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THE LEVEL OF THYROGLOBULIN mRNA IS REGULATED BY TSH BOTH IN VITRO AND IN VIVO

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We have studied the effect of thyrotropin on the intracellular concentration of thyroglobulin and of its mRNA both in a differentiated rat thyroid cell line and in the rat thyroid in vivo. Our data demonstrate that the absence of thyrotropin causes a two fold decrease of both thyroglobulin and of its mRNA suggesting that thyroglobulin synthesis in thyroid cells is 50% constitutive.

Thyroglobulin (TGB) plays a central role in thyroid hormone production in the thyroid gland.In fact it is within the covalent structure of this protein that iodinated tyrosine residues(MIT,DIT) are coupled to synthesize thyroid hormones(T3,T4).The levels of thyroid hormones in the bloodstream are regulated by thyrotropin(TSH) which promotes the reabsorption of thyroglobulin from the thyroid follicle,its subsequent degradation and the secretion of free T3 and T4 in the bloodstream (1). TSH has also a general stimulatory effect on the macromolecular synthesis in the thyroid gland:in fact it has been shown that TSH induces mytosis in the thyroid cells of the whole animal (2), is a growth factor for thyroid cells cultured in vitro (3) and stimulates both total RNA and protein synthesis in the follicular thyroid cells (4). It has been debated for a long time if,in addition to these general trophic effects on the thyroid tissue, the TSH also has a specific effect on TGB biosynthesis. The question has a general interest since the availability of a differentiated rat thyroid cell line (5),of rat TGB mRNA probes (6) and of the cloned rat TGB gene (7) could make the thyroid system an

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attractive one to study hormonal regulation of protein synthesis.In the present study we investigated the effect of TSH starvation on TGB synthesis and on the concentration of TGB mRNA, both in a differentiated rat thyroid cell line and in the rat thyroid gland .The level of TGB mRNA were measured by immunoprecipitation of ^{35}S labeled cell lysate with anti-TGB antibodies and by hybridization to cellular and tissue RNA with a cloned rat TGB cDNA probe. We have found an approximate two fold decrease in the relative concentration of TGB mRNA with both methods and in both experimental systems (cell line,whole rat). On the basis of these results we conclude that 50% of TGB synthesis in the thyroid gland is constitutive while the remaining 50% is under TSH control.

MATERIALS AND METHODS

Labeling of cultured cells with ^{35}S Methionine and measurement of the relative rate of TGB synthesis by immunoprecipitation. Normal differentiated thyroid cells (FRTL) were grown in the usual medium (5) minus TSH for 1 week. The cells were then incubated in the presence of TSH (10 milliunits/ml) (5) for 0, 24, 48, 72 hours. At the end of the incubation time, the cells were washed with phosphate-buffered saline and incubated 30 minutes in 2 ml of methionine-free medium containing 100 µCi of [^{35}S]methionine/ml (600 Ci/mmole; 1 Ci=3.7 x 10^{10} Bq) (Amersham).
The total protein synthesis was measured by TCA precipitation of the whole cell extracts. Thyroglobulin synthesis was measured by immunoprecipitation of the cell extracts (4 x 10^5 cpm) with specific anti TGB antibodies as described (8).
DNA-RNA hybridization.RNA was extracted from both cultured cells and from the thyroid tissue according to a published procedures (8).The polyA content of the RNA preparations was measured by hybridization with [^3H] poly U (50 Ci/mmole). Liquid DNA-RNA hybridization was carried out using as probe a rat TGB cDNA clone, previously described (6), labeled by nick translation (9) to a specific activity of 10^8 cpm/µg. The DNA (1-5 ng) was mixed with different amounts of specific RNAs and yeast RNA , dried down and dissolved in 0.03 ml of 80% formamide (twice recristallized), 0.03M PIPES (pH 6.5), 1 mM EDTA and 0.4 M NaCl (10) to a final RNA concentration of 700 µg/ml. The reaction mixture was incubated at 85°C for 15 minutes and then at 52°C for 15 hrs. At the end of the incubation time the reaction was diluted to 1 ml and divided in 2 aliquots for the determination of the total and S_1 resistant radioactivity (11). S_1 was used at concentrations of 2,000-5,000 U/ml. Filter hybridization was carried out as described by Thomas (12).

