Biochimica et Biophysica Acta, 438 (1976) 449—460

BBA 67846

HUMAN THYROID CYCLIC NUCLEOTIDE PHOSPHODIESTERASE

ITS CHARACTERIZATION AND THE EFFECT OF SEVERAL HORMONES ON THE ACTIVITY *

AKIO NAGASAKA and HIROYOSHI HIDAKA

First Department of Internal Medicine, Faculty of Medicine, University of Nagoya, Nagoya and Deprtament of Biochemistry, Institute for Devlopmental Research, Aichi Prefecture Colony, Kasugai, Aichi (Japan)

(Received December 15th, 1975)

Summary

Cyclic AMP and cyclic GMP phosphodiesterase activities (3′5′-cyclic AMP 5′-nucleotidohydrolase, EC 3.1.4.17) were investigated in the human thyroid gland from patients with hyperthyroidism. Low substrate concentration (0.4 μM) was used. About 60% of the cyclic-AMP and 80% of the cyclic-GMP hydrolytic activities in the homogenate were obtained in the soluble fraction (105 000 × *g* supernatant). The thyroid gland contains two forms of cyclic-AMP phosphodiesterase, one with a K_m of $1.3 \cdot 10^{-5}$ M and the second with a K_m of $2 \cdot 10^{-6}$ M. Cyclic-AMP and cyclic-GMP phosphodiesterase were purified by gel filtration on a Sepharose-6B column. Cyclic-AMP phosphodiesterase activities were found in a broad area corresponding to molecular weights ranging from approx. 200 000 to 250 000 and cyclic-GMP phosphodiesterase activity was found in a single area corresponding to a molecular weight of 260 000. Cyclic-AMP phosphodiesterase activities were stimulated by the protein activator which was found in human thyroid and this stimulation was dependent on Ca^{2+}. Stimulation of cyclic-AMP phosphodiesterase by the activator was not significant even in the presence of enough Ca^{2+}. The effect of D,L-triiodothyronine, D,L-thyroxine, L-diiodotyrosine, L-monoiodotyrosine, L-thyronine, L-diiodothyronine, thyrotropin, hydrocortisone, adrenocorticotropin, cyclic-AMP and cyclic-GMP on the phosphodiesterase activities was studied. Cyclic-AMP, cyclic-GMP, D,L-triiosothyronine, D,L-thyroxine, adrenocorticotropin and hydrocortisone were found to inhibit the phophodiesterase. Triiodothyronine and thyroxine inhibited cyclic-AMP phosphodiesterase more effectively than cyclic-GMP phosphodiesterase. Thyroxine was a more potent inhibitor than triiodothy-

* These data were partly presented at the 7th International Thyroid Conference, 9—13 June 1975, Boston, Mass. U.S.A.

ronine. The concentration of cyclic AMP producing a 50% inhibition of cyclic-GMP phosphodiesterase activity was $5 \cdot 10^{-5}$ M, while the concentration of cyclic GMP producing a 50% inhibition of cyclic-AMP phosphodiesterase was $3 \cdot 10^{-3}$ M. Both cyclic-AMP and cyclic-GMP phosphodiesterase activities in the homogenate of hyperthyroidism, thyroid carcinoma and adenoma were higher than in normal thyroid tissue, when assayed with a low concentration of the substrate (0.4 μM). When a higher concentration (1 mM) of cyclic nucleotides was used as the substrate, cyclic-AMP hydrolytic activity in adenoma tissue was similar to that of normal tissue, while the other activities were higher than normal.

Introduction

Thyrotropin appears to affect the thyroid gland through regulation of cyclic AMP levels in the gland [1—3]. All effects of thyrotropin on thyroid-iodide metabolism, glucose oxidation and protein biosynthesis can be reproduced by cyclic AMP, so that cyclic AMP may play a very important role in the thyroid gland. The intracellular levels of the cyclic nucleotide are controlled by both adenylate cyclase, the enzyme that catalyzes the formation of cyclic AMP, and phosphodiesterase (EC 3.1.4.17) which catalyzes its hydrolysis, both of which were shown to exist in the thyroid gland [1,2,4,5]. The present studies were designated to elucidate in detail the properties of phosphodiesterase in human thyroid gland and the effects of various hormones on its activity in an effort to determine their relative importance in the control of intracellular cyclic-AMP or cyclic-GMP levels.

Materials and methods

Chemicals and reagents

Nucleotides and snake venom (*Crotalus atrox*) in a lyophilized form were obtained from Sigma Chemical Co. Tritium-labelled cyclic nucleotides (specific activity, cyclic AMP, 33.2 Ci/mmol, cyclic GMP, 3.46 Ci/mmol) were purchased from New England Nuclear. All reagents were of analytical grade. Thyroid hormones were purchased from Sigma Chemical Co. Thyrotropin was from Armour Pharmaceutical Co. Adrenocorticotropin (Cortrosyn) was from N.V. Organon Co. Hydrocortisone was obtained from Upjohn Co. and propylthiouracil and methylmercaptoimidazole were from Chugai Pharmaceutical Co.

