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Gene Expression and the Physiological Role of Transforming Growth Factor-α in the Mouse Pituitary

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ABSTRACT—Transforming growth factor-α (TGF-α), a member of the epidermal growth factor (EGF) family, is produced within the mouse anterior pituitaries. However, the cell types of TGF-α-expressing cells and the physiological roles of TGF-α within mouse pituitary glands remain unclear. The aim of the present study was to localize TGF-α mRNA-expressing cells, and to clarify the involvement of TGF-α in estrogen-induced DNA replication in mouse anterior pituitary cells. Northern blot analysis demonstrated TGF-α mRNA expression in adult male and female mouse anterior pituitaries. *In situ* hybridization analysis of the pituitaries in these mice showed that TGF-α mRNA-expressing cells in the anterior pituitary are round, oval, and medium-sized. TGF-α mRNA was colocalized in most of the growth hormone (GH) mRNA-expressing cells, while only some of the prolactin (PRL) mRNA-expressing cells. DNA replication in the anterior pituitary cells was detected by monitoring the cellular uptake of a thymidine analogue, bromodeoxyuridine (BrdU) in a primary serum-free culture system. Estradiol-17β (E2) and TGF-α treatment increased the number of BrdU-labelled mammotrophs, indicating that E2 and TGF-α treatment stimulates the DNA replication in mammotrophs. Immunoneutralization of TGF-α with anti-TGF-α-antibodies nullified the E2-induced increase in DNA replication. RT-PCR analysis of TGF-α mRNA expression in ovariectomized female mice revealed that E2 increases TGF-α mRNA levels. These results indicate that the TGF-α produced primarily in the somatotrophs mediates the stimulatory effects of estrogen on the DNA replication of pituitary cells in a paracrine or autocrine manner.

Key words: pituitary, transforming growth factor-α (TGF-α), somatotroph, mammotroph, estrogen

INTRODUCTION

Many lines of evidence have shown that the development and function of pituitary cells are regulated by a paracrine or autocrine control with hormones, growth factors, and cytokines produced from the pituitary cells (Schwartz and Cherny, 1992; Denef, 1994; Takahashi, 1995; Renner *et al.,* 1996; Ray and Melmed, 1997; Schwartz, 2000). Among the various growth factors synthesized in the anterior pituitary glands, members of the epidermal growth factor (EGF) family appear to be involved in the regulation of corticotrophs and mammotrophs (Childs *et al.,* 1995; Fan and Childs, 1995; Oomizu *et al.,* 2000). Transforming growth factor-α (TGF-α) belongs to the epidermal growth factor (EGF) family and shares the EGF-receptor (EGF-R) with EGF (Marquardt *et al.,* 1983; Massague, 1990). TGF-α is produced in the pituitary glands of several species (Kudlow and Kobrin, 1984; Kobrin *et al.,* 1987; Lazar and Blum, 1992). TGF-α in the pituitary gland has been localized to certain cell types, most notably mammotrophs and somatotrophs (Kobrin *et al*., 1987). In rat pituitary cells, TGF-α mRNA is expressed in somatotrophs, gonadotrophs, and mammotrophs (Fan and Childs, 1995). Immunoreactive EGF-Rs have been observed in subsets of all cell types of rat pituitary secretory cells, but only in some pituitary cells (Fan and Childs, 1995; Armstrong and Childs, 1997). Borgundvaag *et al*. (1992) have reported that chronic estrogen treatment induces a concurrent increase in the pituitary weight and TGF-α production, concluding that TGF-α is involved in estrogen-induced pituitary growth. We have directly demonstrated that TGF-α treatment stimulates DNA replication in mammotrophs and corticotrophs *in vitro* (Oomizu *et al*., 2000). However, in mouse pituitaries, the cell types of TGF-