RESULTS AND DISCUSSION

FRTL cells grow normally in a medium containing 0.5% serum and 6 among hormones and growth factors,including TSH which is essential

for their growth (3,5) .We cultured the FRTL cells for 10 days in the absence of TSH.In these conditions the cells do not divide but they remain viable (data not shown). Protein synthesis in the absence of TSH decreases approximately ten fold(Fig. 1,panel A). After the ten days starvation TSH was added and the cells were cultured for 1,2 and 3 days respectively. At each time point the cells were labeled with [^{35}S] methionine for a period of 30 minutes,lysed and both total protein and TGB synthesis were determined as described in Materials and Methods.The relative rate of TGB synthesis,defined as the percentage of the total counts incorporated in TGB,decreases from 4% in the control cells to 1.5% after TSH starvation and returns to the control value two days after TSH readdition (Fig. 1, panel B). To determine whether the specific TGB induction by TSH was due to an increased stability of the protein or to an effect on the level of TGB mRNA,total RNA was extracted from FRTL cells both before and after TSH starvation.Hybridization with [^{3}H] labeled poly-U showed

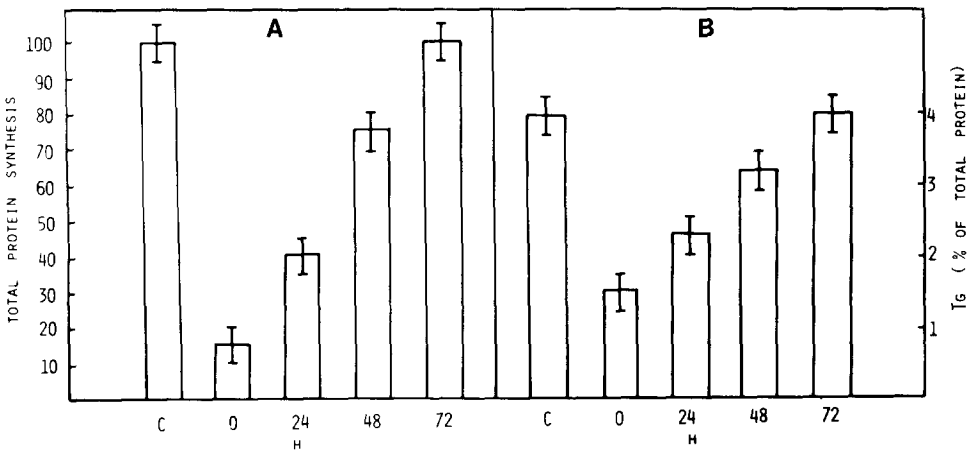

Figure 1. Effect of TSH on the total protein and TGB synthesis. A) Total protein synthesis was measured after incubation of FRTL cells in the presence of [^{35}S] methionine for 30 minutes, as described in "Materials and Methods". The cells were starved 1 week for TSH, the hormone was added and the total protein synthesis was measured by TCA precipitation at time 0, 24, 48 and 72 hours from the readdition of TSH. "C" represents the control value of the total protein synthesis of the cells continuously grown in the presence of TSH. B) TGB synthesis was measured, on the same samples described above, by immunoprecipitation with anti-Tg specific antibodies, as described in "Materials and Methods".

