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General inhibition by transforming growth factor β1 of thyrotropin and cAMP responses in human thyroid cells in primary culture

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Summary

Transforming growth factor β1 (TGFβ1) mRNA has previously been identified in human thyroid cells and this agent has been shown to inhibit DNA synthesis in thyroid cells of some other species.

In normal human thyroid cells in primary culture, TGFβ1 inhibited inconstantly the low basal DNA synthesis and strongly the stimulation of DNA synthesis by equine growth factor (EGF) and serum, and by thyroid-stimulating hormone (TSH) acting through cAMP. This inhibition, by TGFβ1, of the TSH and cAMP-dependent DNA synthesis was associated with an inhibition of PCNA (proliferating cell nuclear antigen) synthesis. TGFβ1 almost completely abolished the cAMP induced stimulation of iodide uptake and thyroperoxidase synthesis. It thus, like EGF, also acts as a dedifferentiating agent. Investigation of the pattern of protein synthesis by two-dimensional gel electrophoresis revealed that while TGFβ1, by itself, increased the synthesis of only one protein, a tropomyosin isoform, it inhibited most of the effects of cAMP on protein synthesis (35 out of 45 cAMP-regulated proteins were affected). It also reversed the effect of cAMP on the morphology of the thyrocytes. The fact that TGFβ1 did not affect the increase in cAMP provoked by TSH in human thyroid cells while inhibiting most of the effects of dibutyryl cAMP in these cells suggests an action at a step distal to cAMP generation. These data reemphasize the potentially major role of TGFβ1 as a local modulator of the thyroid, showing for the first time that it inhibits both the growth and differentiation effects of TSH and cAMP on human thyroid cells.

Introduction

TGFβ is the most potent growth inhibitor known for a wide variety of cell types in vitro including selected cell types of mesenchymal and myeloid origin, as well as nearly all epithelial, lymphoid and endothelial cells (Barnard et al., 1990). Since expression of TGFβ mRNA has been detected in many cell types, including some which are inhibited by it, it has been hypothesized that the polypeptide may mediate a negative autocrine loop limiting normal cell proliferation. Activation of the secreted latent TGFβ molecule could constitute a major regulatory step in the control of its action on cells (Roberts and Sporn, 1988). The molecular mechanism by which TGFβ inhibits cell growth is not known but recent studies indicate that its effects on c-myc expression (Pietenpol et al., 1990) and on the phosphorylation of the p105 RB protein (Laiho et al., 1990) may be central events in this process.

On the other hand, although the effects of TGFβ on cellular differentiation have been examined in several systems, they differ depending on the cell type or marker of differentiation being studied. For example, while TGFβ markedly potentiates the ability of follicle-stimulating hormone (FSH) to stimulate estrogen secretion in granulosa cells, its effect on adrenocortical cells is just the opposite: it strongly antagonizes the ability of adrenocorticotropic hormone (ACTH) to stimulate the secretion of cortisol (Roberts and Sporn, 1988).

TGFβ mRNA has been detected in human thyroid follicular cells (Grübeck-Loebenstein et al., 1989) though immunoreactive TGFβ was only found in malignant thyroid epithelial neoplasia (Jasani et al., 1990). TGFβ itself has been shown to inhibit DNA synthesis

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and proliferation in porcine thyrocytes (Tsushima et al., 1988; Winter et al., 1989) and in the rat thyroid cell line FRTL5 (Morris et al., 1988; Colletta et al., 1989; Pang et al., 1992) and [^{3}H]thymidine incorporation into DNA in human thyrocytes from nontoxic goiter and Graves' disease (Grübeck-Loebenstein et al., 1989). On the other hand, TGFβ was reported to enhance (Morris et al., 1988) or decrease TSH-stimulated iodine trapping (Colletta et al., 1989; Pang et al., 1992) in FRTL-5 cells and to decrease it in porcine thyroid cells (Tsushima et al., 1988). Its effect on cAMP accumulation in response to TSH is also controversial (Tsushima et al., 1988; Winter et al., 1989; Morris et al., 1988; Colletta et al., 1989). In a previous work we have shown that in primary cultures of normal human thyroid cells TSH, acting via cAMP, stimulates growth and promotes the expression of the differentiated functions of these cells (iodide trapping and thyroperoxidase synthesis) (Roger et al., 1988; Lamy et al., 1990). In this study we show that TGFβ1 antagonizes the effects of TSH and dibutyryl cAMP on DNA synthesis and gene expression in human thyroid cells.

Materials and methods

Materials

Bovine TSH was purchased from Armour Pharmaceutical Co (Chicago, IL, USA), collagenase from Worthington Biochemical Co (Freehold, NJ, USA) and dispase from Boehringer Pharmaceutical (Mannheim, Germany). Fetal bovine serum was obtained from Gibco (Paisley, UK); deoxyribonuclease, bovine insulin, human transferrin and dibutyryl cAMP from Sigma (St Louis, MO, USA); TGFβ1 was from British Bio-Technology Ltd. (Oxford, UK); [methyl-^{3}H]thymidine was obtained from the Radiochemical Center (Amersham, UK).

Methods

Primary cultures Normal thyroid tissue was obtained from 3 normal subjects, killed in traffic accidents, at autopsy immediately after death following a protocol approved by the Ethics Committee of the Medical School, and from 3 adult patients undergoing surgery for a hypofunctioning solitary "cold" thyroid nodule. The thyroid cells were cultured as described previously (Roger et al., 1988) in the following mixture, which constitutes the control medium: Dulbecco's Modified Eagle's Medium/ Ham's F12 medium/ MCDB104 medium (2:1:1 vol/vol/vol) containing 2×10^{-3} mol/l glutamine, supplemented by 5 mg/l bovine insulin, 1.25 mg/l human transferrin, 40 mg/l ascorbic acid, 500 mg/l bovine serum albumin and if indicated, 1% fetal bovine serum. Antibiotics (10^5 U/l penicillin, 100 mg/l streptomycin, and 2.5 mg/l amphotericin B) were also added. No systematic differences were observed in the properties of normal thyroid cells prepared from both sources in this and in other studies (Roger et al., 1988; Lamy et al., 1990).

