Volume 232, number 1, 12–16 FEB 05820 May 1988

Effect of Graves' IgG on gene transcription in human thyroid cell cultures

Thyroglobulin gene activation

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Received 24 February 1988

In Graves' disease (GD) the presence of antibodies to the thyroid stimulating hormone (TSH) receptor leads to stimulation of the thyroid gland. The thyroid stimulating activity of Graves' IgG is normally ascertained by bioassays measuring cAMP production. We have investigated the effect of Graves' IgG on the quantitative activation of thyroglobulin (TG) gene in cultured human thyroid cells by RNA hybridisation. TG mRNA expression was activated by TSH and Graves' IgG. Nuclear transcription assays showed that the increase in cytoplasmic mRNA levels was due to increased transcription of TG specific mRNA in nuclei of thyroid cells. Whilst TSH led to a dose dependent increase in TG mRNA levels, Graves' IgG led to a variable activation of TG gene. A significant correlation between the increased TG mRNA transcription and cAMP production was observed with Graves' IgG. Thus the activation of the TG gene by Graves' IgG occurs in parallel with elevation of cAMP.

Thyroid autoimmunity; Graves' disease; Immunoglobulin G; Thyroglobulin; mRNA

1. INTRODUCTION

Thyroid stimulating hormone (TSH) plays a central role in regulating the activity of the thyroid follicular cells. In particular, the TSH-induced release of thyroid hormone is mediated by cyclic AMP (cAMP) which has been shown to modulate the activity of the thyroglobulin (TG) gene [1]. Thus agents which activate the cAMP system or derivatives of cAMP can mimic the effect of TSH on TG gene expression in vitro using rat [2,3] and human [4] thyrocytes.

In Graves' disease (GD), autoantibodies (aAbs) to the TSH receptor can lead to marked stimulation of the gland and therefore hyperthyroidism in autoimmune thyroid disease (AITD) [5]. There is recent evidence that Graves' IgG in vitro leads to the release of TG by cultured human thyroid cells and this stimulation of TG expression correlates with TSH activity [6]. In this study, using cultured human thyroid cells, we have examined the relationship between TG gene activation at the nuclear and cytoplasmic level by hybridisation studies using slot blot techniques and the release of cAMP as assessed by bioassay in response to TSH and Graves' IgG.

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2. MATERIALS AND METHODS

2.1. *Patients' sera*

Serum samples were obtained from 22 patients with AITD. Two sera were from Hashimoto's thyroiditis patients, whilst the remainder were from GD patients. All patients had clinical and biochemical evidence of hyperthyroidism prior to treatment, a diffusely enlarged thyroid gland and diffuse uptake of radioactivity on thyroid scan. Normal sera ($n = 20$) were obtained from

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00145793/88/$3.50

individuals with no history of thyroid disease. Sera were precipitated with ammonium sulphate and the immunoglobulin dialysed overnight against phosphate buffered saline, pH 7.4, filter sterilized and stored at 3.0 mg/ml at −20°C.

2.2. *Culture of human thyroid cells*

Thyroid tissue from a Graves' patient removed at partial thyroidectomy was used and after collagenase dispersion prepared for cryopreservation as described [7]. For in vitro stimulation of thyrocytes, thyroid cells were rapidly thawed at 37°C and grown overnight to allow cells to form a monolayer in RPMI 1640 + 10% fetal calf serum. For measurement of TG gene transcription or cAMP levels approximately 10^6 or 10^5 cells in 500 μl medium, respectively, for each assay were added to the wells of a 24-well costar plate. At this point bovine TSH (bTSH, Armour) or Graves' IgG was added to triplicate wells and incubated for 4 h at 37°C. At the end of incubation, the culture medium was removed, the cells washed once and then prepared for RNA extraction or cAMP release. In other experiments forskolin or 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) were used also.

