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Acidic Fibroblast Growth Factor Modulates Gene Expression in the Rat Thyroid In Vivo

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Abstract We have recently demonstrated that the iv administration of acidic fibroblast growth factor (a-FGF) to rats for 6 days results in a marked increase in thyroid weight with colloid accumulation and flat, quiescent follicular cells. Whereas a-FGF administration consistently increases thyroid weight, there are only minor alterations in serum TSH and thyroid hormones, and no change in intrathyroidal metabolism of ^{125}I metabolism. In the present work, we studied the effects of 1 or 6 daily injections of a-FGF (60 μg/kg BW) or vehicle on the mRNA levels for histone, c-fos, actin, type I 5′ deiodinase (5′D-I), thyroid peroxidase, and thyroglobulin and cathepsin D in the thyroid, liver and bone. Rats were sacrificed 0.5, 2, 4, 8 and 24 h after the 1st or the 6th a-FGF injection and thyroid, liver, and calvarium were removed. The relative amounts of mRNAs were determined by slot blot analysis. There was a 43% increase in thyroid weight in rats treated with a-FGF for 6 days compared to vehicle-treated rats. We observed an increase in c-fos mRNA content in the thyroid gland 0.5 to 4 h after 1 or 6 injections of a-FGF. In contrast, treatment with a-FGF for 1 or 6 days did not affect histone mRNA content, a marker of proliferative activity or actin mRNA levels. Treatment with a-FGF caused a marked decrease in thyroid 5′ D-I mRNA content in the thyroid. The decrease was present 2 h after the first injection and reached a nadir 8 h later. After 6 daily injections, the decrease in 5′ D-I mRNA was present throughout the whole day. In the liver, there was a significant decrease in 5′ D-I mRNA only 2 and 4 h after the 6th daily injection of a-FGF. There was no effect of a-FGF treatment on the mRNA content of thyroid peroxidase, thyroglobulin, or a marker of lysosomal activity, cathepsin D. These data indicate that a-FGF induces colloid accumulation in the rat thyroid without changes in proliferative or lysosomal activities, or alteration in the regulation of the thyroid specific genes thyroid peroxidase and thyroglobulin. Modification in gene expression and induction are reflected by the upregulation of the early response gene c-fos. The marked and persistent decrease in 5′ deiodinase mRNA content after a-FGF treatment suggests that a-FGF may be involved in the regulation of 5′ D-I activity in the thyroid.

Key words: thyroid function, c-fos, type I 5′ deiodinase, histone, cathepsin D, thyroid peroxidase, thyroglobulin, actin

Fibroblast growth factors (FGFs) are single chain proteins which have been shown to induce cellular differentiation with or without mitogenesis in various tissues, both in vitro and in vivo [15]. Two original members of this family, acidic and basic fibroblast growth factors, share a 55% homology in their base sequence, have a similar three-dimensional structure, and have similar but distinct effects on cell regulation [30]. In vitro, FGFs have been shown to be mitogens in primary cultures of dog thyroid cells [24] and in FRTL-5 rat thyroid cells [4,21,23]. Basic fibroblast growth factor has been identified from porcine thyroids [13], and Logan et al. [20] recently suggested that it might act as an autocrine mitogen in rat follicular cells. In contrast to the in vitro data, we have recently shown that, in male rats, iv administration of 60 μg/kg BW per day of human recombinant acidic FGF (a-FGF) for 6 days results in increased thyroid weight without histological signs of cellular proliferation [10,18]. Histologically, thyroids from a-FGF treated rats show colloid accumulation in enlarged follicles with flat, quiescent follicular cells. Radioiodine uptake and intrathyroidal metabolism of radioiodine are unaffected by a-FGF treatment. Treatment with a-FGF for 1 or 6 days caused only minor changes in serum TSH, T_4, and T_3 concentrations, and no changes in

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serum reverse T_3 or thyroglobulin concentrations. The aim of the present study was to investigate the molecular mechanisms leading to such a remarkable increase in thyroid weight without histological signs of cellular proliferation. Using thyroid from rats in which we determined the biological and biochemical effects of a-FGF on thyroid function, we studied the effects of 1 or 6 daily injections of a-FGF or vehicle on the expression of a series of cell growth and tissue related genes as reflected by cellular mRNA levels of c-fos, histone, type I outer ring 5′ deiodinase (5′ D-I), thyroid peroxidase, thyroglobulin, cathepsin D, and actin.

