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Transforming Growth Factor-α Attenuates the Acquisition of Aromatase Activity by Cultured Rat Granulosa Cells

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The effect of transforming growth factor-α (TGFα) on granulosa cell differentiation, as assessed by the acquisition of aromatase activity, was evaluated in vitro by using a primary culture of rat granulosa cells. Harvested from immature, diethylstilbestrol-treated rats, granulosa cells were cultured under serum-free conditions for 72 hr in the presence of saturating concentrations (10^{-7}M) of aromatase substrate androstenedione with or without the specified experimental agents. Basal aromatase activity, as assessed by the generation of radioimmunoassayable estrogen was negligible, remaining unaffected by treatment with TGFα (10 ng/ml) by itself. Whereas treatment with follicle-stimulating hormone (FSH) resulted in a substantial increase in the extent of aromatization, concurrent treatment with TGFα (10 ng/ml) resulted in significant ($P<0.05$), yet reversible inhibition (78 $\pm$ 5.6%) of FSH action. Significantly, this effect of TGFα could not be accounted for by a decrease in cellular viability or plating efficiency nor by a decrease in the number of cells or their DNA content. Although independent of the FSH dose employed, the TGFα effect proved dose- and time-dependent, with an apparent median inhibitory dose (EC_{50}) of 0.33 $\pm$ 0.04 ng/ml, and a minimal time requirement of 48 hr. Capable of substantial inhibition of the forskolin-stimulated accumulation of extracellular adenosine 3′, 5′ cyclic monophosphate (cAMP) and estrogen, TGFα had a measurable albeit limited effect on N^6,2-′O-Dibutyryladensine 3′:5′-cyclic monophosphate-supported estrogen production. Relative potency comparison revealed epidermal growth factor (EGF; EC_{50} = 0.24 $\pm$ 0.03ng/ml) and TGFα to be virtually equipotent as regards the attenuation of FSH-stimulated estrogen biosynthesis. Taken together, our findings indicate that TGFα, like EGF, acting at subnanomolar concentrations, is capable of attenuating the FSH-stimulated (but not basal) accumulation of estrogen. This effect of TGFα proved time- and dose-dependent, involving virtually complete neutralization of FSH action at site(s) both proximal and distal to cAMP generation. As such, these findings provide yet another example of the remarkable qualitative and quantitative similarities between EGF and TGFα, thereby reaffirm-

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ing the prospect that ligands of the EGF/TGFα receptor may play a modulatory role in the course of granulosa cell ontogeny.

Key words: follicular development, transforming growth factor-α, aromatase activity

Epidermal growth factor (EGF) has previously been observed to exert potent regulatory effects on granulosa cell proliferation [1–3] and differentiation [4–8]. Presumably, these effects of EFG are mediated via specific cell membrane receptors, the existence of which on bovine [9], ovine [10], and murine [5,11] granulosa cells has been demonstrated. However, the identity of the ligand occupying the receptor in question under in vivo conditions remains uncertain. Among potential receptor occupants, blood-borne EGF of submaxillary gland origin and locally produced EGF [12] deserve further consideration.

The above notwithstanding, consideration must also be given to transforming growth factor-α (TGFα), a structural analog of EGF capable of binding to an apparently common EGF/TGFα receptor [13], a 1186 residue transmembranous glycoprotein [14,15]. A single-chain, 50-amino acid polypeptide [16,17], TGFα is best known for its ability to produce an acute, albeit reversible phenotypic transformation of normal mammalian cells [13]. However, recent evidence suggests that this member of the EGF-urogastrone family may not only promote autocrine tumoral growth, but may also participate in the regulation of several non-neoplastic processes [18–22]. Specifically, TGFα has recently been reported to accelerate eyelid opening and incisor eruption in newborn mice [19,21] and to stimulate prostaglandin production and bone resorption in cultured mouse calvaria [18,22]. Moreover, it is conceivable that TGFα gene expression under non-neoplastic conditions may not be limited to embryonic tissues [23] and that it may be part and parcel of intense cellular growth processes such as encountered in the course of ovarian follicular development.

To further elucidate the potential relevance of TGFα to granulosa cell ontogeny, we have undertaken to evaluate its effect on granulosa cell differentiation as monitored by the acquisition of aromatase activity. Our findings indicate the TGFα, like EGF, is capable of attenuating granulosa cell estrogen biosynthesis through virtual neutralization of follicle-stimulating hormone (FSH) action at site(s) both proximal and distal to adenosine 3′,5′ cyclic monosphosphate (cAMP) generation.

