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PROPERTIES OF CYCLIC AMP-DEPENDENT PROTEIN KINASE IN NORMAL AND GOITROUS RAT THYROID GLAND

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Summary

Most of the cyclic AMP-dependent protein kinase activity in propylthiouracil-induced goiters and control rat thyroid glands was found in the soluble fraction. The activity in the particulate fractions was cyclic AMP-independent. Protein kinase activity was 2—3-fold higher in all the subcellular fractions of goitrous tissue than of control tissue. In the presence of Triton X-100, both groups showed a significant increase in kinase activity in all subcellular fractions, and the kinase activity in the particulate fractions could now be slightly stimulated by cyclic AMP. Again, enzyme activity in fractions from goiters was significantly higher than in control tissue. Two major peaks, Types I and II, of soluble cyclic AMP-dependent protein kinase activity could be separated by DEAE-cellulose chromatography. Chronic in vivo stimulation by TSH was associated with a selective increase in Type II isoenzyme activity. Elution and pH profiles, dissociation of subunits with 0.5 M NaCl, and activity ratios (—cyclic AMP/+cyclic AMP) for various substrates for Type II isoenzyme in goitrous and control tissue were similar. The elevated activity in goitrous tissue was manifested by an increase in V for histone, ATP, Mg^{2+} and cyclic AMP, with no change in the apparent K_m.

Introduction

Adenosine 3′,5′-monophosphate (cyclic AMP) has been implicated as an intracellular second messenger in mediating the effects of many polypeptide hormones on their target tissues [1,2]. Probably all the cyclic AMP-mediated changes of metabolic activities in cells involve the phosphorylation of sub-

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Abbreviation: TSH, thyroid-stimulating hormone.

strates by cyclic AMP-dependent protein kinases [3—6]. In the thyroid gland, it appears that stimulation of the thyroid cells by thyroid-stimulating hormone is mediated through the cyclic AMP system [7,8]. Several in vitro studies [9—13] demonstrated that treatment of calf thyroid cells with TSH activated protein kinase, presumably through dissociation of holoenzyme. Although Rapoport and DeGroot [14] injected TSH into rats and found no effect on protein kinase activity 20 h after injection, chronic hypersecretion of TSH due to propylthiouracil treatment was associated with an increase in total kinase activity. This was confirmed by Pavlovic-Hournac and Delbauffe [15,16], who used methylthiouracil-treated rats. They also demonstrated that TSH preferentially stimulates some protein kinase activities separated by sucrose gradient ultracentrifugation [16]. By using DEAE-cellulose chromatography, Corbin et al. [17] resolved two distinct isoenzymes of cyclic AMP-dependent protein kinase, Types I and II, from various tissues on the basis of the salt concentration necessary for elution and the association-dissociation behavior of their regulatory and catalytic subunits. Two such isoenzymes have been observed in thyroid tissue by several investigators [18—20]. When it was noted that the proportions of Types I and II protein kinase varied with species and with cell type, investigators began to search for ways in which to correlate enzyme type with cell physiology. Recent studies have shown changes in isoenzyme ratios with changes in hormone-induced cell growth and differentiation. Lee et al. [21] studied the distribution of Types I and II kinase during postnatal development of rat testes and provided some evidence that synthesis of Type II isoenzyme may be involved in cell differentiation and may be under the control of gonadotrophic hormones. Byus et al. [22] showed a selective increase in the specific activity of Type I isoenzyme after isoproterenol-induced cardiac hypertrophy. Roskoski et al. [23] were unable to confirm this increase in soluble heart isoenzyme I activity, but did show a selective decrease in isoenzyme I in salivary glands with isoproterenol-induced hyperplasia.

In this study, we have investigated the subcellular distribution of cyclic AMP-dependent protein kinase activity in propylthiouracil-induced goiters and control rat thyroid tissue. Enzyme activity was 2—3-fold higher in all the subcellular fractions in goitrous tissue. These differences persisted even after solubilization with Triton X-100. Furthermore, TSH hypersecretion was associated with a selective increase in Type II protein kinase activity. This elevated activity was manifested by an increase in V for histone, ATP and Mg^{2+} with no change in the apparent K_m.

