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Alteration in Cyclic AMP-Dependent Protein Kinases and Polyamine Biosynthetic Enzymes during Hypertrophy and Hyperplasia of the Thyroid in the Rat

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SUMMARY

After 2 weeks of goitrogen treatment [propylthiouracil (PTU), 0.02% in drinking water], the thyroids of rats increased to 280% of control wet weight, 270% of dry weight, and 250% of control DNA content. Two phases of growth were apparent, an initial hypertrophy phase lasting 3 days (increase in cell size and gland weight with no detectable increase in DNA) and a hyperplastic phase (increase in DNA with histological evidence of cell proliferation) starting at 3–4 days and continuing through 14 days. The cyclic AMP-dependent protein kinase activity ratio (−cyclic AMP/+cyclic AMP) showed a biphasic pattern during the 2-week thyroid growth period, with maxima at day 1 (132% of control) and day 6 (148% of control). Ornithine decarboxylase (EC 4.1.1.17), the initial enzyme in polyamine biosynthesis, showed a similar biphasic pattern with a 6- to 7-fold elevation in activity at 2–3 days and a 4-fold elevation at 6 days. *S*-Adenosyl-L-methionine decarboxylase (EC 4.1.1.50), the enzyme which catalyzes spermidine synthesis, was elevated 4-fold at 9 days of treatment. The thyroid total supernatant protein kinase activity (+cyclic AMP) increased to 160% of control by 4 days, returning to control by 14 days of PTU treatment. The thyroid had 10% Type I activity and 90% Type II cyclic AMP-dependent protein kinase activity. The specific activity of both Types I and II remained unchanged for the first 2 days of PTU treatment. Both types increased to 150% of control by 4 days. Type I remained elevated throughout the remainder of the 14 days, in contrast to Type II, which decreased conspicuously to control levels by 6 days. A single injection of thyroid-stimulating hormone (TSH, 1.0 unit/100 g of body weight, i.p.) resulted in a 20-fold increase in thyroid ornithine decarboxylase activity by 4 hr. The same dose of TSH produced only a 3-fold induction of ODC in rats hypophysectomized 2 weeks previously. The thyroid specific activity of Types I and II protein kinase was only 55% and 57% of control, respectively, in these unresponsive rats. Thyroids from rats chronically stimulated for 14 days showed an increase in ornithine decarboxylase following TSH administration similar to that of control rats. Changes in the activation as well as specific activity of Types I and II protein kinase during hypertrophy and hyperplasia underlie the complexity of a cyclic AMP-mediated response.

INTRODUCTION

It is generally believed that certain of the actions of TSH[1] as well as thyrotropin-releasing hormone at the level of the thyroid and anterior pituitary, respectively, are mediated by cyclic AMP (1–3). Cyclic AMP exerts its effects in eukaryotes through the activation of cyclic AMP-dependent protein kinases which results in specific protein phosphorylation patterns (4). Two forms of cyclic AMP-dependent protein kinase are apparent following DEAE-cellulose chromatography and are commonly designated Types I and II. They have been found in many different tissues, including rat (5) and bovine thyroid (6, 7). Types I and II cyclic AMP-dependent protein kinases recently have been shown to undergo changes during growth and differentiation (8). The selective activation of Type I cyclic AMP-dependent protein kinase has been implicated in the induction of ornithine decarboxylase (Ec 4.1.1.17) in lectin-stimulated lymphocytes and in other trophic responses (for review, see ref. 8).

Many trophic hormones known to affect RNA and protein synthesis also are capable of inducing ornithine decarboxylase (9). Polyamines have been shown to ac-

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[1] The abbreviations used are: TSH, thyroid-stimulating hormone; PTU, 6-*n*-propylthiouracil; CHO cell, Chinese hamster ovary cell.

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cumulate in parallel with ribosomal RNA in a number of growth-stimulated systems and long have been suspected to have important regulatory functions in cell growth (9, 10). Increased ornithine decarboxylase activity consistently has been one of the earliest marked events occurring during cell proliferation or hormonal stimulation (9). It has been demonstrated in a variety of growth-stimulated tissues and cells that ornithine decarboxylase is induced transcriptionally following an increase in intracellular cyclic AMP and consequent activation of cyclic AMP-dependent protein kinase (9, 11).

The present study was undertaken to document alterations in the amount and activity of Types I and II cyclic AMP-dependent protein kinase and two key polyamine biosynthetic enzymes during the compensatory hypertrophy and hyperplasia which occur in the thyroid following treatment with the goitrogen PTU. In addition, the effect of hypophysectomy on the responsiveness of the thyroid to TSH was investigated.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (250–300 g) were purchased from Charles River Breeding Laboratories (Boston, Mass.). Hypophysectomized rats were purchased from Zivic-Miller (Allison Park, Pa.). The rats were maintained on a 7 a.m.–7 p.m. photoperiod and fed ad litibum.