Preparation of subcellular fraction

The human thyroid tissues were obtained surgically from patients with hyperthyroidism, thyroid carcinoma (papillary, 4 cases; follicular, 3 cases; Hürthle cell, 1 case) and adenoma (follicular, 8 cases). The normal thyroid tissues were obtained by isolation of normal adjacent tissue from a single nodule of thyroid adenoma which was removed by partial thyroidectomy. The thyroid tissues were carefully isolated from connective tissues and weighed, and a small portion of these tissues was taken at the same time and used for histological examination. Thyroid tissue used for most experiments was, unless otherwise stated,

the tissue obtained by thyroidectomy from the hyperthyroid patient. The tissues were homogenized with 9 vol. of 0.32 M sucrose solution containing 1 mM $MgCl_2$, 50 mM Tris · HCl (pH 7.5) using a glass tissue homogenizer. All steps were carried out at 0—4°C. The homogenate was centrifuged at 700 × *g* for 10 min at 4°C, the supernatant decanted and the precipitate washed 3 times by resuspending it in the original homogenate volume of this medium. The 700 × *g* supernatant fraction was then centrifuged at 10 000 × *g* for 10 min at 4°C and the 10 000 × *g* supernatant was also centrifuged at 105 000 × *g* for 60 min at 4°C. These precipitates were washed twice as described for the 700 × *g* precipitate. All precipitates were resuspended in Tris/sucrose medium at the volume of the original homogenate.

Assay procedures

The method is essentially that of Butcher and Sutherland [6], employing snake venom as a source of 5′-nucleotidase. The [^{3}H]adenosine or [^{3}H]guanosine was isolated by cation-exchange resin [7]. The assay for phosphodiesterase activity consisted of a two-step procedure. In the first stage of the incubation, the reaction mixture of 0.5 ml contained 32 mM Tris · HCl (pH 7.5), 4 mM $MgCl_2$, 0.4 μM to 1 mM cyclic [^{3}H]AMP or 0.4 μM to 1 mM cyclic [^{3}H]GMP (100 000 cpm) and an appropriate concentration of enzyme. The reaction was initiated by the addition of cyclic AMP or cyclic GMP. At the end of 15 min at 30°C, the tube containing the reaction mixture was transferred to a boiling water bath for 5 min to terminate the reaction. After thermal equilibration to 30°C, 0.05 ml of snake venom (1 mg/ml) was added for a second 10-min incubation. The reaction was stopped by boiling for 5 min. Then 1 ml of water was added, and the denatured proteins were removed by centrifugation. 1 ml of the clear supernatant fluid was applied to a small ion-exchange column (AG 50W-X4, 200—400 mesh, H^+ form). The product, [^{3}H]adenosine or [^{3}H]guanosine, was eluted with 1.5 ml of 3 M ammonium hydroxide after washing the column with 10 ml of water. The amounts of product were determined in a liquid scintillation spectrometer. Over 95% of adenosine or guanosine is recovered from the column using authentic compounds by this method. About 10% of the added cyclic nucleotide is generally hydrolyzed during the incubation. In this assay condition, over 80% of the products from cyclic AMP by human thyroid preparation and over 90% of the products from cyclic GMP by partially purified thyroid preparation were confirmed to be adenosine or guanosine, respectively, by the method reported previously [7].

Gel filtration

Gel filtration was performed on a Sepharose 6B column (2.6 × 95 cm) with a bed volume of 504 ml at flow rates of 18 ml per h using 50 mM Tris · HCl buffer (pH 7.5), containing 1 M KCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$. The column was calibrated with Blue Dextran 20 000, catalase, aldolase and bovine serum albumin.

Preparation of the activator

The activator of human thyroid was prepared according to Cheung [8]. Thyroid tissue was homogenized with 3 vols. of glass distilled water chilled to 0°C.

The homogenate was adjusted to pH 5.9 and spun at 13 000 × *g* for 30 min. The sediment was discarded. The supernatant was heated in a boiling water bath. After heating for 5 min, the beaker was transferred to an ice-bath for quick cooling. Denatured proteins were removed by centrifugation. The boiled supernatant (pH 5.9) was dialyzed twice against 300 vols. of 10 mM Tris · HCl (pH 7.5) buffer containing 1 mM $MgCl_2$ and 0.1 mM EGTA (ethyleneglycol-bis-(β-aminoethylether)-*N'*,*N'*-tetraacetic acid) for 24 h and used for the experiment. DNA was determined following the method of Schmidt/Thaunhauser/Schneider (micro method) [9]. The protein concentration was determined by the method of Lowry et al. [10].

