# Thyrotropin Increases Prostaglandin Levels in Isolated Thyroid Cells

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Abstract We have shown that two unrelated prostaglandin antagonists block both thyrotropin (TSH) and prostaglandins E ($PGE_1$, $PGE_2$) stimulation of thyroidal adenyl cyclase activation and cyclic 3′,5′-adenosine monophosphate (cAMP) formation, suggesting that prostaglandins play an important role in regulating thyroid function. To further explore this postulate, we measured prostaglandin content by radioimmunoassay in homogeneous bovine thyroid cell preparations in the presence and absence of TSH. Antibodies to albumin-conjugated $PGE_1$ and $PGF_{2\alpha}$ showed specificity for prostaglandins E and F, respectively, but reacted, albeit far less effectively, with heterologous prostaglandins. A double antibody system was used to separate free from antibody-bound $PGE_1$-$^3H$ and $PGF_{2\alpha}$-$^3H$. Thyroid cells were extracted with ethanol/ethyl acetate and the various prostaglandins separated on silicic acid columns. Recoveries of added $PGE_1$-$^3H$ and $PGF_{2\alpha}$-$^3H$ through the extraction and separation procedures ranged from 50–80%. The sensitivity of the method was 10–50 pg. Basal thyroid cell content of $PGE_1$ and $PGF_{2\alpha}$ "equivalents" varied between cell preparations (range = 2–6 ng/0.2 ml cell suspension) but, in each instance, remained constant during 5-30-min incubations at 37°C. TSH, 10-100 mU/ml, increased the levels of cell $PGE_1$ and $PGF_{2\alpha}$ "equivalents" 30-80% above basal during 5-15-min incubations. The stimulatory effect was specific for TSH, no increase in $PGE_1$ or $PGF_{2\alpha}$ "equivalent" levels being seen with luteinizing hormone (LH), human growth hormone (HGH), adrenocorticotropic hormone (ACTH), or glucagon. These data support the thesis that prostaglandins may mediate TSH effects on thyroid.

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## INTRODUCTION

We have recently shown (1, 2) that two unrelated prostaglandin (PG)[1] antagonists block both thyrotropin (TSH) and $PGE_1$ effects on thyroid adenyl cyclase and cyclic 3′,5′-adenosine monophosphate (cAMP) formation, suggesting that PGs may play an important role in regulating thyroid function. In an attempt to validate this thesis, we measured PG content in homogeneous thyroid cell preparations in the presence and absence of TSH.

## METHODS

Antisera used for PG immunoassay were obtained by immunization of rabbits with PG-protein conjugates. $PGE_1$ and $PGF_{2\alpha}$[2] were each conjugated to human serum albumin (HSA) (3). To estimate the number of PG residues incorporated into a molecule of HSA, 4 ng 5,6-$PGE_1$-$^3H$ (88 Ci/mmole)[3] or 0.025 μg 9-$PGF_{2\alpha}$-$^3H$ (14 Ci/mmole)[3] was mixed with 4 mg of the appropriate unlabeled PG before conjugation with HSA. Analysis of the conjugate for tritium content showed that approximately 13 molecules of $PGE_1$ and 15 molecules of $PGF_{2\alpha}$, respectively, were coupled to each molecule of HSA.

Dialyzed conjugates were emulsified with equal volumes of complete Freund's adjuvant. Initially the rabbits received 1 mg immunogen subcutaneously (0.25 mg/footpad); this was repeated 1 wk later by subcutaneous injection at multiple sites along the dosal surface. Booster injections were given at 2- and 4-month intervals. The animals were bled periodically and the relative titer of antisera was measured using a double antibody system to separate free from bound PG as described below. Generally the highest titer of anti-

[1] *Abbreviations used in this paper:* ACTH, adrenocortocotropic hormone; cAMP, cyclic 3′,5′-adenosine monophosphate; HGH, human growth hormone; HSA, human serum albumin; LH, luteinizing hormone; PG, prostaglandin; TSH, thyrotropin.

[2] PGs used in this study were supplied by Dr. John Pike, the Upjohn Co., Kalamazoo, Mich.

[3] New England Nuclear Corp., Boston, Mass.

sera was obtained by bleeding 10–14 days after booster injections at bimonthly intervals.