2.3. *Total RNA extraction*

The single-step acid guanidinium-phenol-chloroform method was used to isolate the total cellular RNA [8].

2.4. *RNA slot blot analysis and hybridization*

The RNA was transferred to Gene Screen Plus membrane (NEN) using a slot blot apparatus (Schleicher and Schuell).

Prehybridization and hybridization were performed according to the manufacturer's instructions. Plasmid cDNA probes to human TG (M4 probe) [9] or chicken brain β-actin (pA1 probe) [10] were labelled by nick translation to a specific activity of $1–2 \times 10^8$ cpm/μg. Hybridization to β-actin cDNA probe was performed to normalise the amount of total RNA applied to each slot as described [11]. Following autoradiography with the TG probe, the membrane was stripped of the labelled probe, autoradiographed to confirm probe removal and the membrane then rehybridized with nick translated β-actin probe. The hybridization signal was ascertained by scanning densitometry in a Shimadzu instrument. For quantification, the density of each band on the β-actin probe blot was expressed as a ratio of an arbitrarily selected band on the same autoradiograph and this ratio was used to calculate the corrected values for the density of the corresponding band on the TG-probed blot [11]. The levels of β-actin mRNA did not change following stimulation with TSH or Graves' IgG (not shown).

2.5. *Nuclear runoff transcription assay*

This was performed by isolating nuclei from human thyroid cells grown in monolayers in the presence or absence of TSH as in [12]. Briefly, nuclei were prepared from the cells following trypsinisation (approx. 5×10^6 viable, isolated thyrocytes) by vortexing the cells gently in 0.5% NP40. Nuclei were incubated for 30 min at 37°C with 500 μCi ^{32}P-UTP (3000 Ci/mmol) followed by purification of the ^{32}P-labelled RNA transcripts [13]. For hybridization, 8.5 μg of linearised plasmid containing the TG or chicken β-actin cDNA were applied to Gene Screen Plus membrane in the slot blot apparatus and prehybridization and hybridization performed exactly as described [14]. As a control, linearized pBR322 was applied to the membrane. Approximately 3×10^6 cpm of runoff products in 1 ml hybridization buffer was applied per filter for nuclei from both the TSH stimulated and unstimulated thyrocytes. Following hybridization, background was reduced by treatment of the filters with RNase A [14] and the filters exposed to Hyperfilm-MP (Amersham International Plc) at −70°C.

2.6. *cAMP assay*

cAMP was measured using a modified procedure as described [15].

3. RESULTS

3.1. *Effect of TSH on TG gene activation*

Increasing concentrations of TSH produced a dose-response stimulation of TG gene activation in human thyroid cells in vitro. At a maximum TSH concentration of 100 mU/ml, an 115.8 ± 45.6%

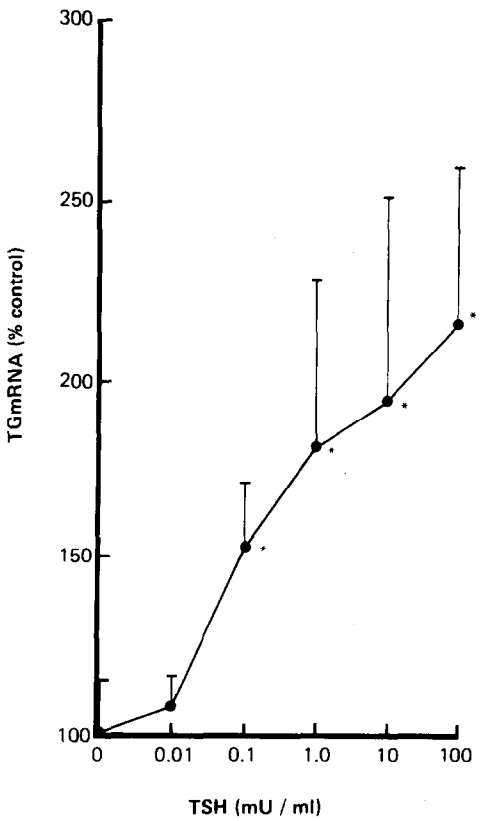

Fig.1. Effect of different concentrations of TSH on transcription of TG mRNA in cultured human thyroid cells. Cultures were exposed to TSH for 4 h. The TG mRNA was quantitated by densitometric scanning of total RNA with nick translated TG probe and normalizing with respect to signal from β-actin probe. The results are derived from three separate experiments.