MATERIALS AND METHODS

Male Sprague-Dawley rats (110–150 g) were obtained from Charles River Laboratories (Kingston, RI) and maintained ad lib on iodine-sufficient Formulab Purina Diet (Richmond, IN). In all experiments, rats were injected iv with either vehicle (PBS containing 60 μg/ml human serum albumin and 180 U/ml heparin), or 60 μg/kg BW recombinant human a-FGF (kindly provided by Merck Sharp and Dohme, West Point, PA). All injections were performed between 0800 and 1100 h.

In the first experiment, the animals (n = 10/group) received a single iv injection and were killed by decapitation 0.5, 2, 4, 8, and 24 h after the injection. In the second experiment, rats were treated daily for 6 days and were killed 0.5, 2, 4, 8, and 24 h after the last injection (n = 10/group). Thyroid (at 1 and 6 days) and calvarium and liver (at 6 days only) were removed, immediately frozen in liquid nitrogen, and stored at −70°C.

RNA Purification and Slot Blot Analysis

Total cellular mRNA was obtained from individual thyroids after guanidinium extraction and centrifugation through a cesium chloride gradient by a modification of the Chirgwin procedure [7]. Samples were suspended in 400 μl TES buffer (220 mM Tris, pH 7.4, 10 mM NaCl, 0.1 mM EDTA) and precipitated on dry ice with 2.5 vol 70% and 95% ethanol before resuspension in 50 μl diethylpyrocarbonate-treated water. For all samples, Northern blot analysis was performed using 5–10 μg of total cellular RNA, to verify the size of the mRNA. The relative abundance of mRNA present in the various tissues (n = 3–6 samples/group) was estimated by slot blot analysis, after 3–5 μg total cellular mRNA were immobilized on a Zetaprobe membrane (Bio-rad, Richmond, CA) using a minifold II slot blot system (Schleicher and Schuell, Inc., Keene, NH), and cross-linked to filters by exposure to UV light for 1 min. The relative amounts of mRNA were normalized for ribosomal 28S RNA content. Plasmid DNAs were labeled with ^{32}P-dCTP by random priming. The rat 5′ deiodinase cDNA, contained in the plasmid G21, in bluescript KS, was kindly provided by P.R. Larsen [3]. The human cathepsin D cDNA, a 1.1 kb EcoRI fragment in pUC19, was kindly provided by J.M. Chirgwin [14]. The mouse c-fos cDNA was obtained from ATCC [22]. The rat histone cDNA (pPS7 H4) was kindly provided by S.R. Grimes [16]. The rat thyroglobulin cDNA, a 0.64 kbp cDNA, was kindly provided by G. Vassart [5]. The thyroid peroxidase probe, a plasmid containing a 2.8 kbp fragment of FRTL-5 thyroid peroxidase cDNA inserted in a pUC9, was kindly provided by S. Kimura [19]. The actin probe is a full-length cDNA for human cytoplasmic β actin [17].

Statistics

Values were expressed as percent ± SE of control values at each time point. The changes in the relative amount of mRNA were analysed using a nonparametric Mann-Whitney test. The size of the region of rejection of the null hypothesis was set by an error of 5%.

RESULTS

As previously reported [10], treatment with a-FGF for 6 days resulted in the expected 43% increase in thyroid weight compared to vehicle-treated animals. Our experimental approach to study the physiological and molecular mechanisms mediating FGF induced increase in thyroid weight was to systematically assay cellular levels of mRNA transcripts for a series of cell growth and thyroid related genes.