Hormone and drug administration. Thyrotropic (TSH; Sigma Chemical Company, St. Louis, Mo.) was dissolved in a glucose-phenol solvent (Sigma Chemical Company) and injected in a volume of 0.5 ml of 0.9% NaCl, i.p. The experimental goiters were produced in rats by the addition of 0.02% PTU (Sigma Chemical Company) plus 2% sucrose to their drinking water. Water intake under these conditions was consistent and varied little among animals. The dose of PTU was approximately 6 mg/rat/day.

Ornithine decarboxylase assay. Ornithine decarboxylase activity was determined by measuring the liberation of $^{14}CO_2$ from L-[1-^{14}C] ornithine as described by Russell and Snyder (12), with minor modifications. The enzymatically liberated $^{14}CO_2$ was trapped by NCS tissue solubilizer (20 μl on Whatman 3 MM paper filters) and counted in 5 ml of toluene-Omnifluor (New England Nuclear Corporation, Boston, Mass.) in a Beckman LS 250 liquid scintillation counter.

The reaction mixture contained 50 μl of enzyme supernatant (approximately 0.3 mg of protein), 140 μl of homogenization buffer, 0.5 μCi of L-[1-^{14}C]ornithine (10 mCi/mmole; Amersham Corporation, Arlington Heights, Il.) and was assayed for 30 min at 37°. The enzyme blank was determined by the addition to the reaction mixture of 4-bromo-3-hydroxy-benzyloxyamine dihydrogen phosphate (NSD), an inhibitor of pyridoxal phosphate-requiring enzymes, and the value was subtracted from all other enzyme determinations. Enzyme activity was assayed in triplicate and expressed as for ornithine decarboxylase in picomoles of $^{14}CO_2$ liberated per milligram of protein.

S-Adenosyl-L-methionine decarboxylase (EC 4.1.1.50) activity. S-Adenosyl-L-methionine decarboxylase activity was determined by measuring the release of $^{14}CO_2$ from S-adenosyl-L-[*carboxyl*-^{14}C]methionine in the presence of 2.5 mM putrescine as the acceptor of the propylamine moiety, as previously described (13).

Cyclic AMP-dependent protein kinase activity. The degree of activation of cyclic AMP-dependent protein kinase(s) represents the relative proportion of the protein kinase present as the holoenzyme (*RC*) compared with that present as the free catalytic subunit (*C*), and it is determined by assaying the supernatant kinase activity in the presence and absence of saturating amounts of cyclic AMP as described previously (14, 15).

Thyroids were rapidly removed, immediately frozen on dry ice, and homogenized in 10 volumes (w/v) of a 10 mM sodium-potassium phosphate buffer (pH 6.8) containing 5 mM NaF, 1 mM EDTA, 0.5 mM 3-isobutyl-1-methylxanthine, and 150 mM KCl. The homogenates were centrifuged at 4° for 3 min at 10,000 × *g* in a Beckman Model B Microfuge. A 5-μl aliquot (approximately 30 μg of protein) of the resulting supernatant was assayed for protein kinase activity for 3 min at 30°. The 5 μl were first pipetted into test tubes containing 45 μl of the homogenization buffer, and the reaction was initiated by the addition of 25 μl of an assay cocktail resulting in the following final assay concentrations: 20 mM sodium-potassium phosphate (pH 6.8), 0.5 mM 3-isobutyl-1-methylxanthine, 50 μg of histone F_{IIb} (Sigma Chemical Company), 10 mM magnesium acetate, 5 mM NaF, and 0.5–1.0 μCi of [γ-^{32}P]ATP (5–10 Ci/mmole; New England Nuclear Corporation) plus sufficient cold ATP to bring the total concentration to 0.1 mM. The assay was terminated after 3 min by pipetting 50 μl of the reaction mixture onto precut strips (1–5 × 5 cm) of Gelman ITLC sheets and chromatographed for 15 min in a tank containing 5% trichloroacetic acid plus 0.2 M KCl according to the method of Huang and Robinson (15). The origin of the strips was prespotted with 70 μl of 20% trichloroacetic acid before spotting the 50 μl to ensure rapid protein denaturation. The strips were air-dried, and the bottom 3-cm portions were cut and counted in 2 ml of a toluene-Omnifluor counting fluid on a Beckman LS 250 liquid scintillation counter. Enzyme blanks were determined by adding 45 μl of 200 mM EDTA to the standard reaction mixture and subtracting from all other measurements. The protein kinase activity ratio (−cyclic AMP/+cyclic AMP) was determined as described by Corbin and Reimann (14) from the amount of ^{32}P incorporated into histone with and without cyclic AMP. The incorporation of ^{32}P into histone was linear for protein concentrations up to approximately 30 μg/assay and for a 5-min incubation at 30°.