Results

Linearity of the enzyme reaction

Cyclic-nucleotide phosphodiesterase activity in human thyroid was investigated using a low substrate concentration (0.4 μM). The linearity of the enzyme reaction with time and enzyme concentration was observed within 15 min when appropriate amounts of enzyme were used. The amount of enzyme which is able to convert below 15% of substrate was added to the incubation mixture. A similar linearity was observed, whichever substrate (cyclic AMP or cyclic GMP) or substrate concentration (low or high) was used.

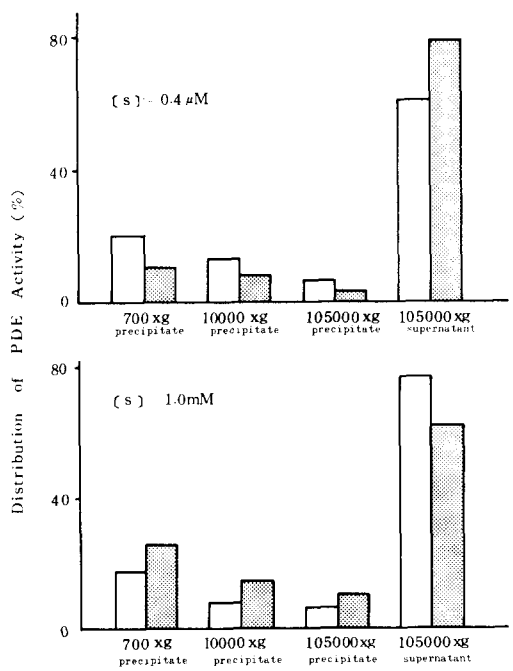

Fig. 1. Subcellular distribution of phosphodiesterase activity (PDE) in the thyroid gland. [S], substrate concentration in the reaction mixture. □, cyclic AMP phosphodiesterase activity. ▨, cyclic GMP phosphodiesterase activity. Recovery of total activity of the homogenate after fractionation was 91%.

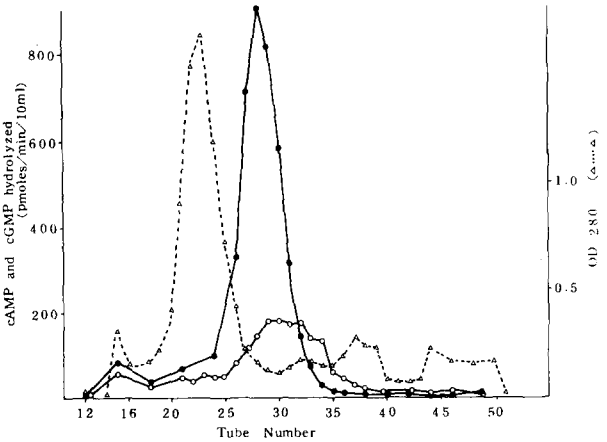

Fig. 2. Sepharose-6B column elution profile of 105 000 × *g* supernatant of the thyroid gland (hyperthyroidism). Volume of each tube was 10 ml. Flow rate was 18 ml/h. Enzyme activity was measured at 0.4 μM of the substrate. Details in the text. ○———○, cyclic AMP phosphodiesterase activity. ●———●, cyclic GMP phosphodiesterase activity. △- - - - -△, absorbance at 280 nm.

Distribution among subcellular fractions

Phosphodiesterase activity in all fractions was measured at both millimolar and micromolar substrate levels. The results from subcellular distribution studies carried out using four glands of four patients were averaged and shown in Fig. 1. Cyclic GMP was hydrolyzed slightly faster than cyclic AMP at the lower substrate level by the 105 000 × *g* supernatant fraction while cyclic AMP was hydrolyzed faster than cyclic GMP at the high substrate concentration. Substantial activity was present in all of the fractions although the most was contained in the supernatant.

Gel filtration on Sepharose 6B

Cyclic-AMP and cyclic-GMP phosphodiesterase were purified through a Sepharose-6B column. Fig. 2 shows the typical elution profile of the phospho-

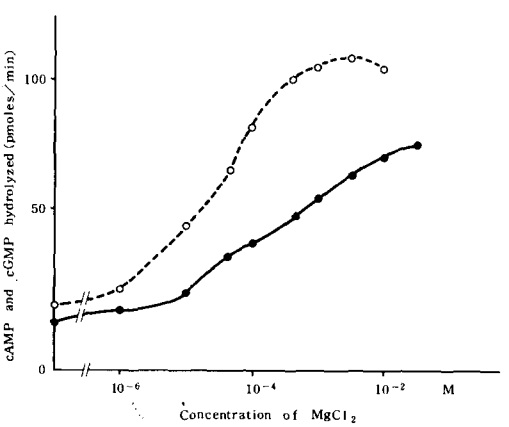

Fig. 3. Effects of Mg ion on phosphodiesterase activity. ●———●, cyclic AMP phosphodiesterase activity. ○- - - - -○, cyclic GMP phosphodiesterase activity. Assay was carried out under standard conditions. Partially purified enzyme by Sepharose 6B chromatography was used.

