Transcriptional control of thyroglobulin gene expression by cyclic AMP

B. Van Heuverswyn*, A. Leriche*, J. Van Sande*, J.E. Dumont* and G. Vassart*+

*Institut de Recherche Interdisciplinaire and +Service de Chimie Clinique, Université Libre de Bruxelles, Campus Erasme, route de Lennik 808, B-1070 Brussels, Belgium

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Transcription of thyroglobulin (Tg) gene is under the positive control of thyrotropin (TSH). The mechanism of this control has been further investigated. Rats were treated with triiodothyronine (T_3) to decrease their endogenous TSH production. Following the intravenous injection of bovine TSH, a 3-fold stimulation of Tg gene transcription could be detected in isolated nuclei as early as 1 h after treatment. The TSH effect was also observed in tissue fragments incubated in vitro under conditions where a concomitant stimulation of cAMP accumulation was detected. Forskolin, a universal activator of adenylate cyclase, was able to mimic TSH action on Tg gene transcription. We conclude that TSH controls transcription of Tg gene directly via its known interaction with receptors on thyrocytes and that cAMP is a physiological mediator of this effect.

Cyclic nucleotide *Forskolin* *Thyrotropin* *Run-off assay*

1. INTRODUCTION

Adenosine 3′,5′-monophosphate (cyclic AMP) is a ubiquitous regulatory molecule active in both prokaryotes and eukaryotes. Transcriptional control of gene expression by cAMP was first demonstrated in *E. coli* (review [1]). Interaction of the lac promoter with cAMP-binding protein is studied as a general model for the binding of regulatory protein to DNA [2]. In eukaryotes, although cAMP is one of the 3 major signal molecules involved in intracellular regulation, evidence of transcription control by cAMP has been provided so far for a few genes only: discoidin I [3], lactate dehydrogenase [4], prolactin [5], phosphoenolpyruvate carboxykinase [6,7] and tyrosine aminotransferase [8].

Abbreviations: cDNA, complementary DNA; prTg, plasmids containing rat thyroglobulin cDNA fragment

The pituitary hormone thyrotropin (thyroid-stimulating hormone, TSH) is the main agent regulating the activity of the thyroid gland. It may be considered as one of the prototype hormones acting via adenylate cyclase activation [9]. Stimulation of thyroid follicular cells by TSH results in their rapid functional activation leading to the synthesis and secretion of thyroid hormones. The latter results from the hydrolysis of a precursor iodoprotein, thyroglobulin (Tg), synthesized in large amounts by the gland [10,11]. The synthesis of Tg is under TSH control [12,13].

We have demonstrated that TSH controls transcription of the Tg gene in a tonic fashion when administered in vivo [14]. In the rat thyroid, the Tg gene was already maximally expressed under physiological conditions. However, the continuous presence of TSH was required to maintain the gene in an activated state as hypophysectomy or triiodothyronine (T_3) administration profoundly depressed its transcription [14]. Here, ex-

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periments were performed to investigate the kinetics of restimulation of Tg gene transcription by TSH in T_3-treated rats and to determine whether cAMP played a role in this regulation.

Our results indicate that the effect of TSH is rapid, is exerted directly on the thyroid cell and can be mimicked by forskolin, an agent known to increase the cytoplasmic level of cAMP.

2. MATERIALS AND METHODS

2.1. *Reagents*

Bovine TSH (bTSH; Thytropar) was obtained from Armour (Kankakee, Il); [^{32}P]UTP, 400 C_i/mmol (1 C_i = 37 GBq) from Amersham; T_3 (triiodo-L-thyronine, sodium salt) from Sigma, forskolin from Hoechst Pharmaceutical (Bombay) and Ro 20-1724 from Hoffman La Roche (Nutley, NJ).

2.2. *Handling of animals*

Albino male rats of the IUPA-OFA strain, weighing 180–240 g, were purchased from Iffa-Credo (St Germain sur l'Arbresle, France). T_3-treated rats received the hormone is a single daily dose intraperitoneally (5 μg/100 g body wt). Alternatively, an equivalent amount was given in the drinking water. Bovine TSH was administered intravenously as a single dose of 0.2 unit/100 g body wt.

Blood was obtained by cardiac puncture under ether anesthesia between 09.00 a.m. and 15.00 p.m.. The animals were subsequently killed by decapitation, and the thyroid glands were removed and dissected free of connective tissue.

2.3. *Measurement of thyroxine in serum*

Thyroxine (T_4) was measured using commercial reagents (Gammacoat-Clinical Assay).

2.4. *In vitro restimulation of the thyroid glands*

The thyroid lobes were cut in 4 pieces at room temperature. Randomly assorted pools of tissue from 5 animals were preincubated for 30 min at 37°C under an atmosphere of O_2:CO_2 (95:5, v/v) in 2 ml Krebs Ringer bicarbonate buffer enriched with 8 mM glucose and 0.5 g/l bovine serum albumin. TSH or forskolin was added and the incubation continued for 150 min. At the end of the incubation, thyroid nuclei were isolated as described [14].