Materials and Methods

6-Propyl-2-thiouracil, kappa-casein, gamma-casein, and beta-casein were purchased from ICN Pharmaceuticals (Cleveland, OH). ATP (from equine muscle; sodium salt), adenosine 3′,5′-monophosphate, theophylline, albumin (bovine fraction V), thyroglobulin (bovine type I), phosvitin (from egg vitellin), alpha-casein, protamine (free base), protamine chloride, histone (from calf thymus; types III-S, II-A, and VIII-S), and DEAE-cellulose (medium mesh anion exchanger; 0.86 mEq/g) were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]cyclic AMP (30—50 Ci/mmol), [γ-^{32}P]ATP (1000—3000 Ci/

mmol), and Econofluor were obtained from New England Nuclear (Boston, MA). 2,5-Diphenyloxazole (PPO), toluene, and Triton X-100 were purchased from Research Products International (Elk Grove Village, IL). Ethylene glycol monomethyl ether and 1,4-bis [2-(4-methyl-5-phenyloxazole)]benzene (dimethyl POPOP) were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Millipore filters (type HA; 0.45 μm pore size; 25 mm diameter) were obtained from Millipore Corporation (Bedford, MA). Gelman filters (type GN-6, 0.45 μm pore size; 25 mm diameter) were obtained from Gelman Instrument Co. (Ann Arbor, MI). All other chemicals were of reagent grade from Fisher Scientific Co. (Fair Lawn, NJ), J.T. Baker Chemical Co. (Phillipsburg, NJ), or Mallinckrodt Incorporated (St. Louis, MO). Distilled water was redistilled in glass and deionized before use.

Experimental animals. Male albino Holtzman rats were used for these studies. Goitrous thyroids were produced by daily subcutaneous injection of 20 mg 6-propyl-2-thiouracil for a minimum of 21 days. This resulted in a 5-fold increase in thyroid wet weight. All rats were fed standard laboratory chow, given water ad libitum, and maintained under similar environmental conditions. Thyroids were removed under ether anesthesia, trimmed of loose connective tissue, and placed in buffer (0.25 M sucrose in 0.05 M Tris-HCl, pH 7.4) at 4°C. Before homogenization they were blotted and weighed (wet weight).

Preparation of partially purified cyclic AMP-dependent protein kinase. Pooled thyroid glands (400—600 mg) were minced and homogenized in buffer (0.01 M Tris-HCl, pH 7.4) at 100 mg wet tissue/ml. Homogenization was carried out at 4°C with a Polytron (Brinkman Instruments Co.) at a setting of 2.6 for four 5-s pulses with interim rest of 2 min. The homogenate was strained through 2 layers of cheesecloth and then centrifuged at 105 000 $\times g$ for 90 min. The supernatant was strained through glass wool to remove lipid particles and applied to a DEAE-cellulose column (0.7 X 5 cm) equilibrated with the homogenization buffer. The columns were washed with five bed volumes of buffer prior to eluting the protein kinase with a 50 ml linear 0—0.5 M NaCl gradient. Approx. 400-μl fractions were collected and assayed for cyclic AMP binding and protein kinase activity. The peaks of enzyme activity were pooled, dialyzed against 0.01 M Tris-HCl (pH 7.4) and concentrated to approx. 3 mg protein/ml in an Amicon model 12 ultrafiltration apparatus with a PM 10 membrane. The total activity for peaks I and II was calculated by addition of activity in individual fractions.

For quantitative recovery and measurement of Types I and II protein kinase, the columns prepared with protein above were first eluted with 4 ml 0.1 M NaCl to remove Type I and then with 4 ml 0.3 M NaCl to remove Type II. The eluted enzymes were dialyzed and concentrated as before.

Preparation of subcellular fractions. Thyroid glands (600—800 mg) were minced and homogenized as described above in 0.25 M sucrose in 0.01 M Tris-HCl, pH 7.4. The homogenate was centrifuged at 500 $\times g$ for 20 min. The supernatant was centrifuged at 9000 $\times g$ for 20 min and the resulting supernatant was centrifuged at 27 000 $\times g$ for 20 min. The 27 000 $\times g$ supernatant was centrifuged at 105 000 $\times g$ for 1.5 h to yield cytosol and a pelleted microsomal fraction. The pelleted fractions were resuspended in 1 ml of cold homogenizing buffer for assay.

