# Differential Effects of Growth Factors on [$^3$H]Thymidine Incorporation and [$^{125}$I]Iodine Uptake in FRTL-5 Rat Thyroid Cells (43085)

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*Abstract.* **We studied the effect of several growth factors on DNA synthesis and function of FRTL-5 rat thyroid cells by simultaneous measurement of [$^3$H]thymidine incorporation and [$^{125}$I]iodide uptake. Endothelial cell growth factor, fibroblast growth factor, platelet-derived growth factor, and insulin-like growth factor I stimulated thymidine incorporation in a dose-dependent manner without the parallel increase of [$^{125}$I]iodide uptake. These growth factors had an additive effect with thyroid-stimulating hormone (TSH) on thymidine incorporation, but they inhibited TSH-stimulated iodide uptake. Bombesin stimulated thymidine incorporation and inhibited TSH-stimulated iodide uptake; epidermal growth factor and gastrin-releasing peptide 10 had neither effect. None of the growth factors studied affected iodide uptake in the absence of TSH. Of the growth factors tested, endothelial cell growth factor, fibroblast growth factor, insulin-like growth factor bombesin, and platelet-derived growth factor all share similar differential effects on FRTL-5 cells: stimulation of DNA synthesis, potentiation of the effects of TSH on DNA synthesis, and attenuation of the effects of TSH on cell function. The data suggest that these growth factors may play important roles in regulation of thyroid function.**

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There are a number of circulating growth factors which are also widely distributed in almost all of the tissues in the body. The regulatory effects of these peptides on thyroid function have received much attention recently. Studies have shown that insulin-like growth factor I (IGF-I), fibroblast growth factor (FGF), and epidermal growth factor (EGF) modulate thyroid function *in vitro* (1–3). Although the receptors for some of the growth factors have been characterized in thyroid cells, the mechanism of action of these growth factors is still unclear (4, 5). We and others have shown that thyrotropin (thyroid-stimulating hormone) and human chorionic gonadotropin stimulate FRTL-5 cell function and DNA synthesis (1, 6). In this study, we tested the effects of several growth factors in comparison to TSH on differentiated FRTL-5 rat thyroid cells. The results show that the effects of these growth factors on cell function and DNA synthesis are dissociated and differ from the effects of TSH and human chorionic gonadotropin

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## Materials and Methods

Human recombinant IGF-I, bovine basic FGF, human recombinant EGF, and human recombinant platelet-derived growth factor (PDGF) were gifts from Amgen, Thousand Oaks, CA. Bovine endothelial cell growth factor (ECGF, acid FGF) was a gift from Vec Tec Inc, Albany, NY. Bombesin and gastrin-releasing peptide (GRP-10) were provided by Dr. Joseph Reeve. Highly purified bovine TSH (bTSH) was obtained from the National Pituitary Program, NIH, Bethesda, MD.

The simultaneous measurement of [$^{125}$I]iodide uptake and [$^3$H]thymidine incorporation was performed. Preliminary study showed that there was no significant interference between measurements of [$^{125}$I]iodide uptake and [$^3$H]thymidine incorporation (see below). [$^{125}$I] Iodide uptake was a well-defined indicator of thyroid cell function (6). In our preliminary experiments, [$^3$H] thymidine incorporation correlated with cellular DNA content and was, therefore, a relative indicator of cell growth. TSH is a strong stimulator of thyroid function ([$^{125}$I]iodide uptake) and growth ([$^3$H]thymidine incorporation, DNA synthesis, and cell replication) (1, 2, 6).

Therefore, TSH served as a positive control for comparison to other growth factors. We did not measure cell number and DNA in this study.