diesterase in the 105 000 × *g* supernatant of human thyroid gland. Purification of cyclic-AMP and cyclic-GMP phosphodiesterase was 12- and 14-fold the homogenate activity with approx. 60% of 105 000 × *g* supernatant activity after Sepharose-6B column chromatography. Cyclic-GMP and cyclic-AMP phosphodiesterase are found in the area corresponding to molecular weights of 260 000 and 200 000—250 000 respectively. The fractions containing cyclic-GMP phosphodiesterase (Fractions No. 24—27) and cyclic-AMP phosphodiesterase (Fractions No. 32—35) were pooled, concentrated in collodion bags at reduced pressure at 4°C and used for experiments. This preparation was referred to as the partially purified preparation.

Properties of partially purified preparation

The maximal activity of both cyclic-AMP and cyclic-GMP phosphodiesterase was observed at pH 7.0—7.5 at low or high substrate levels. However, the enzyme of human platelet and human serum exhibited the optimum at 8.0. The effect of Mg^{2+} on the phosphodiesterase is shown in Fig. 3. Maximal activity was observed at 4 mM for cyclic-GMP hydrolysis, which is comparable to the enzyme of human serum. Maximal of cyclic-AMP hydrolysis was observed with 10 mM of $MgCl_2$. A Lineweaver-Burk plot of a partially purified preparation showed biphasic kinetic behavior (Fig. 4a). Similar results were obtained with the 105 000 × *g* precipitate (4b). Hydrolysis of cyclic GMP by these preparations followed normal Michaelis-Menten kinetics (Fig. 4c,d).

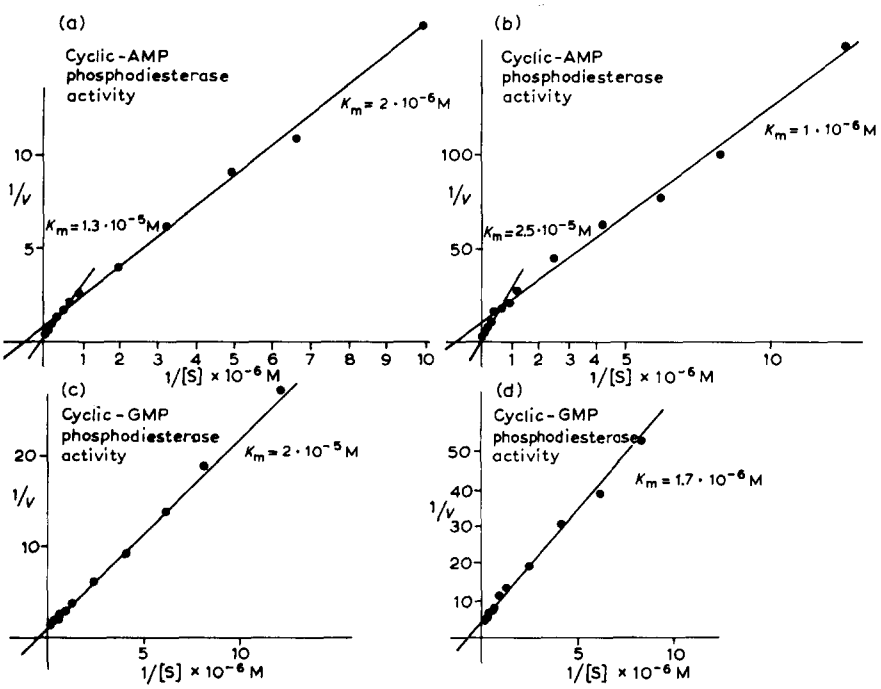

Fig. 4. Kinetic analysis of cyclic-AMP and cyclic-GMP hydrolysis (Lineweaver-Burk plots). Substrate concentrations of the reaction mixture were from 0.16 μM to 1.0 mM. Protein concentrations of the enzyme solutions were (a, c) 4.2 mg/ml (105 000 × *g* supernatant) and (b, d) 5.3 mg/ml (105 000 × *g* precipitate).

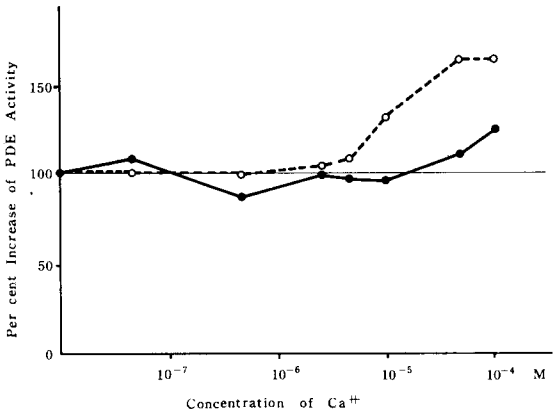

Fig. 5. Effect of Ca^{2+} on the hydrolysis of 0.4 μM cyclic AMP (●———●) and cyclic GMP (○- - - - -○) by a Ca^{2+}-free preparation of the phosphodiesterase. The assay was performed in the presence of saturating amounts of the activator. Ca^{2+}-EGTA buffer was used according to Kakiuchi et al. [17].

