Role and regulation of the fibroblast growth factor axis in human thyroid follicular cells

Helen C. Cocks, Stuart Thompson, Frances E. Turner, Ann Logan, Jayne A. Franklyn, John C. Watkinson, and Margaret C. Eggo

Division of Medical Science, University of Birmingham, Birmingham B15 2TT, United Kingdom

Submitted 22 November 2002; accepted in final form 1 May 2003

FIBROBLAST GROWTH FACTORS (FGFs) are a large family of heparin-binding growth factors, now comprising 23 members, which have been detected in almost all tissues and shown to be mitogenic to many cell types (16, 33). In the human thyroid, FGF-1 and FGF-2 are markedly elevated in cancer (8) and in hyperplasia, when FGF receptor 1 (FGFR1) is also increased (41). The aims of this study were to determine the autocrine significance of these elevations to thyroid growth and function and to identify regulators of FGF and FGFR synthesis in thyroid cells.

There are four high-affinity membrane FGF receptors identified in humans, FGFR1–FGFR4, which have a common structure of extracellular immunoglobulin-like loops, a transmembrane domain, and an intracellular tyrosine kinase domain (33). FGF-1 and FGF-2 signal through FGFR1, which is also found in the nuclear fraction of several cell types (25) including thyroid follicular cells (31), and nuclear FGFR1 is thought to be associated with proliferation in 3T3 (25) and glial cells (38). FGF-1 administration to rats induces a colloid goiter (7), indicating that follicular cells are responsive to exogenous FGFs and in vitro, FGF-2 is a potent mitogen to the FRTL-5 strain of Fisher rat thyroid cells (5, 30). Similar effects have been found in vitro in porcine (11), dog (34), and bovine thyroid cells (14) but, anomalously, not in human thyroid cells (39).

Because FGFR1 is ubiquitous and important in cell growth and differentiation, its synthesis and that of its ligands are likely to be tightly regulated. In endothelial cells, estradiol via protein kinase C (PKC) activation induces FGF-2 secretion within 2 h, as well as exerting genomic effects on its synthesis (1), and oncostatin M similarly increases FGF-2 synthesis (43). Interleukin-1β, epidermal growth factor (EGF), platelet-derived growth factor, estradiol, testosterone, and direct stimulation of PKC or adenylate cyclase have been shown to increase FGF-2 and FGFR1 mRNA and protein expression in cells of diverse origins (21, 29, 35, 40). Whether FGF-2 increases or decreases expression of its receptor varies between tissues. In a pancreatic cell line, FGF-2 decreased receptor expression (13), but in rat testis (23) and in rat brains (15), FGF-2 upregulated FGFR1. In rat goiters induced with methimazole, FGF-2 and FGFR1 mRNA and protein were increased in thyroid follicular cells (4, 31), consistent with the hypothesis that increases in TSH induced by goitrogens are responsible for upregulation of FGF and/or FGFR.

FGF-2 is thus implicated in abnormal human thyroid growth, both as a potential follicular cell mitogen and as a stimulator of thyroid endothelial cell growth (32), but what regulates its expression and that of its receptor is not known. TSH, which is elevated when thyroid hormone levels fall, is the classical regulator of both thyroid growth and function and may play a role in physiological goiter. Similarly, the FGF axis may be implicated in goiters formed in Graves’ disease, due to

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
TSH receptor activation, but in thyroid cancer and in multinodular goiter, where TSH is not elevated, the stimulus is unknown. Furthermore, the absence of conclusive data on the direct effects of FGF-2 on human thyroid cell growth is surprising for a putative goitrogenic agent. FGF effects on human thyroid function are also unclear. In FRTL5 cells and porcine cells, FGF-2 inhibits $^{125}$I uptake (11, 22), but in human cells, we found inconsistent effects (9). We have therefore examined the regulation of growth and function of primary human thyrocytes by FGF-2 and the regulation of FGF-2 and FGFR1 expression by TSH, by FGF-2, and by PKC activation.

MATERIALS AND METHODS

Unless stated otherwise, laboratory reagents were purchased from Sigma Chemical (Poole, UK).