FRTL-5 rat thyroid cells (obtained from Interthyr Research Corp., Baltimore, MD) were maintained by the method described previously (6); $8 \times 10^4$ cells were seeded in each well in a 24-well plate (Linbro, Flow Laboratories, Mclean, VA) and grown in Coon's modified Ham's F-12 medium supplemented with TSH, insulin, somatostatin, hydrocortisone, transferrin, and glycyl-histidyl-lysine (6H) medium with 5% calf serum in 5% $CO_2$-95% air at 37°C for 3 days, then in 5 H medium (deficient in TSH) for 6 to 8 days with medium changes every 2–3 days. For testing the growth factors, the cells were incubated with 0.5 $\mu$Ci of [$^3$H]thymidine with different concentrations of various growth factors alone, bTSH alone, or their combinations in 5H medium containing 1 $\mu M$ thymidine, 0.1% calf serum, and 0.5% bovine serum albumin in 5% $CO_2$-95% air at 37°C for 48 hr. The cells were then washed with 1 ml of phosphate-buffered Hanks' balanced salt solution twice and switched to 0.5 ml of phosphate-buffered Hanks' balanced salt solution containing $15 \times 10^4$ cpm of sodium [$^{125}$I]iodide with 10 n$M$ cold potassium iodide. After 40 min of incubation in 5% $CO_2$-95% air at 37°C, the cells were washed with 1 ml of ice-cold phosphate-buffered saline (PBS, pH 7.5) and incubated with 0.5 ml of PBS with 1 m$M$ potassium perchlorate in 5% $CO_2$-95% air at 37°C for 1 hr. The medium was taken up for counting the $^{125}$I. The cells were washed, collected, homogenized, and the $^3$H was counted in a liquid scintillation counter. The results were expressed as cpm ([$^{125}$I]iodide or [$^3$H]thymidine)/well. The data presented were representative results from two or three experiments.

To test the effect of potassium perchlorate on the discharge of [$^{125}$I]iodide from the cells and the possible interference of [$^{125}$I]iodide on the counting of [$^3$H] thymidine, the cells were prepared and [$^{125}$I]iodide uptake was measured as described above. Then the cells were incubated with 0.5 ml of 1 m$M$ potassium perchlorate in PBS for 1 hr. The medium was collected for counting of [$^{125}$I] on the gamma counter. The cells were washed, collected in 0.2 ml of trypsin (0.75 mg/ml)-PBS, and counted for [$^{125}$I] on the gamma counter. Then the cells were homogenized, 10 ml of Biofluor were added and the $^{125}$I was counted in the liquid scintillation counter. From cells treated with 111 microunits of TSH for 40 hr, the $^{125}$I released into the medium by potassium perchlorate was 7061 ± 698 (SD) cpm/well, $n = 5$. The $^{125}$I remaining in the cells was 159 ± 25 cpm/well; the $^{125}$I of these cells measured in the liquid scintillation counter was 163 ± 34 cpm/well. When 0.5 $\mu$Ci of [$^3$H]thymidine is used, cell counts of $^3$H are approximately 5,000–20,000 cpm/well. Under these conditions, the contribution of the residual $^{125}$I to the $^3$H counts is regarded as neglible.

Nonpaired Student's $t$ test with the Bonferroni correction for multiple comparisons or analysis of variance was used for statistical analysis of the data; $P < 0.05$ was interpreted as significant.

## Results

**Effects of FGF, ECGF, IGF-I, and EGF.** TSH stimulated both [$^3$H]thymidine incorporation and [$^{125}$I] iodide uptake dose dependently (Fig. 1). FGF stimulated [$^3$H]thymidine incorporation by FRTL-5 cells in a dose-related manner but did not affect basal [$^{125}$I] iodide uptake; i.e., [$^{125}$I]iodide uptake in the absence of bTSH (Fig. 1). FGF had a partially additive effect with bTSH on [$^3$H]thymidine incorporation. However, FGF strongly inhibited the effect of TSH on [$^{125}$I]iodide uptake either with a fixed amount of TSH (111 microunits/ml) and varying amounts of FGF or with a fixed FGF concentration of 250 ng/ml and varying amounts of TSH.