Effect of the activator and Ca^{2+} on the phosphodiesterase

Assay of the activator was made according to Kakiuchi et al. [17], based on the ability of the activator to enhance the activity of diluted 105 000 × *g* supernatant fluid of the rat brain homogenate in the presence of 100 μM Ca^{2+}. The amount of activator that doubled the enzyme activity in the standard system was defined as 10 units. Thus, the amount of activator in the thyroid homogenate of hyperthyroidism was found to be 3300 units/ml. The rate of hydrolysis of 0.4 μM cyclic AMP or cyclic GMP by a Ca^{2+}-free preparation of the cyclic-nucleotide phosphodiesterase of the 105 000 × *g* supernatant from the thyroid of a patient with hyperthyroidism, was titrated with Ca^{2+} in the presence of 10 units activator by the method of Kakiuchi et al. [17]. Cyclic-GMP hydrolysis was stimulated with a $CaCl_2$ concentration over 5 μM but no stimulation occurred below 1 μM (Fig. 5). However, cyclic-AMP hydrolysis was not stimulated significantly by the activator in the presence of $CaCl_2$ (Fig. 5). This is comparable to cyclic-AMP phosphodiesterase in rabbit aorta [14].

Effect of various compounds on the phosphodiesterase

The following compounds were used for this study: thyrotropin, hydrocortisone, D,L-thyroxine, D,L-3,5,3′-triiodothyronine, L-3,5-diiodothyronine, L-thyronine, diiodotyrosine, monoiodotyrosine, 6-propylthiouracil, 1-methyl-2-mercaptoimidazole, theophylline, *p*-chloromercuribenzoic acid, adrenocorticotropin, cyclic AMP and cyclic GMP. The effects of the various compounds on partially-purified phosphodiesterase of human thyroid gland are summarised in Table I. Cyclic AMP inhibited cyclic-GMP phosphodiesterase activity by 50% at the concentration of $5 \cdot 10^{-5}$ M, while cyclic GMP produced a 50% inhibition of cyclic-AMP phosphadiesterase at the concentration of $3 \cdot 10^{-3}$ M (Table I). Triiodothyronine and thyrosine were found to be effective inhibitors of cyclic-AMP phosphodiesterase but L-diiodothyronine, L-thyronine, diiodotyrosine and monoiodotyrosine did not affect the phosphodiesterase up to 1 mM. Cyclic-GMP phosphodiesterase was inhibited less effectively by triiodothyronine or thyroxine than cyclic-AMP phosphodiesterase (Table I). Thyroxine was found to be a more potent inhibitor of cyclic-AMP and cyclic-GMP phosphodi-

TABLE I

INHIBITION OF PHOSPHODIESTERASE ACTIVITY BY VARIOUS COMPOUNDS

Inhibitors	Concentration of I_{50} (M)	
	cyclic-AMP phosphodiesterase activity	cyclic-GMP phosphodiesterase activity
L-Thyroxine	$1 \cdot 10^{-5}$	$5 \cdot 10^{-5}$
D-Thyroxine	$2 \cdot 10^{-5}$	$5 \cdot 10^{-5}$
L-Triiodothyronine	$5 \cdot 10^{-5}$	$1 \cdot 10^{-4}$
D-Triiodothyronine	$1 \cdot 10^{-4}$	$4 \cdot 10^{-4}$
L-Thyronine	—	—
L-Diiodothyronine	—	—
L-Diiodotyrosine	—	—
L-Monoiodotyrosine	—	—
KI	—	—
Cyclic AMP		$5 \cdot 10^{-5}$
Cyclic GMP	$3 \cdot 10^{-3}$	
p-Chloromercuribenzoic acid	$1 \cdot 10^{-5}$	$1 \cdot 10^{-3}$
Theophylline	$2.5 \cdot 10^{-4}$	$1 \cdot 10^{-4}$
Hydrocortisone	$5 \cdot 10^{-4}$	$2 \cdot 10^{-3}$
β-Methasone	$8 \cdot 10^{-4}$	$2 \cdot 10^{-3}$
Adrenocorticotropin	$6 \cdot 10^{-4}$	$1 \cdot 10^{-3}$
Thyrotropin	—	—
Propylthiouracil	—	—
Methylmercaptoimidazole	—	—

I_{50}, inhibitor concentration which causes 50% inhibition of the enzyme activity.

esterase than triiodothyronine. The kinetic study indicates that triiodothyronine and thyroxine inhibited cyclic-AMP and cyclic-GMP phosphodiesterase competitively with the substrate (Fig. 6a,b). These inhibitory effects subsided after dialysis of the enzyme preincubated with triiodothyronine and thyroxine. The K_i calculated by Dixon plot was $2.5 \cdot 10^{-5}$ M (thyroxine) and $6 \cdot 10^{-5}$ M (triiodothyronine) for cyclic AMP hydrolysis, and $5 \cdot 10^{-5}$ M (thyroxine) and $6.5 \cdot 10^{-5}$ M (triiodothyronine) for cyclic-GMP hydrolysis (Fig. 6a,b).