Proliferation assay

DNA synthesis was estimated by measuring the incorporation of [^{3}H]thymidine into 10% trichloroacetic acid-precipitable material or by the frequency of [^{3}H]thymidine-labeled nuclei, as estimated by autoradiography. The cells in the Petri dishes were incubated for 24 h in the complete medium containing 3×10^{-5} mol/l thymidine, 10^{-4} mol/l deoxycytidine and 10 mCi/l [^{3}H]thymidine. After removal of the medium, the cells were treated with 10% trichloroacetic acid or, for autoradiography, fixed with methanol. Autoradiography was performed as described previously (Roger et al., 1983), directly in the Petri dishes. The cells were stained with toluidine blue and the proportion of labeled nuclei was determined by counting at least 1000 nuclei in each dish.

Iodide transport

The cells in the Petri dishes were incubated for 2 h with ^{131}I-labeled natrium (2 mCi/l; 10^{-6} mol/l) in Basal Medium Eagle (BME) at 37°C with mercaptomethylimidazole (10^{-3} mol/l) to block organification of iodide to proteins. They were then rapidly rinsed with BME, scraped off the dish and counted in a γ-counter. The radioactivity was normalized to the cellular DNA content measured in the same cells (Roger and Dumont, 1983).

cAMP measurements

Incubation of cells was stopped by scraping them into boiling water. After succinylation, the cellular cAMP concentrations were measured by radioimmunoassay (Brooker et al., 1979) using iodinated 2′-*O*-succinyl-cAMP methyl ester as a tracer.

Two-dimensional (2D) polyacrylamide gel electrophoresis

After 8 h of incubation in the presence of [^{35}S]methionine (100 mCi/l), the cells were rapidly rinsed at room temperature in 0.9% NaCl containing 10^{-3} mol/l $CaCl_2$. In order to lyse the cells and to solubilize the proteins, 240 μl of lysis buffer (O'Farrell, 1975) were added to each Petri dish. Total protein radioactivity was determined as described by Lecocq et al. (1990). Two-dimensional gel electrophoresis was performed as described by Lamy et al. (1990). Proteins were separated by isoelectric focusing on cylindrical gels in the first dimension with 3.2% Servalytes pH 5–7 plus 0.8% Servalytes pH 2–11 and according to molecular mass on sodium dodecyl sulfate, linear gradient (6–16%) polyacrylamide slab gels (total length of the separation gel: 18 cm) in the second dimension.

Results

Effect of TGFβ1 on DNA synthesis in human thyrocytes

Thyroid follicles were seeded with 1% serum. One day after seeding, serum was removed and the cells, which rapidly spread out and developed as a monolayer (Roger et al., 1988), were cultured for 4 days in the presence of different mitogens and of increasing concentrations of TGFβ1. They were incubated for the last 24 h of the culture period with [^{3}H]thymidine in order to evaluate DNA synthesis. Fig. 1 (experiment 1) shows that, in these cells, TGFβ1 inhibited DNA synthesis induced by TSH (250 mU/l), by fetal calf serum (10%) and by EGF (25 μg/l) plus fetal calf serum (1%). This effect was dependent on the TGFβ1 concentration used. It was already noticed at a concentration of TGFβ1 as low as 50 ng/l while a strong inhibition occurred at a TGFβ1 concentration of 5 μg/l.

As shown in our previous work (Roger et al., 1988; Lamy et al., 1990) and in Fig. 1 (experiment 2) dibutyryl cAMP (10^{-4} mol/l) almost completely reproduced the effect of TSH (250 mU/l) on DNA synthesis. Fig. 1 (experiment 2) also shows that TGFβ1 (5 μg/l) inhibited almost as strongly DNA synthesis induced by dibutyryl cAMP as that induced by TSH (67% inhibition versus 85% inhibition respectively) indicating an action downstream of cAMP formation. The extent of inhibition by TGFβ1 of the very weak basal DNA synthesis varied from one culture (i.e. experiment 1) to the other (i.e. experiment 2).

Inhibition by TGFβ1 of TSH effect on DNA synthesis was observed in the six experiments performed while its inhibition of dibutyryl cAMP effect on DNA synthesis was observed in 3 experiments out of 3.

Effect of TGFβ1 on cell morphology

Fig. 2 shows that the retraction of the thyroid cells observed in response to TSH (250 mU/l) (Fig. 2B) was strongly inhibited by TGFβ1 (5 μg/l) (Fig. 2C). This inhibition occurred progressively (not shown) and was maximal after 4 days of treatment of the cells with TSH and TGFβ1. The same effect of TGFβ1 was observed when dibutyryl cAMP (10^{-4} mol/l) was used instead of TSH (not shown). By itself, TGFβ1 did not induce obvious morphological changes (not shown). This effect of TGFβ1 on cell morphology was observed in the six experiments performed.

Effect of TGFβ1 on iodide transport

TSH and dibutyryl cAMP stimulate iodide transport in human thyroid cells seeded and cultured in the absence of serum (Roger et al., 1988). Table 1 shows that TGFβ1 (5 μg/l) completely abolished the accumulation of iodide by cells cultured in the presence of TSH (250 mU/l). This inhibitory effect of TGFβ1 on iodide accumulation was also observed when the cells were cultured with dibutyryl cAMP (10^{-4} mol/l) instead of TSH showing that TGFβ1 acts at a step downstream of cAMP formation. Similar results were obtained in two other experiments.