Bovine thyroid cells were isolated by an intermittent trypsinization technique (4). For PG determinations, 0.2 ml cell suspension (12-20 × $10^6$ cells/ml) in 5 ml Krebs Ringer bicarbonate buffer, pH 7.4, was incubated in 80 × 16 mm covered sterile polypropylene tubes with or without added TSH (Thytropar®, Armour Pharmaceutical Co., Kankakee, Ill.) at 37°C for time periods specified below. After incubation the tubes were quickly transferred to Duall #22 homogenizer tubes, and after a 2 min centrifugation (1000 *g*) at 0°C, the sedimented cells were frozen in dry ice. To the sedimented frozen cells was then added approximately 7000 cpm each $PGE_1$-$^3H$ and $PGF_{2\alpha}$-$^3H$ in 1.5 ml $H_2O$. The cells were homogenized for 15 sec at 23°C with a Potter-Elvehjem homogenizer, 8 ml 100% ethanol was added, and the contents were transferred to a #23 Duall homogenizer tube. The homogenization was repeated. The tubes were centrifuged for 10 min at 1000 *g*, the ethanol supernate was removed by pipetting, and the cell residue was washed with 4 ml 80% ethanol. The combined ethanol supernatant solutions were allowed to stand overnight at 4°C. They were centrifuged for 10 min at 1000 *g* to remove residual sediment, and the supernatant was evaporated to about 2.5 ml volume at reduced pressure. Residual volume was uniformly adjusted to 3 ml with $H_2O$, acidified to pH 3.0 with 0.1 N HCl and extracted three times with half its volume of ethyl acetate (5). The aqueous phase was then discarded; the combined ethyl acetate phases were washed with $H_2O$ to neutral pH and evaporated to dryness under reduced pressure. The efficiency of the extraction procedure was > 90%. The dried residue was dissolved in 3 ml benzene-ethyl acetate (60/40, v/v) immediately before chromatography.

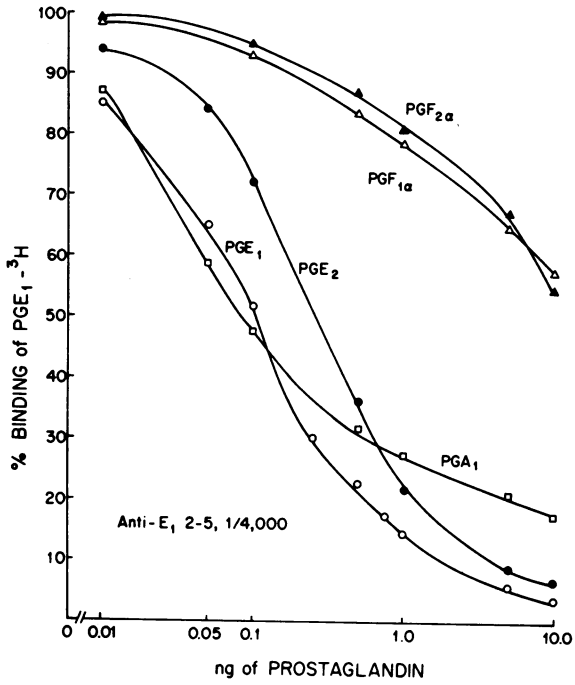


FIGURE 1 Standard curve for $PGE_1$ and cross-reactions of various prostaglandins with antiserum 2-5 at a dilution of 1/4000. Each value represents the mean of closely agreeing (within 5%) triplicate determinations.

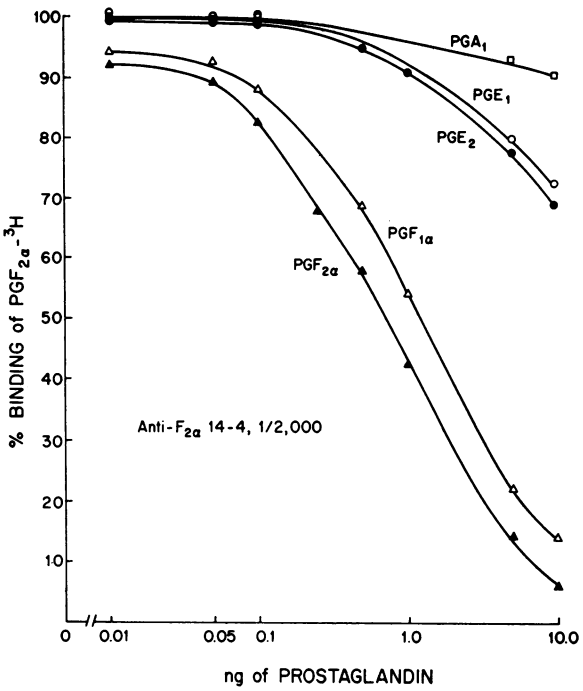


FIGURE 2 Standard curve for $PGF_{2\alpha}$ and cross-reactions of various prostaglandins with antiserum 14-4 at a dilution of 1/2000. Each value represents the mean of closely agreeing (within 5%) triplicate determinations.