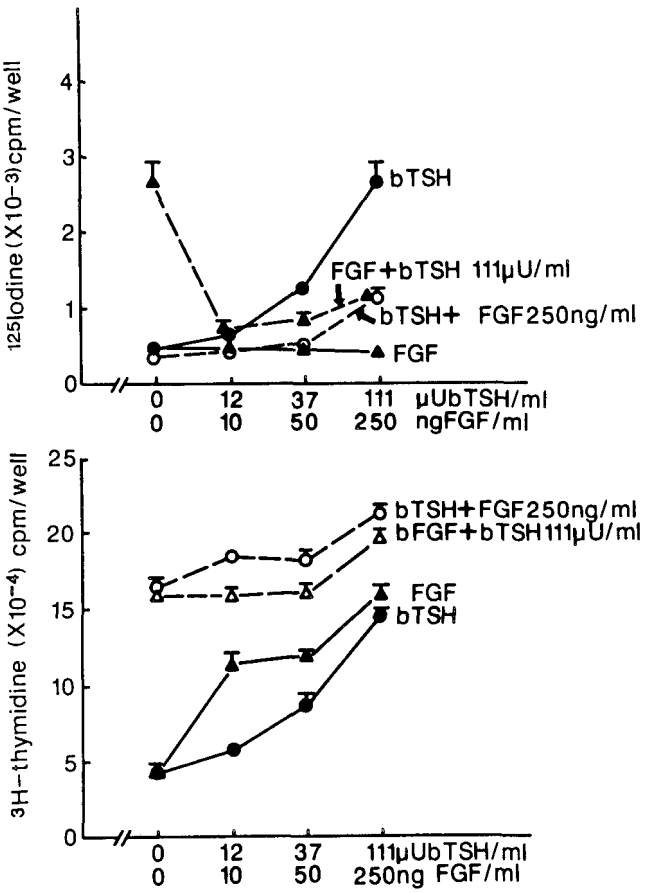


**Figure 1.** Effect of FGF and its interactions with bTSH on [$^{125}$I]iodide uptake (top) and [$^3$H]thymidine incorporation (bottom) by FRTL-5 cells, mean ± SD, $n = 3$. The cells were treated with TSH (0–111 microunits/ml) alone, with FGF (0–250 ng/ml) alone, bTSH (111 microunits/ml) plus various concentrations of FGF (0–250 ng/ml) and FGF (250 ng/ml) plus various concentrations of bTSH (0–111 microunits/ml) at 37°C in 5% $CO_2$-95% air for 48 hr.

ECGF was less potent than FGF in stimulation of [$^3$H]thymidine incorporation and it also blocked TSH-induced [$^{125}$I]iodide uptake (Fig. 2). The effect of ECGF on [$^3$H]thymidine incorporation was partially additive with that of TSH, and the increment caused by ECGF was significant ($P < 0.05$).

IGF-I dose-dependently stimulated [$^3$H]thymidine incorporation by FRTL-5 cells. When combined with TSH, IGF-I had an additive effect with TSH on [$^3$H] thymidine incorporation (Fig. 3). Although IGF-I did not affect basal [$^{125}$I]iodide uptake, it inhibited the effect of TSH on [$^{125}$I]iodide uptake (Fig. 3).

EGF did not show significant effects on [$^{125}$I]iodide uptake or [$^3$H]thymidine incorporation by FRTL-5 cells (data not shown).

**Effects of bombesin, GRP-10, and PDGF.** Bombesin and PDGF each stimulated [$^3$H]thymidine incorporation by FRTL-5 cells in the absence of bTSH ($P < 0.05$) and had no effect on basal [$^{125}$I]iodide uptake (Figs. 4 and 5). Bombesin and PDGF inhibited the [$^{125}$I] iodide uptake stimulated by bTSH. Bombesin and PDGF also had an additive effect with bTSH on [$^3$H]

