POSITIVE AND NEGATIVE REGULATION BY THYROTROPIN OF THYROID CYCLIC AMP RESPONSE TO THYROTROPIN IN PORCINE THYROID CELLS

Nobuyuki TAKASU*, Bernard CHARRIER, Jean MAUCHAMP and Serge LISSITZKY

Laboratoire de Biochimie Médicale et Unité 38 de l'Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine, 27, Bd Jean-Moulin, 13385 Marseille Cédex 4, France

Received 13 October 1977

1. Introduction

It is well established that the pituitary hormone thytropin (TSH) controls many functions of the thyroid gland via a plasma membrane receptor–adenylate cyclase–cyclic AMP system. Recently, increasing evidence from in vitro experiments suggested that previous exposure to TSH blunts the thyroidal cyclic AMP response to subsequent TSH stimulation [1–4]. The physiological significance of this refractory process is not known, since very high concentrations of TSH were used to induce the effect. No reports of induction of refractoriness to TSH by physiological concentrations of the hormone are available. Using porcine thyroid cells, the present experiments were designed to investigate the effect of previous treatment with very low to high concentrations of TSH on the cyclic AMP response to subsequent TSH stimulation. It is shown that 5-day exposure to various concentrations of TSH results in a biphasic modification of the cyclic AMP response to subsequent TSH stimulation: very low concentrations (0.005–0.05 mU/ml) increase thyroid cyclic AMP responsiveness to TSH, whereas higher concentrations induce refractoriness to TSH. To our knowledge, this is the first report of a positive regulation of TSH-stimulated cyclic AMP response by the homologous hormone in vitro.

*On leave from the Department of Medicine, Institute of Adaptation Medicine, School of Medicine, Shinshu University, Matsumoto, Japan

2. Materials and methods

2.1. *Thyroid cell*

Thyroid cells were isolated from porcine glands of adult animals by a discontinuous trypsinization technique [5]. Freshly isolated cells, suspended in Eagle minimum essential medium, pH 7.4, with 10% (v/v) calf serum, penicillin (200 units/ml) and streptomycin sulfate (50 μg/ml), were incubated at a concentration of 3×10^6 cells/ml in Falcon plastic Petri dishes not treated for tissue culture. Cells were incubated at 35°C in 95% air–5% CO_2 with or without thyrotropin.

2.2. *Cell washing*

At the conclusion of the incubation period, cells were centrifuged at 400 × *g* for 5 min at 4°C, the supernatant was discarded and the pellet was resuspended in a phosphate-buffered saline, pH 7.4 (PBS) of the following composition (in mg/l): NaCl 8000, KCl 200, $Na_2HPO_4 . 2H_2O$ 2890, KH_2PO_4 200, $CaCl_2 \cdot 2H_2O$ 66.6 and $MgCl_2 \cdot 6H_2O$ 100. Cells were centrifuged again as before. This washing procedure was repeated 2 times. The total volume of PBS used for washing was 10 times the original volume of cell suspension. After the last washing, the cells were suspended in phosphate-buffered saline containing 0.1% glucose (PBSG).

2.3. *Cyclic AMP assay*

Aliquots (120 μl, 0.1–0.2 mg protein) of washed thyroid cell suspension were incubated in air for 5 min at 37°C in final vol. 150 μl containing PBSG, 10 mM

theophylline and TSH (50 mU/ml). The incubation was ended by immersing the tubes into a dry ice–acetone bath until frozen, followed by subsequent immersion in a boiling water bath for 2 min. The tubes were allowed to cool to room temperature. The cells were homogenized and centrifuged. The pellets were solubilized in NaOH for protein determinations. To 100 μl of the supernatant, 100 μl of an alumina slurry (0.5 g in 1 ml of cyclic AMP-binding assay buffer) was added, followed by vortexing and centrifugation. Aliquots of the supernatant were diluted appropriately and used for assay of cyclic AMP by the competitive protein binding assay [6]. Alumina was used to remove ATP and other nucleotides. Cyclic AMP was not adsorbed.

2.4. *Other methods and chemicals*

Protein estimation was performed according to [7] using bovine serum albumin in NaOH as standard. The pellets from the cyclic AMP determination samples were solubilized in 0.1 N NaOH (final concentration) and protein was estimated.

Porcine TSH (2.3 U/mg) was kindly donated by Dr G. Hennen (Liège, Belgium). Purchases were made from the following sources: cyclic [^{3}H]AMP (spec. act. 26 Ci/mmol) from the Radiochemical Center (Amersham, England), trypsin from Grand Island Biological Company (Grand Island, NY), fetal calf serum from Flow Laboratories (Irvine, Scotland), minimum essential medium from Eurobio (Paris, France) and cyclic AMP from Boehringer (Mannheim, FRG). All other chemicals were of the highest purity available commercially.