Silicic acid chromatography was based on methods previously reported (5). All solvents were spectrograde quality and redistilled before use. Silicic acid (100 mesh, Mallinckrodt Chemical Works, St. Louis, Mo.) was activated at 115°C for 1 hr before use, slurried in benzene-ethyl acetate (9/1, v/v), poured into a 0.7 × 10 cm glass column, and allowed to settle to a height of 3.3 cm. The cell extract in 3 ml benzene-ethyl acetate (60/40, v/v) was then introduced onto the column; residual cell extract in the tube was washed onto the column with an additional 1 ml benzene-ethyl acetate. "A + E group" PGs were further eluted from the column with an additional 8 ml benzene-ethyl acetate-methanol (60/40/2, v/v). Thereafter, 4 ml of this solvent was passed through the column, the eluate discarded, and the "F group" PGs eluted with 6 ml benzene-ethyl acetate-methanol (60/40/15). The respective "A + E group" (12 ml) and "F group" (6 ml) eluates were evaporated at reduced pressure and dissolved in 800 μl of 0.005 M phosphate-buffered saline, pH 7.45, for radioimmunoassay. Recoveries of $PGE_1$-$^3H$ and $PGF_{2\alpha}$-$^3H$ with this chromatographic technique ranged from 60-80% and 50-70%, respectively.

Radioimmunoassay was performed in 10 × 56 mm polypropylene tubes; 100 μl antiserum at a dilution which gave 25-35% binding of labeled PG in the absence of unlabeled PG (1/4000 dilution of anti-$PGE_1$, 1/2000 dilution of anti-$PGF_{2\alpha}$) was added to an 100 μl portion (in triplicate) of PG standard or cell extract. 100 μl $PGE_1$-$^3H$ or $PGF_{2\alpha}$-$^3H$ (approximately 6000-8000 cpm) was added to bring total volume to 300 μl. The tubes were incubated for 2 hr at 23°C, followed by addition of 100 μl 5% normal rabbit serum and an excess of goat anti-rabbit gamma globulin (100 μl, 50%) and overnight incubation at 4°C. The tubes were then centrifuged at 1000 *g* for 40 min at 4°C, the precipitates dissolved