Effect of a-FGF on Cell Growth and Cell Structure Related Gene Expression

In order to determine the effects of a-FGF on gene expression associated with cellular differentiation and mitogenesis, we treated rats with a-FGF for 1 or 6 days and observed a 2–4-fold increase in c-fos mRNA in the thyroid gland. As illustrated in Figure 1 (top panel), 30 min after a single injection of a-FGF, there was a significant increase in c-fos mRNA, and the increase per-

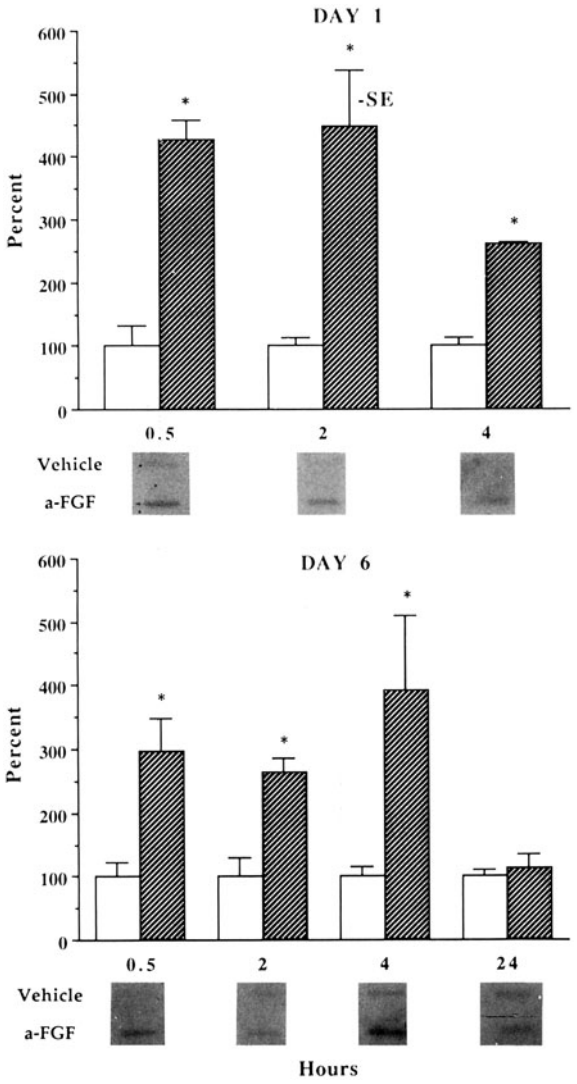

Fig. 1. Relative abundance of c-fos mRNA (normalized for 28S RNA) in the thyroid gland of rats treated with vehicle (open bars) or a-FGF (hatched bars) 0.5, 2, 4, and 24 h after 1 (upper panel) or 6 (lower panel) daily injections. Values are expressed as percent (mean ± SE) of values in vehicle-treated rats at each time point (n = 3/group). Below each panel, a representative sample of c-fos slot blot analysis is shown for each time point. *$P < 0.05$ compared to vehicle-treated control rats.