Protein kinase assay. Histone IIA was used as the substrate for all studies unless stated otherwise. Protein kinase activity was measured by a modification of the method of Kuo and Greengard [24]. The standard incubation volume of 0.1 ml contained 42 mM sodium acetate, pH 6.0, 17 mM NaF, 3.5 mM theophylline, 0.2 mg histone IIA, 11 mM $MgCl_2$, 55 μM [γ-^{32}P]ATP (70 cpm/pmol), 6 μM cyclic AMP, and 50—125 μg protein. The reaction was initiated by the addition of the enzyme. Incubation was for 5 min at 30°C unless otherwise stated. The reaction was terminated by the addition of 20% trichloroacetic acid, after which the sample was filtered through cellulose acetate discs and washed three times with 3 ml 20% trichloroacetic acid. All assays were carried out within ranges of time and protein concentration in which reactions were linear. The radioactivity was determined by liquid scintillation counting. Enzyme activity was expressed as pmol ^{32}P incorporated into histone IIA per min per mg protein (and per mg wet tissue) at 30°C. Protein was measured by the method of Lowry et al. [25], with bovine serum albumin standard.

Analysis of data. Since protein composition may be different in control and goitrous thyroid glands owing to differences in thyroglobulin concentration, enzyme activity in unpurified subcellular fractions was expressed per mg wet tissue. Activity of partially purified enzyme was expressed per mg protein (specific activity) since most of the contaminating proteins were eliminated. Kinetic data were plotted as substrate concentration (S) vs. enzyme activity (V). The values for apparent K_m and V were calculated from the S vs. V graphs all of which displayed saturability. The apparent K_a for cyclic AMP was calculated from the cyclic AMP concentrations vs. enzyme activity curve as recommended by Swillens et al. [26]. Data were analyzed statistically by means of Student's t test for unpaired samples. In all cases, a P value equal to or less than 0.05 was taken to denote a statistically significant difference between the means of groups.

Results

Subcellular distribution of protein kinase activity

Table I shows the activity of cyclic AMP-independent and cyclic AMP-dependent protein kinase in major subcellular fractions of control and goitrous rat thyroid tissue and the effect of membrane solubilization with Triton X-100. In both control and goitrous tissue under all of the conditions shown, the cytosol (105 000 $\times g$ supernatant) contained the highest activity.

In all non-solubilized membrane fractions the protein kinase activity was cyclic AMP-independent (except the 500 $\times g$ pellet from goitrous thyroids). The activity in the corresponding cytosol fractions was cyclic AMP-dependent, with activity in the presence of cyclic AMP increased 6-fold in control cytosol and 4-fold in goitrous thyroid cytosol. In a comparison of activity from non-solubilized fractions, it was observed that propylthiouracil treatment resulted in at least a 3-fold increase in activity both in the presence and absence of cyclic AMP.

Upon solubilization with Triton X-100, control tissue showed a 6—10-fold increase in protein kinase activity of the membrane fractions both in the presence and absence of cyclic AMP. Control cytosol fractions showed a 3-fold increase in

TABLE I

SUBCELLULAR DISTRIBUTION OF PROTEIN KINASE ACTIVITY IN NORMAL AND GOITROUS RAT THYROID GLANDS: EFFECT OF SOLUBILIZATION

Protein kinase activity was measured in the presence (+cyclic AMP) and absence (—cyclic AMP) of 6 μM cyclic AMP. Activity is expressed as fmol ^{32}P incorporated into histone IIA per mg wet tissue per min (Mean ± S.E.M. for $n = 4$). The activity of each subcellular fraction is expressed as a percentage of the activity in all the fractions of equal treatment and is shown in parentheses. Values obtained in the presence of cyclic AMP and marked with * are significantly different from corresponding values without cyclic AMP. All comparable values in control vs. goitrous fractions are significantly higher in the latter. Significance is $P < 0.05$ by Student's *t*-test.