(mean ± SD) increase in TG mRNA transcripts was apparent compared to cells cultured in the absence of the hormone (fig.1). This stimulatory effect of TSH was obtained 2 h following addition of the hormone and persisted for at least 48 h in the presence of 10 mU/ml TSH (fig.2A). Withdrawal of TSH from the culture medium led to a 50% decrease in TG mRNA levels after 5 days which following restimulation of the cells with TSH readily restored transcription rates of the TG gene to comparable levels (fig.2B). When TSH was substituted either with forskolin (10^{-5} M) or TPA (10^{-7} M) an increase in TG mRNA levels of 85.1 ± 39% and 52.8 ± 16.8%, respectively, was apparent. Elevation in cAMP levels with increasing dose of TSH was also observed (not shown).

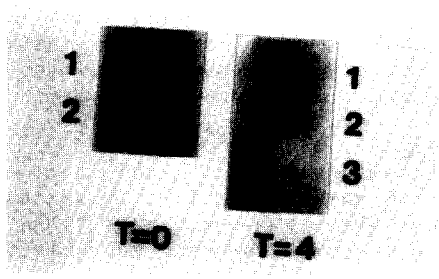

Fig.3. Nuclear runoff transcription assay on nuclei prepared from primary human thyroid cell cultures in the absence (T = 0) or presence of TSH (10 mU/ml) for 4 h (T = 4). ^{32}P-UTP labelled transcribed RNA was purified and hybridised to linearised plasmids: (1) *Eco*RI digested M4 plasmid containing 1260 bp TG cDNA in pBR322; (2) *Eco*RI digested pA1 plasmid containing 2100 bp chicken brain β-actin cDNA in pBR322; (3) *Eco*RI digested pBR322 plasmid as control.

3.2. *TG mRNA is regulated transcriptionally by TSH*

In order to determine whether the cytoplasmic TG mRNA increase observed with TSH was due to activation of the TG gene or changes in degradation of cytoplasmic mRNA, nuclear runoff transcription assays were performed. TSH induced approximately a 7-fold increase in TG gene transcription rate within 4 h compared to cells cultured in the absence of the hormone (fig.3).