3. Results

3.1. *Effect of chronic exposure to low (0.05 mU/ml) or high (10 mU/ml) concentrations of TSH on the cyclic AMP response of thyroid cells to subsequent TSH stimulation*

Isolated thyroid cells were incubated with or without TSH (10 mU/ml) for 1, 3 and 5 days. After the indicated periods of incubation, cells were washed and again incubated with TSH (50 mU/ml) in the presence of 10 mM theophylline in room air (PBSG buffer) for 5 min. A concentration of 50 mU/ml TSH was shown to induce maximum cyclic AMP response (data not shown). After exposure for 1 or 3 days to 10 mU/ml TSH, refractoriness to further TSH stimulation was clearly observed (fig.1). In control cells, TSH responsiveness was maximum after 1 day incubation in culture conditions and then, decreased gradually. In contrast, incubation with 0.05 mU/ml TSH did not induce refractoriness to subsequent TSH stimulation (fig.2) but augmented TSH-stimulated cyclic AMP response gradually up to 5 days. The augmentation of TSH-stimulated cyclic AMP response was a very slow process which needed more than 1 day incubation with 0.05 mU/ml TSH (fig.2) while refractoriness to TSH was a rapid process clearly demonstrated after 1 day incubation with 10 mU/ml TSH (fig.1). After exposure to 10 mU/ml TSH, refractoriness was observed within 1 h and was maximum after 12–24 h (manuscript in preparation).

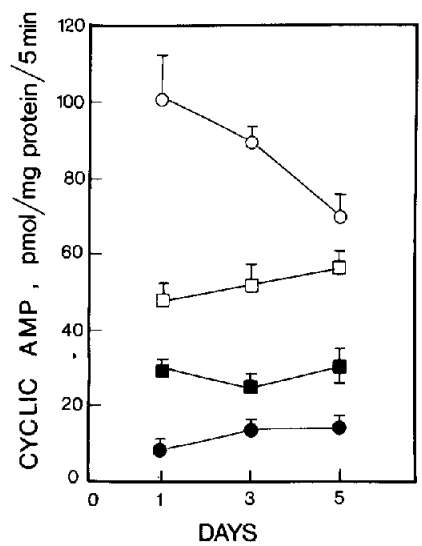

Fig.1. The evolution of TSH-stimulated cyclic AMP response in control and TSH (10 mU/ml) cells. Isolated porcine thyroid cells were incubated for 1, 3 or 5 days in the presence (□,■) or absence (○,●) of TSH (10 mU/ml). The cells were washed at the indicated times and again incubated with (○,□) or without (●,■) 50 mU/ml TSH for 5 min at 37°C in the presence of 10 mM theophylline. The cyclic AMP concentrations after the final 5 min incubation are shown. Each point is the mean ± SE of triplicate determinations.

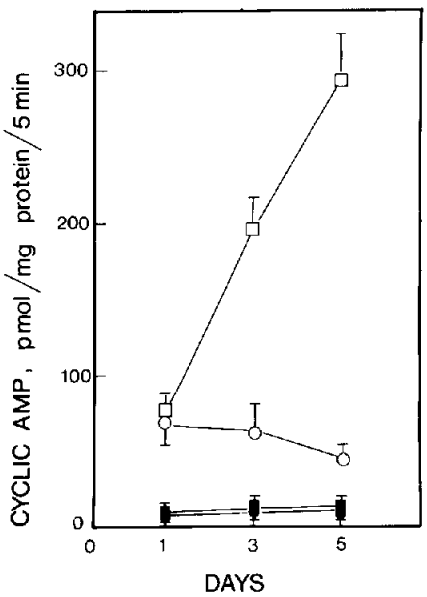

Fig.2. Same experiment as in fig.1 but the cells were incubated in the presence of 0.05 mU/ml of TSH. Same symbols.

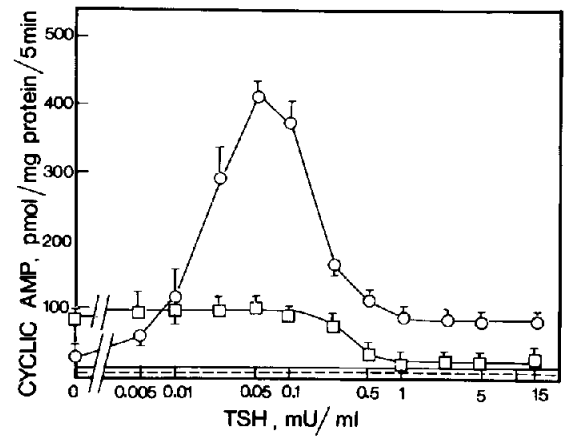

Fig.3. Effects of 1 day and 5 days exposure to different concentrations of TSH on TSH-stimulated cyclic AMP response of isolated thyroid cells. Isolated cells were incubated with TSH (0.005–15 mU/ml) for 1 day (□, - - -) or 5 days (○, ——). The cells were washed and again incubated with (□,○) or without (- - -, ——) 50 mU/ml TSH for 5 min at 37°C in the presence of 10 mM theophylline. Each point is the mean ± SE of triplicate determinations. For the sake of clarity, the experimental points corresponding to curves (——) and (- - -) have not been represented.

