x 10⁶ cell concentration per cm² in each well of 2 cm² multiwell plates. Cultures were carried out in McCoy's 5a medium containing sodium bicarbonate 2.2 g/l and 20% FCS, in humidified air with 10% CO₂ at 37°C for 7 days. Fibroblasts contamination of thyroid cultures was evaluated by the leucineaminopeptidase stain according to Jacquet-mont & Pruniéras (1969). After 7 days of culture fibroblasts never exceeded 2% of cell population.

**Incubation procedures**

Incubations were carried out at 37°C in air containing 10% CO₂ in Krebs Ringer bicarbonate (KRB) buffer, pH 7.4, containing glucose 2.0 g/l, bovine serum albumin 2.0 g/l, and 0.5 mM 3-isobutyl-1-methyl xanthine (IMX). Bovine TSH and catecholamine responsiveness were tested by adding aliquots, appropriately diluted in KRB buffer, to the medium. The effect of α- and β-adrenergic blockers were studied by adding aliquots of appropriate dilutions to the medium 10 min before stimuli. When additive effects of different stimuli were studied, substances were added simultaneously. Final incubation volume was 220 μl in all cases. Incubation was stopped by adding cold absolute ethanol (200 μl) and standing overnight at −20°C. Thawed broken cells were then detached by a rubber scraper and the suspension was centrifuged at 2000 x g at 4°C. cAMP levels of the freeze-dried supernatant and DNA content of the pellet were then determined. All experiments were carried out in quadruplicate for each experimental point and repeated three times with the same results in replicate studies. Results are the average of these. Statistical analysis was performed by one way analysis of variance.

**cAMP and DNA measurement**

cAMP levels were measured in triplicate by a protein binding assay (Brown et al. 1971) and corrected by the DNA content, measured by a fluorometric method (Kissane & Robins 1958) adapted by us for use with cell culture (Toccafondi et al. 1980).

**Drugs**

Bovine TSH (Ambinon, Organon, Oss, Holland) was purified by gel filtration on Ultrogel AcA 44 (LKB, Sweden), collecting the 28000 mw peak with the maximal activity in stimulating cAMP accumulation in thyroid culture cells (Toccafondi et al. 1980) and a specific activity of 8 IU/mg calculated in comparison with the International Standard of Biological Activity (NIBSC 53/11). Multiwell and plastic Petri dishes were obtained from Falcon, Oxnar, Calif., USA. McCoy's medium and FCS were purchased from Gibco, Grand Island, New York, USA; dexoxygenase I from Boehringer Biochemia, Mannheim, W. Germany; [2,8-3H]adenosine-3',5'-monophosphate from Radiochemical Centre, Amersham, UK; adenosine 3',5' monophosphoric acid, IMX, L-epinephrine (E), L-norepinephrine (NE) and DL-isopropenthalol (IPNE) from Sigma, St. Louis, Mi., USA. Terbutaline (Ph.A) was purchased from Ciba-Geigy Co., Basel, Switzerland; collagenase (CLS 6HB) from Worthington Bio. Co., Freehold, N.J., USA; all other reagents of analytical grade from Merck, Darmstadt, W. Germany.

![Fig. 1.](https://www.bioscientifica.com) Cyclic AMP response in normal human thyroid cultured cells exposed to different amounts of bovine TSH (●), epinephrine (●), isopropenthalol (●) and norepinephrine (●). Thyroid cells were incubated, in the presence of 0.5 mM IMX, for 30 min with TSH, and for 20 min with other stimulators. Results are the mean of triplicate determinations for quadruplicate experimental points.
Results

Characteristics of cAMP response to catecholamines

In preliminary experiments there was little change in intracellular cAMP between 5 and 60 min incubation after 7 days of culture. Extracellular cAMP was constantly 10-15% of the concomitant intracellular one. Total cAMP (cell plus medium), therefore, was a reasonable approximation of intracellular cAMP and ranged from 0.4 to 0.8 pmol/μg DNA (mean ± SDM: 0.6 ± 0.2 pmol/μg DNA, n = 54) in different normal human glands.