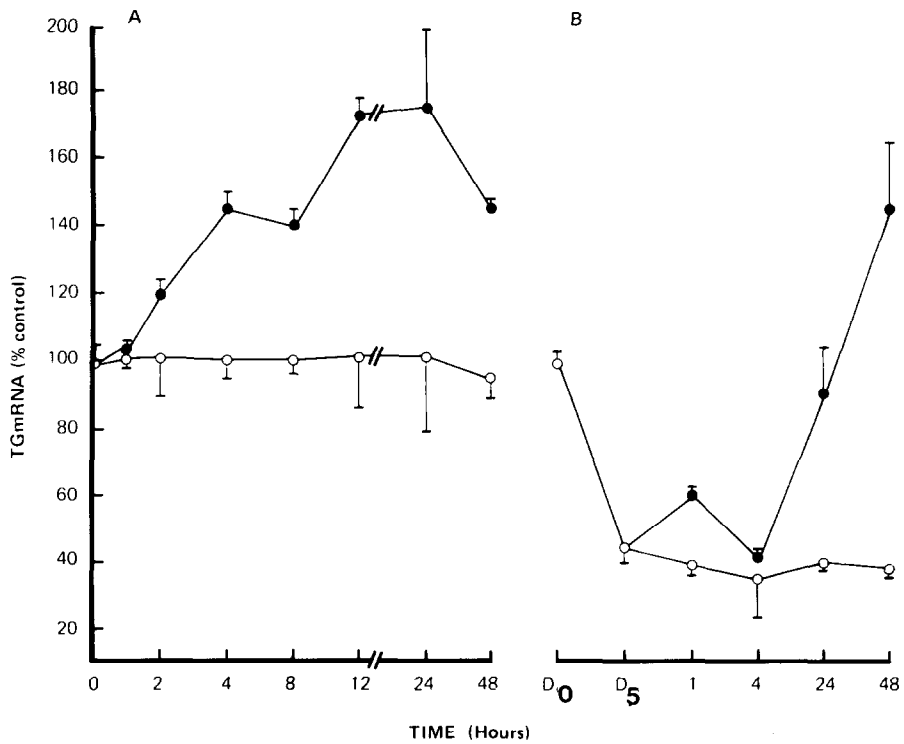

Fig.2. Effect of time on TG mRNA transcription following stimulation of cultured human thyroid cells with TSH. (A) Cells exposed to 10 mU/ml TSH (●) or cultured in the absence of the hormone (○). (B) Cells cultured in the absence of TSH for 5 days before addition of 10 mU/ml TSH (●) or cultured without the hormone (○).

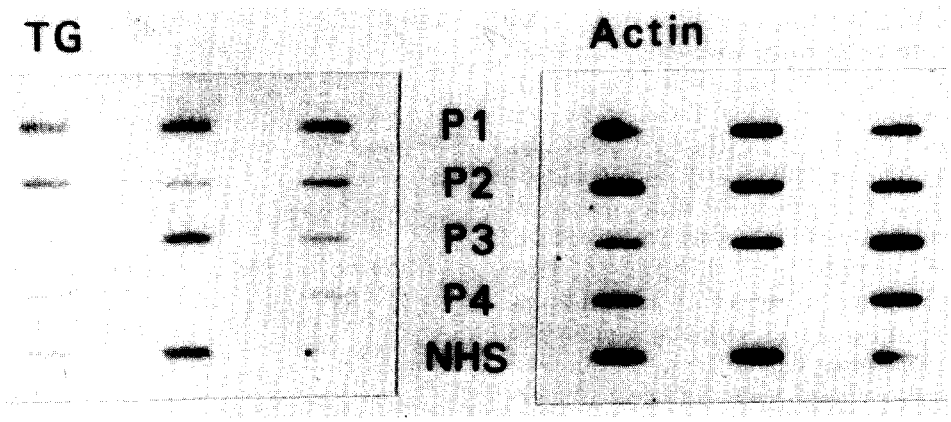

Fig.4. RNA slot blot analysis from human thyroid cells treated with Graves' IgG for 4 h. The figure shows the effect of 4 different sera from patients with Graves' disease (P1, P2, P3 and P4) in comparison to the effect of IgG preparation from a pool of 20 sera from normal individuals (NHS). The slots were hybridized with nick translated TG probe, exposed for autoradiography (4 days) dehybridized and exposed to nick translated β-actin probe for autoradiography (1 day).

