BIOPHYSICS AND BIOCHEMISTRY

Mechanisms of Regulation of Lysosomal Thiol Protease Activity in Thyroid Gland: Effects of Protein Synthesis Inhibitors

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Triiodothyronine administered for 7 days reduces the contents of total cathepsin B and L (but not cathepsin C) in rat thyroid gland. Cycloheximide reduces the contents of cathepsins B, C, and L, while actinomycin D induces a less pronounced decrease in cathepsin C and L activity, cathepsin B activity being unchanged. Against the background of triiodothyronine, both agents equally inhibit these proteases.

Key Words: *lysosomal cysteine proteases; thyroid gland; triiodothyronine; regulation*

Despite the well-known fact that secretion of triiodothyronine (T_3) and thyroxine (T_4) is strongly limited by the rate of lysosomal processing of thyroglobulin [1], regulation of proteolysis in the thyroid gland is considerably less studied in comparison with the mechanisms controlling prohormone synthesis and secretion. An important role in hormonogenesis is played by lysosomal cysteine proteases. The cleavage sites within thyroglobulin molecule for these enzymes [3] and regulation of enzyme activity with pituitary thyrotropic hormone (TSH) have been previously established [2,7]. In comparison with other lysosomal hydrolases, thiol cathepsins are characterized by maximum sensitivity to factors stimulating and inhibiting thyroid function [2], which is indicative of a specific control of expression of these enzymes, probably, during biogenesis. The present study explores the effect of inhibitors of protein synthesis acting at the level of transcription and translation on the activity of lysosomal thiol proteases in intact and T_3-suppressed rat thyroid gland.

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MATERIALS AND METHODS

Experiments were carried out on 54 random-bred male albino rats weighing 210-230 g. The inhibitors of protein synthesis actinomycin D and cycloheximide (Reanal) were injected intraperitoneally in doses of 0.2 and 1 mg/kg, respectively, every 12 h (4 injections). The animals were sacrificed 6 h after the last injection. Control animals were injected with a solvent (5% ethanol). Triiodothyronine (Reanal) was dissolved in 0.025 M NaOH and injected subcutaneously in a dose of 10 μg/kg/day for 7 days. The animals were sacrificed on day 8 of the experiment. Control rats received the vehicle according to the same schedule. T_3-treated rats were injected with actinomycin D and cycloheximide on days 6 and 7 after the start of hormone treatment. Thyroid status was assessed by serum hormone level on the day of sacrifice. T_3 and T_4 were measured by radioimmune assay using RIA-T_3-ST and RIA-T_4-ST kits (Beloris); TSH was measured by enzyme-linked immunosorbent assay (Umelisa kits). Thyroidectomy, homogenization and fractionation of the tissue were performed as described elsewhere [8]. Sedimenting and

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nonsedimenting protease activities were determined spectrofluorometrically [4] on a Jobin & Yvon ME-431SC spectrometer. The total activity was calculated as the sum of sedimenting and nonsedimenting activities. The following substrates were used: Z-Arg-Arg-AMC (cathepsin B), Z-Phe-Arg-AMC (cathepsin L), and Gly-Phe-2NA (cathepsin C). Standard fluorophores 7-amino-4-methylcoumarin and 2-naphthylamine were used. Protein concentration in the homogenates was measured by the Bradford method. The data were processed by ANOVA using the Student's *t* test.

RESULTS

Exogenous T_3 inhibited cathepsins B and L in rat thyroid gland (Fig. 1), which attests to the involvement of these enzymes in a feed-back regulation of thyroid function with iodothyronines. Cathepsin C was resistant to T_3-regulation, as it was previously shown for dipeptidyl peptidase II [2].

Experiments with inhibitors of protein synthesis demonstrated different sensitivity of the studied enzymes to transcription and translation inhibitors (the effect of actinomycin and cycloheximide on the rate of RNA and protein synthesis in the thyroid gland has been documented previously [6]). Cycloheximide induced more pronounced changes: being injected in a maximum toxic dose, it sharply decreased total activity of all studied proteases. The effect of cycloheximide may at least in part be mediated through the pituitary: although in the series with cycloheximide injections changes in the content of TSH were statistically insignificant (Table 1), inhibition of TSH secretion by this agent has been observed *in vivo* [5]. However, in cycloheximide-treated rats, the activity of cathepsins B and C is lower than that in T_3-treated rats ($p<0.05$ and $p<0.001$, respectively), although the level of TSH is much less changed. Thus, the observed inhibition of thiol proteases caused by cycloheximide is a result of the ribosomal site of protein synthesis in the thyroid gland.