Cell culture. Thyroid tissue was obtained from operative specimens in accordance with local ethical committee approval and prepared as described previously (9). For normal thyroid, specimens were taken from the isthmus or contralateral lobe of patients undergoing surgery for diagnostic or complete thyroid lobectomy. For multinodular goiter, nodules were separated, connective tissue was removed, and the nodules were pooled. All thyrotoxic patients were euthyroid for a minimum period of 1 mo before surgery. Follicles were isolated by digestion in 0.2% collagenase (Worthington, Tewford, Berks, UK). Cells were plated in Corning's modified Ham's F-12 Medium (3) (GIBCO-BRL, Paisley, Scotland) supplemented with penicillin (10$^5$ U/ml), streptomycin (100 mg/ml), insulin (10$^{-4}$ g/l), bovine TSH (300 pmol/ml) (molarities calculated using formula, 30 U/mg pure TSH, i.e., 1 nM is equivalent to 1 U/ml), and 1% bovine calf serum (First Link UK, Brierley Hill, UK). Serum was removed after 3 days in culture, and medium was changed every 3 days. Cell layers were harvested in sample buffer (2% SDS and 62.5 mmol/l Tris·HCl, pH 6.8) (10).

Growth studies. After isolation, cells were cultured for 72 h in medium containing 1% newborn calf serum. Medium was changed, and cells were cultured in serum-free medium containing insulin and TSH and incubated for 72 h with FGF-2 (human recombinant, PeproTech, Totam Biologicals, Northants, UK) at concentrations of 0, 1, 3, 10, 30, and 100 ng/l, and 10$^{-4}$ g/l was used when effects to EGF (6$^{13}$I/H9262) were compared. After a wash with HBSS, cells were incubated for 5 min with 1 ml of HBSS containing 20 mM HEPES and 1 drop of Zapoglobin (Coulter Electronics, Luton, UK), which releases nuclei from cells. Isolated nuclei were suspended in 8 ml of formal saline (10% formalin, 0.9% NaCl) and counted using a Coulter counter (Coulter Electronics).

Thyroid function. Cells were incubated with 1 $\times$ 10$^6$ cpm of $^{125}$I (Amersham International, Amersham, UK) in 100 mmol/l NaI for 2 h. The cell layer was rapidly washed with HBSS to remove unincorporated iodide, and the cells were lysed in 1% SDS. Incorporated radioactivity was counted in a gamma counter. Primary human thyroid cell cultures isolated and cultured in this way generally take 40–50% of the given iodide, and of this, 50% is organified as iodoprotein. Iodide uptake and organification confirm that cells express functional sodium iodide symporter, thyroperoxidase, and thyroglobulin. All of the data were obtained from cultures that expressed TSH-dependent iodide uptake. Nonfunctional cultures (i.e., those taking up <5% of given radioactivity after a 2-h incubation and not showing TSH dependence for uptake) were not used.

Separation of nuclei from cytosol. To isolate nuclei, cell layers were incubated in 0.05% Triton X-100, and membrane rupture was observed under the light microscope. The lysate that contained nuclei was immediately removed from the cell culture dishes and centrifuged at 2,000 rpm for 5 min to pellet the nuclei. The supernatant was removed, SDS was added to a final concentration of 2%, and samples were heated with 10% 2-mercaptoethanol. The nuclei were dissolved in reducing sample buffer containing 10% 2-mercaptoethanol. Nuclei prepared in this way do not contain cyto-keratin, a cytoplasmic marker, when analyzed by Western blotting.