MATERIALS AND METHODS

Animals

Immature (23–25 days old) Sprague-Dawley female rats were from Johnson Laboratories Inc. (Bridgeview, IL). Silastic capsules containing diethylstilbestrol (DES) were implanted sc by the vendor and the animals delivered on the second postoperative day. The animals were housed in air-conditioned quarters and given powdered rodent laboratory chow 5001 from Ralston Purina Co. (St. Louis, MO) and water ad libitum. A 14 hr light, 10 hr dark cycle was maintained, with the light cycle starting at 06:00 hr. The animals were killed by cervical dislocation between 3–4 days after surgery.

Reagents and Hormones

McCoy's 5a medium (modified, without serum); penicillin-streptomycin solution; L-glutamine, trypsin/EDTA, 0.05% and 0.02% (wt/vol) in calcium-, and mag-

nesium-free phosphate-buffered salt solution; and Trypan blue stain (0.4%; wt/vol), were obtained from Grand Island Biological Co. (Grand Island, NY). Forskolin (7β-acetoxy-8,13-eppoxy-1α, 6β, 9α trihydroxy-labd-14-en-11-one) and bisbenzimide H 33258 fluorochrome (Hoechst dye 33258; 2-[2-(4-hydroxyphenyl)-6-benzimidazoyl]-6-(1-methyl-4-piperazyl)-benzimidazole trihydrochloride) were from Calbiochem-Behring (La Jolla, CA). N^6, 2'O-Dibutyryladenosine 3':5'-cyclic monophosphate (Bt_2cAMP), DES, highly polymerized calf thymus DNA (sodium salt) and androstenedione, were from Sigma Chemical Co. (St. Louis, MO). ZK 62711 [Rolipram; the racemate of 4-(3'-cyclopentyloxy-4'-methoxyphenyl)-2-pyrrolidone(4-RS)], a potent inhibitor of cAMP-phosphodiesterase (PDE) activity was the generous gift of Mr. N. Sprzagala, Schering Actiengesellschaft, Berlin, West Germany.

Synthetic rat TGFα was from Peninsula Laboratories, Inc. (Belmont, CA). EGF from Collaborative Research, Inc. (Lexington, MA) was further purified as previously described [25]. Ovine FSH —oFSH;NIH-FSH-S14; FSH potency equal to 9 NIH-FSH-S1 units/mg; luteinizing hormone (LH) activity, 0.02 NIH-LH-SI units/mg; prolactin activity $<0.1\%$ by weight— was the generous gift of the National Pituitary Agency, Pituitary Hormone Distribution Program, NIADDK.

In Vitro Studies

Granulosa cells (1×10^5 viable cells/culture) were plated onto tissue culture dishes (35 × 10 mm; Falcon Plastics, Oxnard, CA) containing 1 ml McCoy's 5a medium (modified, without serum) supplemented with L-glutamine (2mM), penicillin (100 U/ml), and streptomycin sulfate 100 μg/ml). Cell cultures were maintained at 37°C under a water-saturated atmosphere of 95% air and 5% CO_2. All agents were dissolved in sterile culture medium and applied in 50μl aliquots. Forskolin and androstenedione were initially dissolved in 99.5% (vol/vol) ethanol, followed by subsequent dilution with sterile culture medium such that the final ethanol concentration in the dish did not exceed 0.5% (vol/vol).

In all experiments (unless indicated otherwise), the cells were cultured for 72 hr in the presence of saturating concentrations (10^{-7} M) of the aromatase substrate androstenedione, with or without the specified experimental agents. At the conclusion of the incubation period, the media were collected and stored frozen at −20°C until assayed for their total estrogen content by RIA. The conversion of unlabeled androstenedione to radioimmunoassayable estrogen during the 72 hr incubation (expressed in terms of ng/culture) was taken to reflect the relative level of aromatase activity acquired during the experimental period. In some experiments, collected media were boiled for 10 min in a water bath and stored frozen at −20°C until assayed for their cAMP content by RIA.

Determination of Cellular Plating Efficiency and Viability

Following aspiration of the medium and its replacement of trypsin/EDTA, 0.05% and 0.02% (wt/vol) in calcium-, and magnesium-free phosphate-buffered salt solution, the aspirated medium and the trypsinized (10 min at 37°C) cell solution of quadruplicate dishes were spun down and the cellular pellets counted in a hemacytometer. Viability was assessed by the trypan blue dye exclusion test. Plating efficiency and cellular viability are expressed in terms of percent cells attached/dish and percent viable cells/dish, respectively.