Effect of TGFβ1 on intracellular cAMP levels

Table 2 shows that a 4-day incubation with TGFβ1 (5 μg/l) did not modify cAMP levels in thyroid cells cultured in control conditions, in the presence of TSH (250 mU/l) or in the presence of forskolin (10^{-5} mol/l). This lack of effect of TGFβ1 on cAMP levels was observed in 3 experiments out of 3.

Effect of TGFβ1 on protein synthesis

The effect of TGFβ1 on protein synthesis in thyroid cells was analyzed by using two-dimensional gel electrophoresis. Proteins were labeled with [^{35}S]methionine for the last 8 h of a 4-day culture in the absence or presence of TGFβ1 (5 μg/l) with or without TSH (250 mU/l). In a previous paper (Lamy et al., 1990) we

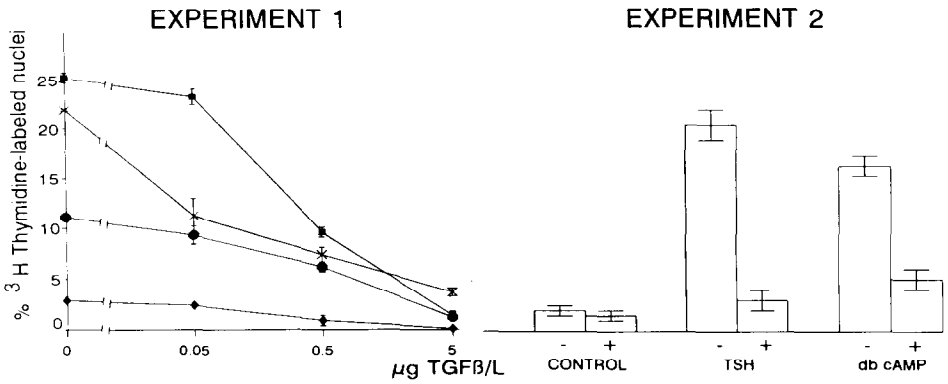

Fig. 1. Effect of TGFβ1 on DNA synthesis in thyroid cells stimulated to proliferate by different mitogens. Thyroid cells were seeded in the presence of 1% serum. After 24 h they were cultured for 4 additional days in serum-free medium in the presence, in experiment 1, of 25 μg/l EGF and 1% fetal calf serum (FCS) (●), 10% fetal calf serum (×), 250 mU/l bovine TSH (■) or control medium (♦) with increasing concentrations of TGFβ1 and, in experiment 2, of 250 mU/l bovine TSH or of 10^{-4} mol/l dibutyryl cAMP with (+) or without (−) 5 μg/l TGFβ1. [^{3}H]Thymidine was present for the last 24 h of the culture period. The cells were fixed with methanol, rinsed, and processed for autoradiography. The results of each experiment are the means ± range of values of duplicate Petri dishes.

TABLE 1

EFFECT OF TGFβ1 ON IODIDE TRANSPORT

Thyroid cells were seeded and cultured without serum for 5 days. After one day, TSH (250 mU/l) or dibutyryl cAMP (10^{-4} mol/l) were added to the culture medium with or without TGFβ1 (5 μg/l) and their presence was maintained throughout the experiment. Iodide transport was measured at equilibrium on cells at day 5 as described in Methods. The results are the mean ± range of values of duplicate culture dishes.

Treatment	Iodide uptake (cpm ^{131}I/ng DNA)	
	−TGFβ1	+TGFβ1 5 μg/l
Control	2.4 ± 0.4	2.7 ± 0.2
TSH (250 mU/l)	37 ± 1.5	2.2 ± 0.2
Dibutyryl cAMP (10^{-4} mol/l)	30 ± 5.0	6.7 ± 0.9

TABLE 2

EFFECT OF TGFβ1 ON cAMP LEVELS

Thyroid cells were seeded in the presence of 1% serum. 24 h later they were cultured for 4 additional days in serum-free medium in the presence of TSH (250 mU/l) or forskolin (10^{-5} mol/l) with or without TGFβ1 (5 μg/l). The results are the mean ± standard deviation of the mean (SEM) of values of triplicate culture dishes.

Treatment	cAMP (pmol/μg DNA)	
	−TGFβ1	+TGFβ1 5 μg/l
Control	0.5 ± 0.1	0.6 ± 0.1
TSH 250 μU/ml	17.6 ± 2.0	20.2 ± 2.3
Forskolin 10^{-5} mol/l	8.1 ± 1.0	7.4 ± 1.0

showed that TSH enhanced, in these cells, the [^{35}S]methionine labeling of 26 proteins (1–26) and decreased the labeling of 19 others (1′–18′; actin). The position of these proteins in a two-dimensional gel pattern is shown in Fig. 3A. The same results were obtained when the cells were incubated with dibutyryl cAMP (10^{-4} mol/l) instead of TSH.

When added to the culture medium together with TSH (250 mU/l) or with dibutyryl cAMP (10^{-4} mol/l), TGFβ1 (5 μg/l) partly or completely inhibited a great number of the effects of these agents on protein syn-