Western blotting. The cell layer dissolved in 1% SDS was sonicated and passed through a needle to shear DNA. The protein concentration was assayed using Bio-Rad Protein Assay (Bio-Rad Laboratories, Herts, UK). Equal amounts of protein were heated with 10% 2-mercaptoethanol in sample buffer at 70°C for 30 min and analyzed by electrophoresis on a denaturing SDS-polyacrylamide gel in the Tris-glycine buffers described by Elgin (10). The protein was transferred by Western blotting onto Hybond-P polyvinylidene difluoride membrane (Amersham). Membranes were blocked in 10% milk in TBS-T buffer (10 mmol/l Tris·HCl, 100 mmol/l NaCl, and 0.1% Tween 20, pH 7.5) overnight at 4°C and incubated with primary antibody (FGF-2, rabbit polyclonal antibody 1:1,000 dilution from Sigma; FGFR1, rabbit polyclonal antibody to COOH terminus from Santa Cruz, 1:500 dilution) in 5% milk in TBS-T for 90 min at room temperature. Membranes were washed 3 $\times$ 15 min in TBS-T and incubated with secondary antibody conjugated to horseradish peroxidase (HRP, The Binding Site, Birmingham, UK) in TBS-T for a further 90 min at room temperature. After three further 15-min washes, HRP activity was detected using enhanced chemiluminescence (ECL; Kirkengaard and Perry Laboratories, Insight Biotechnology, Wembley, UK) and exposed to Kodak MXB film (G.R.I., Rayne, UK). An antibody to cytokeratin 8 (The Binding Site) was used to assess equality of protein loading. Intensity of bands on Western blots was determined by densitometry on UVP gelbase image analysis software (UVP, Cambridge, UK).

Real-time quantitative RT-PCR. Total RNA was isolated using TRIzol reagent by following the manufacturer's instructions (GIBCO-BRL). cDNA was synthesized, and quantitative PCR was performed as described previously (28). ELISA for FGF-2. ELISA was performed on cell lysates harvested in 1.0% Triton X-100 by use of the ultrasensitive ELISA from R&D Systems (Abingdon, Oxon, UK). Results were corrected for protein content of the cell lysate. Conditioned medium from cells was also analyzed using this kit.

Statistics. In each experiment to measure growth or function of thyrocytes, quadruplicate wells were used, and the experiments were repeated on $\geq$3 different functional cell preparations except where noted. The means ± SE or SD are shown, and the data were analyzed by ANOVA with Tukey-Kramer's post hoc test.

RESULTS

FGF-2 is mitogenic to normal primary human thyrocytes and those from multinodular goiter. To determine whether thyroid follicular cells from normal thyroid and those from multinodular goiter responded to exogenous FGF-2 in growth and function assays, primary cultures of three different preparations of normal thyrocytes and two preparations from multinodular goiter were not used.
In the absence of heparin, FGF-2 stimulated mitogenesis in normal primary thyroid follicular cells (2.97 ± 0.89-fold increase in cell number, P < 0.001) compared with control, as shown in Fig. 1A. This was comparable to the effects of EGF (2.62 ± 0.33-fold, P < 0.001). Heparin had no significant effect on the mitogenic effect of either FGF-2 (3.27 ± 0.25-fold) or EGF (2.72 ± 0.51-fold).

Data from pooled nodules from a multinodular goiter are shown in Fig. 1B. Quadruplicate wells were used to determine growth from two preparations, and both are shown. In preparation 1, a 4.4-fold increase in cell number was seen (P < 0.001), and in preparation 2, a 2-fold increase was seen (P < 0.05). The effects seen with FGF were comparable to the effects of EGF in both preparations.

FGF-2 inhibits iodide uptake by normal primary human thyrocytes and those from multinodular goiter. Cells were cultured, as described, from three different normal thyroid cell preparations. At the first medium change, serum was removed and cells were treated with FGF-2 (10 µg/l) and EGF (6 µg/l), as described for the growth studies. Before harvesting, quadruplicate wells of cells were incubated with 10^5 cpm of ^125I for 2 h. Radioactivity in the cell lysate was determined with a gamma counter, data were normalized to control values without FGF-2, and the results of the means of the three preparations, with the SD, are shown in Fig. 2A. In the absence of heparin, FGF-2...
inhibited iodide uptake by 74.0 ± 11.0% (P < 0.05), and the magnitude of the effects was comparable to that of the effects of EGF (68.5 ± 7.8% inhibition, P < 0.05). Although the addition of heparin significantly increased iodide uptake in the control (137.0 ± 31.5% of control without heparin, P < 0.05), heparin had no effect on the inhibition of iodide uptake by FGF-2.