Determination of Cellular DNA Content

Following aspiration of the medium and its replacement with "DNA buffer" (100 mM NaCL, 10 mM EDTA, and 10 mM Tris, pH 7.0), the cells were scraped off the dish with a rubber policeman and the total cellular DNA content determined for triplicate dishes as previously described [26]. Briefly, the DNA concentration of a sonicated cellular preparation was measured using fluorescent enhancement of Hoechst dye 33258 consequent upon its binding to DNA. The fluorescent enhancement is highly specific for DNA with no other cell component producing significant fluorescence. The intensity of the fluorescence is read in a spectrophotometric cell (spectrosil) with the use of a spectrofluorometer, at excitation and emission wave lengths of 350 and 454 NM, respectively. The results are expressed in terms of the calf thymus DNA standard as micrograms per culture.

RIA

Medium total estrogen content was determined using an antiserum raised against estradiol-17β-O- carboxymethyloxime-bovine thyroglobulin, generously provided by Dr. Delwood C. Collins, Emory University School of Medicine, (Decatur, GA). Medium (extracellular) cAMP content was determined with a cAMP RIA kit (NEX 132) obtained from New England Nuclear (Boston, MA). Sample acetylation for enhanced assay sensitivity was routinely employed. Assay procedures and performance characteristics were as previously described [26].

Data Analysis

All experimental data are present as the mean $\pm$ SE of duplicate measurements of triplicate cultures. RIA data analysis, dose-response curve fitting, and determination of the median inhibitory dose (EC_{50}), were carried out with the use of CURVE FIT software, a package based on the 4-parameter logistic equation designed to fit the results to a sigmoidal function curve. Statistical significance was determined by the student's paired 2-tailed t-test and analysis of variance (ANOVA), as indicated.

RESULTS

Effect of Treatment of TGFα on Basal and FSH-Induced Aromatase Activity of Cultured Rat Granulosa Cells: Reversibility Studies

To investigate the possibility that TGFα may regulate aromatase activity, granulosa cells were cultured in the absence or presence of FSH (100 ng/ml), with or without TGFα at the 10ng/ml dose level (Fig. 1). Basal aromatase activity, as assessed by the generation of radioimmunoassayable estrogen, was negligible, remaining unaffected by treatment with TGFα alone. Whereas treatment with FSH resulted in a substantial increase in the extent of aromatization, concurrent treatment with TGFα resulted in significant ($P < 0.05$) inhibition of FSH action ($78 \pm 5.6\%$). These findings indicate that TGFα is capable of attenuating the FSH-stimulated (but not basal) accumulation of estrogen by cultured rat granulosa cells.

To examine the reversibility of the TGAα effect, granulosa cells were initially cultured for 72 hr in the presence of FSH (100 ng/ml), with or without TGFα (10 ng/ml). The media were then removed, and the cells were washed and reincubated in the absence or presence of FSH (100 ng/ml) for additional 24 hr. This sequence was

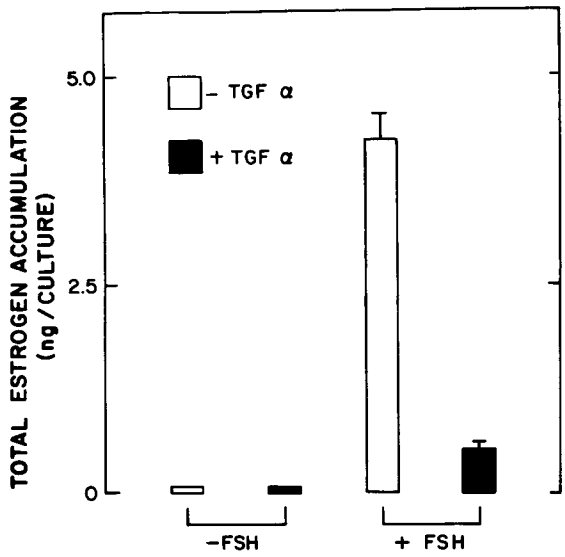

Fig. 1. Effect of treatment with TGFα on basal and fsh-induced aromatase activity of cultured rat granulosa cells. Granulosa cells (1×10^5 viable cells/culture) were obtained from immature, DES-treated rats and cultured under serum-free conditions for 72 hr in the absence or presence of FSH (100 ng/ml), with or without TGFα (10 ng/ml). Androstenedione (10^{-7}M) served as the aromatase substrate. At the conclusion of the incubation period, the media were collected and assayed for their total estrogen content by RIA. The results represent the mean ± SE of three separate determinations.

repeated on a daily basis for a total of 3 days. In data not shown, FSH-pretreated cells responded promptly to FSH retreatment with sustained increases in the accumulation of estrogen. In contrast, cells pretreated with FSH and TGFα displayed progressive daily increments in response to FSH retreatment, the accumulation of estrogen on day 3 (1.8 ± 0.6 ng/culture) approximating that of cells pretreated with FSH alone (2.2 ± 0.9 ng/culture). These findings suggest that the inhibitory effect of TGFα is reversible upon removal of the peptide.