Thyroid cyclic-nucleotide phosphodiesterase activity in various thyroid disorders

Thyroid cyclic-nucleotide phosphodiesterase activity was determined in the thyroid homogenate of hyperthyroidism, thyroid carcinoma, adenoma and normal. Both cyclic-AMP and cyclic-GMP phosphodiesterase activities were measured at millimolar and micromolar substrate levels. The results are summarised in Table II. Both the cyclic-AMP and the cyclic-GMP hydrolytic activity per DNA were found to be higher in hyperthyroidism, thyroid adenoma and carcinoma than in normal when activities were measured at low substrate level (0.4 μM). However, at high substrate level (1.0 mM) these hydrolytic activities were found to increase significantly only in hyperthyroidism. Table II shows the ratio of cyclic-GMP to cyclic-AMP hydrolysis by the 105 000 × *g* supernatant of thyroid homogenate in various thyroid disorders. This ratio varied among the preparations from various source (Table III). At a lower substrate level, the ratio was significantly higher in hyperthyroidism than in other thyroid disorders or normal. But at high substrate level, it was higher than normal in all disorders.

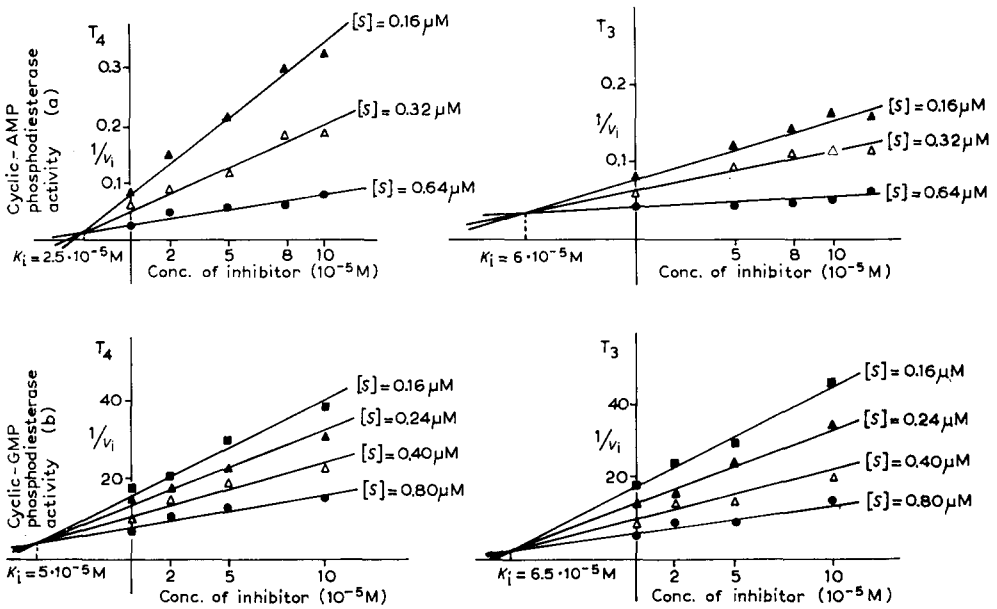

Fig. 6. Inhibition of cyclic-AMP and cyclic-GMP phosphodiesterase activity by the thyroid hormones (Dixon plots). Triiodothyronine and thyroxine were dissolved in dimethylsulfoxide (5%). Final concentration of dimethylsulfoxide in the reaction mixture was 0.5%. Cyclic-AMP and cyclic-GMP phosphodiesterase activities were not inhibited by lower concentrations of dimethylsulfoxide (below 1.0% in final concentration). S, substrate concentration.

TABLE II

CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ACTIVITY IN VARIOUS THYROID DISORDERS

The values are mean ± SE (a) per mg protein (b) per μg DNA.