Cells from two different multinodular goiters were cultured. At the first medium change, serum was removed and cells were exposed to human recombinant FGF-2 at concentrations of 0, 1, 3, 10, 30, and 100 μg/l for 72 h. Before harvesting, quadruplicate wells of cells were incubated with 10^5 cpm of 125I for 2 h. FGF-2 inhibited iodide uptake in primary human thyrocytes from multinodular goiter in a concentration-dependent manner (Fig. 2B). Significant effects were seen at concentrations of 10 μg/l (30% inhibition of uptake, P < 0.01). At 100 μg/l, inhibition of 40% was seen.

FGFR synthesis is maintained in cells in culture. These effects of FGF-2 on both thyroid growth and function were demonstrable only in the first week after isolation. We therefore wondered whether FGFR1 synthesis was reduced or whether the receptor was degraded with time in culture, and so we performed Western blotting to examine FGFR1 expression throughout the culture period. Cell lysates were analyzed by Western blot probing with antibodies to COOH-terminal FGFR1 (Santa Cruz Biotechnology, Santa Cruz, CA). Equal amounts of cell protein were analyzed at each time point. Blots were stripped and reprobed with an antibody to cytokeratin 8 (The Binding Site), a cytoskeletal protein found in 98% of thyrocytes that is not regulated by TSH (6). This experiment was repeated on three different cell preparations. The data from one such preparation are shown in Fig. 3. At all time points examined, FGFR1 expression was maintained, and a single band of 120 kDa was detectable.

TSH increases the expression of FGFR1 protein. Regulation of FGFR1 expression by TSH in functional primary human thyrocytes (normal and multinodular goiter) was determined by Western immunoblotting. Cells were cultured in serum-free medium at the first medium change (day 4). After 7 days in culture, TSH was omitted from the culture medium for 72 h before challenge with TSH (0.01–10 U/l) after a 72-h period of TSH deprivation. Fifty micrograms of total cell protein were analyzed by SDS-PAGE and probed for FGFR1 using an antibody to the COOH terminus. Cytokeratin 8 (CYTO 8) probing of the same blot, shown beneath, shows equivalence of protein loading.

Fig. 3. FGF receptor 1 (FGFR1) expression with time in culture. Follicular cells were terminated on the days postisolation, which are indicated by lysis in SDS sample buffer. Fifty micrograms of total cell protein were analyzed by SDS-PAGE and probed for FGFR1 using an antibody to the COOH terminus. Cytokeratin 8 (CYTO 8) probing of the same blot, shown beneath, shows equivalence of protein loading.

Fig. 4. Effect of TSH on FGFR1 expression. A: human primary follicular cells from a normal thyroid were incubated in TSH at various concentrations for 72 h (0, 0.01, 0.1, 0.3, 1, and 10 U/l) after a 72-h period of TSH deprivation. Fifty micrograms of total cell protein were analyzed by SDS-PAGE and probed for FGFR1 using an antibody to the COOH terminal. A representative Western blot from 6 experiments is shown. Cytokeratin 8 probing of the same blot, shown beneath, shows equivalence of protein loading. B: cells were treated as in A. Nuclear and cytosolic fractions were separated and analyzed by SDS-PAGE and probed for FGFR1 using the COOH-terminal antibody. A representative Western blot from 3 is shown. C: Western blot shows human primary follicular cells incubated in 1 U/L TSH (lanes 2 and 3) and recombinant human FGFR1 (lanes 1 and 4) probed with antibody to COOH terminus of FGFR1. Lanes 3 and 4 show effects of preabsorbing the antibody with an excess of antigen.
FGF-2 AND FGFR1 IN HUMAN THYROID CELLS

**E464**

FGF-2 AND FGFR1 IN HUMAN THYROID CELLS

**Fig. 5.** Time course of effects of TSH on FGFR1. A: primary human thyrocytes were cultured in the presence and absence of TSH (1 U/l) and harvested at time points of 4, 8, 24, and 72 h. Cell proteins were analyzed by Western blotting and probed with the antibody to the COOH terminus of FGFR1. B: RNA from primary human thyrocytes incubated with TSH (1 U/l) was extracted at time points of 0, 4, 8, 16, 24, and 72 h and assayed for levels of FGFR1 mRNA by real-time quantitative RT-PCR. Values are means ± SD; n = 3. **P < 0.01, **+P < 0.001.