TGFα-Attenuated Aromatase Activity: TGFα Dose-Dependence

To evaluate the dose requirements of TGFα as regards the attenuation of estrogen biosynthesis, granulosa cells were cultured in the absence or presence of FSH (100 ng/ml), with or without increasing concentrations (1–100 ng/ml) of TGFα (Fig. 2). Whereas estrogen biosynthesis by control and TGFα (100 ng/ml)-treated cells was relatively low and comparable, concomitant treatment with increasing concentrations of TGFα resulted in dose-dependent decrements in the FSH-stimulated accumulation of estrogen with an apparent EC_{50} of 0.33 ± 0.04 ng/ml, and a maximal inhibitory effect of 91 ± 2%. These findings indicate that the ability of TGFα to inhibit FSH-supported aromatase activity is dose-dependent producing a virtually complete neutralization of the FSH effect at the high end of the TGFα dose range.

TGFα-Attenuated Estrogen Biosynthesis: FSH Dose-Dependence

To further evaluate the ability of TGFα to attenuate the FSH-mediated acquisition of aromatase activity, granulosa cells were cultured in the absence or presence of

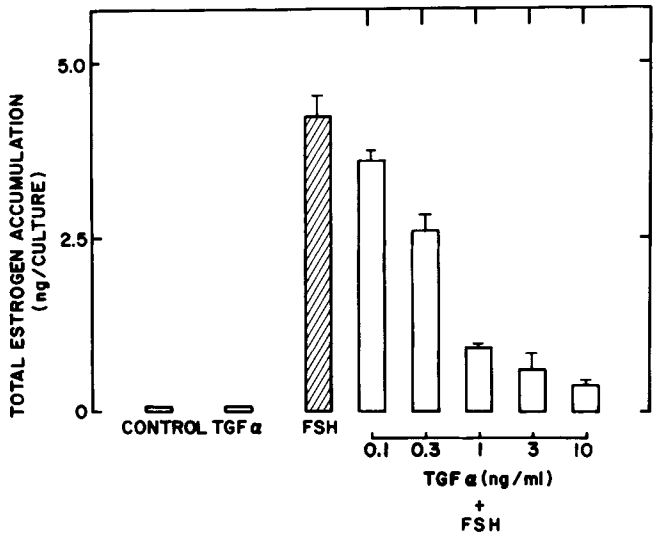

Fig. 2. TGFα-Attenuated estrogen accumulation: TGFα dose-dependence. Granulosa cells were cultured as described in Figure 1 in the absence or presence of FSH (100 ng/ml), with or without increasing concentrations (0.1–10 ng/ml) of TGFα. Androstenedione (10^{-7}M) served as the aromatase substrate. At the conclusion of the incubation period, the media were collected and assayed for their total estrogen content by RIA. The results represent the mean ± SE of three separate determinations.

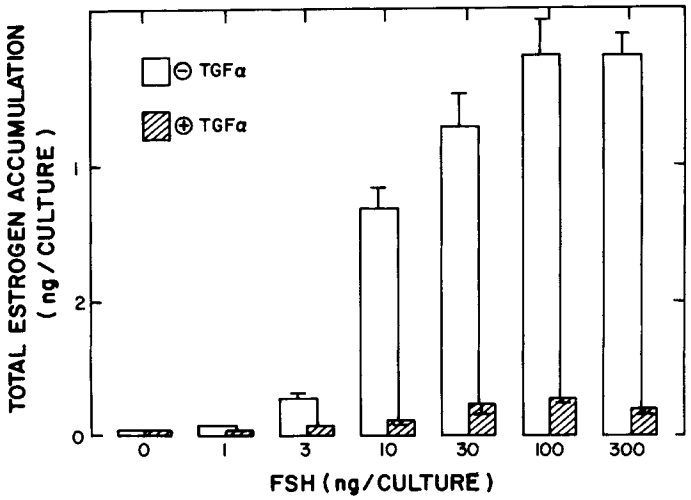

Fig. 3. TGFα-Attenuated estrogen accumulation: FSH dose-dependence. Granulosa cells were cultured as described in Figure 1 in the absence or presence of increasing concentrations of FSH (1–300 ng/ml), with or without TGFα (10 ng/ml). Androstenedione (10^{-7}M) served as the aromatase substrate. At the conclusion of the incubation period, the media were collected and assayed for their total estrogen content by RIA. The results represent the mean ± SE of three separate determinations.