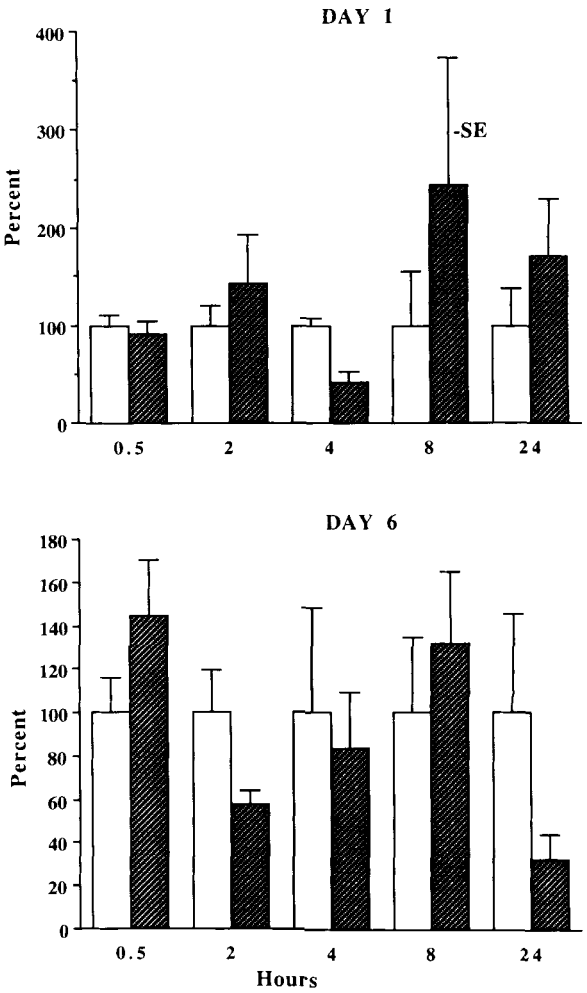

Fig. 2. Relative abundance of histone mRNA (normalized for 28S RNA) in the thyroid gland of rats treated with vehicle (open bars) or a-FGF (hatched bars) 0.5, 2, 4, 8, and 24 h after 1 (upper panel) or 6 (lower panel) daily injections. Values are expressed as percent (mean ± SE) of values in vehicle-treated control rats at each time point (n = 3–6/group).

sisted 2 and 4 h after the injection. After 6 daily injections of a-FGF, a similar increase in c-fos mRNA levels was observed. c-fos mRNA levels had returned to basal levels 24 h after the last injection. c-fos mRNA levels 8 and 24 h after the 1st injection and 8 h after the 6th injection of a-FGF were extremely low and could not be detected. There was no change in the relative amount of c-fos mRNA present in the liver or bone after 6 daily injections of a-FGF (data not shown). In contrast to what was observed for c-fos, treatment with a-FGF did not affect the amount of histone mRNA, a marker for proliferative activity, in the thyroid (Fig. 2). Similarly, there was no change in the relative amount of histone mRNA present in the liver, long bone, or calvarium (data not shown). Acidic-FGF treatment did not affect the actin mRNA content of the thyroid gland (data not shown).

Effect of a-FGF on Type I 5′ Deiodinase mRNA

The effects of a-FGF treatment on the content of the mRNA for the thyroid type I 5′ deiodinase, the enzyme responsible for the deiodination of T_4 to generate T_3, is illustrated in Figure 3. Treatment with a-FGF for 1 or 6 days caused a significant decrease in thyroid type I 5′ deiodi-