The activity ratio (−cyclic AMP/+cyclic AMP) is expressed as the amount of ^{32}P incorporated into histone in the absence of cyclic AMP divided by the amount of ^{32}P incorporated in the presence of cyclic AMP. (Using these assay conditions, the amount of acid-precipitable ^{32}P was linear with or without cyclic AMP throughout the 3-min assay.) Sephadex G-100 chromatography of certain supernatant preparations (16) as well as addition of activated charcoal to absorb any free cyclic AMP were used to verify that the protein kinase activity ratio accurately reflected the relative amount of free catalytic subunit and of holoenzyme present in the tissue prior to homogenization.

Determination of soluble Type I and Type II cyclic AMP-dependent protein kinase. Thyroids were removed, frozen on dry ice, and stored at −70° until assay. The glands were homogenized in 10 volumes of a 5 mM Tris-HCl buffer (pH 7.5) containing 2 mM NaF and 2 mM EDTA in a motor-driven dual glass homogenizer. Usually three thyroids were pooled for each determination. The homogenate was centrifuged at 10,000 × *g* in a Beckman Model B Microfuge for 5 min at 4°, and 0.5 ml of the supernatant was applied to a DEAE-cellulose column (10 × 1 cm) (Whatman DE-52). A small aliquot of the supernatant applied to the column was assayed for protein kinase activity. The column was washed with 15 ml of the above buffer prior to initiation of a 30-ml (15 ml in each beaker) linear salt gradient from 0 to 400 mM NaCl in the same washing buffer. Thirty 1-ml fractions were collected by a Gilson fraction collector, and a 50-μl aliquot of each fraction was assayed for protein kinase activity in the presence of cyclic AMP (10 μM) as described earlier. The column flow rate was approximately 20 ml/hr. This method of tissue preparation in low ionic strength buffer and the slow application of a relatively small sample size on a proportionately large column under low-salt conditions ensures complete reassociation of the kinase subunits so that the total pool sizes of the kinase isozymes can be measured accurately by the chromatographic separation. This methodology prevents run-through of free catalytic subunit as has occurred in many previously reported studies, which leads to underestimation of total kinase present, especially the Type I isozyme which, without these precautions, tends to stay in the dissociated state. The Type I isozyme peak of kinase activity was shown to represent true reassociated holoenzyme and not to be contaminated with free catalytic subunit by its dependence on the addition of exogenous cyclic AMP for maximal activity. As further assurance, the elution profile of the free catalytic subunit was determined and found not to overlap with the Type I isozyme elution profile (0.015–0.035 M versus 0.060–0.12 M NaCl,

respectively). Controls for complete association were run on each column separation, and the flow-through wash and initial gradient fractions were assayed to determine that no free catalytic subunit elutes from the column. These procedures, along with estimation of kinase recovery, ensure that the isozyme changes detected are accurate. The cyclic AMP-independent protein kinases are excluded as they do not use F_{IIb} histone as a substrate under these conditions (less than 1% of measured activity).

To assess the recovery rate, the cyclic AMP-dependent protein kinase activity in a 10-μl aliquot of the supernatant also was determined. After chromatography, 75–90% of the supernatant kinase activity was consistently recovered. The determination of the recovery includes a factor to compensate for the salt inhibition of the protein kinase activity in the eluted peaks. This inhibition was determined to be 39% for Type I and 52% for Type II by comparing the activity of pooled peak fractions before and after dialysis to remove the NaCl. The specific activities of the isozymes were calculated for the total kinase activity (picomoles per minute) eluted in the respective peak (corrected for salt inhibition) on the basis of the original supernatant protein applied to the column. Changes in specific activity were taken to represent changes in total relative tissue kinase pool size. Conductivity measurements of the salt gradient fractions indicate the Type I and Type II cyclic AMP-dependent protein kinase elute at 20–60 mM NaCl and 120–200 mM NaCl, respectively.

DNA, protein, wet and dry weight determination. DNA in thyroid tissue was determined by a modified fluorometric method described by Setaro and Morley (17). The quantitation involves the reaction of 3,5-diaminobenzoic acid dihydrochloride (Aldrich Chemical Company, Milwaukee, Wisc.) with deoxyribose liberated from DNA by acid hydrolysis. Fluorescence was measured by an Aminco-Bowman spectrofluorometer (excitation 420 nM, emission 520 nM) using calf thymus DNA (Sigma Chemical Company) as standard.