3.2. *Effects of different concentrations of TSH in chronic condition on adenylate cyclase–cyclic AMP response to subsequent TSH stimulation*

Isolated thyroid cells were incubated in the presence of TSH (0.005–15 mU/ml) for 1 day or 5 days (fig.3). The cells were washed and again stimulated with or without 50 mU/ml TSH as described above.

Treatment of cells with TSH for 1 day produced refractoriness to TSH (fig.3, open squares). The degree of refractoriness was dose dependent. Maximum and half-maximum effects were observed for concentrations of 1 mU/ml and 0.25–0.50 mU/ml, respectively. These values are about 5–10% of the TSH concentrations which produced half-maximum and maximum cyclic AMP increase in acute stimulation conditions (data not shown). Low concentrations of TSH (less than 0.05 mU/ml) for 1 day did not induce refractoriness to subsequent TSH stimulation.

Treatment with various concentrations of TSH for 5 days (fig.3, open circles) resulted in a biphasic modification of adenylate cyclase–cyclic AMP response to subsequent TSH stimulation: low concentrations of TSH ($\leqslant$ 0.05 mU/ml) augmented the thyroid cell cyclic AMP response to TSH (positive regulation), whereas higher concentrations decreased subsequent TSH-stimulated cyclic AMP synthesis (negative regulation). In these conditions of culture, maximum cyclic AMP response to TSH was observed around a TSH concentration of 0.05 mU/ml, i.e., about 30 pM on the basis of spec. act. 40 U/mg for porcine TSH and mol. wt 30 000.

4. Discussion

The phenomenon demonstrated in this paper is that the TSH-sensitive adenylate cyclase-cyclic AMP system of porcine thyroid cell is under the control of both positive and negative regulation by TSH. Depending on the dose of hormone and the duration of cell incubation, previous treatment with TSH is able to augment or to decrease the degree of cyclic AMP response to subsequent TSH stimulation. Positive regulation is operating in the range of physiological TSH levels (0.005–0.05 mU/ml), whereas negative regulation can only be demonstrated with high con-

centrations of the hormone. Negative regulation is a rapid process occurring within several hours while positive regulation is a slow process, which develops gradually up to 5 days. These differences suggest that the underlying mechanism might be different in both cases. The possible mechanism for positive and negative regulation will be discussed elsewhere (manuscript in preparation).

Physiological concentrations of TSH preserve the sensitivity of the adenylate cyclase–cyclic AMP system and keep thyroid function to an optimum level (positive regulation). On the other hand, negative regulation or refractoriness to TSH protects the sensitive thyroid cells from acute changes of TSH levels.

In mice experiments in vivo, [8] TSH was unable to induce thyroidal refractoriness to TSH but restored responsiveness to TSH from the partial refractory state provoked by previous treatment with triiodothyronine. The results suggested that the prolonged absence of an adequate level of TSH might be the cause of thyroidal unresponsiveness to acute TSH administration. The present observation that physiological concentrations of TSH maintain thyroidal responsiveness to acute TSH stimulation in isolated thyroid cells might explain the situation observed in vivo.

Acknowledgements

The excellent technical assistance of Miss C. Pelassy is gratefully acknowledged. N.T. was supported by a fellowship from the Institut National de la Santé et de la Recherche Médicale.

References

[1] Kaneko, Y. (1976) Horm. Metab. Res. 8, 202–206.
[2] Takasu, N., Sato, S., Yamada, T., Makiuchi, M., Furihata, R. and Miyakawa, M. (1976) Horm. Metab. Res. 8, 206–211.
[3] Shuman, S. J., Zor, U., Chayoth, R. and Field, J. B. (1976) J. Clin. Invest. 57, 1132–1141.
[4] Rapoport, B. and Adams, R. J. (1976) J. Biol. Chem. 251, 6653–6661.
[5] Fayet, G., Pacheco, H. and Tixier, R. (1970) Bull. Soc. Chim. Biol. 52, 299–306.
[6] Gilman, A. (1970) Proc. Natl. Acad. Sci. USA 67, 305–312.
[7] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[8] Gafni, M., Sirkis, N. and Gross, J. (1975) Endocrinology, 97, 1256–1262.