Cyclic AMP hydrolyzed		Cyclic GMP hydrolyzed	
[S] = 0.4 μM (pmol/15 min)	[S] = 1.0 mM (nmol/15 min)	[S] = 0.4 μM (pmol/15 min)	[S] =1.0 mM (nmol/15 min)
Normal (9 cases)			
(a) 53.7 ± 6.2	10.1 ± 1.0	140 ± 32	2.7 ± 0.5
(b) 1.7 ± 0.8	0.17 ± 0.06	2.4 ± 0.7	0.05 ± 0.02
Hyperthyroidism (8 cases)			
(a) 140 ± 15 *	18.7 ± 3.1 *	435 ± 67 *	9.1 ± 2.1 *
(b) 5.0 ± 1.2 *	0.5 ± 0.08 *	14.6 ± 1.5 *	0.27 ± 0.03 *
Adenoma (8 cases **)			
(a) 143 ± 29 *	10.5 ± 3.2	513 ± 120 *	7.9 ± 2.1 *
(b) 5.1 ± 1.6 *	0.18 ± 0.04	9.0 ± 2.4 *	0.15 ± 0.08
Carcinoma (8 cases ***)			
(a) 214 ± 36 *	19.5 ± 5.2 *	565 ± 102 *	10.0 ± 2.8 *
(b) 5.8 ± 1.4 *	0.27 ± 0.09	9.8 ± 2.3 *	0.13 ± 0.06

* Statistically significant ($P < 0.05$) compared to normal (t-test).

** Follicular adenoma.

*** Papillary, 4; follicular, 3; Hürthle cell, 1.

TABLE III

RATIO OF CYCLIC GMP PHOSPHODIESTERASE ACTIVITY TO CYCLIC AMP PHOSPHODIESTERASE ACTIVITY

	[S] = 0.4 μM (pmol/μg DNA)	[S] = 1.0 mM (nmol/μg DNA)
Normal	1.4	0.3
Hyperthyroidism	2.9	0.5
Adenoma	1.8	0.8
Carcinoma	1.7	0.5

Discussion

The properties of human thyroid cyclic-nucleotide phosphodiesterase are not essentially different from those of the other tissue enzyme [11—15]. Substantial activity was present in all the fractions, although mostly in the 105 000 × *g* supernatant. Mg^{2+} was essential for cyclic-AMP and cyclic-GMP hydrolysis by the thyroid enzyme. The existence of the protein activator [8,16,17] was also demonstrated in the thyroid tissue. The pH optimum at 7.0—7.5 was different from the pH optimum of the enzyme in the other tissues; rabbit aorta [14], dog heart [6]: 9.0; rat brain [31], bovine brain [30], human platelet [32], human serum [7] and frog erythrocyte [33]: 8.0. The effect of cyclic GMP on thyroid cyclic-AMP phosphodiesterase was also similar to the effect obtained from other tissues [16,18]. It is interesting that cyclic-GMP hydrolysis was more effectively inhibited by cyclic AMP than cyclic-AMP hydrolysis was by cyclic GMP. Many investigators demonstrated the existence of two K_m values for cyclic-AMP phosphodiesterase of various tissues [11—15,19]. The present paper shows that the thyroid enzyme also exhibits two K_m values, but cyclic-GMP phosphodiesterase in the thyroid gland has only one K_m value. Apparent K_m values of the soluble fraction and the precipitated fraction were not different from each other. Triiodothyronine and thyroxine inhibit both thyroid cyclic-AMP and cyclic-GMP phosphodiesterases reversibly, but thyroxine is a more potent inhibitor than triiodothyronine. These thyroid hormones inhibit cyclic-AMP phosphodiesterase more effectively than cyclic-GMP phosphodiesterase. 20 μM of thyroxine produced a 50% inhibition of thyroid cyclic-AMP phosphodiesterase, but the same concentration of triiodothyronine did not inhibit the cyclic-GMP phosphodiesterase at all. Several investigators have reported that triiodothyronine and thyroxine inhibit the phosphodiesterase in adipose tissue, beef heart [20], isolated fat cell [21] and bone tissue [22]. There is a possibility that triiodothyronine or thyroxine affects cyclic-AMP level in the thyroid gland through its inhibition of phosphodiesterase. This suggests the existence of a short feedback mechanism in the thyroid gland, which was also proposed by Shishiba et al. [23] and Burke et al. [24]. But to draw conclusion on the regulation of cyclic nucleotides in the thyroid gland by triiodothronine or thyroxine is not possible until definite results of in vivo experiments are obtained. Although there are reports that thyrotropin and propylthiouracil stimulate cyclic-AMP phosphodiesterase in vivo [5] and in vitro