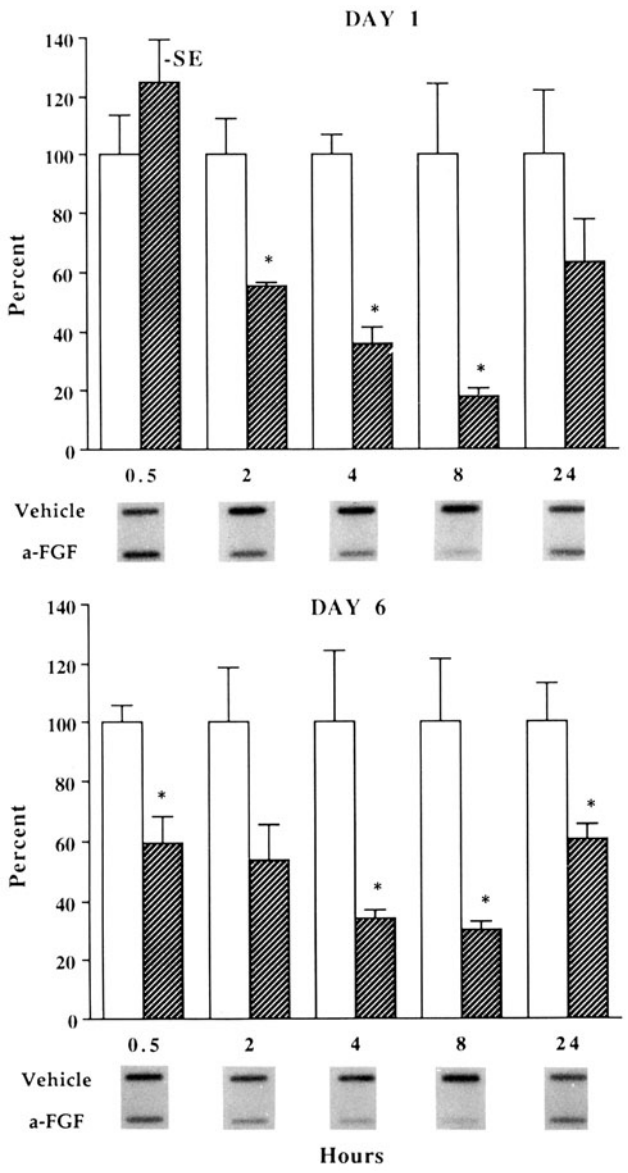

Fig. 3. Relative abundance of 5′ deiodinase mRNA (normalized for 28S RNA) in the thyroid gland of rats treated with vehicle (open bars) or a-FGF (hatched bars) 0.5, 2, 4, 8, and 24 h after 1 (upper panel) or 6 (lower panel) daily injections. Values are expressed as percent (mean ± SE) of values in vehicle-treated rats at each time point (n = 3/group). Below each panel, a representative sample of 5′D-I slot blot analysis is shown for each time point. *$P < 0.05$ compared to vehicle-treated control rats.

nase mRNA levels compared to the values observed in the vehicle-treated animals. The decrease was evident 2 h after a single injection of a-FGF and maximal inhibition was observed at 8 h (Δ 82%). Twenty-four hours after the first injection, 5′D-I mRNA levels were similar to those of vehicle-treated animals (Fig. 3, upper panel). After 6 daily injections of a-FGF, the decrease in 5′D-I mRNA was present throughout the whole day and was maximal 8 h after a-FGF injection (Δ 70%) (Fig. 3, lower panel). In the liver, there was a significant decrease in 5′D-I mRNA only 2 and 4 h after the 6th daily injection of a-FGF. 5′ deiodinase mRNA was not detected in the long bone or calvarium, used as a negative controls (data not shown).

Effect of a-FGF on Gene Expression Associated With Thyroid Function

Cellular levels of peroxidase and thyroglobulin mRNAs were used as markers of the organification of iodide and of the synthesis of the