Protein was determined by the method of Lowry *et al.* (18), using bovine serum albumin as standard. The wet weight of the thyroid after blotting was determined on an Ainsworth balance. Thyroid dry weight was determined after 12 hr in a drying oven at 50°.

Thyroid histology. Thyroid glands were fixed in 10% formalin and embedded in paraffin blocks. Samples were sectioned (5 μm) and stained with hematoxylin and eosin. Slides were examined microscopically (×400 enlargement) and photographed.

Statistics. Data were analyzed for statistical significance by Student's *t*-test.

RESULTS

Production of thyroid growth by PTU administration. Administration of 0.02% PTU for 14 days in the drinking water of rats resulted in a marked enlargement of the thyroid gland, with wet and dry weights and DNA per gland increasing to 280%, 270%, and 250% of control, respectively. Marked changes in thyroid histology were apparent during the progressive stages of gland hypertrophy and hyperplasia. Thyroid epithelial cells were enlarged and columnar in appearance as compared with the flattened or cuboidal cells characteristic of the unstimulated basal state after only 2 days of PTU treatment. A decrease in follicular colloid content also was seen after 2 days, indicating enhanced thyroid hormone secretion involving endocytosis and lysosomal digestion of the colloid. The columnar cells at 2 days had conspicuously enlarged nuclei but showed no signs of cell division. By 4 days, cells were still enlarged, little follicular colloid remained, and there was evidence of increased cell number. By 14 days, the thyroid appeared quite different from the normal state, with little evidence of follicular structure. No colloid was present, and marked hyperplasia was apparent.

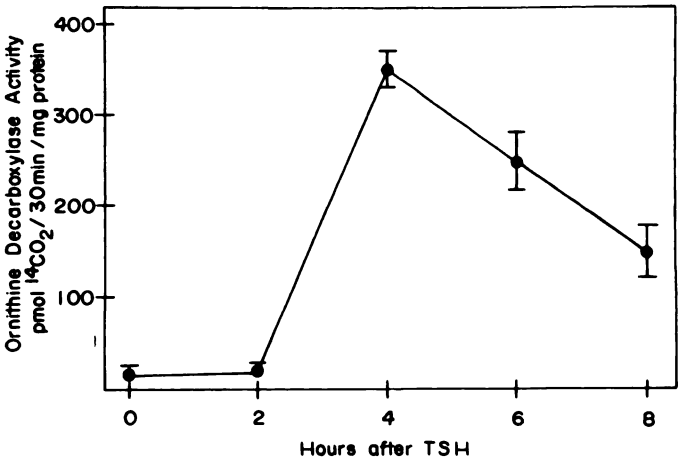

FIG. 1. *Effect of TSH administration (1 unit/100 g of body weight, i.p.) on the time course of ornithine decarboxylase activity in thyroids of intact rats at various times after administration*

Each point represents the mean ± standard error of the mean of duplicate determinations in five separate rats.

Polyamine biosynthetic enzymes in hypertrophy and hyperplasia of the rat thyroid. A single injection of TSH (1 unit/100 g of body weight, i.p.) resulted in a 20-fold elevation of thyroid ornithine decarboxylanse activity, reaching a maximum at 4 hr (Fig. 1). This increase in ornithine decarboxylase activity has been shown to be blocked by actinomycin D or cycloheximide, indicating *de novo* enzyme synthesis rather than activation of preexisting enzyme. Ornithine decarboxylase activity, as well as *S*-adenosyl-L-methionine decarboxylase activity, increased in a biphasic pattern during the 14 days of PTU administration (Fig. 2). Ornithine decarboxylase activity was elevated at the first time measured (24 hr) and reached a maximum (7-fold elevation) by 3 days, followed by a marked decline to near control levels at 4 days. A

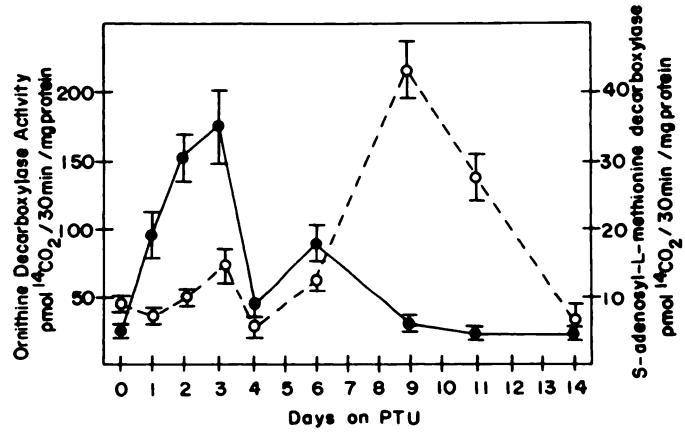

FIG. 2. *Effect of PTU on the activities of ornithine decarboxylase (●—●) and S-adenosyl-L-methionine decarboxylase (○- - -○) in thyroids of rats*