increasing concentrations of FSH (1–300 ng/ml), with or without TGFα at the 10 ng/ml dose level (Fig. 3). Treatment with increasing concentrations of FSH resulted in dose-dependent increments in the accumulation of estrogen, the 100 ng/ml dose resulting in saturation at 2.8 ± 0.16 ng/culture. Although TGFα by itself was without

effect on the basal accumulation of estrogen, it nevertheless led to significant ($P < 0.05$) inhibition of all (but the 1 ng/ml) doses of FSH (71%, 93%, 89%, 90%, and 93% for the 3, 10, 30, 100, and 300 ng/ml of FSH, respectively). These findings indicate a relatively constant degree of inhibition, suggesting that the magnitude of the TGFα effect may be independent of the concentration of FSH employed.

TGFα-Attenuated Estrogen Accumulation: Time-Dependence

To study the time-dependence of TGFα-attenuated estrogen biosynthesis, granulosa cells were cultured for the duration indicated in the absence or presence of FSH (100 ng/ml, with or without TGFα at the concentration of 10 ng/ml (Fig. 4). In the absence of FSH, basal estrogen accumulation was relatively low throughout the 72 hr incubation period, remaining unaffected by treatment with TGFα by itself. In contrast, treatment with FSH produced time-dependent increments in estrogen accumulation, a significant ($P < 0.05$) increase being noted by 24 hr of treatment, the peak effect (2.5 ± 0.19 ng/culture) occurring at the 72 hr time point. However, concomitant treatment with TGFα produced significant ($P < 0.05$) attenuation of the FSH-stimulated accumulation of estrogen for all time points studied, the maximal inhibitory effect being noted at the 72 hr time point (87.7 ± 3.4% inhibition).

Effect of Treatment with EGF or TGFα on the FSH-Stimulated Accumulation of Extracellular cAMP and Estrogen: Relative Potency Comparison

To evaluate the relative potencies of EGF and TGFα in attenuating FSH-stimulated estrogen accumulation, granulosa cells were cultured in the absence or presence of FSH (100 ng/ml), with or without increasing concentrations (0.01–100 ng/ml) of EGF or TGFα (Figs. 5A and 5B). Concurrent treatment with increasing concentrations of either EGF or TGFα produced dose-dependent decrements in the FSH-stimulated accumulation of estrogen with apparent EC_{50} values of 0.24 ± 0.03

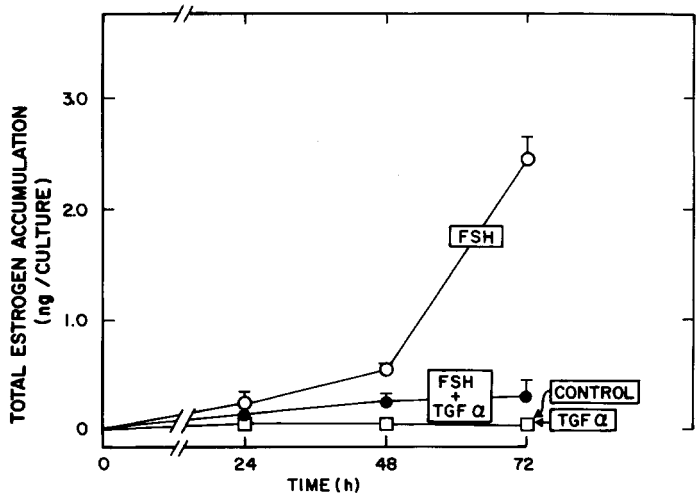

Fig. 4. TGFα Attenuated estrogen accumulation: time-dependence. Granulosa cells were cultured as described in Figure 1 for the duration indicated (24–72 hr) in the absence or presence of FSH (100 ng/ml), with or without TGFα (10 ng/ml). Androstenedione (10^{-7}M) served as the aromatase substrate. At the conclusion of the incubation period, the media were collected and assayed for their total estrogen content by RIA. The results represent the mean ± SE of three separate determinations.