TSH increases the expression of FGFR1 in both the nucleus and cytosol. We found previously that FGFR1 is expressed in nuclei of rat thyroid follicular cells in tissue sections (31). To study the molecular forms of FGFR1 in different subcellular compartments and the effects of TSH on this distribution, we used Western blotting. Nuclei and cytosol were separated, as described in MATERIALS AND METHODS, and analyzed by Western blot probing with antibody to the FGFR1 COOH terminus as shown in Fig. 4B. This experiment was repeated on three different cell preparations. The blot shown is from cells derived from a multinodular goiter, whereas the data shown in Fig. 4A are from normal cells. Sensitivity to TSH is similar in Fig. 4, A and B, with increased expression evident at a TSH concentration of 0.1 U/l. Normal thyroid follicular cells and those from goiters apparently do not differ in vitro in TSH regulation of expression of FGFR1. In Fig. 4B, the blot was exposed to allow comparison of the nuclear and cytoplasmic expressions of FGFR1. FGFR1 was predominantly expressed in the cytosol, although nuclear expression was detectable. TSH increased expression of the receptor in both cellular fractions. Stimulation was evident at 0.1 U/l TSH.

Confirmation of the identity of the band labeled as FGFR1 was shown using human recombinant FGFR1 (Sigma) (lane 1, Fig. 4C) as a positive control compared with thyrocyte lysate (lane 2, Fig. 4B). Preincubation of the antibody with a blocking peptide to FGFR1 resulted in prevention of labeling of bands from both the recombinant protein and the thyrocyte lysate (lanes 3 and 4, respectively; Fig. 4C).

TSH increases expression of FGFR1 protein by 8 h in culture, which is sustained at 72 h. Time-course studies to determine when TSH exerted its stimulatory effect on FGFR1 synthesis were performed and are shown in Fig. 5A. TSH was used at a concentration of 1 U/l, and incubation times of challenge were 4, 8, 24, and 72 h. Western blotting was performed on cell lysates by using the antibody to FGFR1. Control lysates from cells incubated without TSH are shown at each time point, and equal amounts of protein were loaded for each paired sample. TSH increased the expression of FGFR1 by 8 h (3-fold) when assessed by densitometry, which was sustained at 72 h.

TSH increases expression of FGFR1 mRNA by 4 h in culture. To determine whether the effects of TSH were mediated through increases in mRNA, quantitative RT-PCR was performed. Cells were harvested at time points indicated in Fig. 5B and lysed in TRIzol. Total RNA was extracted, cDNA was synthesized, and PCR was performed for FGFR1 on the ABI PRISM 7700 as described. Data are expressed as expression of FGFR1 mRNA relative to expression of 18S mRNA. TSH increased the expression of FGFR1 mRNA by 4 h (P < 0.01; Fig. 5B). At 24 h, the expression had declined to control values (n = 3, means ± SD).

FGF-2 decreases the expression of its receptor. To determine whether FGF-2 regulated the expression of its receptor FGFR1, three different primary cultures of normal thyrocytes were cultured and treated with FGF-2 (0, 1, 3, 10, 30, and 100 μg/l) for 72 h. Cell lysate was analyzed by Western blotting, as shown in Fig. 6. FGF-2 decreased expression of the receptor FGFR1 to 60–85% of control value at 100 μg/l in the three preparations when analyzed by Western blotting.

