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# Adenylate Cyclase Activity of Normal and Goitrous Rat Thyroids[1] (39941)

OSCAR HABHAB, R. C. BHALLA, AND N. S. HALMI

*Department of Anatomy, University of Iowa, Iowa City, Iowa 52242*

There is ample evidence that stimulation of the thyroid by TSH involves the adenylate cyclase-cyclic AMP system. TSH activates thyroid adenylate cyclase in many *in vitro* systems, and addition of cyclic AMP or its congeners such as dibutyryl cyclic AMP to thyroid slices, isolated thyroid cells, or thyroid homogenates simulates many of the effects of TSH (1). The effect of endogenous TSH on thyroid adenylate cyclase activity is less clear. Some studies have shown that propylthiouracil (PTU) treatment, which causes hypersecretion of TSH, results in increased basal activity of thyroid adenylate cyclase (2, 3); others found a decrease (4).

We recognized that a more detailed study of the ionic and other requirements of adenylate cyclase in normal and chronically TSH-stimulated rat thyroid tissue is necessary before discrepancies in the literature concerning the basal activity of this enzyme in normal vs goitrous thyroid tissue can be evaluated. This paper deals with the effects of $Mg^{2+}$, $Mn^{2+}$, $Ca^{2+}$, $F^-$, GTP, and $PGE_1$ on adenylate cyclase activity in thyroid tissue from normal and PTU-treated (goitrous) rats. Our study shows that differences exist between control and goitrous thyroids in the level of adenylate cyclase activity with respect to the response to $Mg^{2+}$ and stimulation by $PGE_1$ in the presence of GTP.

*Materials and Methods. Materials.* 6-Propyl-2-thiouracil (PTU) was purchased from ICN Pharmaceuticals. Adenosine 5′-triphosphate (ATP, from equine muscle, sodium salt), cyclic adenosine 3′,5′-monophosphate (cAMP), theophylline, Dowex 50W-X8 (200-400 mesh, 8% cross linked, $H^+$ form), and guanosine 5′-triphosphate (GTP) were obtained from Sigma Chemical Co. Scintisol Complete was purchased from Isolab Inc. 2-Phosphoenol pyruvate (PEP; trisodium salt) and pyruvate kinase (from rabbit muscle) were obtained from Calbiochem. [$\alpha^{32}$P]ATP (TEA salt) and [$^3$H]cAMP ($NH_4$ salt) were purchased from New England Nuclear. Prostaglandin $E_1$ ($PGE_1$, courtesy of the UpJohn Company), was dissolved in absolute ethanol (10 mg/ml) and $Na_2CO_3$ (0.2 mg/ml, to pH 7.0) and then diluted with incubation buffer to give the desired concentrations.

*Tissue preparation.* Male albino rats weighing 90-120 g were purchased from the Holtzman Company, Madison, Wisconsin, and were divided into control and treated groups. Both groups were fed standard laboratory chow, given water *ad libitum*, and were maintained under similar conditions. The treated rats were given daily sc injections of 20 mg of PTU for 21 to 35 days before they were killed. The thyroids were removed under ether anesthesia, quickly trimmed of loose connective tissue, and weighed on a Mettler torsion balance. The tissue was homogenized in buffer (0.25 *M* sucrose in 0.05 *M* Tris-HCl, pH 7.4) at a concentration of 50 mg wet tissue/ml in glass Dounce homogenizers at 4°, then strained through two layers of cheesecloth to remove connective tissue and particulate material. The whole homogenate was used for assaying adenylate cyclase activity.