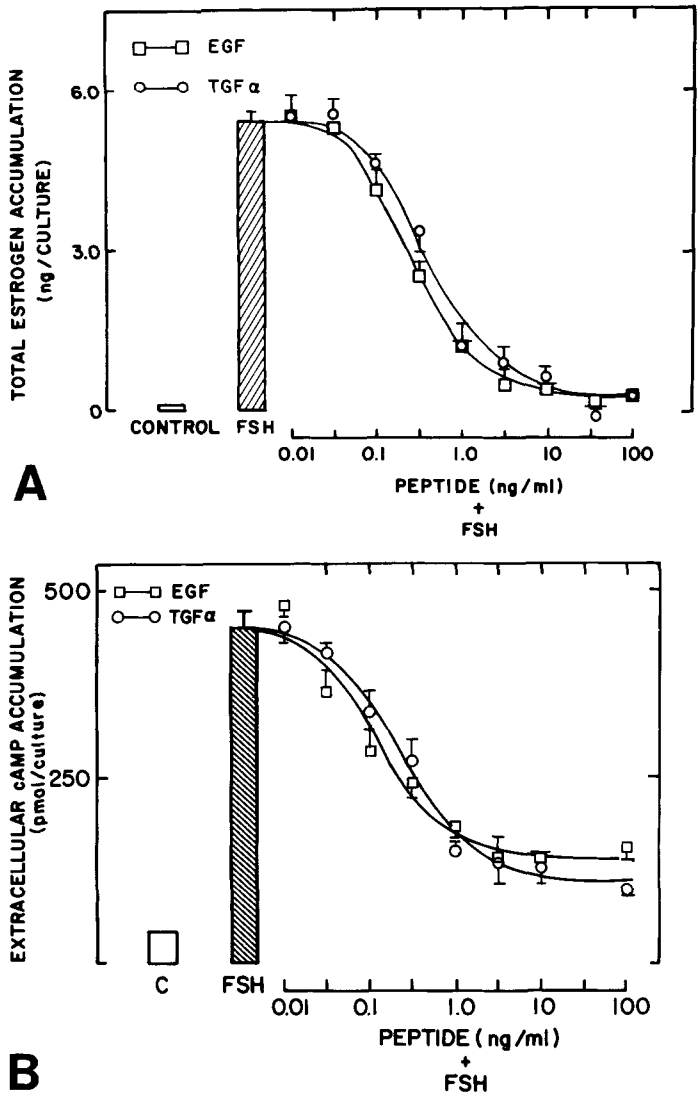

Fig. 5. Effect of treatment of EGF or TGFα on the FSH-stimulated accumulation of extracelllular cAMP and estrogen: relative potency comparison. Granulosa cells were cultured as described in Figure 1 in the absence or presence of FSH (100 ng/ml), with or without increasing concentrations (0.01–100 ng/ml) of EGF or TGFα. Androstenedione (10^{-7}M) served as the aromatase substrate. At the conclusion of the incubation period, the media were collected and assayed for their total estrogen (A) or cAMP (B) content by RIAs. The results represent the mean ± SE or three separate determinations.

and 0.42 ± 0.07 ng/ml, respectively (Fig. 5A). Treatment with maximal inhibitory doses (100 ng/ml) of either EGF or TGFα produced indistinguishable attenuation of the FSH effect (approximately 94% inhibition). Treatment with higher doses of EGF or TGFα did not yield additional decrements in the FSH-stimulated accumulation of estrogen. Qualitatively comparable results were obtained upon monitoring of the FSH-stimulated accumulation of extracellular cAMP (Fig. 5B). These findings indi-

cate that EGF and TGFα are virtually equipotent as regards the attenuation of FSH-stimulated estrogen biosynthesis and that their effects are exerted, at least in part, at site(s) proximal to cAMP generation.

Effect of Treatment with TGFα on the Basal and Forskolin-Stimulated Accumulation of Extracellular cAMP and Estrogen

To further evaluate the possibility that TGFα-attenuated FSH action may involve a decrease in stimulatable adenylate cyclase activity (independent of FSH receptor involvement), use was made of forskolin, a diterpene alkaloid capable of rapid, reversible, and potent stimulation of adenylate cyclase activity in intact cultured granulosa cells. In this experiment, Rolipram (3×10^{-6}M) treated granulosa cells were cultured in the absence or presence of forskolin (10^{-5}M), with or without increasing concentration (0.1–10 ng/ml) of TGFα (Fig. 6). Concurrent treatment with increasing concentrations of TGFα, yielded dose-dependent decrements in the forskolin-stimulated accumulation of estrogen with an EC_{50} of 0.71 ± 0.15 ng/ml and a maximal inhibitory effect of 73.8 ± 6.5%. Qualitatively comparable results were obtained when monitoring the accumulation of extracellular cAMP (not shown). These results further suggest that the ability of TGFα to attenuated FSH action is due, at least in part, to TGFα acting at sites(s) proximal to cAMP generation resulting in a decrease in stimulatable adenylate cyclase activity.