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α expressing cells are unknown, and the relationship between estrogen-induced DNA replication and TGF-α-induced replication is unclear. The aim of the present study was to identify the type of cells that express TGF-α mRNA and to clarify the mechanism of estrogen-induced DNA replication in the mouse pituitary. Expression of TGF-α mRNA was studied by Northern blot analysis. The types of cells expressing TGF-α mRNA were studied by *in situ* hybridization, and the effects of E2 on TGF-α synthesis were studied by reverse transcription-polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Animals

Adult male and female mice of the ICR strain (CLEA Japan Inc., Osaka, Japan) were housed in a temperature-controlled room with free access to the CE-7 commercial diet (CLEA Japan Inc.) and water. All animal care and experiments were carried out according to the Guidelines of Animal Experimentation, Faculty of Science, Okayama University, Japan.

Northern blot analysis

Total RNA from the anterior pituitaries of ovariectomized female mice was extracted using the method of Chomczynski and Sacchi (1987). Total cellular RNA was denatured by incubation at 65°C for 15 min with 1×MOPS, (0.02 M MOPS, 8 mM sodium acetate, 1 mM EDTA), 2.2 M formaldehyde and 50% (w/v) formamide. Total RNA (10 μg) was electrophoresed on a 1% agarose gel. The RNA was transferred to a nylon membrane (hybond-N+ Amersham, Arlington Hights, IL) by electroblotting (30 V, 16 hr). The transferred RNA was fixed with an UV lamp for 5 min. The membrane was prehybridized in 5×SSPE, 50% formamide, 5×Denhardt's solution, 10% dextran sulfate, and 1 mg/ml herring sperm DNA at 42°C for 2 hr. Hybridization was carried out at 42°C for 16 hr in hybridization buffer containing 50 ng [α-^{32}P]dCTP-labelled probe. After the hybridization, the membrane was washed with 1×SSC and 0.1% SDS at 42°C for 20 min and 0.5×SSC and 0.1% SDS at 42°C for 20 min. Hybridized signals were detected by autoradiography.

In situ hybridization analysis of TGF-α mRNA-expressing cells

Pituitaries were quickly fixed overnight in 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.6) at 4°C. They were then embedded in paraffin and sectioned at a thickness of 5 μm thick. The sections were digested with 10 mg/ml proteinase K (Merck, Darmstad, Germany) at 37°C for 30 min. The reaction of proteinase K was blocked by 0.2% (w/v) glycine in 0.01 M PBS. The sections were fixed with 4% paraformaldehyde in 0.01 M PBS and were incubated in 0.25% acetic anhydride in 0.1M triethanolamine for 10 min at room temperature. The mouse TGF-α cDNA probe (pcrmTGFα-ST1), rat GH cDNA probe, and rat PRL cDNA probe were labelled with digoxigenin (DIG)-11-UTP at 37°C overnight (20 hr) by random primer using the DIG DNA labeling and detection kit (Boehringer Mannheim, Germany). pBR328 plasmids were labelled and used as a negative control for *in situ* hybridization.

The sections were hybridized at 42°C overnight in a solution of 5×SSPE, 1×Denhardt's solution, 10% (w/v) sodium dextran sulfate, 50% (v/v) deionized formamide, 120 μg/ml denatured herring sperm DNA, and 0.3 ng/μl DIG-labelled probe. The slides were washed in 1×SSC at room temperature for 10 min followed by 1×SSC and 0.5×SSC at 45°C for 15 min each. The final wash was carried out in 0.2 × SSC at room temperature for 1 min. Nonspecific binding was blocked by 0.5% (w/v) BSA in TN buffer (0.1 M Tris-HCl, 0.3 M NaCl, pH 7.5) for 30 min. Anti-DIG-alkaline phosphatase conjugate (1:1000) was applied to the slides and kept at room temperature for 1 hr. The slides were washed twice in 0.2% (v/v) Tween 20 TN buffer for 10 min. Nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) were used as chromogens for hybridization detection. Color development was carried out for 20 hr at room temperature.