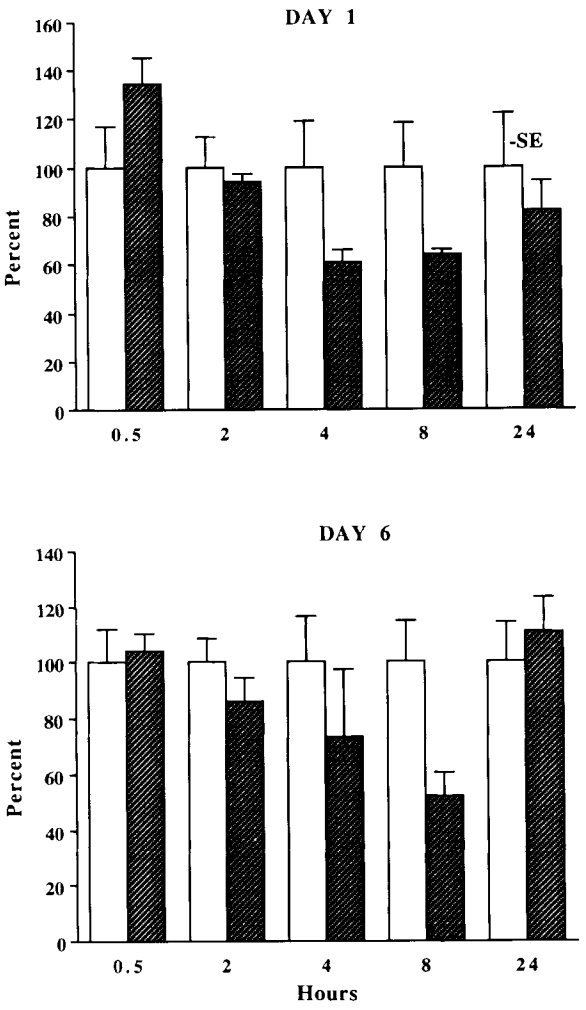

Fig. 4. Relative abundance of thyroid peroxidase mRNA (normalized for 28S RNA) in the thyroid gland of rats treated with vehicle (open bars) or a-FGF (hatched bars) 0.5, 2, 4, 8, and 24 h after 1 (upper panel) or 6 (lower panel) daily injections. Values are expressed as percent (mean ± SE) of values in vehicle-treated control rats at each time point (n = 3–5/group).

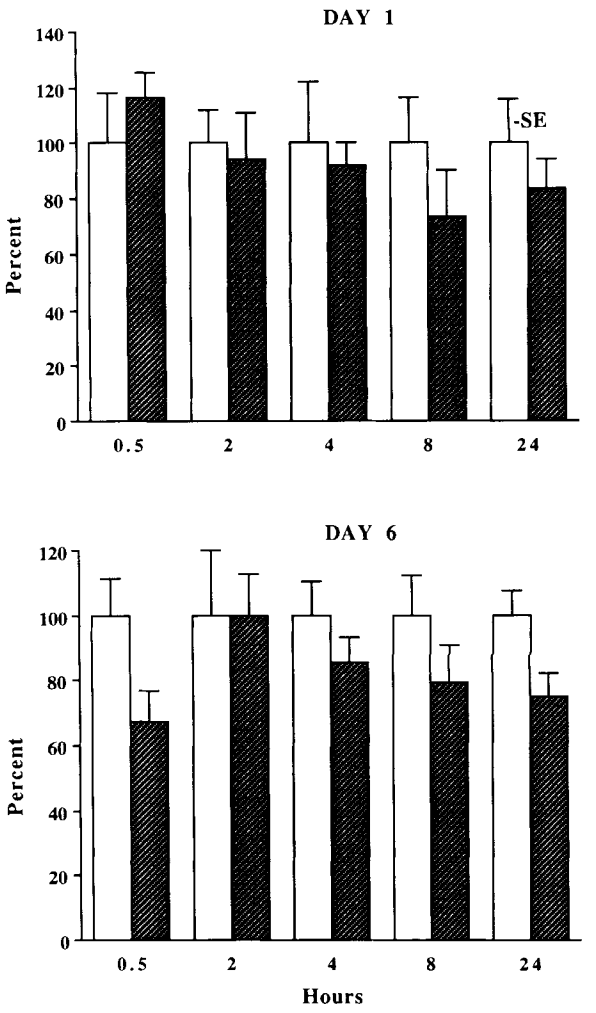

Fig. 5. Relative abundance of thyroglobulin mRNA (normalized for 28S RNA) in the thyroid gland of rats treated with vehicle (open bars) or a-FGF (hatched bars) 0.5, 2, 4, 8, and 24 h after 1 (upper panel) or 6 (lower panel) daily injections. Values are expressed as percent (mean ± SE) of values in vehicle-treated control rats at each time point (n = 3–5/group).

protein matrix for thyroid hormones, respectively. In contrast to what was observed for 5′D-I mRNA, injection of a-FGF for 1 or 6 days did not cause consistent changes in the relative amount of thyroid peroxidase (Fig. 4) or thyroglobulin (Fig. 5) mRNA.

Effect of a-FGF on Cathepsin D mRNAs

The mRNA levels of cathepsin D were measured in order to determine if the increase in colloid accumulation in a-FGF treated rats was associated with decreased synthesis in the lysosomal enzyme involved in the removal of T_3 and T_4 from thyroglobulin. Cathepsin D mRNA levels were detected in control and a-FGF treated animals, but were not affected by a-FGF administration for 1 or 6 days at any time point (data not shown).