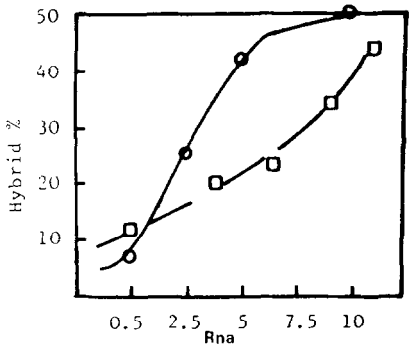

Figure 2. Effect of TSH on the TGB mRNA levels in thyroid cells. Total RNA was extracted from FRTL cells grown either in the presence (○) or in the absence(□) of TSH for 6 days. The RNA was then hybridized with the insert of a TGB specific cDNA clone, pRT575 (6), labeled to a specific activity of 1 x 10^8 cpm/µg. The hybridization was carried out as described in "Material and Methods". The amount of poly A^+ RNA (ngr/ 0.03 ml reaction mixture), measured by hybridization with [^{3}H] poly U, is indicated. The maximal value of the hybrid was 50% of the input DNA since the probe used was labeled in the double stranded form.

that there was no significant change in the poly-A content of the two RNA preparations(data not shown).On the other hand,hybridization with a nick-translated,cloned rat thyroglobulin cDNA fragment, extracted from plasmid pRT575(6), shows an approximate two fold decrease in TGB mRNA content in the cells starved for TSH(Fig.2), an effect similar in magnitude to the one observed by immunoprecipitation(Fig. 1, panel B).We thought worthwhile at this point to determine whether the effect of TSH on the TGB mRNA level in the rat thyroid gland in vivo was comparable to the effect observed in the cultured cell system. This would allow to use the FRTL cell line as a more convenient"analog" of the thyroid gland to study the regulation of TGB mRNA synthesis. A group of 40 rats was artificially made hyper-thyroid by daily injection of T_4(10µg /100g body weight/day).After 10 days of treatment the TSH in the serum was undetectable by a radioimmunoassay against human TSH(data not shown).At the end of the ten days period 20 rats were killed and total RNA was extracted from their thyroid gland.The remaining rats were injected with T_4 for two additional days and received at the same time two subcutaneous

Table I.

RAT TREATED WITH	TG M RNA
T4	1
--	2.5 ± 0.6
TSH	1.9 ± 0.4
PTU	3.0 ± 1.4

Effect of TSH on the TGB mRNA levels in vivo. Total thyroid RNA was extracted from the thyroids of rats, untreated or treated as described in the text, and spotted on nitrocellulose filter. The TGB mRNA was measured by hybridization of the filter to the [^{32}P] labeled insert of plasmid pRT575. The filter was then washed and exposed to an X-Ray film. The hybridization values were normalized by integration of the peak areas of the densitometry scanning and are expressed relatively to the level of the T_4 treated rats, arbitrarily set to 1.

injections/day of bovine TSH(30 mU/100g body weight/injection).A third group of rats was not treated at all to serve as a control,and to a fourth group was given the anthithyroid drug Propylthiouracil (PTU) to raise the concentration of TSH in their serum(13).

Total RNA was extracted from the thyroids of rats either untreated or treated as described above, and the levels of TGB mRNA measured by dot-blot hybridization(Table 1).Table 1 shows the level of TGB mRNA in the four RNA samples described above relative to the level of TBG mRNA in the T_4 treated animals(whose level was arbitrarily set to 1). Untreated,PTU treated and TSH injected animals have comparable levels of TGB mRNA which are in all cases about two fold higher then those of T_4 treated animals.These values are in good agreement with data obtained both in primary cultures of hog thyroid cells (14) and in whole rats(15). In the above mentioned studies the hybridizations were performed with non cloned TGB cDNA probes. Our data indicate that TGB mRNA steady-state levels are regulated by TSH. In addition they also suggest that the basal serum TSH level is sufficient to support maximal TGB synthesis, since the PTU treatment does not cause an additional increase in TGB mRNA level.This last evidence is in agreement with the results of Scherberg (16). We cannot at the

present indicate if the TSH increases the stability of TGB mRNA or affects the rate of transcription of the rat TGB gene. Preliminary data by Van Heuverswyn et al.(17)seem to indicate an effect at the trancriptional level.We believe that the analogy we found between the effect of TSH in cultured thyroid cells and in the thyroid gland of whole animals strenghtens the notion that this cell line can be conveniently used to study the regulatory regions of the thyroglobulin gene,which promises to be an interesting one both for its hormonal regulation and for its tissue specific expression.

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