Preparation of cDNA Probes

A 502-bp mouse TGF-α cDNA clone (pcrmTGFα-ST1) was generated as follows. DNA fragments encoding a portion of mouse TGF-α was obtained from total RNA from ICR mice by RT-PCR. Oligonucleotide primers for TGF-α were designed based upon the mouse sequence and were synthesized by the Gibco BRL Custom Primers, Life Technologies Asia Pacific (Yokohama, Japan). The primer sequences and the location on the nucleotide sequences were as follows: TGF-α 5' (AGCCAGAAGAAGCAAGCCATCACT, 367–390), TGF-α 3' (CTCATTCTCGGTGTGGGTTAGCAA, 845–868). The cDNA fragments were subcloned into pGEM3zf(+). Clones were sequenced using fluorescent primers and an automated DNA sequencer, and were confirmed to be cDNAs encoding a portion of the mouse TGF-α.

The rat GH cDNA (790 bp) cloned to the *Hind* III site of the plasmid pBR322, and rat PRL cDNA (823 bp) cloned to the *Pst* 1 site of the plasmid pBR322 were kindly provided by Dr. J.A. Martial (University of Liege, Belgium).

Isolation of pituitary cells

Anterior pituitaries obtained from 2-month-old male mice were dissociated with 0.5% trypsin (0.5% w/v; DIFCO, Detroit, MI) as previously described (Oomizu *et al.,* 1998). Cell viability was checked using the trypan blue exclusion test and was usually found to be more than 95%. The isolated pituitary cells were suspended in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium without phenol red (DMEM/F12 medium, Sigma Chemical Co., St. Louis, MO) containing fetal calf serum (FCS, 10%, v/v; Gibco BRL, Life Technologies, Inc., Rockville, MD). The cells were seeded on poly-L-lysine-(Sigma Chemical Co.) coated plastic coverslips (diameter 13 mm; Sumitomo, Tokyo, Japan) in 24-well tissue-culture plates (Becton Dickinson, Lincoln Park, NJ) at a cell density of 5×10^5 cells/ml per well. The culture was allowed to proceed in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Exposure to a TGF-α-specific antibody

After a 3-day culture in DMEM/F12 medium containing 10% FCS, the pituitary cells were cultured in serum-free DMEM/F12 medium supplemented with hydrocortisone (100 μg/l), triiodothyronine (400 ng/l), transferrin (10 mg/l), bovine glucagon (10 ng/ml), parathyroid hormone (200 ng/l), insulin (1 μg/l), and sodium selenite (5 μg/l) for another 3 days. Each treatment was performed on three plastic coverslips. An antibody specific for human TGF-α (1 μg IgG, Ab-3, Oncogene Science, Uniondale, NY) was added to the medium (500 μl) for 5 days with estradiol-17β (E2, 10^{-9} M). The TGF-α antibody treated with excess human TGF-α (1 ng) for 30 min at room temperature was also added to the medium with E2. The medium containing the antibody and E2 was changed daily. After a 5-day exposure to the antibody specific to TGF-α, a 5-bromo-2'-deoxyuridine (BrdU) solution of the cell proliferation kit (Amersham) was added to the culture media (3 mg/ml) for 12 hr to assess DNA-replicating cells. The cultured cells were fixed in Bouin's solution for 30 min at room temperature, and immunocytochemical detection was performed as described previously (Oomizu *et al.,* 2000).

Quantification of the immunolabelled cells

Cells were observed under a light microscope at ×600 magnification. The fields were scanned across the coverslip to avoid overlap. In each field, immunopositive and immunonegative cells were counted, and more than 1000 cells were observed from each cov-

erslip. The percentages of BrdU-labelled cells indicate those of cells that had a densely labelled nucleus in relation to the total cells. Each group consisted of three coverslips.