DISCUSSION

The thyroid gland is the only source of T_4. The process leading to synthesis and secretion of T_4 is complex and involves several steps: active transport of iodide into the follicular cell, peroxidation of iodide by the enzyme peroxidase at the apical membrane of the cell allowing its incorporation in the tyrosyl residues of thyroglobulin (colloid), coupling of the iodotyrosines residues on the thyroglobulin, colloid resorption by endocytosis, digestion of the thyroglobulin by lyso-

somal enzymes, and secretion of thyroid hormones at the basal membrane of the thyrocyte. The thyroid cell is also rich in type I 5′ deiodinase, an enzyme that catalyses the deiodination of the prohormone T_4 into the active hormone T_3. All these fundamental steps of intrathyroidal metabolism are actively stimulated by TSH [29]. Furthermore, the proliferation and differentiation expression of the thyroid cell is also modulated by various growth factors [11]. In order to further understand the mechanisms by which a-FGF induces a TSH-dependent increase in colloid accumulation and thyroid weight in the rat [10], we studied the effects of 1 and 6 injections of a-FGF on the expression of several genes encoding a cell cycle regulated protein and an early response transactivation factor that mediates cell signaling mechanisms related to proliferation. In addition, specific markers of intrathyroidal metabolism were evaluated. The results demonstrate that a-FGF resulted in increased c-fos mRNA levels without changes in histone or actin mRNA levels. Acidic-FGF decreased 5′D-I mRNA levels but had no effect on thyroid peroxidase, thyroglobulin, or cathepsin D mRNA levels.

In vitro studies have shown that a-FGF stimulates thymidine incorporation in various cell types, including FRTL-5 cells [21]. Similarly, basic-FGF stimulates DNA synthesis in FRTL-5 and cultured porcine thyroid cells [13]. In vivo studies have also demonstrated a mitogenic effect of a-FGF in the arterioles of ischemic myocardium in dogs [1]. In the present study, a rapid increase in c-fos gene expression was observed after a-FGF administration, indicating activation of genes associated with early events in the proliferative process [8]. However, as indicated by the lack of a-FGF related changes in histone mRNA content, there was an absence of subsequent events necessary for competency in initiating DNA synthesis and mitotic activity [28]. Upregulation of c-fos expression may, therefore, reflect activation of cellular signaling mechanisms associated with nonproliferative components of gene expression related to thyroid function [22]. These findings are consistent with the absence of histological signs of cellular proliferation in the thyroid of a-FGF treated rats, in which follicular cells are quiescent and flat. The reason for the contrast between induction of cellular differentiation and mitogenesis by a-FGF in thyroid cells in vitro and in vivo is unclear. One possibility is that cultured thyroid cells may have lost part of their regulatory mechanisms so that a proliferate response observed in vitro following administration of a-FGF might not reflect the in vivo situation.

Consistent with the histological data, there was no increase in actin mRNA levels in the thyroid of rats treated with a-FGF, suggesting that there was no increase in cytoskeletal components and cell volume. However, the absence of increased actin mRNA levels in the thyroid does not preclude the possibility of cytoskeleton reorganization.

We have previously demonstrated that the increase in thyroid weight following a-FGF administration in male or female rats was characterized by colloid accumulation, without an increase in serum thyroglobulin concentrations [10]. Therefore, a possible explanation for this effect of a-FGF on the thyroid was an increase in thyroglobulin synthesis without increase in thyroglobulin digestion. However, we did not observe a change in thyroglobulin mRNA content in the thyroid at any time point after 1 or 6 daily injections of a-FGF. Similarly, thyroid peroxidase mRNA levels were unaffected by a-FGF administration. This extends our previous observation of a lack of a-FGF induced change in peroxidase and thyroglobulin mRNA levels 24 h after the 6th injection [10], and suggests that impaired oxidation of iodide and/or impaired thyroglobulin synthesis do not play a role in the pathogenesis of a-FGF induced colloid goiter. Consistent with our results, Roger and Dumont [24] observed no effect of basic-FGF on thyroglobulin induced gene expression in cultured dog thyroid cells and Black et al. [4] found only a small increase in thyroglobulin mRNA in FRTL-5 cells incubated for 24 h with basic-FGF.