*Adenylate cyclase assay.* Adenylate cyclase activity was measured by the method of Krishna *et al.* (5), with the use of a PEP-pyruvate kinase ATP-regenerating system. The incubation volume of 200 μl contained 4-5 mg of wet tissue, 10 m*M* theophylline, 10 m*M* PEP, 12.5 IU/ml of pyruvate kinase, 1.23 or 2 m*M* [$^{32}$P]ATP (5-10 cpm/pmole), 1-16 m*M* $Mg^{2+}$ or $Mn^{2+}$, as required by assay conditions, and 0.05 *M* Tris-HCl, pH 7.4, to volume. The reaction was initiated by the addition of a mixture containing theophylline, PEP, pyruvate kinase, [$^{32}$P]ATP, and $MgCl_2$ or $MnCl_2$. The incubation time was 5 min (except where

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indicated), after which the reaction was stopped by the addition of 100 $\mu$l of a diluting solution which contained 40 m*M* ATP, 0.02 *M* Tris-HCl buffer, pH 7.4, and 12 m*M* [$^3$H]cAMP (approximately 17 cpm/nmole). The tubes were then immediately placed in a 100° dry bath for 3 min. Cyclic AMP formed was separated by Dowex column chromatography followed by two precipitations with $ZnSO_4$-$Ba(OH)_2$.

The activity present in homogenates that were boiled before assay was subtracted from the experimental values. Protein was measured by the Lowry method, with bovine serum albumin as the standard.

Experiments were repeated at least three times, each experiment being run in triplicate. The adenylate cyclase activity was expressed per milligram of wet tissue, but was also calculated on a per milligram of protein and per milligram of DNA basis. Statistical analyses were performed by Student's *t* test for unpaired samples and analysis of variance.

*Results. Effect of PTU injection on thyroid weight.* After the course of PTU injections, the thyroid wet weight increased on the average from 15 to 75 mg.

*Effect of $Mg^{2+}$ concentration.* The effects of increasing the concentration of $Mg^{2+}$ from 1 to 16 m*M* on basal (panel A) and fluoride-stimulated (panel B) adenylate cyclase activity are shown in Fig. 1. It has been a general observation that $Mg^{2+}$ at concentrations above that necessary to convert most of the ATP to a MgATP complex increases basal and hormone-stimulated adenylate cyclase activity (6). We also observed that the basal adenylate cyclase activity increased with increasing $Mg^{2+}$ concentration; the activity at 4 m*M* $Mg^{2+}$ and higher concentrations was significantly greater ($P < 0.05$) than the activity at 2 m*M* $Mg^{2+}$, the concentration equimolar with the ATP present in the incubation mixtures.

The adenylate cyclase activity of the control group showed a steep 3.5-fold rise as the $Mg^{2+}$ concentration increased from 1 to 4 m*M*; a further increase in $Mg^{2+}$ concentration did not raise enzyme activity significantly. The adenylate cyclase activity of goitrous thyroids continued to rise as $Mg^{2+}$