The effect of catecholamines on cAMP accumulation of monolayer thyroid cells was rapid. Indeed, E (4×10⁻⁷ M), IPNE (5×10⁻⁷ M) and NE (1×10⁻⁵ M) produced a significant increase after 5 min (mean ± SDM: 39.0 ± 3.0; 22.3 ± 2.8; 20.7 ± 2.5 pmol/μg DNA, respectively).

To delineate the responsiveness to catecholamines, cAMP accumulation was studied at varying concentrations from 1×10⁻⁹ M to 1×10⁻⁴ M during incubation of thyroid cells for 20 min. As shown in Fig. 1, E and IPNE doubled cAMP basal levels at a dose of 1×10⁻⁸ M, and NE at a dose of 4×10⁻⁷ M. The half maximum and maximum responses were found with a dose of 4×10⁻⁷ M and 1×10⁻⁵ M for E; of 5×10⁻⁷ M and 1×10⁻⁵ M for IPNE; of 1×10⁻⁵ M and 1×10⁻⁴ M for NE.

Table 1.
Effect of 10 min preincubation with phentolamine (1×10⁻⁵ M) on cAMP accumulation induced by bovine TSH (0.5 mU/ml), epinephrine (1×10⁻⁷ M) and norepinephrine (1×10⁻⁵ M) in human thyroid cultured cells.

<table>
<thead>
<tr>
<th></th>
<th>cAMP pmol/μg DNA</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.6 ± 0.1</td>
<td>-</td>
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<tr>
<td>Bovine TSH</td>
<td>3.6 ± 0.7</td>
<td>500</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>16.2 ± 2.1</td>
<td>2600</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>19.3 ± 1.2</td>
<td>3116</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>0.6 ± 0.2</td>
<td>0</td>
</tr>
<tr>
<td>Phentolamine + bovine TSH</td>
<td>6.6 ± 0.2</td>
<td>1000**</td>
</tr>
<tr>
<td>Phentolamine + epinephrine</td>
<td>14.0 ± 2.7</td>
<td>2233*</td>
</tr>
<tr>
<td>Phentolamine + norepinephrine</td>
<td>42.8 ± 0.9</td>
<td>7033**</td>
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* Not statistically different from epinephrine alone.
** Significantly different (P < 0.01) from bovine TSH or norepinephrine alone.

Table 2.
Effect of 10 min preincubation with propranolol (1×10⁻⁵ M) on cAMP accumulation induced by bovine TSH (0.5 mU/ml), epinephrine (1×10⁻⁷ M), isoproterenol (1×10⁻⁶ M) and norepinephrine (1×10⁻⁵ M) in human thyroid cultured cells.

<table>
<thead>
<tr>
<th></th>
<th>cAMP pmol/μg DNA</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.4 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>Bovine TSH</td>
<td>2.7 ± 0.7</td>
<td>575</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>14.4 ± 1.2</td>
<td>3500</td>
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<td>Isoproterenol</td>
<td>10.0 ± 0.6</td>
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<tr>
<td>Norepinephrine</td>
<td>18.1 ± 1.1</td>
<td>4425</td>
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<tr>
<td>Propranolol</td>
<td>0.4 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>Propranolol + bovine TSH</td>
<td>2.2 ± 0.7</td>
<td>450*</td>
</tr>
<tr>
<td>Propranolol + epinephrine</td>
<td>0.4 ± 0.2</td>
<td>0</td>
</tr>
<tr>
<td>Propranolol + isoproterenol</td>
<td>0.4 ± 0.1</td>
<td>0</td>
</tr>
<tr>
<td>Propranolol + norepinephrine</td>
<td>0.4 ± 0.1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Not statistically different from bovine TSH alone.