Injection of a-FGF resulted in a striking decrease in the content of mRNA for type I 5′ deiodinase in the thyroid, and, to a much lesser extent, in the liver. To our knowledge, this is the first example of a relatively selective inhibition of 5′ D-I in the thyroid, a major source of circulating T_3 in the rat [6]. 5′ deiodinase catalyzes the conversion of T_4, secreted only by the thyroid, into T_3. Two isozymes are responsible for this reaction. Type I 5′ deiodinase is abundant in the thyroid, liver, and kidneys, and its recent cloning has shown that it contains a selenocystein in the active center [3]. Type II 5′ deiodinase is present mainly in the brain, pituitary, and brown adipose tissue. Although the nucleic acid sequence is not yet available for 5′D-II, it

has been shown that the substrate binding subunit of this enzyme does not contain selenium [25]. In the rat, the thyroid is a major source of circulating T_3, mostly through intrathyroidal T_4 to T_3 type I 5′ deiodination, while type I 5′ deiodination in peripheral tissues, including the liver and kidney, plays a less important role [6]. Type II 5′ deiodination of T_4 in the brown adipose tissue might also account for 20–25% of serum T_3 concentrations [26].

Consistent with our finding of a marked decrease in 5′D-I mRNA content in the thyroid, Lombardi et al. [21] have recently shown that incubation of FRTL-5 cells with a-FGF decreased both basal and TSH-stimulated T_3 production, suggesting that the activity of 5′D-I is also decreased. In contrast, Courtin et al. [9] reported that incubation of cultured astroglial cells for 4–24 h with a-FGF resulted in a marked increase in 5′D-II activity. Furthermore, an effect of basic-FGF has also been demonstrated in cultured pituitary cells on TRH-induced TSH secretion [2]. We have recently reported that treatment with a-FGF was associated with a small but significant 10–15% decrease in serum T_3 concentrations 2 to 4 h after a single injection of a-FGF, while serum T_3 concentrations were increased 8 to 24 h after the 6th injection [10]. It is conceivable that the specific effects of FGFs on the pituitary-thyroid axis and on the expression of the two major enzymes involved in T_3 production may affect serum T_3 concentrations.

Removal of T_4 and T_3 from the thyroglobulin matrix is the last step leading to T_4 secretion into the blood by the thyroid gland. Although the exact mechanisms of this phase have not been completely elucidated, it is thought to occur in lysosomes. Several lysosomal endopeptidases, cathepsins B, D and L, have been isolated from rabbit and human thyroids. Selective in vitro inhibition of these lysosomal enzymes has been shown to result in a marked decrease in iodoamino acid release from rabbit thyroglobulin [12]. We postulated that impaired thyroglobulin digestion might play a role in the accumulation of colloid observed in a-FGF treated animals [27]. However, treatment with a-FGF did not change the mRNA content of cathepsin D, used as a marker of lysosomal activity, suggesting that a-FGF did not alter the integrity of this pathway, although other lysosomal enzymes such as endopeptidases [29] might be more essential for thyroglobulin hydrolysis.

In conclusion, iv administration of a-FGF results in a marked increase in thyroid weight with colloid accumulation. The mechanisms involved in the effects of a-FGF on thyroid homeostasis remain unclear, but do not seem to include cellular proliferation or alteration in the regulation of the thyroid specific genes thyroid peroxidase and thyroglobulin or the lysosomal enzyme cathepsin D. Modifications in gene expression and induction are reflected by the upregulation of the early response gene c-fos. The marked and persistent decrease in 5′ deiodinase mRNA content after a-FGF treatment suggests that this growth factor may play a role in the regulation of 5′D-I activity in the thyroid.

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