Ovariectomy and estrogen treatment

Two-month-old female mice were ovariectomized under ether anesthesia. Seven days after the ovariectomy a single subcutaneous injection of E2 (Sigma Chemical Co.) was given at a dose of 250 ng in 0.05 ml of sesame oil. Ovariectomized female mice treated with sesame oil only served as controls. Pituitaries were obtained 48 hr after the E2 injection.

cDNA synthesis and PCR amplification

Total RNA (2 μg) in a final volume of 20 μl was subjected to reverse transcription using Superscript II Reverse Transcriptase (Gibco-BRL) with random primers according to the manufacturer's instructions. Each PCR was performed using primer pairs specific for TGF-α, as described, and primer pairs specific for β-actin, as based on a previous report (Matsuda and Mori, 1997). The size of the resulting DNA fragment following PCR amplification was 502 base pairs (bp) for TGF-α and 469 bp for β-actin. These primers generate PCR products of 502 bp for TGF-α and 469 bp for β-actin respectively. These primers were synthesized by Gibco BRL Custom Primers, Life Technologies Asia Pacific (Yokohama, Japan). PCR was performed using Takara Taq (Takara, Otsu, Japan) and a thermal cycler (Gene Amp PCR System 9700, Applied Biosystems, Branchburg, NJ). The conditions for PCR were as follows: after activation of DNA polymerase by a 20-sec incubation at 95°C, 40 cycles (for TGF-α cDNA) and 25 cycles (for β-actin cDNA) of reactions, including denaturation for 30 sec at 95°C and extension for 1 min at 60°C, followed by an additional extension for 10 min at 60°C.

A 10-μl aliquot of each reaction was electrophoresed on 2% agarose gel, stained with ethidium bromide, and photographed under ultraviolet rays.

Statistics

Data are presented as the mean±S.E.M and were analyzed by analysis of variance followed by Duncan's multiple range tests.

RESULTS

Detection of TGF-α mRNA in the anterior mouse pituitary by Northern blot analysis

Northern blot analysis using the mouse TGF-α cDNA probe revealed that a single band of TGF-α mRNA (4.7 kb) was expressed in the mouse anterior pituitary gland (Fig. 1).

In situ hybridization analysis of TGF-α mRNA-expressing cells

TGF-α mRNA-expressing cells were found to be evenly distributed throughout the anterior pituitary gland, but not the intermediate or posterior lobes (Fig. 2A). TGF-α mRNA-expressing cells were round, oval, and medium-sized (Fig. 2B). In control experiments for *in situ* hybridization, no signal was detected when DIG-labelled pBR328 probes were used as controls (Fig. 2C). In adult male pituitaries, TGF-α mRNA-expressing cells accounted for 65% (±2.1, n=3) of the total pituitary cells, and for 55% (±1.7, n=3) in adult female pituitaries.

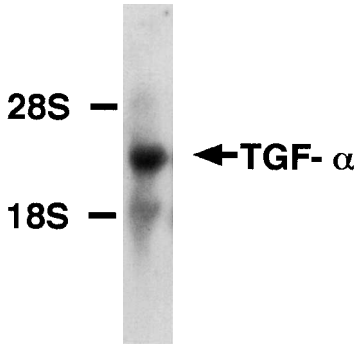

Fig. 1. Northern blot analysis of pituitary TGF-α mRNA. Total RNA (10 μg) obtained from adult female mice was electrophoresed on a 1% agarose gel and then transferred to a nylon membrane. The membrane was hybridized with ^{32}P-dCTP-labelled mouse TGF-α cDNA probes. The relative positions of the 28S and 18S ribosomal RNAs are shown.