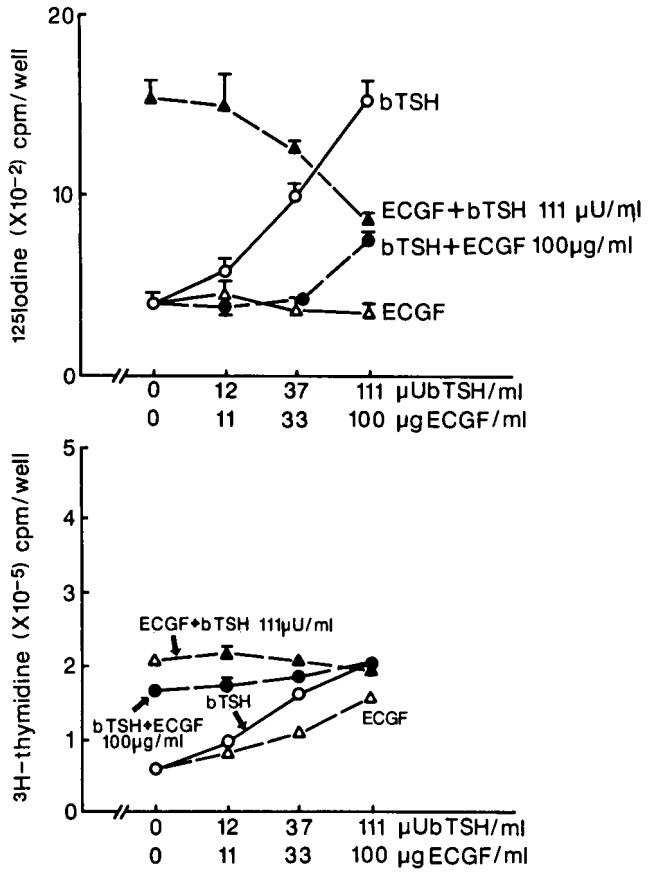


**Figure 2.** Effect of ECGF on [$^{125}$I]iodide uptake and [$^3$H]thymidine incorporation by FRTL-5 cells, mean ± SD, $n = 3$. The cells were treated with bTSH (0–111 microunits/ml) alone, with ECGF (0–100 μg/ml) alone, bTSH (111 microunits/ml) plus various concentrations of ECGF (0–100 μg/ml) and ECGF (100 μg/ml) plus various concentrations of bTSH (0–111 microunits/ml) at 37°C in 5% $CO_2$-95% air for 48 hr.

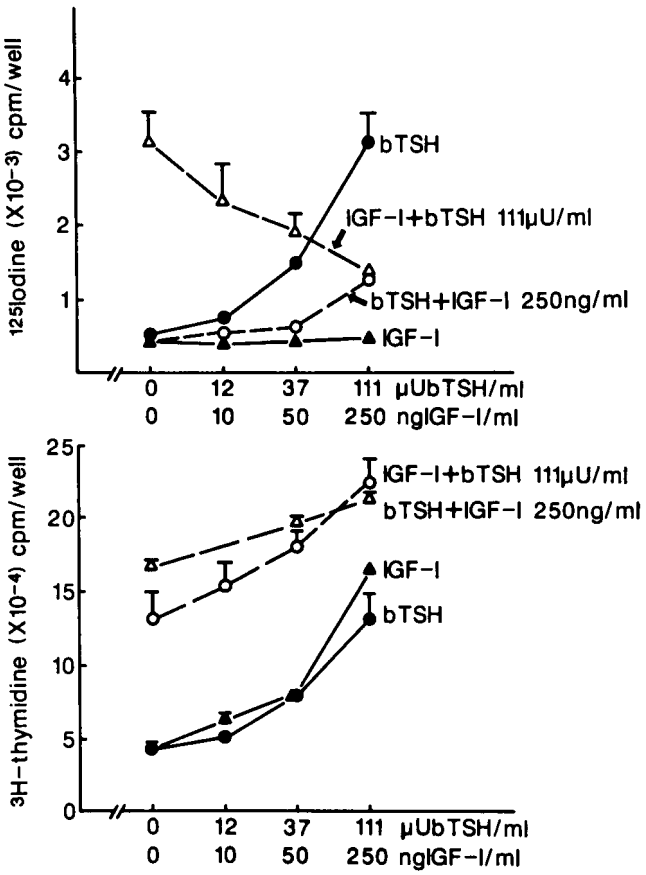


**Figure 3.** Effect of IGF-I and its interactions with bTSH on [$^{125}$I]iodide uptake and [$^3$H]thymidine incorporation by FRTL-5 cells, mean ± SD, $n = 3$. The cells were treated with bTSH (0–111 microunits/ml) alone, with IGF-I (0–250 ng/ml) alone, bTSH (111 microunits/ml) plus various concentrations of IGF-I (0–250 ng/ml) and IGF-I (250 ng/ml) plus various concentrations of bTSH (0–111 microunits/ml) at 37°C in 5% $CO_2$-95% air for 48 hr.

thymidine incorporation by FRTL-5 cells (Figs. 4 and 5).

GRP-10, a peptide related to bombesin, did not affect [$^{125}$I]iodide uptake and [$^3$H]thymidine incorporation by FRTL-5 cells (data not shown).

## Discussion

We have tested several growth factors for their effects on thyroid function and growth utilizing rat FRTL-5 cells. In this study, TSH was used in all of the experimental conditions as a positive control. The use of simultaneous measurement of [$^{125}$I]iodide uptake and [$^3$H]thymidine incorporation enabled us to evaluate FRTL-5 thyroid cell function as well as growth at the same time. This study confirmed the previous reports showing mitogenic effects of IGF-I and FGF on FRTL-5 cells (2, 4, 5), but we also found that these growth factors inhibit FRTL-5 cell function ([$^{125}$I]iodide uptake).