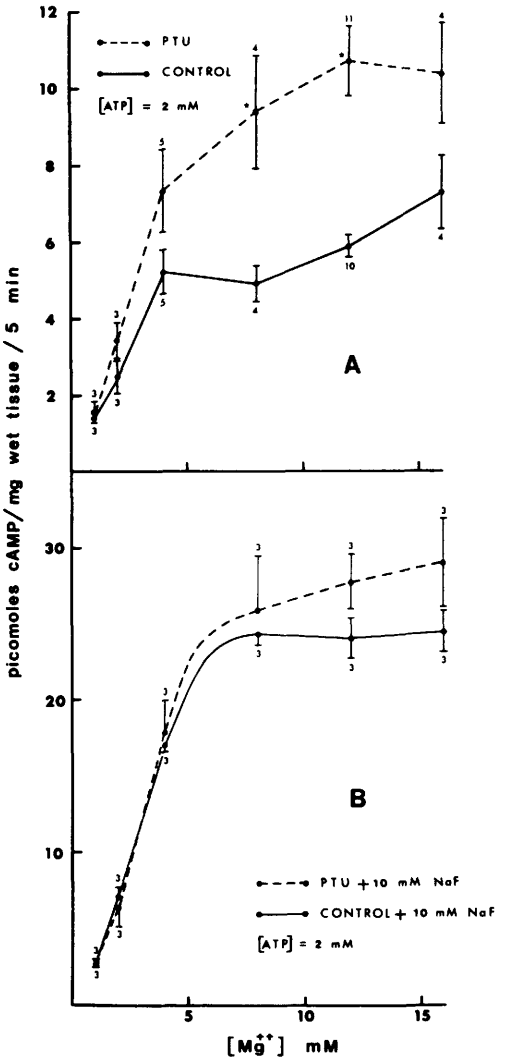


FIG. 1. The effect of magnesium concentration on adenylate cyclase activity in thyroid tissue of normal (control) and PTU-treated rats in the absence (A) and presence (B) of 10 m*M* NaF. Values are the means ± SEM. The number of experiments is given at each point. The asterisks represent statistical significance ($P < 0.05$) of a PTU value above its corresponding control value (Student's *t* test).

was elevated above 4 m*M*, and reached a plateau at 12 m*M* $Mg^{2+}$. The thyroid adenylate cyclase activity of PTU-treated animals was greater than that of the control group at all points, with significant differences ($P < 0.05$ by *t* test) observed at 8 and 12 m*M* $Mg^{2+}$. Analysis of variance (Table I) showed significant effects of treatment with PTU and $Mg^{2+}$ concentration as

TABLE I. ANALYSIS OF VARIANCE FOR THE EFFECTS OF PTU TREATMENT AND $Mg^{2+}$ CONCENTRATION ON THYROID ADENYLATE CYCLASE ACTIVITY.

| Source of variation | Degrees of freedom | Mean squares | $F$ | Significance of $F$ |
|---|---|---|---|---|
| Treatment (PTU)[a] | 1 | 19055.7 | 47.6 | 0.001 |
| $Mg^{2+}$ concentration[b] | 5 | 6104.0 | 15.3 | 0.001 |
| Two-way interaction[c] (PTU × $Mg^{2+}$) | 5 | 1031.3 | 2.6 | 0.05 |
| Residual | 41 | 400.2 | | |

[a] PTU > control.
[b] Addition of $Mg^{2+}$ had a significant effect.
[c] The $Mg^{2+}$ effect was not the same in the PTU group as in controls.

well as a significant interaction between PTU treatment and $Mg^{2+}$ concentration. The differences between adenylate cyclase activity in normal and goitrous thyroid described in this report were still present when the results were expressed on the basis of per milligram of protein or per milligram of DNA.

The stimulation of adenylate cyclase by 10 m*M* NaF was not significantly greater in the PTU-treated animals than in the control group. In both groups, near-maximal stimulation of adenylate cyclase by $F^-$ occurred at $Mg^{2+}$ concentrations of 6 m*M* and higher (Fig. 1B).

*Time course of adenylate cyclase activity.* This was examined with 2 m*M* ATP and 12 m*M* $Mg^{2+}$. The adenylate cyclase activity of the PTU group was significantly ($P < 0.05$ by $t$ test) greater than that of the control group at 1, 3, 5, and 10 min of incubation (data not presented). The initial velocity for the PTU group was 2.6 pmole of cAMP/mg wet tissue/min compared with 1.5 pmole of cAMP/mg wet tissue/min for the control group. The velocity at 5 min was not substantially different from the initial velocity for either group, which justifies the use of this time period for incubation in all other experiments.

*Effect of $Mn^{2+}$ and $Ca^{2+}$.* Adenylate cyclase activity was also studied at 2 m*M* ATP, with $Mn^{2+}$, at concentrations varying from 1 to 16 m*M*, substituted for $Mg^{2+}$. Enzyme activity in the PTU-treated group appeared slightly greater than in the control group at all concentrations of $Mn^{2+}$ (Fig. 2), but the difference was not of statistical significance by analysis of variance. At $Mn^{2+}$ concentrations of 1 and 2 m*M*, there was higher adenylate cyclase activity than at