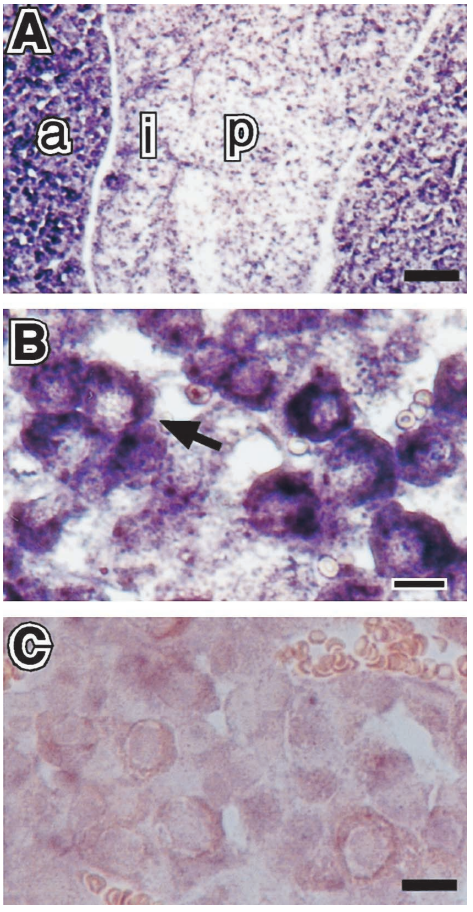

Fig. 2. TGF-α mRNA expression in the male mouse pituitary was analyzed by *in situ* hybridization. A: TGF-α mRNA-expressing cells were evenly distributed throughout the anterior pituitary. TGF-α mRNA-expressing cells were observed in a subpopulation of secretory cells in the anterior lobe (a), but not in the intermediate (i) or posterior lobes (p). Bar=50 μm B: TGF-α mRNA-expressing cells in the anterior pituitary (arrow) were round, oval and medium sized. Bar=10 μm C: *In situ* hybridization using pBR328 DIG-labelled probes detected no signals in the anterior pituitary cells. Bar=10 μm

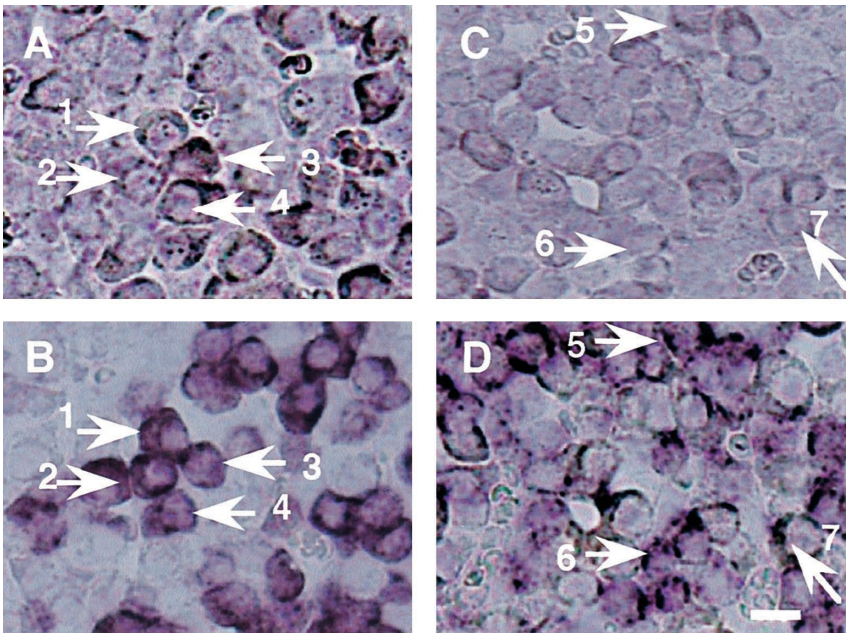

Fig. 3. Determination of TGF-α mRNA-expressing cell types by *in situ* hybridization using serial sections of the anterior pituitary glands from adult male (A and B), and female (C and D) mice. *In situ* hybridization was carried out using cDNA probes for TGF-α mRNA (A and C), GH mRNA (B) and PRL mRNA (D). Paired photographs of A and B, and those of C and D are adjacent sections obtained from the serial sections of mouse pituitary glands (5 μm thick), respectively. The cells (#1, #2, #3 and #4) expressed both TGF-α mRNA and GH mRNA. The cells (#5) expressed both TGF-α mRNA and PRL mRNA, while the cells (#6 and #7) expressed PRL mRNA, but not TGF-α mRNA. Bar=20 μm