	Non-solubilized				Solubilized (0.1% Triton X-100)			
	Control		Goitrous		Control		Goitrous	
Fraction	—cyclic AMP	+cyclic AMP	—cyclic AMP	+cyclic AMP	—cyclic AMP	+cyclic AMP	—cyclic AMP	+cyclic AMP
500 × *g*	7.3 ± 0.9 (2.4%)	8.5 ± 1.9 (0.5%)	42.6 ± 1.2 (3.7%)	79.5 ± 9.7 * (1.9%)	44.9 ± 2.7 (3.5%)	88.9 ± 12.8 * (2.1%)	116.0 ± 3.4 (4.8%)	254.3 ± 21.0* (3.2%)
9000 × *g*	12.7 ± 4.1 (4.1%)	17.5 ± 5.1 (1.1%)	90.1 ± 13.0 (7.8%)	83.1 ± 8.8 (2.0%)	113.9 ± 16.1 (9.0%)	185.4 ± 40.8 (4.3%)	253.3 ± 27.3 (10.0%)	395.5 ± 49.4* (4.9%)
27 000 × *g*	—	1.9 ± 0.6 (0.1%)	27.9 ± 0.7 (2.4%)	25.3 ± 4.4 (0.6%)	122.3 ± 9.6 (9.7%)	181.0 ± 19.7 * (4.2%)	231.2 ± 28.4 (9.5%)	290.3 ± 37.9 (3.6%)
105 000 × *g* pellet	17.5 ± 1.5 (5.7%)	21.3 ± 2.0 (1.3%)	76.3 ± 5.6 (6.6%)	80.1 ± 5.3 (1.9%)	190.0 ± 7.1 (15.0%)	259.7 ± 15.4 * (6.0%)	437.3 ± 25.4 (18.0%)	543.3 ± 36.5 (6.8%)
105 000 × *g* supernatant	269.4 ± 30.4 (87.8%)	1492.5 ± 157.4 * (97.0%)	925.5 ± 44.2 (79.6%)	3942.5 ± 378.6 * (93.6%)	794.3 ± 61.5 (62.8%)	3595.0 ± 422.9 * (83.4%)	1392.5 ± 50.7 (57.3%)	6562.5 ± 443.0* (81.6%)

activity. Goitrous tissue showed a 3—12-fold increase in membrane-associated activity with and without cyclic AMP and a 1.6-fold increase in cytosol activity. After the solubilization, the kinase activity in most membrane fractions showed significant cyclic AMP-dependency although the extent of stimulation was minimal. Thus, there was a significant release of both cyclic AMP-dependent and -independent protein kinase activity from subcellular membranes by Triton X-100 treatment, more so in control tissue than in goitrous tissue. As in the non-solubilized fractions, protein kinase activity in solubilized fractions was greater in goitrous than in control tissue.

In the non-solubilized preparations, over 80% of the kinase activity was present in the cytosol fraction. After solubilization, a greater percentage of the enzyme activity was distributed throughout the membrane fractions, particularly the 105 000 $\times g$ pellet. There were no significant differences in kinase distribution in the subcellular fractions between control and goitrous tissue, whether solubilized or not.

DEAE-cellulose chromatography

The major forms of rat thyroid protein kinase from 105 000 $\times g$ supernatant were separated by DEAE-cellulose chromatography and defined as Type I or II according to the salt concentration necessary for elution and their dissociation characteristics in 0.5 M NaCl, by the criteria of Corbin et al. [17]. Both control (Fig. 1A) and goitrous tissue (Fig. 1B) contained two major peaks of cyclic AMP-dependent protein kinase which eluted from the columns at NaCl concentrations of 0.06—0.08 M (Type I) and 0.15—0.20 M (Type II). These correspond to the salt concentrations for elution of Types I and II protein kinase seen in other tissues [3,17]. Although Type I in Fig. 1B does not appear to be cyclic AMP dependent, it showed a 3-fold stimulation with cyclic AMP after dialysis and concentration. Fig. 2 shows the effects of preincubating these enzymes in 0.05 M NaCl prior to determining their activity ratios. As defined by Corbin et al. [17] and observed in these experiments, Type I protein kinase is rapidly dissociated in salt, whereas the regulatory and catalytic subunits of Type II kinase are not dissociated after 20 min in 0.5 M NaCl. It also appears that kinase from control tissue does not differ from that in goitrous tissue with respect to its holoenzyme stability in salt.