Rats were given PTU at 0.02% in their drinking water for various time periods. Data are expressed as means ± standard error of the mean of 5–10 animals. Ornithine decarboxylase activity was significantly elevated above controls at days 1, 2, 3, 4, and 6 ($p < 0.01$), whereas *S*-adenosyl-L-methionine decarboxylase activity was significantly different from controls only at days 3, 9, and 11 ($p < 0.01$).

second peak of ornithine decarboxylase activity (3-fold elevation) occurred at 6 days, with a subsequent return to basal activity throughout the remainder of the treatment period. *S*-Adenosyl-L-methionine decarboxylase activity was highest at day 9 with a 4-fold elevation of enzyme activity.

Cyclic AMP-dependent protein kinases in rat thyroid hypertrophy and hyperplasia. The cyclic AMP-dependent protein kinase activity ratio (−cyclic AMP/+cyclic AMP) increased maximally within 1 day (Fig. 3), with maximal ornithine decarboxylase activity expressed within 3 days of initiation of PTU treatment, as indicated above (Fig. 2). The decrease in cyclic AMP-dependent protein kinase activity ratio at 3 days preceded the marked decline in ornithine decarboxylase activity at 4 days. The highest activity ratio occurred at 6 days (150% of control) and was maintained at this level through 11 days.

In contrast to the biphasic pattern in cyclic AMP-dependent protein kinase activity ratio, the total supernatant cyclic AMP-dependent protein kinase activity (+cyclic AMP) showed a different pattern. Total cyclic AMP-dependent protein kinase activity increased to

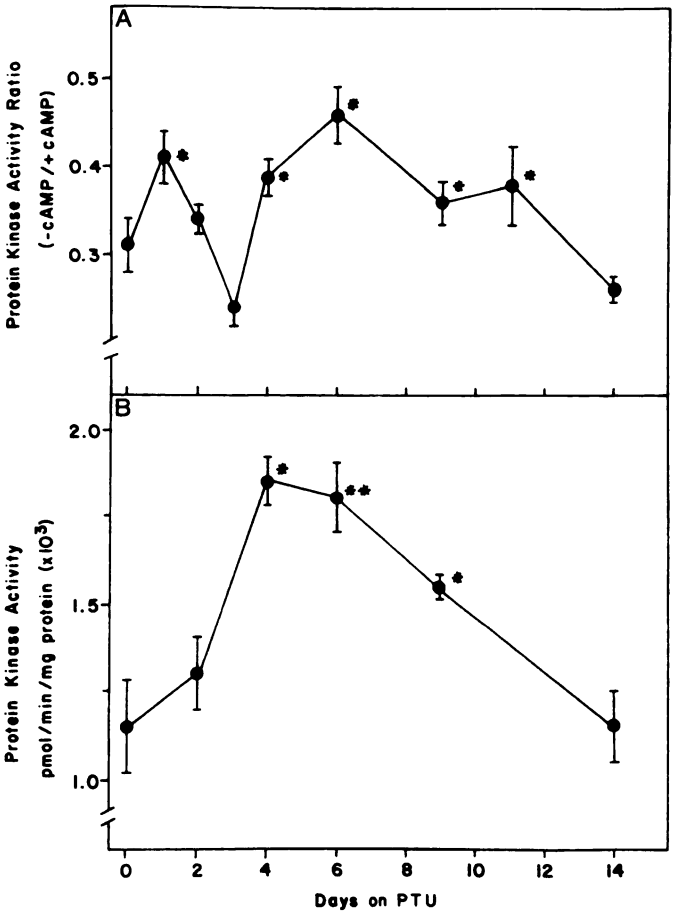

FIG. 3. *Effect of administration of PTU on the cyclic AMP-dependent protein kinase activity ratio (A) thyroids of rats, and total protein kinase activity (+cyclic AMP) (B) as a function of days after PTU administration*

Each point represents the mean ± standard error of the mean of at least five rats. *Asterisks* indicate data that differ significantly from controls ($p < 0.05$).