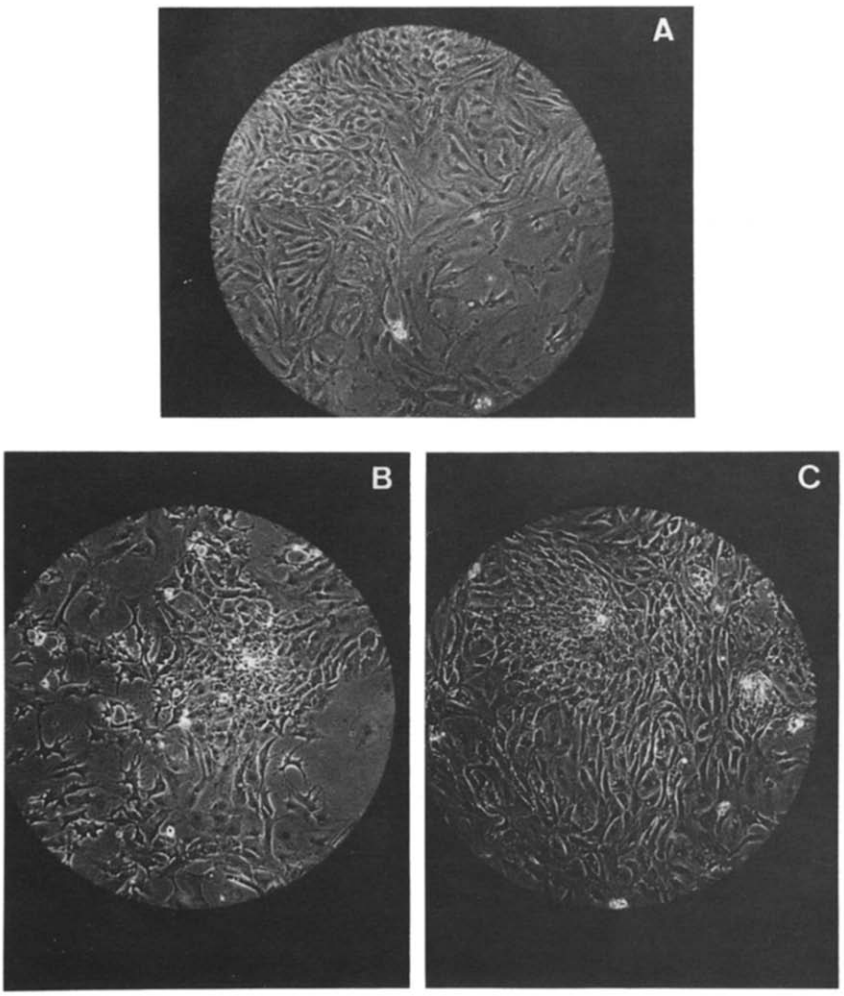

Fig. 2. Reversal by TGFβ1 of cell retraction induced by TSH. Thyroid cells were cultured as described in Fig. 1 with 250 mU/l bovine TSH in the presence (Fig. 2C) or absence (Fig. 2B) of 5 μg/l TGFβ1. Phase contrast microscopy was used (×100). Fig. 2A = control.

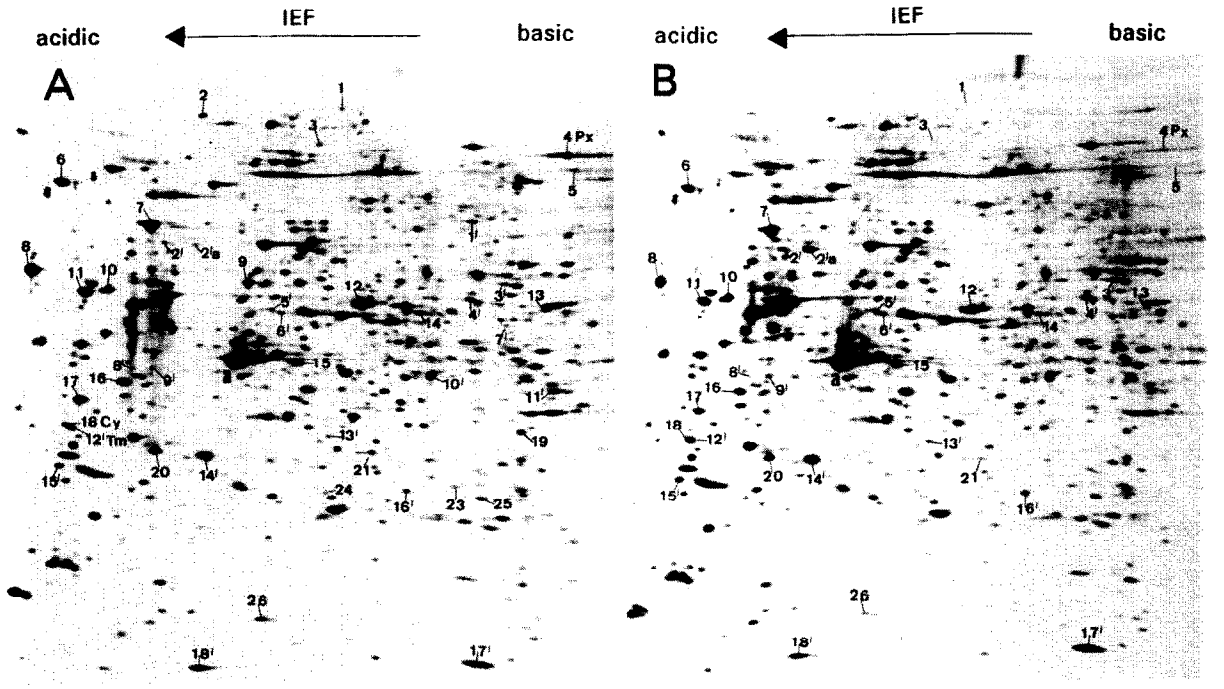

Fig. 3. Autoradiographs of two-dimensional gel electrophoresis of total proteins extracted from thyroid cells exposed to TSH (A) or to TSH and TGFβ1 (B). The cells were seeded in the presence of 1% serum. One day after seeding, serum was removed and TSH (250 mU/l) or TSH (250 mU/l) plus TGFβ1 (5 μg/l) were added to the culture medium and maintained for 4 days. 100 μCi [^{35}S]methionine was added to each Petri dish 8 h before the end of the culture period. The proteins whose synthesis is modulated by TSH are identified on Fig. 3A. 1 to 26: proteins whose synthesis is increased by TSH; 1′ to 18′ and actin: proteins whose synthesis is decreased by the hormone. The TSH-regulated proteins whose synthesis is sensitive to TGFβ1 are indicated in Fig. 3B. In this experiment the synthesis of proteins 17′ and 18′ was not decreased by TSH and protein 22 was not detectable. Px: thyroperoxidase; Cy: PCNA/cyclin; Tm: tropomyosin; a: actin.