2.5. *Measurement of thyroglobulin gene transcription in isolated nuclei*

The procedures have been detailed [14]. Thyroglobulin gene transcription was measured by hybridization of labeled nuclear RNA to immobilized plasmids containing fragments of rat Tg cDNA (prTg) [14]. The total length of the cDNA inserts used in this study (prTg 32 and prTg 39) amounts to 70% of the Tg mRNA.

2.6. *Measurement of cAMP in the thyroid tissue*

Thyroid lobes were cut into 2 pieces at room temperature. Four pieces were incubated in 1 ml at 37°C as described above. After 30 min preincubation, TSH, forskolin or Ro 20-1724 was added to the medium and the tissue incubated for another 30 min. The incubation was ended by boiling the tissue in water for 5 min and the cAMP concentration determined by the method of Gilman, as described [15].

3. RESULTS AND DISCUSSION

3.1. *Kinetics of TSH effects on Tg gene transcription*

In previous experiments the regulation of thyroglobulin gene transcription was studied in rats subjected to various manipulations leading to suppression or stimulation of their endogenous TSH production. Following the killing of the animals Tg gene transcription was measured in isolated thyroid nuclei using the 'run-off assay' [16]. Lowering the level of circulating TSH by hypophysectomy or T_3 administration led to a dramatic decrease of Tg gene transcription which could be restored by administration of the hormone [14]. However, early times of the kinetics of restimulation by TSH could not be studied properly because the level of Tg gene transcription in T_3 suppressed animals frequently dropped below the detection limit of our assay (around 100 ppm). This was mainly due to the fact that the cDNA probes used here represented only 20% of the thyroglobulin mRNA length [17].

To provide larger probes, a rat thyroid cDNA library (Leriche, A., submitted) was screened with bovine Tg cDNA and 2 clones, prTg 32 and prTg 39, were selected. Together, their insertions represent 70% of the rat Tg m RNA. Using these new clones, the limit of detection of the transcription

Table 1

Effect of the intravenous administration of TSH on transcription of thyroglobulin (Tg) gene in rats chronically treated with T_3

Rat treatment[a]	[^{32}P]RNA input hybridization (cpm × 10^{-6})	[^{32}P]RNA to filters (pBR322)	Hybridized cpm (prTg)	Tg gene transcription (ppm[b])	Serum T_4[c] (μg/dl)
	2.40	12	166	208	
T_3 (7 days)					0.7 ± 0.1
	2.69	7	157	147	
	1.81	8	389	546	
T_3 (7 days)					1.3 ± 0.3
+ TSH (1 h)	2.19	7	475	565	
	2.99	18	830	718	
T_3 (7 days)					2.6 ± 0.6
+ TSH (3 h)	0.88	6	227	662	

[a] Rats received a daily dose of 5 μg T_3/100 g body wt for 7 days given in their drinking water. Thyroid tissue was obtained on the last day of T_3 treatment, 1 or 3 h after a single injection (i.v.) of bTSH (0.2 IU/100 g body wt). Control animals were killed 1 h after administration of a saline solution (0,9% NaCl)

[b] The rate of Tg gene transcription in ppm was calculated as the difference between the cpm hybridized to prTg and pBR322 filters. The difference was divided by the total input radioactivity, corrected for the efficiency of hybridization between 25 and 50%, see [14]) and multiplied by 1.4 to account for the size ratio between the mRNA to the cDNA inserts

[c] T_4 in serum was determined on blood samples obtained from individual rats and expressed as the mean ± 1 SD

Nuclei were prepared from 2 pools of 10 thyroid glands for each group of animals and transcription of the Tg gene was measured as described [14]. The ^{32}P-labeled transcripts were hybridized to filters containing immobilized DNA from pBR322 plasmid or from recombinant prTg plasmids. Results from individual transcription reactions are presented

assay was reduced to 18 ppm. This allowed accurate measurement of Tg gene transcription in T_3 suppressed animals (178 + 43 ppm) and the detection of an effect of TSH as early as 1 h after the intravenous administration of the hormone (table 1). If one takes into account the exceptional size of the Tg gene (~ 200 kb [18,19] and its expected transcription time (around 60 min [20]), this may be considered as a very rapid effect of TSH. At earlier times our measure of Tg transcription would be biased by the delay involved in the progression along the gene of the stimulation wave.

3.2. *Stimulation by TSH of Tg gene transcription in incubated tissue*

To determine whether the observed effect resulted from a direct action of TSH on thyroid tissue, thyroid fragments from rats pretreated with T_3 were incubated in vitro and challenged with TSH. As a preliminary to the measurement of Tg gene transcription we checked that under the incubation conditions used, intracellular cyclic AMP accumulation could be stimulated by TSH and by the universal adenylate cyclase activator, forskolin as in thyroids of other species [21]. Although modest when compared to the effect observed in dog thyroid [21], both agents exerted a clear stimulatory effect which could be amplified by the addition of a phosphodiesterase inhibitor (Ro 20-1724) to the incubation medium (fig.1).