The activity distribution for each isoenzyme was determined by addition of the activity in all fractions comprising each major peak. In the control group the distribution of Type I and Type II was 28 ± 2.3% and 72 ± 2.3% respectively as compared with 7.0 ± 2.2% and 93.0 ± 2.2% in the goitrous group (means ± S.E.M. for five experiments). Quantitative recovery of these isoenzymes was also carried out by batch-elution from DEAE-cellulose and the percent distribution of activities was identical to that obtained by the addition procedure. In addition to a shift in distribution of enzymes, chronic TSH stimulation also resulted in a significant increase in the activity of Type II protein kinase from a control value of 25.5 ± 2.7 to a goitrous value of 97.4 ± 14.8 pmol ^{32}P incorporated per mg protein per min. The activity of Type I also increased (24.0 ± 2.1 to 47.3 ± 9.9) in goitrous tissue but not significantly so and when the activity was expressed per mg wet tissue, there was little change in the activity of Type I while Type II showed a 3-fold increase in activity. In

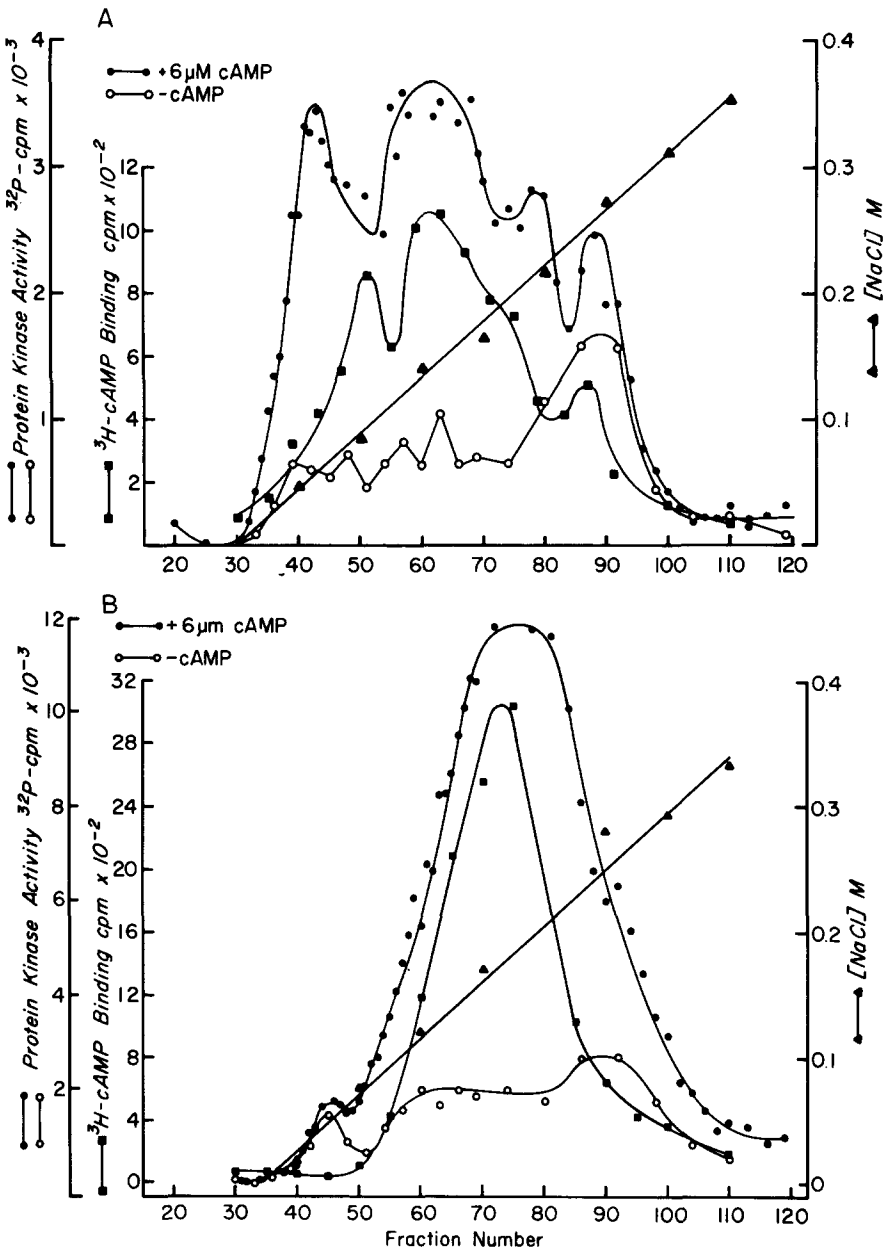

Fig. 1. DEAE-cellulose chromatography of protein kinase in the 105 000 × *g* supernatant of normal (A) and propylthiouracil-treated (B) rat thyroid tissue. Preparation of partially purified cyclic AMP-dependent protein kinase is described under Materials and Methods. The fractions were assayed for protein kinase activity in the absence and presence of 6 μM cyclic AMP. They were also assayed for binding of [^{3}H]cyclic AMP. Salt concentration was measured using a conductivity bridge.

view of the fact that Type II was the major isoenzyme in both groups and showed marked increase in its activity with chronic TSH stimulation, it was further characterized as to its physical and kinetic properties in order to determine whether the change was qualitative or quantitative.

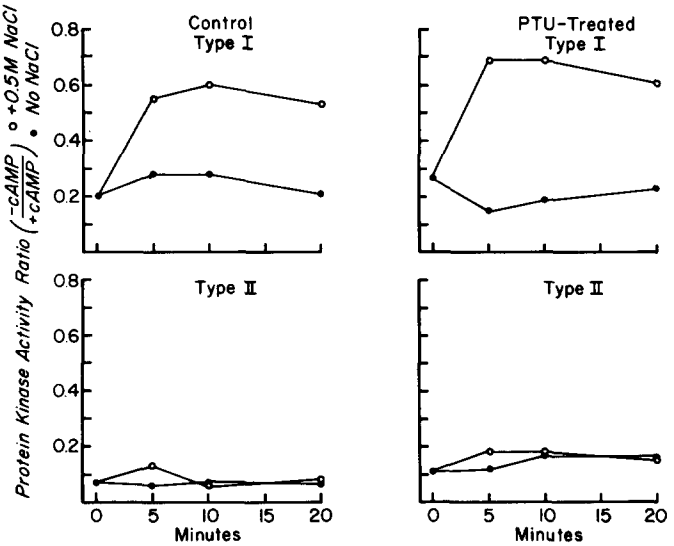