A maximum drop of enzyme activity occurred when actinomycin D and cycloheximide were administered to T_3-pretreated animals. Actinomycin D and T_3 acted synergically (Fig. 1): actinomycin D alone only slightly decreased the activity of cathepsins B, C, and L to 89.5±18.3, 75.22±7.04, and 68.22±9.40% of the control, respectively, while in T_3-pretreated rats, it inhibited the activity of these enzymes to 38.53±3.82, 49.23±16.22, and 42.0±2.78% of the mean values observed in animals injected with T_3 alone. The degree of cycloheximide-induced inhibition of cathepsins did not depend on T_3-pretreatment, i.e., cycloheximide and T_3 exerted

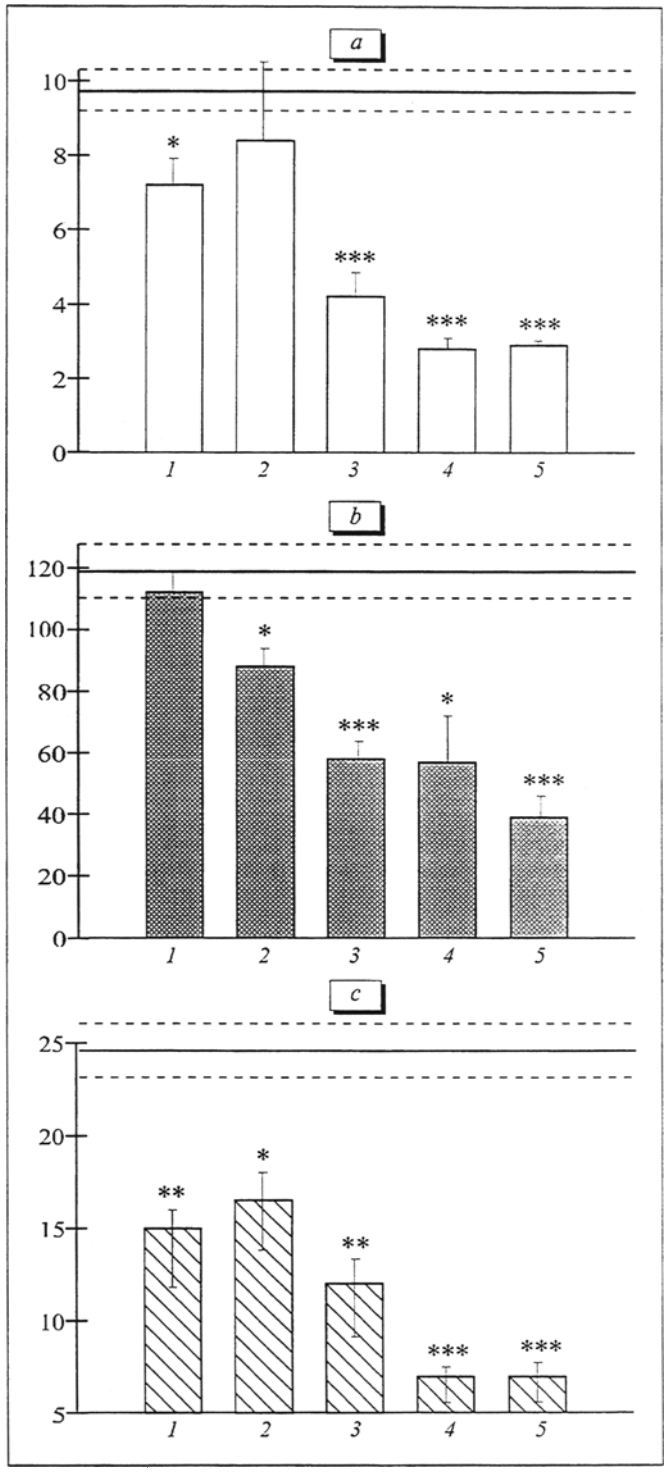

Fig. 1. Effect of triiodothyronine (T_3) and protein synthesis inhibitors on activity of lysosomal cysteine proteases. Total activity of cathepsin B (*a*), cathepsin C (*b*), and cathepsin L (*c*). Ordinate: enzyme activity, nmol 7-amino-4-methylcoumarin/mg protein×h× liter. Horizontal line: activity of the control series. *1*) T_3; *2*) actinomycin D; *3*) cycloheximide, *4*) T_3+actinomycin D; *5*) T_3+cycloheximide. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with the control.

an additive effect. These findings confirm the hypothesis that inhibition of lysosomal cysteine proteases

TABLE 1. Serum Level of TSH and Thyroid Hormones

Experimental series	Serum hormone level		
	T_3, nmol/liter	T_4, nmol/liter	TSH, IU/liter
Control (n=18)	1.665±0.195	36.668±6.433	1.530±0.555
T_3 (n=8)	1.256±0.433	1.060±0.977*	0.074±0.008**
Actinomycin D (n=8)	0.824±0.401	16.960±8.596	0.701±0.283
Cycloheximide (n=8)	0.302±0.098*	12.792±1.771**	0.350±0.171
T_3+actinomycin D (n=6)	1.738±0.224	0.984±0.655*	0.072±0.011**
T_3+cycloheximide (n=6)	1.568±0.356	1.023±0.820*	0.091±0.008**

Note. *p<0.001, **p<0.05 compared with the control.

in the thyroid gland of rats treated with T_3 is related to suppression of cathepsin biosynthesis. Previous studies have demonstrated [7] modulation of the cathepsin B mRNA in cultured FRTL-5 thyrocytes by TSH [4]. The interaction between actinomycin D and T_3 indicates nontranscriptional regulation of the cathepsin mRNA level (a similar effect of TSH has been previously shown for thyroid NADP-dependent malate dehydrogenase [1]). In this case, synergism in the action of actinomycin D and T_3 results from simultaneous inhibition of transcription and shortening of thiol cathepsin mRNA half-life in T_3-induced suppression of TSH level.

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