thesis: 35 cAMP-regulated proteins out of 45 were affected (Fig. 3 and Table 3). Among these proteins four were identified in a previous work (Lamy et al., 1990) by their characteristic migration in two-dimensional gel electrophoresis and by immunodetection. They include two cytoskeleton proteins, actin and a high molecular weight isoform of tropomyosin (protein 12′), thyroperoxidase (protein 4), a key enzyme in the synthesis of thyroid hormones, and the proliferating cell nuclear antigen (PCNA/cyclin; protein 18) which is the auxiliary protein activating DNA polymerase δ and which is required for cell proliferation.

Fig. 4 illustrates the fact that TGFβ1 (5 μg/l) strongly inhibited the stimulation by TSH (250 mU/l)

TABLE 3

POLYPEPTIDES WHOSE SYNTHESIS IS MODULATED BY TSH AND DIBUTYRYL cAMP AND INFLUENCE OF TGFβ1 ON THIS ACTION: SUMMARY OF TWO-DIMENSIONAL GEL ELECTROPHORESIS ANALYSES

Thyroid cells from 3 different subjects were cultured as described in Fig. 3 in the presence of TSH (250 mU/L), dibutyryl cAMP (10^{-4} mol/l) and TGFβ1 (5 μg/l) as indicated in the table. Proteins were labeled with [^{35}S]methionine, separated by two-dimensional gel electrophoresis and identified by the same reference number as in Fig. 3.

Polypeptide reference number	Effect of TSH or dibutyryl cAMP	Inhibition by TGFβ1 of TSH or dibutyryl cAMP effect
2, 9, 19, 23, 24, 25	↑	0
4 (Px), 6, 7, 8, 10, 11, 12, 15, 16, 17, 20, 22	↑	+
1, 3, 5, 13, 14, 18 (Cy), 21, 26	↑	+ +
1′, 7′, 10′, 11′	↓	0
5′, 6′, 8′, 9′, 13′, 14′, 15′	↓	+
2′, 3′, 4′, 12′ (Tm), 16′,17′,18′, actin	↓	+ +

(↑) (↓) Level of [^{35}S]methionine incorporation increased or decreased compared to the one observed in control cells. 0, no inhibition; +, partial inhibition; + +, complete inhibition

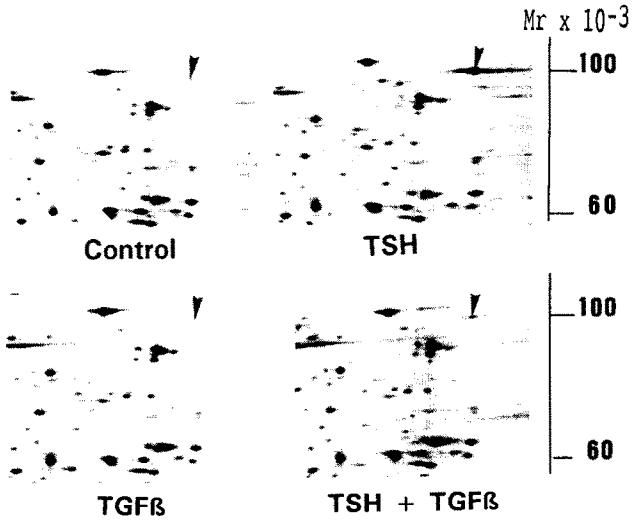

Fig. 4. Autoradiographic detail of [^{35}S]methionine-labeled proteins separated by two-dimensional gel electrophoresis. A characteristic section of the two-dimensional gel was chosen to illustrate the effect of TSH (250 mU/l) and TGFβ1 (5 μg/l) on thyroperoxidase synthesis in thyroid cells. Thyroid cells were processed as described in Fig. 3. Arrowheads indicate the position of thyroperoxidase.

of thyroperoxidase synthesis and shows that it even suppressed the weak expression of this protein in control cells. This constitutes the second example in human thyroid cells of the inhibition by TGFβ1 of the expression of a differentiated function of these cells.

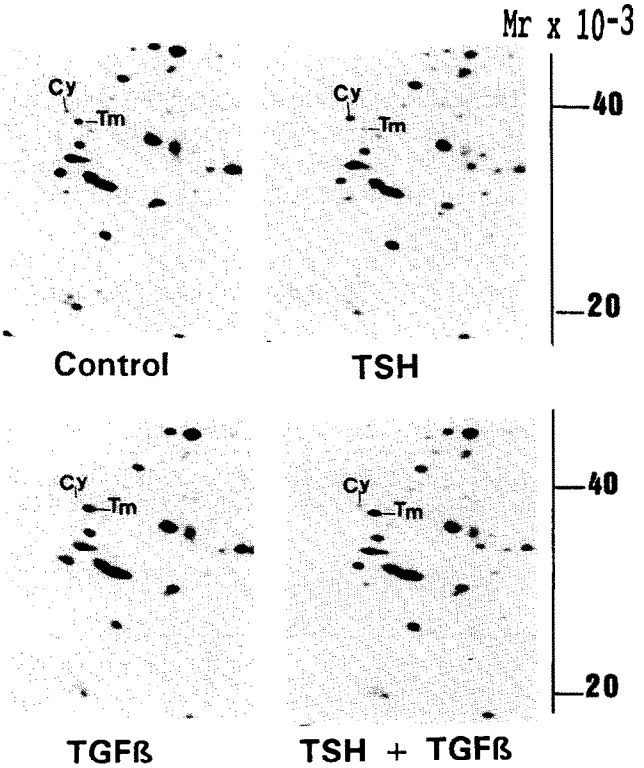

Fig. 5. Autoradiographic detail of [^{35}S]methionine-labeled proteins separated by two-dimensional gel electrophoresis. A characteristic section of the two-dimensional gel was chosen to illustrate the effect of TSH (250 mU/l) and TGFβ1 (5 μg/l) on PCNA/cyclin and tropomyosin synthesis in thyroid cells. Thyroid cells were processed as described in Fig. 3. Cy: PCNA/cyclin; Tm = tropomyosin.