Characterization of TGF-α mRNA-expressing cells

For the determination of the cell types of TGF-α mRNA-expressing cells, serial sections were studied by non radioisotopic *in situ* hybridization using cDNA probes for TGF-α mRNA (Fig. 3A and 3C), GH mRNA (Fig. 3B) and PRL mRNA (Fig. 3D). Most of the GH mRNA-expressing cells contained TGF-α mRNA (79–83%), whereas only a small population of PRL mRNA-expressing cells contained TGF-α mRNA (1–3%).

Effects of anti-TGF-α antibody treatment on DNA replication in pituitary cells

E2 treatment for 5 days significantly increased the percentage of BrdU-labelled cells ($p<0.01$). Treatment of pituitary cells with a combination of E2 and anti-TGF-α antibodies did not increase the number of BrdU-labelled cells (Fig. 4). The TGF-α antibody with excess TGF-α did not affect E2-induced increase in the percentage of BrdU-labelled cells.

RT-PCR analysis of TGF-α mRNA levels

To determine the effects of E2 on TGF-α gene expression, the relative TGF-α mRNA content was measured using semiquantitative RT-PCR. The technical validation of this assay is depicted in Fig. 5A,B. Various amounts of RT products (in volume, 0.5, 1, 1.5, 2 μl) for TGF-α and β-actin cDNAs were amplified to ensure linearity for the PCR-amplification (Fig. 5A). The PCR products for TGF-α mRNA and β-actin mRNA increased in accordance with the increase in RT products (templates). TGF-α mRNA levels were deter-

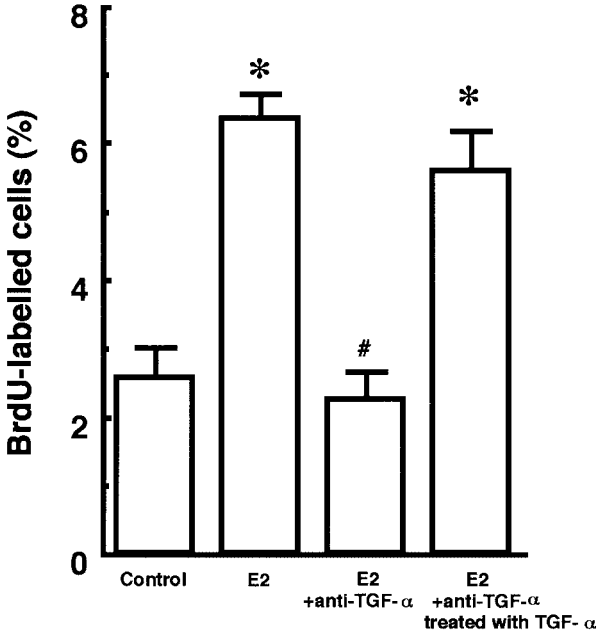

Fig. 4. Effect of E2 and anti-TGF-α antibody on the number of BrdU-labelled pituitary cells. After a 3-day culture in serum-free DME/F12 medium, an antibody specific for human TGF-α (1 μg IgG1) was added to the medium (500 μl) for 5 days with estradiol-17β (E2, 10^{-9} M). After the exposure to E2 and the antibody, BrdU solution was added to the culture media for 12 hr to assess DNA synthesizing cells. E2 treatment increased the number of BrdU-labelled cells compared with the control (*$p<0.01$). Treatment with a combination of anti-TGF-α antibody and E2 decreased the number of BrdU-labelled cells compared with E2 treatment (#$p<0.01$).