Effect of Treatment with TGFα on the Basal and Bt_2 cAMP-Stimulated Accumulation of Estrogen

To further localize the TGFα sites (s) of action relative to the generation of cAMP, the effect of treatment with TGFα on events related to cAMP action were

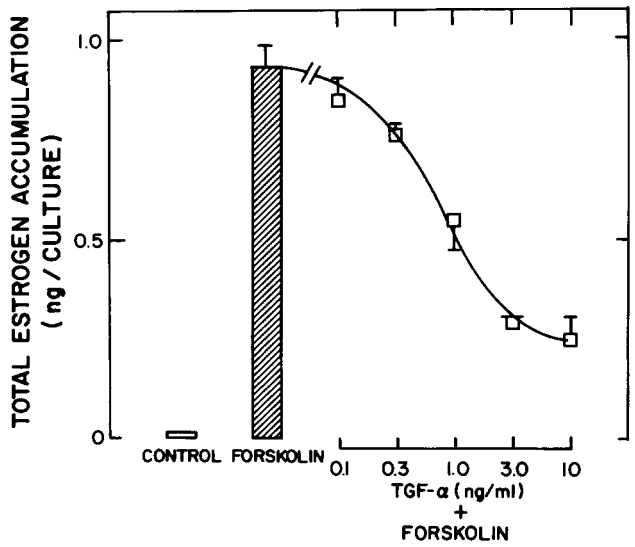

Fig. 6. Effect of Treatment with TGFα on the basal and forskolin-stimulated accumulation of estrogen. Rolipram (3×10^{-6}M)-treated granulosa cells were cultured as described in Figure 1 in the absence or presence of forskolin (10^{-5}M), with or without increasing concentrations (0.1–10 ng/ml) of TGFα. Androstenedione (10^{-7}M) served as the aromatase substrate. At the conclusion of the incubation period, the media were collected and assayed for their or total estrogen content by RIA. The results represent the mean ± SE of three separate determinations.

evaluated. To this end, granulosa cells were cultured in the absence or presence of Bt_2cAMP (10^{-3}M), with or without increasing concentrations (0.1–10 ng/ml) of TGFα (Fig. 7). Whereas treatment with Bt_2 cAMP produced a significant ($P < 0.05$) increase over controls in estrogen accumulation, concurrent treatment with increasing concentrations of TGFα had a measurable, albeit limited inhibitory effect on Bt_2cAMP action. These findings suggest that the ability of TGFα to attenuate FSH hormonal action can be attributed, in part, to TGFα acting at site(s) distal to cAMP generation.

Direct Effect of Treatment with TGFα on Cellular DNA Content, Plating Efficiency, and Viability

To evaluate the possibility that TGFα action may be attributable to a decrease in cell number, granulosa cells were cultured in the absence or presence of FSH (100 ng/ml), with or without 10 ng/ml of TGFα (Table I). However, treatment with TGFα proved to be without significant effect on granulosa DNA content, plating efficiency, or viability, suggesting that the inhibitory effect of TFGα cannot be accounted for by a decrease in the number of cells present.

DISCUSSION

The effect of TGFα on granulosa cell differentiation, as assessed by the acquisition of aromatase activity, was evaluated in vitro using a primary culture of rat granulosa cells. Our findings indicate that TGFα, like EGF, is capable of attenuating the FSH-stimulated accumulation of estrogen and that the two peptides are equipotent in this regard. These observations provide yet another example of the remarkable

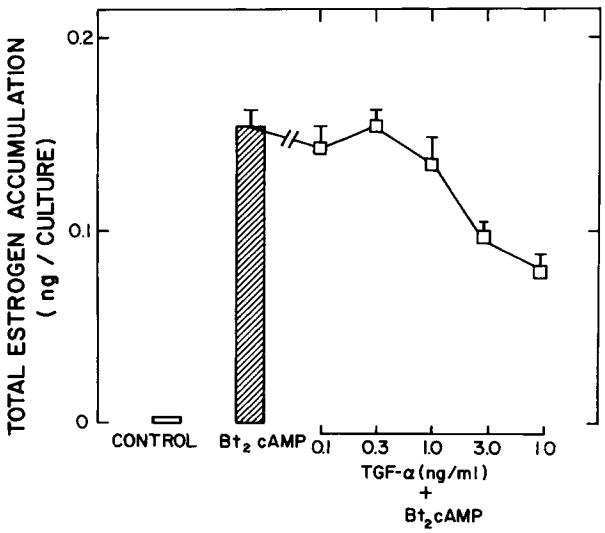

Fig. 7. Effect of treatment with TGFα on the basal and Bt_2 cAMP-stimulated accumulation of estrogen. Granulosa cells were cultured as described in Figure 1 in the absence or presence of Bt_2 cAMP (10^{-3} M), with or without increasing concentrations (0.1–10 ng/ml) of TGFα. Androstenedione (10^{-7} M) served as the aromatase substrate. At the conclusion of the incubation period, the media were collected and assayed for their total estrogen content by RIAs. The results represent the mean ± SE of three separate determinations.