Fig. 5 shows that TGFβ1 (5 μg/l) not only inhibited the stimulation by TSH (250 mU/l) of the synthesis of the PCNA/cyclin but that it even inhibited the basal level of expression of this protein. The effect of TGFβ1 on the level of PCNA/cyclin synthesis thus correlates well with its effect on DNA synthesis (Fig. 1). By itself TGFβ1 had little effect on protein synthesis. Apart from its effect on thyroperoxidase and PCNA/cyclin synthesis, it stimulated the synthesis of only one protein (protein 12′) which is an isoform of tropomyosin (Fig. 5). TSH and dibutyryl cAMP decreased the synthesis of this protein but this inhibition was relieved by the simultaneous addition of TGFβ1 to the culture medium (Fig. 5). The inhibition by TGFβ1 of the effect of TSH on actin (Fig. 3) and tropomyosin synthesis might be implicated in the reversal, by this agent, of the effect of TSH on the morphology of thyroid cells (Fig. 2).

Discussion

A growing body of evidence indicates that normal cell proliferation is controlled by a balance between positive and negative intracellular and extracellular signals. In previous studies using dog thyrocytes in primary culture, we have shown and characterized the quite distinct positive signalings of proliferation by either TSH acting through cAMP or by EGF independently of cAMP (Roger et al., 1987; Lamy et al., 1989; Dumont et al., 1989, 1992). While TSH via cAMP stimulates and maintains the functional capacity of thyroid cells (iodide transport, thyroglobulin and thyroperoxidase gene expression), EGF antagonizes these effects (Roger and Dumont, 1982; Roger et al., 1985; Pohl et al., 1990). We and others have recently extended these conclusions to human thyroid cells in culture (Roger et al., 1988; Kasai et al., 1987; Williams et al., 1988; Nagayama et al., 1989; Lamy et al., 1990). In the present study we characterized the negative effects of TGFβ1, the most potent growth inhibitor for epithelial cells, on the growth and functional capacity of normal human thyrocytes in primary culture. Since TGFβ mRNA and activity have been demonstrated in normal or pathological thyroid tissues or cultures (Grübeck-Loebenstein et al., 1989; Jasani et al., 1990; Morris et al., 1988), this factor might well be involved in a negative autocrine loop, as suggested in other systems (Arteaga et al., 1990; Wu et al., 1992).

As shown by Tsushima and coworkers on porcine thyrocytes (Tsushima et al., 1988) and Grübeck-Loebenstein et al. (1989) and Wyllie et al. (1991) on human thyrocytes we observed that TGFβ1 potently inhibited the stimulation of DNA synthesis by serum and EGF. In some experiments, we also detected an

inhibition by TGFβ1 of the low basal level of DNA synthesis. In addition, we showed for the first time in human thyrocytes that TGFβ1 almost completely inhibited the mitogenic effect of TSH. A 95% inhibition of DNA synthesis was obtained with 200 pM of TGFβ1 and half-maximum inhibitory effects were reached below 20 pM, which is comparable to the concentrations of TGFβ1 inhibiting the TSH-dependent growth in the FRTL-5 rat thyroid cell line (Morris et al., 1988; Colletta et al., 1989; Pang et al., 1992) and the growth of other epithelial cells (Barnard et al., 1990). It should be noted that the present results were obtained in serum-free medium, thus avoiding the possible interferences with serum factors documented in other systems (Barnard et al., 1990). No cytotoxic effect was detected even on a 4-day exposure to 5 μg/l TGFβ1 as judged by microscopic examination, DNA measurements, absence of inhibition of [^{35}S]methionine incorporation into total proteins (not shown) and absence of alteration in the pattern of protein synthesis in two-dimensional gel separation.

The mechanisms of the inhibition, by TGFβ1, of the TSH-dependent proliferation remains to be determined. TGFβ1 also markedly inhibited the stimulation of DNA synthesis by dibutyryl cAMP, which in large part reproduces the mitogenic effect of TSH (Roger et al., 1988). This result, which contradicts a recent report on FRTL5 cells (Pang et al., 1992), indicates that, in human cells, the growth inhibition occurred downstream from the cAMP accumulation. In some but not all systems (Pietenpol et al., 1990; Chambard and Pouysségur, 1988), the growth inhibitory effects of TGFβ1 have been associated with a rapid inhibition of c-myc transcription, an early event considered to be necessary in the mitogenic cascade. TGFβ1 may also inhibit DNA synthesis at late G1 stages, possibly through inhibition of p34^{cdc2} kinase activity (Howe et al., 1991) and of phosphorylation of the p105^{RB} protein (Laiho et al., 1990). We observed that TGFβ1 also inhibits the TSH/cAMP-dependent synthesis of PCNA/cyclin, the auxiliary protein of DNA polymerase δ that is necessary for DNA synthesis (Liu et al., 1989), which is a late event associated with TSH-stimulated mitogenesis in dog (Lamy et al., 1989) and human thyrocytes (Lamy et al., 1990). Moreover, inhibition of the cAMP effects on the synthesis of other proteins might be instrumental in the growth inhibition by TGFβ1.