TSH does not increase the expression of FGF-2 protein. To determine the expression and regulation of FGF-2 in human thyroid cells, lysates were prepared using the protocol described in Fig. 5 and analyzed by Western blot probing with antibody to FGF-2 (Santa Cruz) (Fig. 7). Cytokeratin 8 is shown as a control for loading. FGF-2 was detectable as multiple bands of 18, 22, 24, 28, and 32 kDa on Western blots. The higher molecular mass bands predominated with barely discernible bands at 18 kDa. Preincubation of the antibody to FGF-2 with a blocking peptide resulted in loss of labeling of all of these bands. The antibody cross-reacted with a positive control of recombinant human FGF-2 of 17.2 kDa, but a dimer of 34 kDa was also seen in this preparation even under reducing conditions. In all experiments (3 different cell preparations), TSH did not increase FGF-2 expression. When the nuclei and cytosol were separated, FGF-2 was seen mainly in the cytosol.
TSH inhibited FGF-2 expression at 0.1–

Fig. 8 in the line graph. In four of the cells to TSH in 125I uptake assays is also shown in the absence of TSH, as shown in Fig. 8. The response of the cells to TSH in 125I uptake assays is also shown in Fig. 8 in the line graph. In four of five preparations, TSH inhibited FGF-2 expression at 0.1–0.3 U/l, the concentration of TSH that stimulates thyroid function maximally, but in one preparation TSH had no significant effect on FGF-2 expression. Pooled data from the four preparations showed that there was a significant decrease to 0.9 ± 0.3 pg/µg protein (means ± SE) after incubation in 0.3 U/l TSH for 72 h. Measurement of FGF-2 released into the medium over 72 h from 106 cells gave values ~6 pg. TSH did not affect the release of FGF-2.

**PKC activation induces FGF-2 synthesis and secretion.** Because TSH did not directly affect FGF-2 synthesis, we examined the role of signaling pathways that we have previously shown to regulate thyroid growth and function. We performed these experiments in the presence and absence of TSH, because TSH programs the cell for differentiation and changes the protein expression profiles of the cells. The effects of 72-h treatment with the tumor-promoting phorbol ester tetradecanoyl phorbol 13-acetate (TPA, 30 nmol/l) on the content of FGF-2 in the media and cell layer of cells cultured with and without TSH are shown in Table 1. In the cell layer, TPA had no significant effect on FGF-2 content when cells were cultured without TSH. Secreted FGF-2 was increased slightly by TPA treatment in the absence of TSH. In the presence of TSH, TPA treatment significantly increased FGF-2 secretion more than fourfold. Effects on the cell layer were more modest.

A time course of the effects of TPA on FGF-2 secretion and on FGF-2 and FGFR1 mRNA is shown in Fig. 9. To avoid pleiotypic effects, medium was changed 48 h before the start of the experiment, and TPA was added 6, 4, and 2 h before the termination of the experiment. Conditioned medium was removed and assayed for FGF-2 by ELISA, and the RNA was isolated from the cell layer. FGF-2, FGFR1, and 18S rRNAs were analyzed by quantitative RT-PCR. The effects of TPA on two different cell preparations are shown. TPA induced rapid changes in FGF-2 mRNA,

---

**Table 1. Effects of TPA with and without TSH on FGF production in primary cultures of human thyroid cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>FGF [pg/ml]</th>
<th>TPA Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>In medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-TSH</td>
<td>3.0 ± 0.6</td>
<td>*4.8 ± 0.5</td>
</tr>
<tr>
<td>+TSH</td>
<td>4.3 ± 0.8</td>
<td>*18.2 ± 2.6</td>
</tr>
<tr>
<td>In cell layer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-TSH</td>
<td>2.6 ± 0.3</td>
<td>*3.3 ± 0.2</td>
</tr>
<tr>
<td>+TSH</td>
<td>2.4 ± 0.3</td>
<td>*3.8 ± 0.4</td>
</tr>
</tbody>
</table>

Effects of 72-h treatment with TPA (30 nmol/l) on content of fibroblast growth factor (FGF)-2 in medium and cell layer of cells cultured with (+) and without (−) TSH. Two preparations of cells were used, and data from triplicate wells were assayed by ELISA. *P < 0.05 in both preparations for effects of TPA in medium and cell layer.
increases being seen within 2 h, the maximum effect occurring 4 h after addition, when there was a mean 10-fold increase. FGFR1 mRNA was also increased 2.0-fold at this time point, but there were no changes at the other time points. In both preparations, TPA produced a rapid increase in FGF-2 secretion within 2 h of addition. A further increase was seen at 4 h, but no further increases were seen between 4 and 6 h in either preparation.