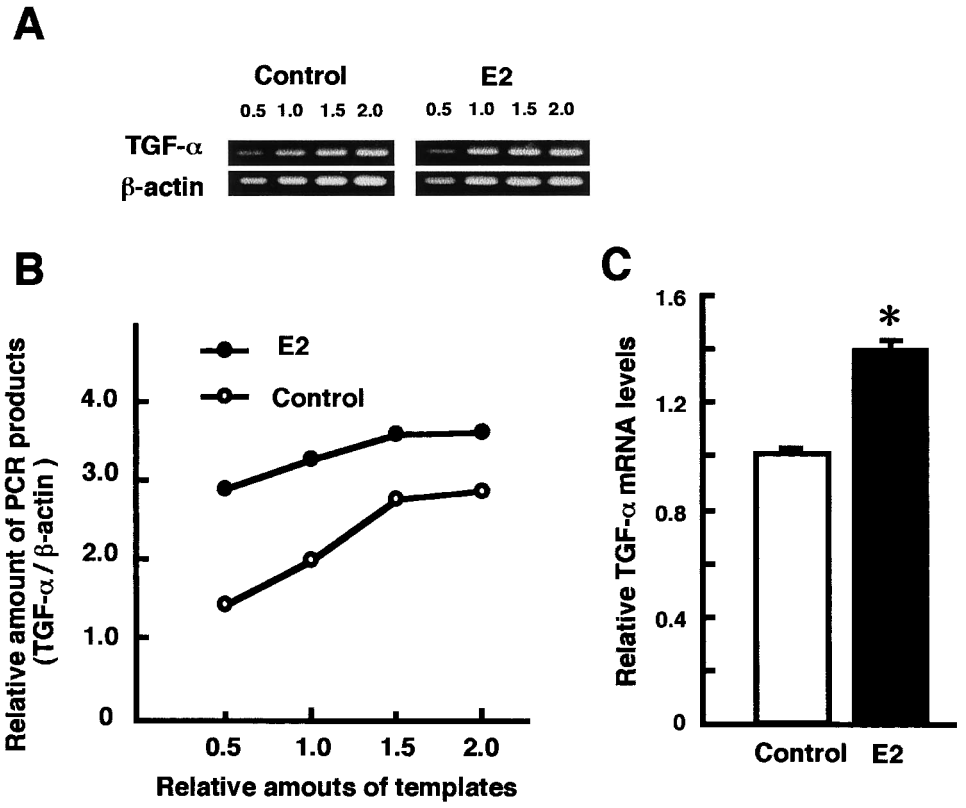

Fig. 5. Analyses of TGF-α and β-actin mRNAs amplified by RT-PCR. Effect of E2 treatment on the amount of TGF-α mRNA was studied in the pituitary cells of ovariectomized mice. E2 was subcutaneously injected to the ovariectomized mice at a dose of 250 ng/mouse. Pituitaries were obtained after 48 hr of the injection. RNA samples were collected and subjected to RT-PCR. A: The analysis of the linearity of TGF-α and β-actin cDNAs in relation to the amount of RT products. PCR amplification was carried out with various amounts of (0.5–2 μl) of the RT products (for TGF-α and β-actin) from controls and E2-treated mice. Each PCR product was electrophoresed on a 2% agarose gel, stained with ethidium bromide, and photographed under UV irradiation. The number above the gel pictures depict the amount of RT products (μl). The band intensities of TGF-α and β-actin cDNAs were increased with increasing amount of RT products. B: The analysis of quantiataive changes in the bands depicted in A. The relative amounts of TGF-α mRNA were normalized according to the β-actin mRNA levels in each sample. C: The summary of the change of TGF-α mRNA levels induced by E2 treatment. The TGF-α mRNA levels were normalized as performed in B, and the relative values were expressed by regarding the control value as 1.0. Each column represents the mean (±S.E.M) of three independent experiments. For the semiquantitative analysis of the relative amounts of TGF-α mRNA the band intensities of 1 μl of RT product were used. *p<0.01, significantly different from the control.

mined using 1 μl of RT product, and were normalized according to the intensity of the co-amplified β-actin cDNA value. PCR analysis under this condition revealed an increase in TGF-α mRNA levels in response to E2 treatment compared with the control treatment (Fig. 5C).