TGFβ1 has a growth inhibitory action, and is also well known as a multifunctional factor modulating various specialized functions in various systems including endocrine tissues. In thyrocytes, its effects on specialized functions and TSH responses are controversial in animal culture models and have not been studied in human cells. The present observations that TGFβ1 potently antagonized the stimulation by TSH and dibutyryl cAMP of iodide transport and thyroperoxidase synthesis in human thyrocytes are in agreement with reports by Tsushima et al. (1988) on porcine cells and Colletta et al. (1989) and Pang et al. (1992) on rat thyroid FRTL-5 cells, but they strikingly contrast with the observation by Morris (1988) of a stimulation, by TGFβ1, of iodide transport in FRTL-5 cells. However, a 4-day exposure to TGFβ1 did not affect the stimulation by TSH of cAMP accumulation in human thyrocytes, which is in accordance with Tsushima's report on porcine cells and that of Colletta on FRTL-5 cells, but contradicts data of Winter et al. (1989) on porcine cells and Morris et al. (1988) on FRTL-5 cells. TGFβ1, like EGF (Roger et al., 1988; Lamy et al., 1990) thus appears as a potent local antagonist of TSH effects on the functional capacity of human thyrocytes, acting distal to cAMP accumulation.

As in FRTL-5 cells (Garbi et al., 1990), the morphological effects of TSH and dibutyryl cAMP were progressively inhibited by TGFβ1, although TGFβ1 alone did not obviously influence the morphology of human thyrocytes at variance with its effect on FRTL-5 cells (Garbi et al., 1990). The morphological effects of TSH and cAMP are associated with a disorganization of microfilament bundles and a decrease in the synthesis of microfilament proteins including actin and high M_r isoforms of tropomyosin (Westermark and Porter, 1982; Roger et al., 1989; Lamy et al., 1990). By contrast, TGFβ1 induces stabilization of microfilament bundles in FRTL-5 cells (Garbi et al., 1990) and, in the present experiments, it stimulated the synthesis of a high M_r tropomyosin isoform (T_m 3) and it counteracted the inhibitory effects of TSH and cAMP on the synthesis of this tropomyosin isoform and of actin. Whether the reversal by TGFβ1 of TSH effects on morphology and cytoskeleton proteins is related to the abundantly documented effects of this agent on the extracellular matrix composition and organization as in other cell types (Massagué, 1990) is unknown.

While TGFβ1 per se had only very little effect on the pattern of gene expression as revealed by the two-dimensional gel electrophoresis of neosynthesized proteins, it was unexpected to find that it reversed a large majority of the effects of TSH and dibutyryl cAMP on the synthesis of proteins. TSH, through cAMP, positively or negatively modulated the synthesis of at least 45 proteins in human thyrocytes (Lamy et al., 1990), and TGFβ1 antagonized the cAMP effect on 35 of these proteins. On the combined basis of the effects on growth, differentiation, morphology and protein synthesis, it can therefore be concluded that TGFβ1 appears as an almost general antagonist of the TSH and cAMP cascade in the human thyroid. Although TGFβ1 is known to have various effects on gene expression in different systems [such as induction of c-jun and jun B (Pertovaara et al., 1989), autoinduc-

tion of TGFβ1 (Kim et al., 1990), inhibition of growth factor activation of myc and KC genes (Coffey et al., 1988) and of some metalloprotease genes (Kerr et al., 1990)], to our knowledge, there is no precedent for such a general inhibitory effect. According to proposals that the spatial organization and morphology of cells may influence their growth and differentiation characteristics, as well as gene expression (Ben-Ze'ev, 1989), we have suggested that the positive or negative effects of TSH via cAMP on the synthesis of different proteins (28 out of 45) may depend in part on the morphological changes that cAMP induces (Lamy et al., 1990). The inhibitory action of TGFβ1 on the modulation, by cAMP, of the synthesis of many proteins might thus reflect its reversion of the morphological action of cAMP. Nevertheless, TGFβ1 also inhibited the TSH effects on the synthesis of several proteins (12 out of 35 that are affected by TGFβ1), including PCNA and thyroperoxidase, which are not responsive to morphological changes (Lamy et al., 1990). The wide range of inhibition, by TGFβ1, of cAMP responses should also be compared to the situation we found previously with EGF, which appears as another antagonist of TSH responses in human thyrocytes (Lamy et al., 1990). Interestingly, inhibitory effects of TGFβ1 and EGF overlap on 19 proteins. However, TGFβ1 potently repressed the TSH effects on the synthesis of a few proteins that are unaffected by EGF, and conversely. Unlike TGFβ1, EGF weakly influenced TSH effects on growth (Lamy et al., 1990). Whether the unidentified proteins whose synthesis is similarly affected by TGFβ1 and EGF are related to differentiation and morphology is unknown.

The present study therefore illustrates the fact that the stimulatory effects of TSH on human thyrocytes, not only on growth but even on their functional capacity, cannot be considered outside the context of local modulators such as TGFβ1 (or EGF).

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References

Arteaga, C.L., Coffey Jr, R.S., Dugger, T.C., McCutchen, C.M., Moses, H.L. and Lyons, R.M. (1990) Cell Growth Differ. 1, 367–374.

Barnard, J.A., Lyons, R.M. and Moses, H.L. (1990) Biochim. Biophys Acta 1032, 79–87.

Ben-Ze'ev, A. (1989) in Cell Shape: Determinants, Regulation and Regulatory Role, pp. 95–119, Academic Press, New York.

Brooker, G., Harper, J.F., Teraski, W.L. and Moylan, R.D. (1979) Adv. Cyclic Nucleotide Res. 10, 1–34.

Chambard, J.C. and Pouysségur, J. (1988) J. Cell. Physiol. 135, 101–107.