In studies examining signaling pathways coupled to TSH receptor activation, extraordinarily high TSH concentrations are used to show coupling to phospholipase C, which is upstream of PKC activation (2). We therefore used TSH at 100 U/l (100 nmol/l) to determine whether these pharmacological concentrations would increase FGF-2 secretion and synthesis. Even at these concentrations, TSH did not significantly affect either FGF-2 synthesis or secretion in three different preparations of thyroid cells.

**DISCUSSION**

We have previously shown that FGFR1 is increased in human goiter, and in this study we show that TSH increases the expression of FGFR1 mRNA and protein in primary human thyrocytes. These effects are rapid, FGFR1 mRNA increases being seen by 4 h and protein by 8 h. The mRNA had returned to control values by 24 h, whereas the increased protein expression was sustained for 72 h, implying that FGFR1 has a long half-life. Western blotting for FGFR1 produced a single band of 120 kDa. The sensitivity and magnitude of the effects of TSH varied between preparations, which may reflect variable TSH receptor function or expression, or autocrine FGF-2 effects. The regulation of FGFR1 by TSH was found both in cells from normal thyroids and in those from multinodular goiter, suggesting that aberrations in the control of FGFR1 synthesis by TSH are not directly responsible for the development of multinodular goiter. The stimulation of FGFR1 synthesis by TSH suggests a mechanism for the goiter seen in Graves' disease, where the TSH receptor is stimulated by autoantibodies. The increase in follicular cell FGFR1 induced with TSH may increase the sensitivity of the cells to local FGF levels, or it may increase the proportion of cells able to respond to FGFs.

We have shown that FGF-2 inhibits thyroid function measured by $^{125}$I uptake in primary human thyrocytes when examined shortly after their isolation, significant effects being seen at 10 µg/l. In this regard FGF-2 acts similarly to EGF and PKC activation with TPA (9). We also found that it was mitogenic to primary human thyrocytes isolated from both functional normal and multinodular glands. The growth effects of FGF-2 in human thyrocytes are similar to those reported for FGF-2 in porcine thyrocytes (11), dog thyrocytes (34), and the rat thyroid cell line FRTL-5 (5, 30), but this is the first report of a mitogenic effect of FGF-2 in primary human thyrocytes. We did not find evidence for differing sensitivity to FGF-2 between the cells from the multinodular goiters and the normal follicular cells.

We found that the effects of FGF-2 on growth and function were not dependent on addition of heparin and were seen only within the first week of culture, when the cells were plated at low cell density. This probably explains why Taylor et al. (39) were unable to show FGF-2-induced mitogenesis in human thyrocytes. Why human thyroid cells lose sensitivity to FGF-2 with time in culture is not clear. The loss of response to FGF-2 is not due to loss of FGFR1, because we found no profound change in expression of FGFR1 on Western
blots throughout the culture period extending over 2 wk, nor did we see evidence for FGFR1 degradation. This loss of effect with time in culture was not found with the EGF receptor, which, when activated, remained a potent inhibitor of thyroid function throughout culture (9). FGFR1 function is known to be influenced by the local environment in the membrane, and gangliosides as well as glycosaminoglycans influence its bioactivity. A recent report showed that cell membrane GM1 ganglioside binds FGF-2 and is required for its mitogenic effects (36). There have been many detailed studies of the ganglioside composition of thyroid cells, some of which show changes in thyroid disease states (27). Whether ganglioside composition changes with culture remains to be determined. Alternatively, members of the Sprouty family, which inhibit signaling by receptor tyrosine kinases (17), or the protein tyrosine phosphatase LAR, which selectively inhibits tyrosine phosphorylation of signaling intermediates (42), may be active. Again, whether this means to control FGFR1 activity operates in vivo and in vitro in thyroid cells needs to be determined.