Fig. 2. Effect of prior incubation with 0.5 M NaCl on the protein kinase activity ratio of DEAE-cellulose purified Type I and Type II protein kinase from normal and goitrous rat thyroid tissue. The pooled peaks of Types I and II protein kinase from DEAE-cellulose chromatography of 105 000 × *g* supernatant were dialyzed, concentrated and then used for these experiments. At zero time an aliquot of 5.0 M NaCl was added to a quantity of enzyme to give a final concentration of 0.5 M NaCl. An equal aliquot of buffer was added to the same quantity of enzyme to give a no-salt control. These samples were incubated at 30° C for the times indicated on the graph (0, 5, 10, and 20 min). After this incubation 25—40-μl aliquots (70—150 μg protein) were added to 70 μl of reaction mixture (histone IIA plus [^{32}P]ATP) with and without 6 μM cyclic AMP. The reaction was incubated 15 min at 30° C and terminated by addition of 20% trichloroacetic acid. The samples were filtered through Millipore filters, and activity was measured by liquid scintillation spectrometry. Values were expressed as the activity in the absence of cyclic AMP divided by the activity in the presence of cyclic AMP.

pH optimum and apparent K_a (cyclic AMP)

The pH optimum of Type II protein kinase in both control and goitrous thyroids was approx. 6.3 with nearly identical pH profiles. The apparent K_a for cyclic AMP (the concentration of cyclic AMP required for half-maximal increase in enzyme activity), determined under standard assay conditions, was $5 \cdot 10^{-8}$ M for both treatment groups. However, the V for cyclic AMP stimulation was 3-fold higher for goitrous kinase (9.7 nmol/mg protein per 20 min) than for control enzyme (3.2 nmol/mg protein per 20 min).

Kinetic constants for histone IIA, ATP, and Mg^{2+}

The apparent K_m values of Type II protein kinase from both control and goitrous tissue were nearly identical for histone IIA, ATP, and Mg^{2+} (0.55 mg/ml, 11.0 μM, and 1.5 mM respectively). However, the V values were increased 2—3-fold in the goitrous group.