When Tg gene transcription was measured in tissue incubated for 150 min in the presence of TSH, a 2-fold stimulation was detected over the levels observed in control conditions (control, 101 + 36 ppm; stimulated, 195 + 83 ppm). Results from individual hybridization reactions are reported in table 2. In spite of some dispersion and of the expected lower magnitude of TSH effect when measured under in vitro conditions, they demonstrate that stimulation of Tg gene transcrip-

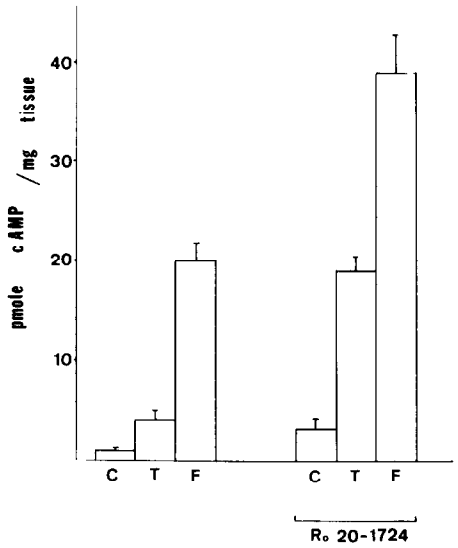

Fig.1. Effect of bTSH and forskolin on cAMP accumulation in rat thyroid cells in vitro. cAMP accumulation in thyroid cells was measured after incubation of the tissue in presence of the agents for 30 min, as described in section 2. The thyroid glands from rats treated for 3 days with T_3 were incubated under control conditions (C) or in the presence of 50 mU/ml bTSH (T) or 10 μM forskolin (F). The effect of Ro 20-1724 (8×10^{-5} M), a phosphodiesterase inhibitor, was also investigated. Results are expressed as means ± 1 SD in 1 typical experiment out of 3.

tion is a direct effect of thyrotropin on the thyroid gland.

3.3. *Forskolin mimics TSH effect on Tg gene transcription*

While most TSH effects are mediated by cAMP in the dog thyroid [22], no such demonstration is available in the rat species. Also, there are clear examples of TSH actions which are independent of cAMP [23]. Therefore, it was of interest to investigate whether elevation of the intracellular concentration of cAMP would alter Tg gene transcription. Thyroid fragments from T_3 treated rats were incubated for 150 min in the presence or absence of forskolin (10 μM). This agent clearly mimicked the TSH effect by producing 2-3-fold stimulation of gene transcription (control 77 + 17 ppm; stimulated, 218 + 46 ppm) (table 3). Interestingly, the fact that forskolin stimulated cAMP accumulation to a larger extent than TSH under our ex-

Table 2

Effect of TSH on transcription of the Tg gene in rat thyroid fragments incubated in vitro

Incubation conditions	[^{32}P]RNA input/ hybridization (cpm × 10^{-6})	[^{32}P]RNA to filters (pBR322)	hybridized cmp (prTg)	TG gene transcription (ppm[a])
	2.49	17	69	65
Control	3.52	12	153	138
	3.43	15	131	100
	2.41	11	92	100
TSH	3.71	16	304	240
(50 mU/ml)	4.52	10	365	246

[a] Results are expressed as in table 1

Rats received a daily dose of 5 μg T_3/100 g body wt for 5 days given in their drinking water. Thyroid tissue was obtained the last day of T_3 treatment and was incubated for 150 min in control medium or in the presence of bTSH (50 mU/ml) as described in section 2. Nuclei were isolated from the incubated tissue and used in in vitro transcription experiments as described in table 1

Table 3

Effect of forskolin on transcription of Tg gene in rat thyroid fragments incubated in vitro

Incubation conditions	[^{32}P]RNA input/ hybridization (cpm × 10^{-6})	[^{32}P]RNA to filters (pBR322)	hybridized cmp (prTg)	TG gene transcription (ppm[a])
	3,76	11	117	84
Control	4.17	11	60	58
	3.52	11	116	90
Forskolin	4.28	34	280	171
(10 μM)	4.22	27	332	222
	2.38	25	228	262

[a] Results are expressed as in table 1

Rats were treated as described in table 2. Tissue was incubated for 150 min in control medium or in the presence of forskolin (10 μM) as described in section 2. Nuclei were isolated from the incubated tissue and used in in vitro transcription experiments as described in table 1

perimental conditions (see fig.1) was not accompanied by a stronger effect on Tg gene transcription. Similar observations have been made for other cAMP mediated effects of TSH [21,24].

4. CONCLUSIONS

Our results confirm and extend previous data demonstrating a transcriptional control of Tg gene expression by TSH. Thyrotropin effect is rapid and results from a direct interaction of the hormone with thyrocytes. The observation that forskolin is able to mimic the TSH effect strongly suggests that cAMP is the physiological mediator of the transcriptional action of the hormone. It clearly demonstrates that the Tg gene belongs to the family of transcription units subjected to regulation by cyclic nucleotides [3-8]. Confirmation of this conclusion has recently been obtained in cultured dog thyroid cells by measurement of steady state levels of Tg mRNA (Van Heuverswyn, B., in preparation). The availability of clones DNA segments containing the Tg promoter [25] makes the thyroid system an attractive model to investigate the molecular mechanisms involved in gene control by cAMP.

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