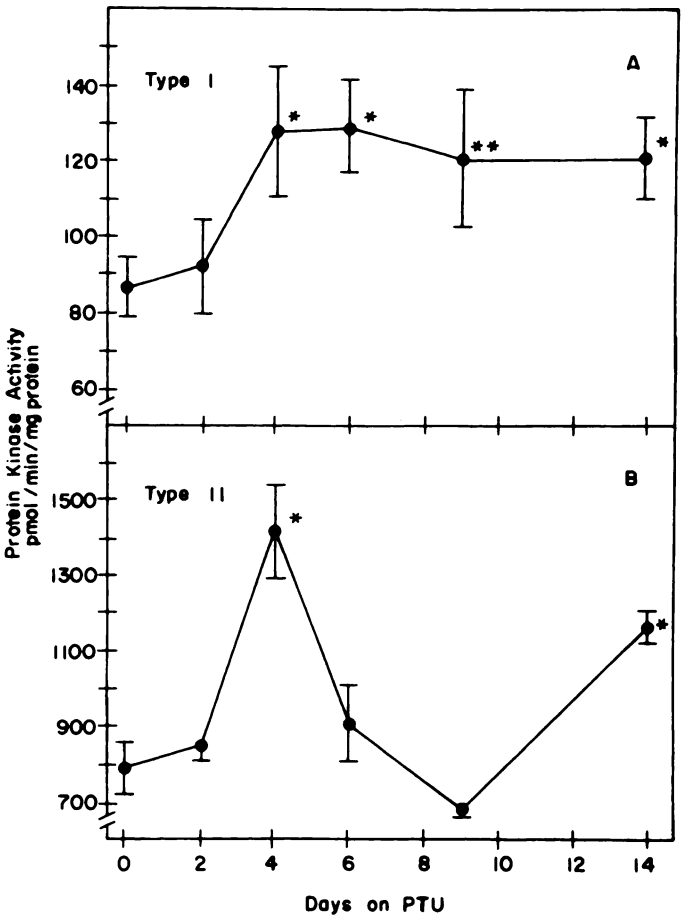

FIG. 4. *Effect of PTU administration on the total amounts of Types I and II cyclic AMP-dependent protein kinase in rat thyroid*

Data are presented as the means ± standard error of the mean of five columns (each column had applied to it a supernatant containing extracts of three thyroid glands). Each value was determined by adding the total enzyme activity under each peak following chromatography on DEAE-cellulose and is expressed as picomoles of $^{32}PO_4$ incorporated into histone per minute per milligram of protein added to the column. *Asterisks* represent data significantly different from control(* $p < 0.01$; ** $p < 0.05$).

150% of control by 4 days and remained elevated until 14 days, when it dropped to control levels (Fig. 3B).

The predominant form of cyclic AMP-dependent protein kinase in thyroid was Type II, which comprised 90% of the total cyclic AMP-dependent protein kinase. Marked and selective alterations in the amounts of Type I and Type II protein kinase in the thyroid occurred during the 14-day growth period (Fig. 4). Both Type I and Type II activities remained at control levels for the first 2 days, a time when an increased activity ratio occurred, but were increased conspicuously from 2 to 4 days, reaching 150% and 175% of control, respectively. Type I remained elevated throughout the 14-day period, whereas Type II declined sharply after 4 days. Type II increased again to 150% of control at 14 days.

Responsiveness of the thyroid following hypophysectomy and chronic thyrotropin stimulation. Thyroids from rats hypophysectomized for up to 2 weeks showed a significant decrease in total cyclic AMP-dependent protein kinase activity (Table 1). Thyroid supernatants

TABLE 1

DEAE-Cellulose separation and determination of cyclic AMP-dependent protein kinase Type I and Type II activities in rat thyroid following hypophysectomy

N designates the number of separate thyroids in the experiment. Seven to ten thyroids were pooled in order to measure reliably cyclic AMP-dependent protein kinase holoenzyme patterns.

Experimental group (*N*)	Thyroid protein kinase activity			
	Type I		Type II	
	Mean ± SEM	% Control	Mean ± SEM	% Control
	pmoles/min/mg protein		*pmoles/min/mg protein*	
Control (21)	46.9 ± 0.96	100.0	570.0 ± 12.8	100.0
Hypophysectomy				
1 wk after (21)	28.6 ± 1.41[a]	61.0	438.6 ± 57.7	77.0
2 wk after (10)	25.7	57.1	313.6	55.0

[a] Significantly different from control ($p < 0.05$).

from rats hypophysectomized for 1 week showed a total cyclic AMP-dependent protein kinase activity of 80% of control (data not shown) with a corresponding decrease in the amounts of both Type I and Type II (61% and 77% of control, respectively; $p < 0.05$). Absence of TSH for 2 weeks resulted in a further decrease in cyclic AMP-dependent protein kinase activity. Type I and Type II cyclic AMP-dependent protein kinase activity decreased to 57% and 55% of control, respectively (Table 1).

The well-known subsensitivity of the thyroid to exogenously administered TSH following removal of the trophic effect of endogenous TSH was tested in rats hypophysectomized for 2 weeks. There was a dramatic blunting of the ornithine decarboxylase response to a single injection of TSH (3-fold compared with 20-fold in control) (Fig. 5). Basal thyroid ornithine decarboxylase activity was similar in both control and hypophysectomized rats. The thyroid gland, despite being deprived of its trophic maintenance via TSH for 2 weeks, showed no detectable decrease in wet weight (data not shown).