FGF-2 was expressed at low levels in the cells and in the medium (1–2 pg/μg cell protein and 2 pg·ml⁻¹·10⁶ cells⁻¹·day⁻¹, respectively) in all of the cell preparations from normal and multinodular goiter tissue. The elevated expression of FGF-2 in multinodular goiter tissue was not maintained when follicles were isolated and maintained in culture. This contrasts with our data with cells from thyroid cancer, which maintained their elevated FGF-2 expression in culture (9). TSH inhibited FGF-2 expression, which was inversely correlated with thyroid function. The inhibition of autocrine FGF-2 production by TSH may help to sustain maximal ¹²⁵I uptake and organization. In sheep thyroid cells, Hill et al. (19) found that FGF-2 production was increased by low concentrations of TSH, but in human thyroid cells, we found no evidence that TSH mediates its mitogenic effects through increasing follicular cell FGF-2 production. We found, however, that PKC activation induced a rapid FGF-2 secretion and a rapid increase in FGF-2 mRNA synthesis. The effects on secretion were seen within 2 h, suggesting release from the extracellular matrix as well as increased synthesis. This mechanism was previously shown for estradiol effects on FGF-2 release from endothelial cells (1). Interestingly, although TSH by itself did not increase FGF-2 secretion, it potentiated the effects of PKC activation on FGF-2 secretion. TSH induces differentiation in these cells, which results in profound changes in protein expression and cell architecture (26). Conceivably, differentiation makes FGF-2 more available for release from the cells. Consistent with our in vitro findings, Emoto et al. (12) recently showed that FGF-2 free from extracellular matrix was increased in Graves’ disease and in papillary thyroid carcinoma.

In the thyroid, as elsewhere, PKC activation will follow downstream from receptor tyrosine kinase (RTK) activation and subsequent phospholipase C activation. Whether FGF-2 synthesis is under the control of a particular PKC isoenzyme and whether the isoenzyme profile of PKC changes with thyroid tumorigenesis are unknown. TSH, even at supraphysiological concentrations, did not increase FGF-2 production, indicating that the human TSH receptor is not functionally coupled to phospholipase C. This is contrary to other reports (2) but consistent with our data for FRTL5 cells (37), where we found no evidence of this coupling. There is convincing evidence, however, that the thyroid hormones iodothyronine and thyroxine (T₃ and T₄, respectively), which are synthesized and secreted by our preparations of human thyroid cells (9), activate MAPKp44/42, and that this effect is blocked by PKC inhibition (24). This raises the possibility that prevailing intrathyroidal T₄ levels could regulate synthesis and release of FGF-2. Other means to activate PKC and thus increase follicular cell FGF-2 are via RTK-linked receptors, whose synthesis or that of their ligands may be controlled by TSH or thyroid hormones.

In conclusion, we have shown that FGFR1 is present in human thyroid cells and that, when activated, it stimulates growth and inhibits thyroid function. However, within a week of culture, cells become insensitive to the effects of FGF-2 in both growth and function assays. Whether this is a property of cell culture or whether thyroid cells in vivo are able to switch off FGFR1 signaling is unknown. TSH increases FGFR1 expression but is not responsible for the increased expression of FGF-2 seen in goiter. PKC activation, which may be traded through thyroid hormones as well as RTK-linked receptors, can increase FGF-2 synthesis and secretion. TSH enhances this secretion. These relationships are summarized in Fig. 10. Effects of TSH and PKC activation on FGF-1, which is also elevated in cancer (8) and hyperplasia (41), or on other members of the FGFR family that are secreted, e.g., FGFs 4–8 and FGF-10, remain to be explored. Similarly, the other FGFR receptors and receptor isoforms, in particular FGFR2, which has been shown to be expressed in human thyroid cells (20), and FGF-binding proteins (33) are of unknown importance.

DISCLOSURES

The support of The Wellcome Trust, project grant number 49772 (F. E. Turner, A. Logan, J. A. Franklyn, and M. C. Eggo), and of a fellowship award from the Royal College of Surgeons and the Get-A-Head Charity (H. C. Cocks and S. Thompson) is gratefully acknowledged.

REFERENCES