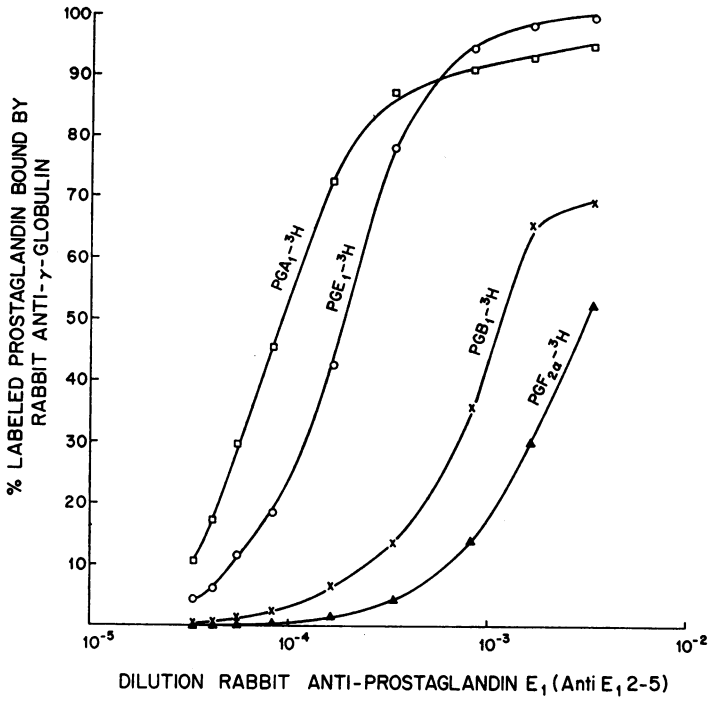


FIGURE 3 Binding of $^3$H-labeled PGs $A_1$, $E_1$, $B_1$, and $F_{2\alpha}$ by the serum of a rabbit immunized with $PGE_1$-HSA (antiserum 2-5). $PGA_1$-$^3$H (approximately 1.18 pmoles), $PGE_1$-$^3$H (approximately 1.5 pmoles), $PGB_1$-$^3$H (approximately 1.5 pmoles), and $PGF_{2\alpha}$-$^3$H (approximately 0.74 pmoles), each about 7000 cpm per reaction mixture, were incubated with dilutions of antiserum (final dilutions) in a 0.3 ml reaction volume for 1 hr at 37°C. After addition of 5% carrier normal rabbit serum in a 0.1 ml volume, goat anti-rabbit gamma globulin (50%, 0.1 ml) was added and immune precipitation was allowed to proceed overnight at 4°C. Results are means of closely agreeing (within 5%) triplicate determinations.

in 300 μl solubilizer (NCS, Amersham Searle, Des Plaines, Ill.), and counted in toluene scintillation solution.

## RESULTS

Radioimmunoassay standard curves showed a significant difference between 10 and 50 pg for $PGE_1$ (Fig. 1) and between 50 and 100 pg for $PGF_{2\alpha}$ (Fig. 2). When 0.2 ml $H_2O$ was carried through the entire procedure, virtually no displacement ($\leq 2\%$) was recorded on the curve for either $PGE_1$ or $PGF_{2\alpha}$. Although there was only a 1-2% relative cross-reaction between PGs E and F with the antisera to $PGE_1$-HSA and $PGF_{2\alpha}$- HSA

TABLE I

*Radioimmunoassay of Known Amounts of $PGE_1$ and $PGF_{2\alpha}$ Added to Isolated Thyroid Cells*

| Thyroid cell volume | Prostaglandin added | | Level of $PGE_1$* measured‡ | | | Level of $PGF_{2\alpha}$§ measured‡ | | |
|---|---|---|---|---|---|---|---|---|
| | $PGE_1$ | $PGF_{2\alpha}$ | Mean ±SD | No.‖ | Recovery | Mean ±SD | No.‖ | Recovery |
| *ml* | *ng* | | *ng* | | | *ng* | | |
| 0.1 | 0 | 0 | 2.959 ±0.453 | 9 | — | 2.128 ±0.216 | 9 | — |
| 0.1 | 1.0 | 1.0 | 4.055 ±0.229 | 6 | 102.4 | 3.196 ±0.396 | 6 | 102.2 |
| 0.1 | 10.0 | 10.0 | 12.918 ±0.679 | 9 | 99.7 | 13.065 ±0.524 | 6 | 107.7 |

* $PGE_1$ "equivalents".

‡ Corrected for extraction and separation recoveries.

§ $PGF_{2\alpha}$ "equivalents".

‖ No. = number of experimental observations, each experimental determination performed in triplicate.

TABLE II

*Radioimmunoassay of $PGE_1$ and $PGF_{2\alpha}$ in Varying Volumes of Thyroid Cell Suspension*

| Cell suspension volume | $PGE_1$ "equivalents" | | $PGF_{2\alpha}$ "equivalents" | |
|---|---|---|---|---|
| | Mean ±SD | No.* | Mean ±SD | No.* |
| *ml* | *ng* | | *ng* | |
| 0.05 | 1.64 ±0.28 | 8 | 1.48 ±0.29 | 9 |
| 0.1 | 3.44 ±0.32 | 5 | 2.92 ±0.30 | 9 |
| 0.2 | 6.06 ±0.47 | 7 | 5.70 ±0.56 | 8 |

* No. = number of experimental observations, each experimental determination performed in triplicate.

(based on mass of $PGF_{1\alpha}$ or $PGF_{2\alpha}$ required to displace 50% of bound $PGE_1$-$^3H$ [Fig. 1] and mass of $PGE_1$ or $PGE_2$ required to displace 50% of bound $PGF_{2\alpha}$-$^3H$ [Fig. 2]), $PGE_1$ antiserum was unable to effectively distinguish between $PGE_1$, $PGE_2$, and $PGA_1$ (Fig. 1) while $PGF_{2\alpha}$ antiserum recognized both $PGF_{1\alpha}$ and $PGF_{2\alpha}$ (Fig. 2).[4] Thus, while we are reporting $PGE_1$ and $PGF_{2\alpha}$ levels, these are in fact $PGE_1$ and $PGF_{2\alpha}$ "equivalents" (6).