Chronically stimulated thyroids also have been shown to be subsensitive or refractory to exogenous TSH administration (19). However, thyroids stimulated for 14 days by high endogenous TSH (via PTU administration) responded in a normal manner when ornithine decarboxylase induction was measured 4 hr following injection of TSH (Fig. 6). The ornithine decarboxylase activity in the 14-day treated glands had returned to control levels.

DISCUSSION

The trophic response of the thyroid to a chronic elevation in TSH following goitrogen treatment has long been studied as an *in vivo* growth model. Our observation of a distinct hypertrophy phase lasting for several days preceding cell division enabled us to study biochemical events comparable to the G_1 and S phases of the cell cycle. The most dramatic elevation of ornithine decarboxylase activity occurred in the hypertrophy phase (0–

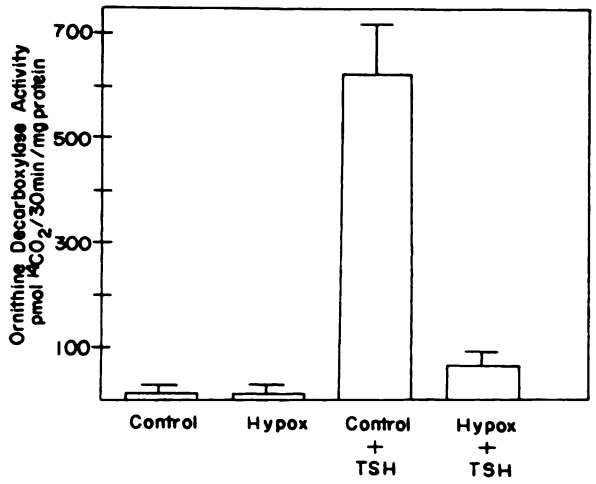

FIG. 5. *Effect of hypophysectomy (Hypox) on the thyroid ornithine decarboxylase response to exogenous TSH*

Male Sprague-Dawley rats (200–250 g) hypophysectomized for 2 weeks were administered TSH (1 unit/100 g of body weight, i.p.), and ornithine decarboxylase was measured 4 hr after injection. Bars represent the mean ± standard error of the mean of five determinations. Both the control + TSH groups and the hypox + TSH groups are significantly different from controls ($p < 0.05$).

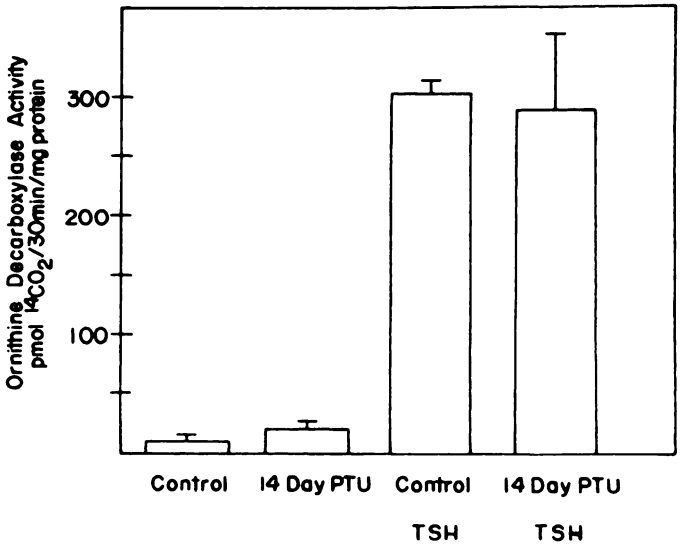

FIG. 6. *Effect of chronic PTU stimulation on the ornithine decarboxylase response of the thyroid to exogenous TSH in rats*

Rats received 0.02% PTU in their drinking water for 14 days and were given TSH (1 unit/100 g of body weight, i.p.). Ornithine decarboxylase activity was measured at 4 hr. Data are expressed as means ± standard error of the mean of five determinations. There were no significant differences between control + TSH and 14 day PTU + TSH or between control and 14 day PTU groups.

3 days) and was consistent with ornithine decarboxylase induction as a G_1-specific event in the cell cycle (11, 20). The expression of *S*-adenosyl-L-methionine decarboxylase, an enzyme related to spermidine synthesis, reached maximal activity during the time of DNA synthesis, i.e., S phase (4–14 days). The *S*-adenosyl-L-methionine decarboxylase expression was somewhat different, although more time points were measured, than that reported previously (21). An elevation in *S*-adenosyl-L-methionine decarboxylase activity in CHO cells also occurs during the S phase of the cell cycle (20).