[15], the present study using human thyroid tissue indicates that thyrotropin and propylthiouracil do not affect the phosphodiesterase activity in vitro. Our results were identical to the report of Miyai et al. [25]. It has been reported that glucocorticoid inhibited the phosphodiesterase in beef heart (K_i, $1.1 \cdot 10^{-3}$ M) [26] and adrenocorticotropin activated the phosphodiesterase [27]. Our data show that glucocorticoid and adrenocorticotropin inhibited the phosphodiesterase in the thyroid, but the concentration of these compounds which inhibit the enzyme was too high to be a physiological dose. Further studies are necessary to come to a conclusion. The present paper shows that thyroid cyclic-nucleotide phosphodiesterase activity of hyperthyroidism increased significantly compared to the activity of normal tissue. This result somewhat disagrees with a previous report [28]. The increased activities in hyperthyroidism are more prominent when they are compared to the normal activity by the activity per DNA content. This agrees with histological findings that thyroid cells become hypertrophic in hyperthyroidism compared to the normal cells. This increase is thought to be due to an increased level of phosphodiesterase activity in thyroid cell. The low K_m phosphodiesterase activity per DNA in thyroid carcinoma and adenoma increased over the normal activity. The increased activities of the high-K_m enzyme in thyroid carcinoma and adenoma were observed as a function of thyroid wet weight or mg protein, but no increment was found as a function of DNA content. This result agrees with the fact that cell population density in thyroid carcinoma or adenoma increases [29]. On the other hand, elevated phosphodiesterase activities as a function of DNA content in thyroid carcinoma or adenoma were observed when the activities were measured at lower substrate level (0.4 μM). This suggests that the low-K_m enzyme may have a certain role in the thyroid.

Acknowledgment

We thank Drs. N. Nakagami, S. Hayami and H. Hasegawa (Nagoya First Red Cross Hospital) for supplying us with thyroid specimens and for their advice on this work, and Dr. Leslie J. DeGroot (University of Chicago, U.S.A.) for his valuable comments.

References

1 Pastan, I. and Katzen, R. (1967) Biochem. Biophys. Res. Commun. 29, 792—798
2 Zor, U., Kaneko, T., Lowe, I.P., Bloom, G. and Field, J.B. (1969) J. Biol. Chem. 244, 5189—5195
3 Dumont, J.E. (1971) Vitam. Horm. 29, 287—412
4 Monn, E. and Christiansen, R.O. (1971) Science 173, 540—542
5 Bastomsky, C.H., Zakarija, M. and McKenzie, J.M. (1971) Biochim. Biophys. Acta 230, 286—295
6 Butcher, R.W. and Sutherland, E.W. (1962) J. Biol. Chem. 237, 1244—1250
7 Hidaka, H. and Shibuya, M. (1974) Biochem. Med. 10, 301—311
8 Cheung, W.Y. (1971) J. Biol. Chem. 246, 2859—2869
9 Schneider, W.C. (1946) J. Biol. Chem. 164, 747—751
10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
11 Song, S.Y. and Cheung, W.Y. (1971) Biochim. Biophys. Acta 242, 593—605
12 Lagarde, A. and Colobert, L. (1972) Biochim. Biophys. Acta 276, 444—453
13 Schröder, J. and Rickenberg, H.V. (1973) Biochim. Biophys. Acta 302, 50—63
14 Hidaka, H., Asano, T. and Shimamoto, T. (1975) Biochim. Biophys. Acta 377, 102—116
15 Zsabo, M. and Burke, G. (1972) Biochim. Biophys. Acta 284, 208—219
16 Cheung, W.Y. (1970) Biochem. Biophys. Res. Commun. 38, 533—538

17 Kakiuchi, S., Yamazaki, R., Teshima, Y. and Uenishi, K. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3526—3530
18 Sakai, T., Thompson, W.J., Lavis, U.R. and Williams, R.H. (1974) Arch. Biochem. Biophys. 162, 331—339
19 Thompson, W.J. and Appleman, M.M. (1971) J. Biol. Chem. 246, 3145—3150
20 Mandel, L.R. and Kuehl, F.A. (1967) Biochem. Biophys. Res. Commun. 28, 13—18
21 Challoner, D.R. and Allen, D.O. (1970) Metabolism 19, 480—487
22 Marcus, R. (1975) Endocrinology 96, 400—408
23 Shishiba, Y., Shimizu, T. and Ozawa, Y. (1975) in International Congress Series 361, 3 (Abstract) Excerpta Medica
24 Burke, G., Yu. S. and Richman, R. (1975) in International Congress Series 361, 3 (Abstract) Excerpta Medica
25 Miyai, K., Amino, N., Azukizawa, M. and Kumahara, Y. (1971) Acta Endocrinol. 67, 209—215
26 Senft, G., Schultz, G., Munske, K. and Hoffmann, M. (1968) Diabetologia 4, 330—335
27 Pawlson, L.G., Lovell-Smith, C.J., Manganiello, V.C. and Vaughan, M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 1639—1642
28 Field, J.B., Larsen, P.R., Yamashita, K. and Chayoth, R. (1974) J. Clin. Endocr. Metab. 39, 942—949
29 Anderson, W.B., Russell, T.R., Carchman, R.A. and Pastan, I. (1973) Proc. Natl. Acad. Sci. U.S. 70, 3802—3805
30 Cheung, W.Y. (1969) Biochim. Biophys. Acta 191, 303—315
31 Drummond, G.I. and Perrott-Yee, S. (1961) J. Biol. Chem. 236, 1126—1129
32 Salzman, E.W. (1970) New Engl. J. Med. 286, 358—363
33 Rosen, O.M. (1970) Arch. Biochem. Biophys. 137, 435—441