The effect of different doses of bovine TSH on cAMP intracellular levels was studied and a dose-dependent increase in cAMP levels was found after 30 min incubation from a dose of 4.4×10⁻¹⁰ M, while the maximum was reached with a dose of 1.1×10⁻⁸ M (Fig. 1).

Effects of adrenergic blockers on catecholamine- and TSH-induced cAMP accumulation

To investigate whether the effect of catecholamines on cAMP accumulation in human thyroid cells could be mediated through α-adrenergic receptors, the effect of a 10 min preincubation with phentolamine (an α-blocking agent) on the influence of E and NE was studied. As shown in Table 1, PhA (1×10⁻⁵ M) enhanced the potency of NE (1×10⁻⁵ M) (+140%), leaving the response to E (1×10⁻⁷ M) unchanged, while no effect was elicited by PhA (1×10⁻⁵ M) alone. The effect of preincubation with PhA was also studied on TSH-induced cAMP accumulation, to verify whether the effect of TSH could involve an α-adrenergic mechanism. PhA (1×10⁻⁵ M) significantly increased (+100%)
the potency of a submaximal dose of bovine TSH (0.5 mU/ml) (Table 1).

The role of β-adrenergic receptors in catecholamine action on human thyroid cells was evaluated by studying the effect of a 10 min preincubation with propranolol (a β-blocking agent), on catecholamine-stimulated cAMP accumulation (Table 2). Pro (1 × 10⁻⁵ M) completely inhibited the stimulatory effect of E (1 × 10⁻⁷ M), IPNE (1 × 10⁻⁶ M) and NE (1 × 10⁻⁵ M), while no effect was elicited on cAMP by Pro alone. On the contrary, Pro (1 × 10⁻⁵ M) failed to inhibit the effect of a submaximal dose of bovine TSH (0.5 mU/ml) (Table 2).

![Figure 2](image-url)

**Fig. 2.** Effect of simultaneous stimulation with bovine TSH (0.5 mU/ml) and epinephrine (2 × 10⁻⁸ M; A) norepinephrine (5 × 10⁻⁷ M; B) and isoproterenol (2 × 10⁻⁸ M; C) on cAMP accumulation in normal human thyroid cultured cells. Incubation with stimulators was carried out for 20 min in the presence of 0.5 mM IMX. Results are the mean (± SD) of triplicate determinations for quadruplicate experimental points.

**Effects of catecholamines plus TSH on cAMP accumulation**

Human cultured thyroid cells were simultaneously incubated with submaximal doses of E (2 × 10⁻⁸ M), IPNE (2 × 10⁻⁸ M) or NE (5 × 10⁻⁷ M) and bovine TSH (0.5 mU/ml) to find a possible α-adrenergic-like effect of TSH. As depicted in Fig. 2, the effect of E and IPNE was additive to that of bovine TSH.
In contrast the increase of cAMP accumulation produced by the simultaneous stimulation with NE and bovine TSH was nearly 50% lower than expected.

*Effects of β1- and β2-adrenergic agonists on thyroid cell cAMP accumulation*

To clarify whether the β-adrenergic receptors of human thyroid cells could be β1- or β2-receptors or both, the effect of a β1-agonist, prenalterol, and of a β2-stimulator, terbutaline, on thyroid cell cAMP accumulation was studied. Prenalterol (1 \times 10^{-6} M) weakly stimulated cAMP accumulation, the maximum response to the β1-agonist being observed at a dose of 1 \times 10^{-5} M. In contrast, terbutaline doubled cAMP baseline levels at a dose of 1 \times 10^{-7} M, with a maximum response being observed with 1 \times 10^{-4} M (Fig. 3 A). This suggest that the β-adrenergic receptors of follicular cells are mainly of the β2 character. Indeed, metoprolol, a selective β1-adrenergic blocker, used at a concentration of 2 \times 10^{-6} M, did not affect the response of thyroid cells to IPNE (5 \times 10^{-7} M) (Fig. 3 B).