While these studies were in progress, Levine, Gutierrez-Cernosek, and Van Vunakis (7) reported that immunization with $PGE_1$ produced antibodies directed to $PGB_1$. We therefore carried out similar studies with our $PGE_1$ antiserum and $PGB_1$. $PGB_1$-$^3H$ was obtained by heating $PGE_1$-$^3H$ or $PGA_1$-$^3H$ in 0.02 N NaOH at 100°C for 5 min; Andersen (8) has demonstrated that under these conditions $PGE_1$ and $PGA_1$ are quantitatively converted to $PGB_1$. Fig. 3 illustrates the binding capacity of our $PGE_1$ antiserum. Of the four labeled antigens used, the greatest binding occurred with $PGA_1$ and $PGE_1$. Although cross-reaction with $PGB_1$-$^3H$ was observed, binding was much less effective.

Table I shows the correlation between PG added to 0.1 ml cell suspension and that recovered in the radioimmunoassay. When either 0.05, 0.1, or 0.2 ml thyroid cells was extracted, the levels recorded in the radioimmunoassay were proportional to the volume of cell suspension used (Table II).

During a 15 min incubation at 37°C, TSH effected an increase in thyroid cell levels of both $PGE_1$ and $PGF_{2\alpha}$ "equivalents" (Fig. 4). Although the increase in PG levels was dose-related, the TSH effect on $PGE_1$ was biphasic, and both the $PGE_1$ and $PGF_{2\alpha}$ "TSH dose-response" curves were relatively flat. Thus, the maximum TSH-induced increase in $PGE_1$ levels was approximately 80% over control and that in $PGF_{2\alpha}$ levels approximately 40% over control. The increases were, however, highly reproducible and specific for TSH (Table III).

[4] It should be noted, however, that presence of a double bond at position 5,6 reduced ability of $PGE_2$ to cause displacement to one-third that of $PGE_1$ (Fig. 1) and that loss of a double bond at position 5,6 reduced ability of $PGF_{1\alpha}$ to cause displacement to 70% that of $PGF_{2\alpha}$ (Fig. 2).

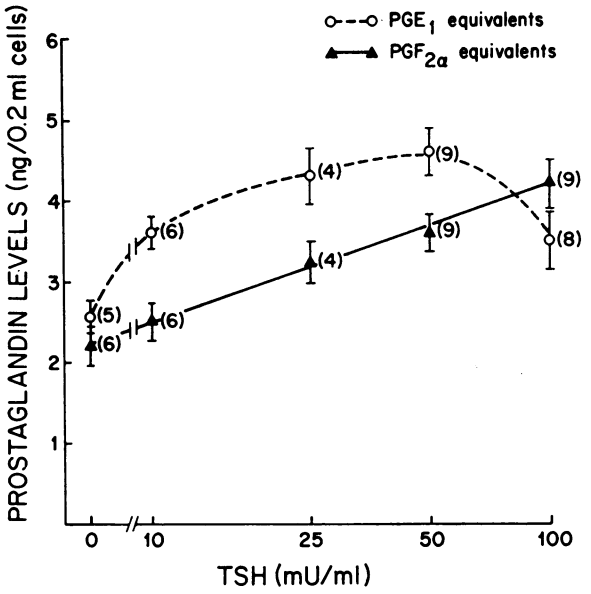


FIGURE 4 Effects of TSH on prostaglandin content of isolated bovine thyroid cells during 15-min incubation at 37°C. Results are mean ± SD of 4–9 experimental determinations (number of experimental observations given in parentheses), each experimental determination performed in triplicate.

The stimulatory effect of TSH on cell PG content was evident within 5 min of incubation at 37°C and increased through 30 min of incubation. Basal levels of PGs did not change during this period. TSH, incubated with thyroid cells at 0°C for periods up to 30 min did not influence cell PG content (Table IV).