Coffey, R.J., Bascom, C.C., Sipes, N.J., Graves-Deal, R., Weissman, B.E. and Moses, H.L. (1988) Mol. Cell. Biol. 8, 3088–3093.

Colletta, G., Cirafici, A.M. and DiCarlo, A. (1989) Cancer Res. 49, 3457–3462.

Dumont, J.E., Jauniaux, J.C. and Roger, P.P. (1989) Trends Biochem. Sci. 14, 67–71.

Dumont, J.E., Lamy, F., Roger, P. and Maenhaut, C. (1992) Physiol. Rev. 72, 667–697.

Garbi, C., Colletta, G., Cirafici, A.M., Marchisio, P.C. and Nitsch, L. (1990) Eur. J. Cell. Biol. 53, 281–289.

Grübeck-Loebenstein, B., Buchan, G., Sadeghi, R., Kissonerghis, M., Londei, M., Turner, M., Pirich, K., Roka, R., Niederle, B., Kassal, H., Waldhäusl, W. and Feldmann, M. (1989) J. Clin. Invest. 83, 764–770.

Howe, P.M., Draetta, G. and Leof, E.B. (1991) Mol. Cell. Biol. 11, 1185–1194.

Jasani, B., Wyllie, F.S., Wright, P.A., Lemoine, N.R., Williams, E.D. and Wynford-Thomas, D. (1990) Growth Factors 2, 149–155.

Kasai, K., Hiraiwa, M., Sijuki, Y., Emoto, T., Banba, N., Nakamura, T. and Shimoda, S. (1987) Acta Endocrinol. 114, 396–402.

Kerr, L.D., Miller, D.B. and Matrisian, L.M. (1990) Cell 61, 267–278.

Kim, S.J., Angel, P., Lafyatis, R., Hattori, K., Kim, K.Y., Sporn, M.B., Karin, M. and Roberts, A.B. (1990) Mol. Cell. Biol. 10, 1492–1497.

Laiho, M., De Caprio, J.A., Ludlow, J.W., Livingston, D.M. and Massagué, J. (1990) Cell 62, 175–185.

Lamy, F., Roger, P., Lecocq, R. and Dumont, J.E. (1989) J. Cell. Physiol. 138, 568–578.

Lamy, F., Taton, M., Dumont, J.E. and Roger, P.P. (1990) Mol. Cell. Endocrinol. 73, 195–209.

Lecocq, R., Lamy, F. and Dumont, J.E. (1990) Electrophoresis 11, 200–212.

Liu, Y.C., Marraccino, R.L., Keng, P.C., Bambara, R.A., Lord, E.M., Chou, W.G. and Zain, S.B. (1989) Biochemistry 28, 2967–2974.

Massagué, J. (1990) Ann. Rev. Biol. 6, 597–641.

Morris, J.C., Ranganathan, G., Hay, I.D., Nelson, R.E. and Jiang, N.S. (1988) Endocrinology 123, 1385–1394.

Nagayama, Y., Yomashita, S., Hirayu, M., Izumi, M., Uga, T., Ishikawa, N., Ito, K. and Nagataki, S. (1989) J. Clin. Endocrinol. Metab. 68, 1155–1159.

O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007–4021.

Pang, X., Park, M. and Hershman, J.M. (1992) Endocrinology 131, 45–50.

Pertovaara, L., Sistonen, L., Bos, T.J., Vogt, P.K., Keski-Oja, J. and Alitalo, K. (1989) Mol. Cell. Biol. 9, 1255–1262.

Pietenpol, J.A., Holt, J.T., Stein, R.W. and Moses, H.L. (1990) Proc. Natl. Acad. Sci. USA 87, 3758–3762.

Pohl, V., Roger, P.P., Christophe, D., Pattyn, G., Vassart, G. and Dumont, J.E. (1990) J. Cell Biol. 111, 663–672.

Roberts, A.B. and Sporn, M.B. (1988) Adv. Cancer Res. 51, 107–145.

Roger, P.P. and Dumont, J.E. (1982) FEBS Lett. 144, 209–212.

Roger, P.P. and Dumont, J.E. (1983) J. Endocrinol. 96, 241–249.

Roger, P.P., Rickaert, F., Lamy, F., Authelet, M. and Dumont, J.E. (1989) Exp. Cell Res. 182, 1–13.

Roger, P.P., Servais, P. and Dumont, J.E. (1987) J. Cell. Physiol. 130, 58–67.

Roger, P.P., Servais, P. and Dumont, J.E. (1983) FEBS Lett. 157, 323–329.

Roger, P.P., Taton, M., Van Sande, J. and Dumont, J.E. (1988) J. Clin. Endocrinol. Metab. 66, 1158–1165.

Roger, P.P., Van Heuverswyn, B., Lambert, C., Reuse, S., Vassart, G. and Dumont, J.E. (1985) Eur. J. Biochem. 152, 239–245.
Tsushima, T., Arai, M., Saji, M., Ohba, Y., Murakami, H., Ohmura, E., Sato, K. and Shizume, K. (1988) Endocrinology 123, 1187–1193.
Westermark, B. and Porter, K.R. (1982) J. Cell Biol. 94, 42–50.
Williams, D.W., Williams, E.D. and Wynford-Thomas, D. (1988) Br. J. Cancer 57, 535–539.
Winter, H., Bechtner, G. and Greil, W. (1989) Ann. Endocrinol. (Paris) 50, 89 (abstract).
Wu, S., Theodorescu, D., Kerbel, R.S., Willson, K.V., Mulder, K.M., Humphrey, L.E. and Brattain, M.G. (1992) J. Cell Biol. 116, 187–196.
Wyllie, F.S., Dawson, T., Bond, Y.A., Goretzki, P., Game, S., Prime, S. and Wynford-Thomas, D. (1991) Mol. Cell. Endocrinol. 76, 13–21.