**Discussion**

Some previous studies, in which subcellular preparations of various mammalian thyroid tissue were used to study the adenylate cyclase activity suggested that biogenic amines were incapable to modify it (Pastan & Katzen 1967; Wolff & Jones 1971; Kendall-Taylor 1972), but further studies have clearly demonstrated that catecholamines are stimulators of the adenylate cyclase system both of subcellular fractions, organ fragments and in isolated cells of different animal species (Gilman & Rall 1968; Maayan & Ingbar 1970; Marshall et al. 1975; Sato et al. 1976). In these studies the catecholamine effect was tested on heterogeneous cell populations. We used human thyroid cells in primary culture to exclude the catecholamine effect on non-follicular cells. Indeed, differentiated follicular cells in primary culture maintained the responsiveness to TSH and the capacity to secrete thyroglobulin, while the contamination of fibroblasts was negligible.

Catecholamines were found to mimic the effect of TSH on cAMP intracellular accumulation in human thyroid follicular cells, thus supporting what has previously been shown to occur in animal thyroid glands using subcellular preparations (Marshall et al. 1975), organ fragments (Gilman & Rall 1968; Sato et al. 1976) or dispersed cells (Maayan & Ingbar 1970). The catecholamine effect was very rapid, and the efficacy of epinephrine and isoproterenol was 100 times higher than that of norepinephrine while the potency ratio, calculated by comparing the half-maximum effects of epinephrine, norepinephrine and isoproterenol, was 2:1:1. The lower efficacy and potency of norepinephrine may be related to its lower affinity for β-adrenergic receptors. The lower potency of isoproterenol as compared to epinephrine disagrees with that found 'in vivo' by Melander (1970) and 'in vitro' by Marshall et al. (1975) in studies on mammals, but agrees with results on human thyroid slices (Sato et al. 1976). Accordingly, these discrepancies may relate to species differences.

Studies performed using α- and β-adrenergic blockers allowed us to characterize further the adrenergic receptors involved in regulating the adenylate cyclase-cAMP system of human follicular cells. The stimulation of β-adrenergic receptors increases cAMP intracellular accumulation in human thyroid follicular cells, indicating that β-adrenergic receptors are present in thyroid follicular cells. The lack of effect of propranolol on the TSH response of human thyroid cells supports the view that the TSH receptor and the β-adrenergic receptor are distinct in different entities, in agreement with studies in mammals (Davies et al. 1977; Marshall et al. 1975; Tanini et al. 1978).

The stimulatory effect of terbutaline, a β2-adrenergic agonist, the lack of effect of prenalterol, a β1-adrenergic agonist, and the ineffectiveness of metoprolol, a β1-adrenergic blocker, imply that the β-adrenergic receptors in human thyroid follicular cells may be classified as β2 receptors. These data in humans agree with results from animal studies (Melander et al. 1975).

Phentolamine increased the effect of NE. These data disagree with those of others (Sato et al. 1976; Aiyoshi et al. 1978; Van Sande et al. 1980). However, these differences may be due to differences in the experimental model (slices vs primary culture, cf. Patrono et al. 1981). In addition, the potentiating effect by phentolamine may signify that α-adrenergic receptor activation inhibits cAMP intracellular accumulation in human thyroid cells.

The potentiating effect exerted by phentolamine on TSH response together with the inhibitory effect produced by norepinephrine on TSH response in experiments of simultaneous stimulation
with TSH, suggests that an α-adrenergic receptor may be involved in humans in the counter-regulation of TSH stimulation. These results confirm to a large extent what has been hypothesized by Marshall et al. (1974) in studies with bovine plasma membranes and by Yamashita et al. (1979) in dog thyroid slices.

In conclusion, the present data provide evidence for the existence of β-adrenergic receptors in human thyroid follicular cells, apparently different from TSH receptors. In addition, the existence of α-adrenergic receptors involved in TSH action counter-regulation may be postulated.

Acknowledgments

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References


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