TABLE I. Effect of Treatment with Tgfα on Granulosa Cell DNA Content, Plating Efficiency and Viability

Treatment	DNA Content (μg/culture)	Granulosa cell[a] (%)	
		Plating efficiency	Viability
None	4.35 ± 0.4	54 ± 3	85 ± 5
TGFα	4.05 ± 0.7	57 ± 2	89 ± 4
FSH	4.25 ± 0.2	59 ± 6	81 ± 1
FSH + TGFα	4.10 ± 0.9	59 ± 5	79 ± 5

[a]Granulosa cells (5×10^5 cells/culture) were cultured as described in figure 1 in the absence or presence of FSH (100 ng/ml), with or without TGFα (10 ng/ml). At the conclusion of the 72 hr treatment period, measurements of cellular DNA content, plating efficiency, and viability were carried out as described under Materials and Methods. Data points represent the mean ± SE of 12 determinations, representing quadruplicate measurements for each of a total of three separate experiments.

qualitative and quantitative similarities between EGF and TGFα, suggesting that at least some of the actions of these two peptides may be mediated via a common EGF/TGFα receptor site.

Although the exact cellular mechanism(s) whereby TGFα interferes with FSH action remain uncertain, consideration must be given to site(s) of action concerned with cAMP generation, breakdown, or action. Although TGFα-mediated attenuation of FSH receptor binding capacity and/or affinity represents an attractive possibility, the previously reported inability of EGF to alter FSH binding [5] renders such possibility unlikely. Instead, our present findings suggest that the ability of TGFα to attenuate FSH hormonal action may be accounted for, at least in part, by action at site(s) proximal and distal to cAMP generation. Given that TGFα action is closely associated with the phosphorylation of a variety of cellular proteins [13], it is tempting to speculate that one or more membranous proteins concerned with the transduction of the FSH signal may be subject to TFGα-triggered phosphorylation, with consequent modification and inactivation. Whether TGFα is capable of modulating cAMP-PDE activity remains to be investigated.

Given the ability of high doses of progesterone to inhibit granulosa cell differentiation, consideration was also given to the possibility that TGFα enhanced progestin biosynthesis may result in the attenuation of the FSH effect. However, data not shown revealed that TGFα, like EGF, is equally capable of inhibiting FSH-supported progestin biosynthesis thereby suggesting that progesterone is not likely a mediator of the inhibitory TGFα action in this setting.

Although these and previous findings establish the granulosa cell as a site of EGF and TGFα action, their physiologic relevance remains uncertain. For one, the identity of the endogenous ligand capable of occupying the apparently common EGF/TGFα receptor remains elusive. Although circulating members of the EGF-urogastrone family may conceivably play a role in the activation of ovarian EGF/TGFα receptors, it is tempting to speculate local intraovarian provision of the endogenous receptor ligand. In this connection, recent studies suggest limited, albeit measurable prepro EGF gene expression at the level of the ovary [12]. In contrast, comparable information with respect to TGFα gene expression is unavailable at the present time. In either case, this putative ligand may be a membrane-bound protein member of the EGF family for which a role in the mediation of cell-cell communication has been proposed [27]. It is thus anticipated that future studies of the regulation of the EGF/

TGFα receptor and the elucidation of its ligand will shed new light on the relevance of this system to the process of follicular development.

Whereas the pivotal role of FSH in the acquisition of granulosa cell aromatase activity is well established (28), the role of growth factors in the modulation of this process continues to be elucidated. Thus far, two major classes of growth factor receptors have been implicated in this connection. On the one hand, the type I insulin-like growth factor (IGF) receptor has been observed to mediate the generally stimulatory effect of its ligands [29]. Indeed, not only was somatomedin-C/IGF-I found to amplify FSH-induced aromatase acquisition by murine [30] and porcine [31] granulosa cells, high doses of insulin proved equally stimulatory to rat [32] as well as human [33] granulosa cell aromatase activity. In contrast, EGF has been reported to inhibit murine [34] as well a porcine [8] granulosa cell aromatase activity. In this connection, our present findings provide further evidence in keeping with the inhibitory nature of the EGF/TGFα receptor as regards the regulation of estrogen biosynthesis. Given the apparently opposing roles of the IGF-I and EGF/TGFα receptor systems as regards the regulation of aromatase activity, it is tempting to speculate that the net aromatase activity acquired by the granulosa cell may reflect, at least in part, the balance struck between the opposing actions of the different classes of growth factors involved. According to this view, a complex series of growth factor interactions at the level of the granulosa cell may constitute one of several variables responsible for follicular selection and the assertion of follicular dominance.

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