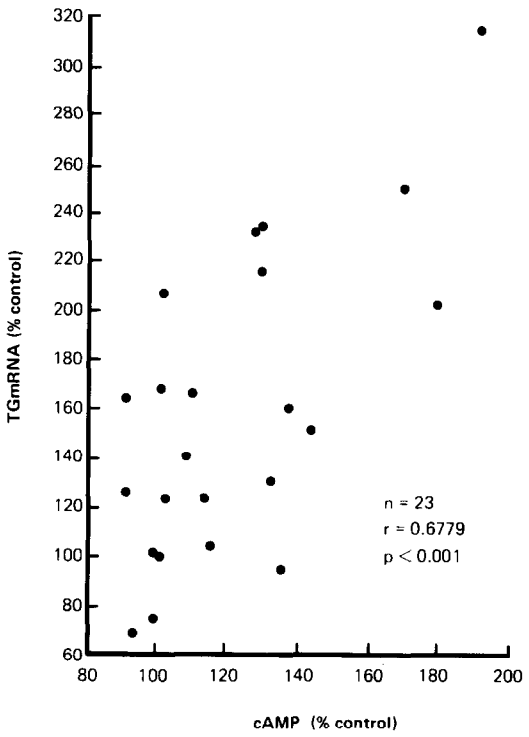

Fig.5. Correlation of transcription of TG mRNA in comparison to cAMP release following treatment of human thyroid cells with Graves' IgG preparations from 22 patients and pooled normal human sera.

This demonstrates that TSH effects the TG gene expression at the nuclear level. Actin transcription did not change with TSH stimulation (fig.3).

3.3. *Effect of immunoglobulin from Graves' disease patients on TG gene activation*

Immunoglobulin preparations from the sera of Graves' disease patients produced variable levels of TG gene activation after 4 h incubation which was not observed with pooled sera from normal individuals (fig.4). When compared to the cAMP increase with the Graves' IgG preparations, a highly significant correlation was obtained ($r = 0.6779$, $p < 0.001$) between the levels of TG gene activation and cAMP release, suggesting that cAMP was intimately involved in the stimulatory activity of the Ig preparations (fig.5).

4. DISCUSSION

Our results show that TSH produces a dose dependent increase in TG mRNA levels in cultured human thyroid cells. Whilst a several fold increase in TG specific RNA transcripts was apparent in the nuclei, the elevation in cytoplasmic TG mRNA levels was approximately 2-fold, which may thus indicate increased degradation of the TG mRNA in the cytoplasm following transcription. The parallel increase in the release of cAMP from these cells in

response to TSH suggests that cAMP acts as the physiological mediator of the TSH induced activation of the TG gene in human thyrocytes. These findings are supported by the effect of forskolin, an activator of the adenylate cyclase system which mimicked the effect of TSH on TG mRNA induction. Comparable observations in vitro also show that an increase in serum TG levels can be related to the TSH dose administered in humans [16]. Our findings contrast with the RNA hybridisation studies by Chebath et al. [17] where maximum TG levels reach a plateau at 0.05 mU/ml TSH in porcine thyroid cell cultures. Patients with hyperthyroid Graves' disease have elevated serum levels of TG which return into the normal range once euthyroidism is achieved with antithyroid treatment [18]. Thyroglobulin levels have been shown to correlate with TSH receptor antibody activity in vitro [7]. We now demonstrate a significant correlation between the activation of TG gene expression and the level of TSH receptor antibody activity as assessed by the generation of cAMP in a human thyroid cell bioassay. The ability to assess the thyroid cell functional response to TSH receptor antibody has always been limited by the inappropriateness of measuring release of total TG (the levels of which are also regulated by other factors) and the difficulty of measuring levels of thyroid hormone in tissue culture supernatants. Indirect measures of parameters such as receptor binding, cAMP generation, iodine uptake and colloid droplet formation give only limited and indirect information on the thyroid synthetic capacity. The demonstration of TG mRNA stimulation by TSH receptor antibody and their correlation with cAMP synthesis suggests a more direct measure of thyroid cell stimulation. The fact that the correlation is incomplete opens avenues for further investigation of other second messenger routes to the activation of the TG gene.

Acknowledgements: We thank Drs Y. Malthiery and S. Whatley for providing us with thyroglobulin and chicken β-actin cDNA probes, respectively, and Drs M. Page and F. Farzaneh for invaluable advice on nuclear runoff assays and hybridization protocols. Many thanks are due to Mrs J. De Groote for expert secretarial assistance. This work was supported by grants from the Medical Research Council and The Wellcome Trust.

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