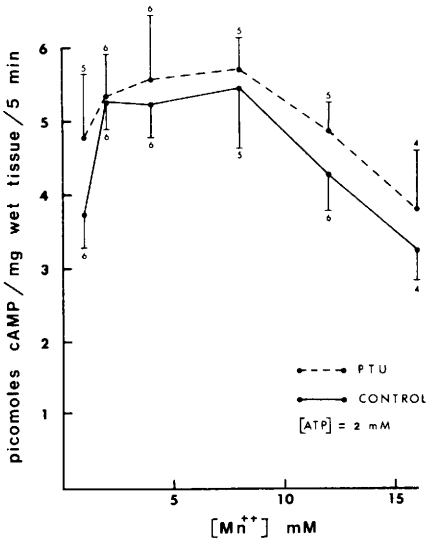


FIG. 2. The effect of manganese concentration on adenylate cyclase activity in thyroid tissue of normal (control) and PTU-treated rats. Values are the means ± SEM. The number of experiments is given at each point.

equimolar $Mg^{2+}$ concentrations (compare Figs. 1A and 2), both for control and goitrous glands ($P < 0.05$). $Mn^{2+}$ concentrations above 2 m*M* did not further stimulate adenylate cyclase activity, and $Mn^{2+}$ in excess of 8 m*M* depressed it, in both normal and goitrous tissue, in contrast with the effect of such concentrations of $Mg^{2+}$ (Fig. 1A).

With 2 m*M* ATP and 12 m*M* $Mg^{2+}$ in the assay reaction, $Ca^{2+}$ was added at concentrations from 0.15 to 3.0 m*M*. Figure 3 shows that both control gland and goiter adenylate cyclase activities were similarly inhibited by the addition of $Ca^{2+}$. The inhibition constant $K_i$, which gives the concentration of $Ca^{2+}$ at half-maximal inhibition,

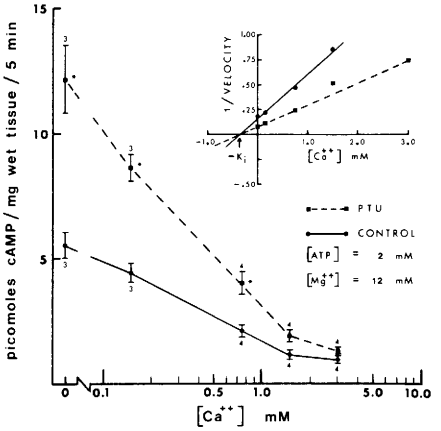


FIG. 3. The effect of calcium concentration on adenylate cyclase activity in thyroid tissue from normal (control) and PTU-treated rats. Values are the means ± SEM. The number of experiments is given at each point. An asterisk represents statistical significance ($P < 0.05$) of a PTU value above its corresponding control value (Student's $t$ test). The inset shows the reciprocal velocity (reciprocal of picomoles of cAMP per milligram of wet tissue per 5 min) vs inhibitor ($Ca^{2+}$) concentration. $K_i$ is the inhibitor concentration at half-maximal velocity.

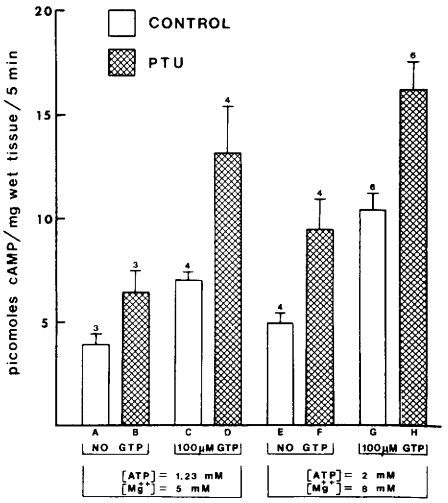


FIG. 4. The effect of 100 $\mu M$ GTP on adenylate cyclase activity at two substrate (ATP:$Mg^{2+}$) concentrations. Each bar is the mean value, with error bars representing SEM. The number of experiments is shown above each bar.

was calculated to be 0.36 m$M$ for both the control and PTU-treated groups.