The addition of PTU to the drinking water of rats previously has been shown to result in a rapid increase in the plasma level of TSH coupled to a decreased pituitary TSH content (22). The reported fluctuations of plasma TSH were strikingly similar to the fluctuations in thyroidal ornithine decarboxylase activity reported in this study; i.e., both ornithine decarboxylase and plasma TSH increased by 1 day and reached a peak level at 3 days, followed by a rapid decline, returning to control levels by 14 days (22). The ornithine decarboxylase activity pattern reported here, with maximal activity at 3 days (7-fold), compares with that reported by other investigators following methylthiouracil treatment. Richman *et al.* (23) reported maximal ornithine decarboxylase activity at 2 days (5-fold), whereas Matsuzaki *et al.* (24) reported maximal ornithine decarboxylase activity occurring at 4 days (8- to 18-fold) following initiation of goitrogen treatment.

The cyclic AMP-dependent protein kinase activity ratio in thyroid followed a biphasic pattern similar to that of ornithine decarboxylase in PTU-treated rats. A tight temporal correlation between cyclic AMP-dependent protein kinase activation and ornithine decarboxylase induction has been reported in rat liver regeneration following partial hepatectomy (25). This temporal correlation, as well as the relationship between the extent of activation of cyclic AMP-dependent protein kinase and the induction of ornithine decarboxylase, has been extensively documented in a variety of growth-stimulated tissues (9, 11).

The mechanism for protein kinase-mediated induction of ornithine decarboxylase is not completely understood but may involve a hormone-stimulated cytoplasmic to nuclear translocation of regulatory and/or catalytic subunits of protein kinase followed by a specific phosphorylation of chromatin-associated proteins related to transcriptional control of the ornithine decarboxylase gene. Translocation has been demonstrated after luteinizing hormone stimulation of the ovary (26) and after cholinergic stimulation of the adrenal medulla (27). Friedman and Chambers (28) detected the regulatory subunits of both Type I and Type II protein kinase bound to nonhistone chromosomal proteins isolated from nuclei of bovine liver cells.

The generality of increased amounts of Type I and Type II protein kinase during thyroid hypertrophy and hyperplasia is supported by similar reports in other growth systems. Habhab *et al.* (29) reported a 2-to 3-fold higher protein kinase activity with an increase in Type II isoenzyme after 21 days of PTU treatment in rat thyroid, and also increased Type II isoenzyme after chronic stimulation *in vivo* with TSH. They looked at cyclic AMP-dependent protein kinase after 21 days of PTU and would have missed the early Type I alteration associated with hypertrophy. Also, they used Holtzman rats rather than Sprague-Dawley rats, which may have different unstimulated levels of cyclic AMP-dependent protein kinase isoenzymes. Type I protein kinase is increased specifically during isoproterenol-induced cardiac hypertrophy (8). Types I and II protein kinase vary markedly during the cell cycle. In synchronized CHO cells, Type II increases in late G_1 followed by a dramatic decline as cells enter the S phase. The dramatic rise and fall in Type II protein kinase reported here in the rat thyroid coincide with the initiation of DNA synthesis. This same rise and fall of Type II protein kinase also was seen during the CHO cell cycle at the G_1/S border (8).

The finding of reduced amounts of Type I and Type II protein kinases in the thyroids from hypophysectomized rats indicates that one trophic effect of TSH could be maintenance of the proper intracellular concentration of cyclic AMP-dependent protein kinase. Friedman *et al.* (30) reported a 50% lower specific activity of cyclic AMP-dependent protein kinase activity in thyroids from hypophysectomized rats than in intact animals. One consequence of the reduced levels of protein kinase could be a compromised response to agents working through the adenylate cyclase/cyclic AMP-dependent protein kinase axis.

That the return of ornithine decarboxylase activity to control levels at 14 days of PTU treatment was due to effects other than a decreased thyroid sensitivity to TSH was demonstrated. Rat thyroids chronically stimulated for 14 days responded normally to exogenous TSH administration when ornithine decarboxylase activity was measured 4 hr after TSH injection. Thyroid glands from rats treated with PTU for up to 6 months failed to show the usual increase in cyclic AMP following the addition of TSH *in vitro* (19).

In conclusion, this investigation represents a physiological study of thyroid biosynthetic processes. It suggests that the TSH released from the adenohypophysis results in significant synchronization of key biochemical events in the thyroid. An early intracellular chain of biochemical events in the response of the thyroid to chronic TSH elevation appears to be the activation of cyclic AMP-dependent protein kinase and induction of ornithine decarboxylase followed by alteration in the isozyme types of protein kinase.

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