Substrate utilization

Various cellular proteins were tested as substrates for the partially purified Type II enzyme (histones IIA, IIIS, VIIIS; protamine free base and chloride;

caseins alpha, beta, gamma, and kappa; phosvitin, thyroglobulin, and albumin). For all the substrates tested, the specific activity of kinase in thyroids from goitrous rats was higher (2—10-fold) than the control kinase activity, both cyclic AMP-dependent and -independent (data not given). The activity ratios (—cyclic AMP/+cyclic AMP), however, were comparable in the two groups for all substrates. Protamines and caseins showed cyclic AMP-independent kinase phosphorylation comparable to that of the histones in both treatment groups. However, the cyclic AMP-dependency was greatest for the histones. Beta-casein proved to be a good substrate for protein kinase in both groups, and thyroglobulin a poor substrate.

Discussion

Examination of the subcellular distribution of cyclic AMP-dependent protein kinase from goitrous and control rat thyroid tissue showed that a large proportion of enzyme activity resides in the cytosol fraction (Table I). This is in agreement with previous observations on thyroid tissue from other species [27,28]. Kinase activity in the membrane fractions was predominantly cyclic AMP-independent, as was recently reported for beef thyroid tissue [29]. As observed previously [14—16], chronic stimulation by TSH in vivo is associated with an increase in kinase activity. In the cytosol fraction of goitrous tissue both cyclic AMP-dependent and -independent kinase were increased; however, in the particulate fractions the increase in activity was only in the cyclic AMP-independent kinase.

As observed by Suzuki and Field [29] in beef thyroid, solubilization of membranes in Triton X-100 also increased the enzyme activity in particulate fractions and resulted in cyclic AMP dependency, although this was slight. The increase in kinase activity due to chronic TSH stimulation persisted even after solubilization. Triton X-100 also caused an increase in the activity of cytosol kinase. This rise was small compared with the marked stimulation in the particulate fractions, and we have no ready explanation for it. Triton may have exposed more phosphorylating sites or blocked inhibitory substances.

Since the cytosol kinase activity comprised the largest fraction of the total, it was further characterized. As found in thyroid tissue from other species [18—20,30], two major peaks of cyclic AMP-dependent kinase activity could be separated by DEAE-cellulose chromatography. According to the salt concentration necessary for their elution (Fig. 1) and the effects of 0.5 M NaCl in dissociating the subunits (Fig. 2), the two peaks could be designated as Type I and Type II by the criteria of Corbin et al. [17]. Type II was found to be the predominant enzyme, comprising about 72% of the total kinase activity in control tissue and 93% in goitrous tissue. A selective increase in Type II kinase in goitrous rat thyroids was observed. Delbauffe and Pavlovic-Hournac [16] separated two peaks (5 S and 6.3 S) of rat thyroid cyclic AMP-dependent protein kinase by sucrose gradient ultracentrifugation and found that chronic TSH stimulation resulted in a selective increase in the 6.3 S peak. It is difficult to compare our findings since the relationship between kinases separated by anion exchange and by sedimentation characteristics has not been established.

Type II cyclic AMP-dependent protein kinase from normal and goitrous

thyroid had similar elution profiles (Fig. 1), dissociation characteristics (Fig. 2) and similar effects on substrates. A comparison of kinetic parameters showed that the apparent K_m values for histone IIA, ATP and Mg^{2+} were the same, but the corresponding V values were markedly increased in the goitrous tissue. The apparent K_m value for histone IIA observed in the present investigation (0.55 mg/ml) was similar to that reported by Rapoport and DeGroot [14] for beef thyroid. The apparent K_m value for ATP (11.0 μM) compared favorably with previously reported values of 15—50 μM [18], 13 μM [31], and 7.5—12 μM [30] for thyroid kinase. Similarly, the apparent K_m for Mg^{2+} (1.5 mM) was similar to that previously reported by Wilson and Malkin [19]. The apparent K_a for cyclic AMP stimulation of control and goitrous protein kinase was comparable to values found in previous studies [19,20,30,31]. The results suggest that chronic TSH stimulation increased the concentration of Type II cyclic AMP-dependent kinase rather than the characteristics of the enzyme in rat thyroids.