*Effect of GTP and $PGE_1$.* The effect of GTP on adenylate cyclase activity was studied at two different ATP:$Mg^{2+}$ concentrations (1.23:5 and 2:8 m$M$). In both conditions, addition of 100 $\mu M$ GTP caused increases of from 80 to 110% in both the control and goiter tissue (Figs. 4 and 5).

The effect of varying concentrations of $PGE_1$ ($3 \times 10^{-7}$ to $3 \times 10^{-4}$ $M$) on adenylate cyclase activity was studied at two ATP:$Mg^{2+}$ concentrations (1.23:5 and 2:8 m$M$). Concentrations of an ethanol-sodium carbonate solution equal to those present in the most concentrated solutions of $PGE_1$ tested had no effect on the basal adenylate cyclase activity (no $PGE_1$) in either the control tissue or that from PTU-treated rats.

Figure 5 shows that $PGE_1$ alone did not stimulate adenylate cyclase activity in normal tissue. In goitrous tissue, $3 \times 10^{-5}$ $M$ $PGE_1$ was required for significant stimulation ($P < 0.05$). When GTP was added, normal tissue still did not respond to $PGE_1$. However, goitrous thyroids now showed significantly elevated adenylate cyclase activity at $PGE_1$ concentrations as low as $3 \times 10^{-6}$ $M$. The findings indicate that adenylate cyclase activity in goitrous thyroid tissue is more responsive to GTP and $PGE_1$ in combination than that in normal thyroid tissue.

*Discussion.* Elevated basal adenylate cyclase activity in thyroids of PTU-treated rats has been observed by Fontaine (3) and Zakarija *et al.* (2). The opposite findings of Granner and Halmi (4) may have been due to technical reasons, namely, the use of purified membranes instead of crude homogenate as the source of enzyme. It is conceivable that washing of membranes deprived these of some factor necessary for the full expression of this enzymatic activity and that this affected enzyme from goiters more severely than enzyme from normal thyroid tissue. It is also possible that PTU treatment caused translocation of adenylate cyclase from membranes to cytosol.

One of the important findings in this study was the dissimilar effect of increasing $Mg^{2+}$ concentrations on adenylate cyclase activity when normal and goitrous thyroid tissue were compared (Fig. 1A). At $Mg^{2+}$:ATP concentration ratios of 4 and 6, a statistically valid difference in enzyme activity in favor of the goitrous tissue was demonstrable, in agreement with the findings of Fontaine (3) and Zakarija *et al.* (2). A significant interaction between PTU

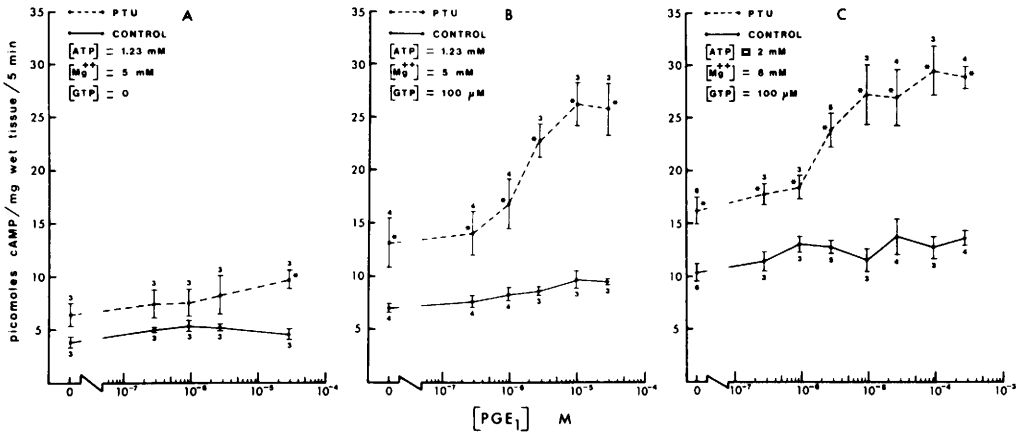


FIG. 5. The effect of $PGE_1$ concentration on adenylate cyclase activity in thyroid tissue of normal (control) and PTU-treated rats. Values are the means ± SEM. The number of experiments is given at each point. Panel C differs from panels A and B with respect to ATP:$Mg^{2+}$ concentration, and panel A differs from panels B and C by the absence of GTP from the reaction mixture. An asterisk represents statistical significance ($P < 0.05$) of a PTU value above its corresponding control value (Student's $t$ test).