TABLE III

*Effect of TSH and Other Hormones on Thyroid Cell Content of Prostaglandins**

| Hormone added | $PGE_1$ "equivalents" | | $PGF_{2\alpha}$ "equivalents" | |
|---|---|---|---|---|
| | Mean ±SD | No.§ | Mean ±SD | No.* |
| - | *ng*‡ | | *ng*‡ | |
| None | 4.717 ±0.549 | 9 | 3.044 ±0.375 | 9 |
| TSH, 50 mU/ml | 7.918 ±0.688 | 9 | 5.652 ±0.519 | 6 |
| Heat inactivated TSH‖, 50 mU/ml | 4.444 ±0.856 | 9 | 3.484 ±0.411 | 8 |
| LH, 0.5 mg/ml | 4.016 ±0.753 | 6 | 3.018 ±0.474 | 5 |
| ACTH, 0.5 mg/ml | 3.785 ±0.184 | 9 | 2.981 ±0.744 | 5 |
| HGH, 0.5 mg/ml | 3.467 ±0.570 | 7 | 3.048 ±0.218 | 6 |
| Glucagon, $10^{-6}$ M | 3.909 ±0.769 | 9 | 2.970 ±0.218 | 5 |

* 0.2 ml thyroid cell suspension, 15 min incubation at 37°C.
‡ ng/0.2 ml cell suspension.
§ No. = number of experimental observations, each experimental determination performed in triplicate.
‖ 100°C for 2 hr prior to addition to thyroid cells.

TABLE IV

*Time-Course of TSH-Induced Increase in Thyroid Cell Prostaglandin Content*

| Incubation time and temperature | $PGE_1$ "equivalents" | | | | | $PGF_{2\alpha}$ "equivalents" | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | TSH, 50 mU/ml | | | Control | | TSH, 50 mU/ml | | |
| | Mean ±SD | No.‡ | Mean ±SD | No.‡ | Percent increase | Mean ±SD | No.‡ | Mean ±SD | No.‡ | Percent increase |
| | *ng** | | *ng** | | | *ng** | | *ng** | | |
| 5 min, 37°C | 2.33 ±0.23 | 9 | 2.92 ±0.22 | 8 | 25 | 2.60 ±0.62 | 6 | 3.68 ±0.65 | 8 | 42 |
| 15 min, 37°C | 2.28 ±0.27 | 9 | 3.35 ±0.60 | 8 | 47 | 2.89 ±0.53 | 7 | 4.02 ±0.48 | 6 | 39 |
| 30 min, 37°C | 2.55 ±0.23 | 9 | 4.21 ±0.50 | 9 | 65 | 2.40 ±0.40 | 6 | 4.33 ±0.91 | 9 | 80 |
| 30 min, 0°C | 2.41 ±0.25 | 6 | 2.29 ±0.31 | 6 | — | 2.53 ±0.48 | 5 | 2.68 ±0.55 | 5 | — |

* ng/0.2 ml cell suspension.

‡ No. = number of experimental observations, each experimental determination performed in triplicate.

## DISCUSSION

Several laboratories have recently reported the production of antibodies to PGs (3, 6, 9). It has been suggested (3) that the inability of antibody to $PGE_1$ to distinguish between $PGA_1$ and $PGE_1$ may be due to conversion of protein-bound $PGE_1$ to $PGA_1$ in vivo or in vitro. Levine et al. (7) noted that methodologic variables might influence the apparent specificity of PG antisera, so that divergent findings with the various PG radioimmunoassays were to be anticipated. The inability of our anti-$PGE_1$ antiserum to effectively distinguish between $PGE_1$ and $PGE_2$ or of our anti-$PGF_{2\alpha}$ antiserum to distinguish between $PGF_{2\alpha}$ and $PGF_{1\alpha}$, or the ability of our $PGE_1$ antiserum to recognize $PGE_1$ but not $PGB_1$ (contrasting sharply with the findings of Levine et al. (7)) should thus be viewed in this light.

PGs E and F simulate a variety of TSH effects on thyroid and are considerably more potent in this regard than other PGs (10). Demonstration that TSH increases thyroid cell content of these "thyrotropic" PGs supports our earlier contention that PGs may play an important role in mediating TSH effects on thyroid (1, 2, 11). It should be noted in this regard that the thyroid cell preparations used in this study respond functionally (e.g., adenyl cyclase activation, iodide trapping (1, 2, 11)) in similar fashion to both TSH and $PGE_1$. Moreover, the TSH-induced increase in cell $PGE_1$ and $PGF_{2\alpha}$ occurred promptly after addition of TSH, paralleling in this respect the temporal character of TSH- (and $PGE_1$-) induced activation of thyroid adenyl cyclase and increase in cAMP (1, 2). (The variation in basal cell PG content is consistent with the considerable variability in other aspects of cell function (12).) Although one cannot, from these findings, determine whether the TSH-induced increase in cell PG content reflects increased synthesis, decreased degradation, or both, this study represents, to our knowledge, the first reported instance of hormone-induced increase in tissue PGs.


## ACKNOWLEDGMENTS

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