EGF did not affect rat thyroid cell function and growth in this study, possibly due to the lack of EGF receptors in this isolated clone of FRTL-5 cells. Pre-

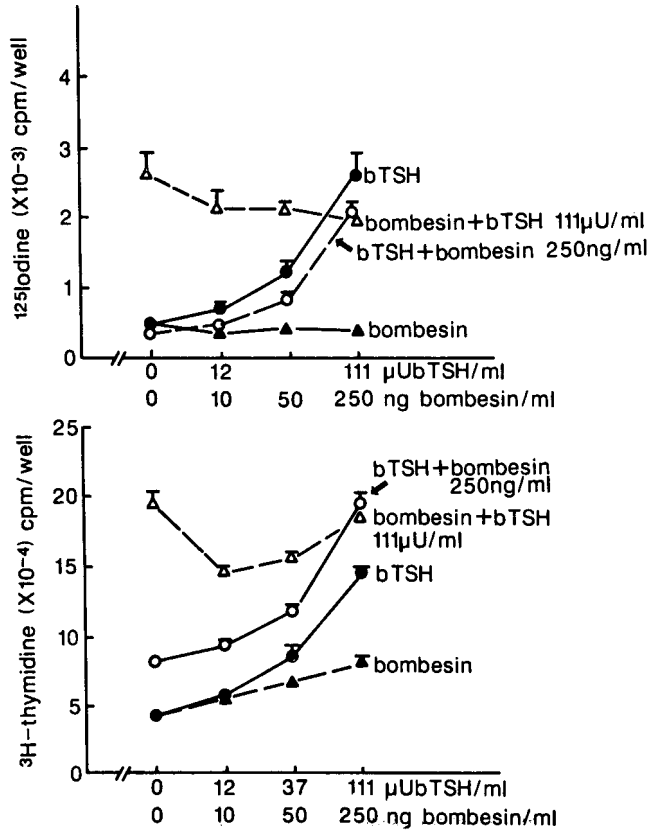


**Figure 4.** Effect of bombesin and its interaction with bTSH on [$^{125}$I] iodide uptake and [$^{3}$H]thymidine incorporation by FRTL-5 cells, mean ± SD, $n$ = 3. The cells were treated with bTSH (0–111 microunits/ml) alone, with bombesin (0–250 ng/ml) alone, bTSH (111 microunits/ml) plus various concentrations of bombesin (0–250 ng/ml) and bombesin (250 ng/ml) plus various concentrations of bTSH (0–111 microunits/ml) at 37°C in 5% $CO_2$-95% air for 48 hr.

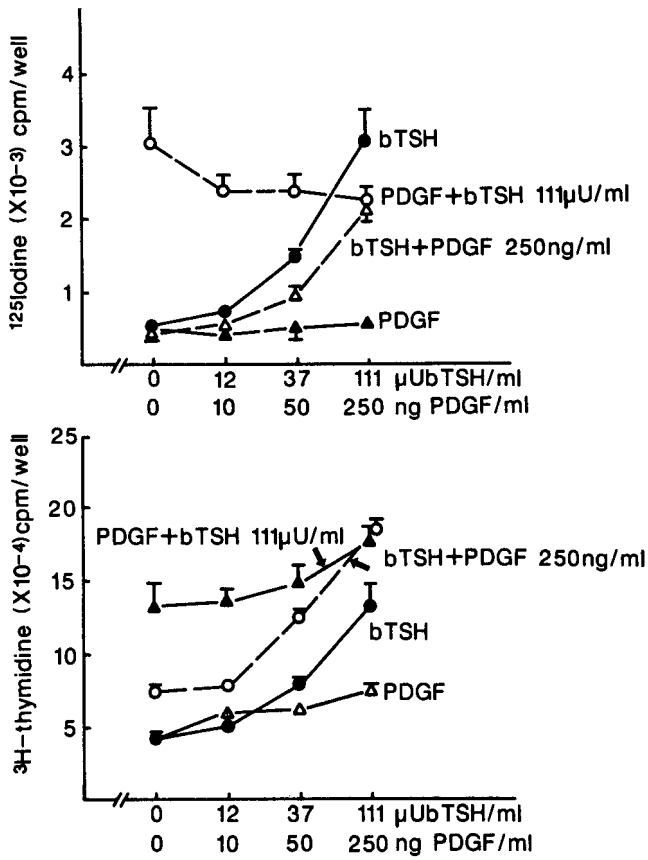


**Figure 5.** Effects of PDGF and its interaction with bTSH on [$^{125}$I] iodide uptake and [$^{3}$H]thymidine incorporation by FRTL-5 cells, mean ± SD, $n$ = 3. The cells were treated with bTSH (0–111 microunits/ml) alone, with PDGF (0–250 ng/ml) alone, bTSH (111 microunits/ml) plus various concentrations of PDGF (0–250 ng/ml) and PDGF (250 ng/ml) plus various concentrations of bTSH (0–111 microunits/ml) at 37°C in 5% $CO_2$-95% air for 48 hr.