It is becoming increasingly evident that Types I and II isoenzymes may have separate functions in the cell, and so it was most interesting to observe the selective increase in Type II protein kinase in goitrous tissue. The change observed in Type I isoenzyme in goitrous tissue was not statistically significant. The interpretation of these findings is not clear at present. However, they are comparable to those of Lee et al. [21], who related stimulation of rat testis differentiation by gonadotropins to synthesis of Type II protein kinase. Further study will be necessary to determine whether activation of Type II protein kinase in goitrous thyroids is causally related to the tissue hyperplasia, or merely a concomitant event.

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References

1 Robison, G.A., Butcher, R.W. and Sutherland, E.W. (1968) Ann. Rev. Biochem. 37, 149—174
2 Robison, G.A., Butcher, R.W. and Sutherland, E.W. (1971) Cyclic AMP, Academic Press, Inc., New York
3 Rubin, C.S. and Rosen, O.M. (1975) Ann. Rev. Biochem. 44, 831—887
4 Krebs, E.G. (1972) Curr. Top. Cell. Regul. 5, 99—133
5 Greengard, P. and Kuo, J.F. (1970) in Role of Cyclic AMP in Cell Functions (Greengard, P. and Costa, E., eds.), p. 287, Raven Press, New York
6 Kuo, J.F. and Greengard, P. (1969) J. Biol. Chem. 244, 3417—3419
7 Dumont, J.E. (1971) Vitam. Horm. 29, 287—412
8 Field, J.B. (1975) Metabolism 24, 381—393
9 Field, J.B., Bloom, G., Kerins, M.E., Chayoth, R. and Zor, U. (1975) J. Biol. Chem. 250, 4903—4910
10 Lamy, F. and Dumont, J.E. (1974) Eur. J. Biochem. 45, 171—179
11 Roques, M., Tirard, A. and Lissitzky, S. (1975) Mol. Cell. Endocrinol. 2, 303—316
12 Spaulding, S.W. and Burrow, G.N. (1974) Biochem. Biophys. Res. Commun. 59, 386—391
13 Spaulding, S.W. and Burrow, G.N. (1975) Endocrinology 96, 1018—1021
14 Rapoport, B. and DeGroot, L.J. (1972) Endocrinology 91, 1259—1266
15 Pavlovic-Hournac, M. and Delbauffe, D. (1975) Biochimie 57, 1245—1247

16 Delbauffe, D. and Pavlovic-Hournac, M. (1976) FEBS Lett. 69, 59—62
17 Corbin, J.D., Keely, S.L. and Park, C.R. (1975) J. Biol. Chem. 250, 218—225
18 Spaulding, S.W. and Burrow, G.N. (1972) Endocrinology 91, 1343—1349
19 Wilson, B.M. and Malkin, A. (1974) Can. J. Biochem. 52, 319—326
20 Rappaport, L., Leterrier, J.F. and Nunez, J. (1971) Biochimie 53, 721—726
21 Lee, P.C., Radloff, D., Schweppe, J.S. and Jungmann, R.A. (1976) J. Biol. Chem. 251, 914—921
22 Byus, C.V., Chubb, J.M., Huxtable, R.J. and Russell, D.H. (1976) Biochem. Biophys. Res. Commun. 73, 694—702
23 Roskoski, R., Ngan, P., Mettenburg, R. and Lund, D.D. (1978) Biochem. Biophys. Res. Commun. 82, 641—647
24 Kuo, J.F. and Greengard, P. (1970) J. Biol. Chem. 245, 4067—4073
25 Lowry, O.H., Rosebrough, N.H., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
26 Swillens, S., Van Cauter, E. and Dumont, J.E. (1974) Biochim. Biophys. Acta 364, 250—259
27 Roques, M., Tirard, A. and Torresani, J. (1973) Biochimie 55, 1421—1430
28 Sand, G. and Verhaegen, M. (1972) Soc. Belge Biochim. 21, 984—985
29 Suzuki, S. and Field, J.B. (1978) Endocrinology 103, 1783—1793
30 Leonard, J.L. and Rosenberg, L.L. (1977) Biochim. Biophys. Acta 484, 336—347
31 Yamashita, K. and Field, J.B. (1972) Metabolism 21, 150—158