treatment of the animals and $Mg^{2+}$ concentrations in the assay mixture was also documented by analysis of variance (Table I). Such a differential effect of $Mg^{2+}$ on tissue from goitrous thyroids was no longer demonstrated when $F^-$-activated, rather than basal, adenylate cyclase was studied (Fig. 1B). This may indicate that the difference between the adenylate cyclase of normal and goitrous thyroid with regard to activation by $Mg^{2+}$ has been somehow cancelled out by addition of $F^-$ or that the excessive stimulation by $F^-$ has masked this difference. As shown in Fig. 2, the substitution of $Mn^{2+}$ for $Mg^{2+}$ also abolished significant differences between the enzyme from hyperplastic and normal glands, which suggests that the apparent qualitative change in adenylate cyclase brought about by PTU treatment was cation specific. The inhibitory effect of $Ca^{2+}$ on thyroid adenylate cyclase was unaffected by PTU treatment (Fig. 3).

GTP had a comparable effect in activating adenylate cyclase from normal or goitrous thyroids (Figs. 4 and 5). As shown in Fig. 5, $PGE_1$ had no effect on the enzyme from normal thyroids. High concentrations ($3 \times 10^{-5}$ $M$) caused some stimulation of adenylate cyclase from PTU goiters. The addition of GTP to the medium both sensitized the enzyme of goitrous tissue to $PGE_1$, so that significant activation occurred with $3 \times 10^{-6}$ $M$ $PGE_1$, and increased its response to higher $PGE_1$ concentrations. It has been a general observation that GTP can enhance $PGE_1$-stimulated adenylate cyclase activity (7-9), but the unusual feature in this study was that the magnitude of this effect was altered by the state of the tissue, being greater in the hyperplastic condition than in the normal state.

The results of this study show that adenylate cyclase of PTU-induced goiters shows qualitative differences from that of control glands, revealed by a different pattern of activation in response to elevated concentrations of $Mg^{2+}$ and an increased sensitivity to stimulation by $PGE_1$ in the presence of GTP.

*Summary.* The influence of $Mg^{2+}$, $Mn^{2+}$, and $Ca^{2+}$ as well as the effects of guanosine triphosphate (GTP) and prostaglandin $E_1$ ($PGE_1$) on adenylate cyclase activity in homogenates of thyroid glands from rats treated with propylthiouracil (PTU) and from normal control rats were compared. Increasing concentrations of $Mg^{2+}$ in the medium stimulated adenylate cyclase activity: at 8 and 12 m$M$ $Mg^{2+}$, cyclase activity was significantly higher in the PTU-treated group. The effect of $Mn^{2+}$ on adenylate cyclase activity was essentially the same in goitrous and normal thyroids. Calcium inhibited adenylate cyclase activity; the $K_i$ for $Ca^{2+}$ (about 0.4 m$M$) was approximately

equal for normal and goitrous glands. There was also no significant difference between goitrous and control thyroid tissue in fluoride-stimulated adenylate cyclase activity determined at various $Mg^{2+}$ concentrations. Addition of GTP to the assay mixture stimulated adenylate cyclase activity in both groups. GTP also made tissue from both groups of rats responsive to stimulation of adenylate cyclase by $PGE_1$; the effect occurred at lower $PGE_1$ concentrations and was greater in the goitrous thyroids. These results suggest a qualitative difference between the basal adenylate cyclase of normal and goitrous thyroids with regard to (a) activity in the presence of excess $Mg^{2+}$ and (b) responsiveness to $PGE_1$ in the presence of GTP.

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