vious studies showed that EGF is a potent growth factor and inhibitor of iodine metabolism in porcine, sheep, and canine thyroid cells (3, 7, 8). Although species specificity for EGF may have influenced the results, others have shown that murine EGF had no effect on [$^{3}$H]thymidine uptake by cultured rat thyroid follicles (9).

Interestingly, we observed that PDGF and bombesin are also very potent stimulators of DNA synthesis by FRTL-5 cells. Like IGF-I and FGF, both PDGF and bombesin also potentiate the effects of TSH on [$^{3}$H] thymidine incorporation and attenuate the effects of TSH on [$^{125}$I]iodide uptake by FRTL-5 cells. ECGF, an acidic fibroblast growth factor which belongs to the FGF family (10), stimulated [$^{3}$H]thymidine incorporation and attenuated the TSH-stimulated [$^{125}$I]iodide uptake. Both ECGF and FGF bind to the same receptors and share a similar spectrum of biologic activity (10). The much lower biologic activity of ECGF compared with FGF observed in this study is consistent with previous studies which showed that basic FGF is about 30–100 times as potent as acidic FGF (10). Bombesin is a peptide with 14 amino acids and is a potent mitogen for Swiss $3T_3$ fibrocytes and small cell lung cancer cells (11). GRP-10, a mammalian decapeptide which shares the C-terminal sequence of bombesin, had no effect on FRTL-5 cells, but we did find effects with bombesin. This suggests that the N-terminal sequence of bombesin is essential for its biologic action on FRTL-5 cells.

Thyroid cells have both IGF-I and insulin receptors; the mitogenic effect of insulin may be mediated partially through the IGF-I receptor (4, 12). In the present work, the 5H medium and 6H medium contained human insulin (10 μg/ml) so that interaction of insulin with the IGF-I receptor could have been involved. In addition, the effects of insulin could have influenced the actions of the various growth factors. Nevertheless, we observed dose-related mitogenic effects of the various growth factors added to the 5H medium. The action of IGF-I was especially marked. Others have recently reported that insulin and IGF-I have effects on growth and iodide uptake in FRTL-5 cells similar to those described here (13); the mitogenic

effect of 50 ng of IGF-I/ml was comparable to that of 10 $\mu$g of insulin/ml.

Of the growth factors tested, IGF-I, FGF, ECGF, bombesin, and PDGF all stimulate [$^3$H]thymidine incorporation which is similar to the effect of TSH, and all of them potentiate TSH-stimulated [$^3$H]thymidine incorporation. However, they attenuate cell function ([$^{125}$I]iodide uptake) stimulated by TSH. These differential effects suggest that these growth factors affect FRTL-5 cells in a way that is different from that of TSH and human chorionic gonadotropin which act through cAMP (1, 2, 6, 14, 15). The various growth factors share similar differential effects on DNA synthesis and cell function, although they bind to their own specific membrane receptors. A potential mechanism to explain these effects was suggested by the report that several growth factors can stimulate a common protein kinase (16). Studies have shown that TSH increases cellular calcium, turnover of inositol phosphates, and diacylglycerol levels. The increase of diacylglycerol may activate protein kinase C and thus stimulate growth of FRTL-5 cells (17–19). IGF-I itself can increase the diacylglycerol level in FRTL-5 cells and synergize with TSH to increase the diacylglycerol level further (19). The exact mechanism of action of these growth factors and their interaction with TSH on the growth and function of thyroid cells is still unclear. The data on the interaction of TSH with these growth factors in this study may reflect the interaction between cAMP-protein kinase A and calcium-inositol phosphates-diacylglycerol-protein kinase C systems. Our results also indicate the complexity of the regulation of thyroid function. Further studies will be interesting in regard to the mechanism of action of these growth factors on the function and growth of thyroid cells and their potential significance in the regulation of thyroid function.

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