DISCUSSION

It is well known that estrogen stimulates the mammotroph proliferation (Lloyd *et al.,* 1975; Takahashi and Kawashima, 1987). We have recently shown that estrogen-induced growth is inhibited by treatment with RG-13022, a specific inhibitor for EGF receptors in adult mouse pituitaries. TGF-α treatment stimulates DNA replication of pituitary cells (Oomizu *et al.,* 2000). Accordingly, these results suggest that the estrogen-induced DNA replication of pituitary cells is mediated by TGF-α through the EGF-receptor signaling pathway. However, as far as we know, the cell types of TGF-α-expressing cells have not previously been studied in mouse pituitaries, and the mechanism of TGF-α leading to DNA replication within the pituitary gland has remained unclear. We have demonstrated in the present study that TGF-α mRNA is expressed only in the anterior lobe, but not the intermediate and posterior lobes. Treatment with an antibody specific for TGF-α appears to reduce estrogen-induced DNA replication, clearly indicating that TGF-α acts on mammotrophs as paracrine regulator for pituitary functions through EGF receptors.

We found a 4.7-kb band for TGF-α mRNA in the anterior pituitary by Northern blot analysis using mouse TGF-α cDNA. TGF-α mRNA of similar size has previously been detected in other mouse tissues (Tamada *et al.,* 1991). In the present study, TGF-α was detected primarily in somatotrophs and in some of the mammotrophs in the mouse pituitaries. We did not study the other types of pituitary cells for TGF-α mRNA expression. TGF-α mRNA-expressing cells made up 55–65% of anterior pituitary cells in the present study, and most of the somatotrophs expressed

TGF-α mRNA. It is well known that somatotrophs account for almost half of the total anterior pituitary cells (Sasaki and Iwama, 1988). Therefore, most TGF-α mRNA-expressing cells seem to be somatotrophs in mouse pituitaries. On the other hand, Kobrin *et al*. (1987) has localized immunoreactive TGF-α in mammotrophs and possibly in somatotrophs in the bovine anterior pituitary. In human pituitary TGF-α is localized in somatotrophs (Finley *et al.,* 1994). Fan and Childs (1995) have reported based on *in situ* hybridization results that somatotrophs, gonadotrophs, and mammotrophs express TGF-α mRNA in the rat pituitaries. The discrepancy in the cell types of TGF-α-mRNA expressing cells among these reports may in part be due to the difference in the animal species examined. In rat and mouse pituitaries, somatotrophs and mammotrophs are closely located and are distributed evenly throughout the anterior lobes of the pituitary glands. Based on these findings, it is very likely that the TGF-α produced in somatotrophs acts on adjacent mammotrophs in a paracrine manner to stimulate DNA replication of mammotrophs.

Pituitary TGF-α expression is regulated under the influence of hormones, neurotransmitters, and other growth factors (Kobrin *et al*., 1987; Borgundvaag *et al*., 1992). Acute estrogen treatment increases TGF-α mRNA levels in the mouse anterior pituitary. Similarly, chronic estrogen treatment stimulates TGF-α gene expression in rat pituitaries (Borgundvaag *et al*., 1992). Furthermore, blockage of TGF-α synthesis by treatment with antisense oligodeoxynucleotide for TGF-α mRNA decreases estrogen-induced DNA replication in mammotrophs (Oomizu *et al*., 2000). Altogether, these findings have led us to hypothesize that estrogen-induced TGF-α expression is involved in the DNA replication in pituitary cells.

Cell proliferation in the females has been found to fluctuate during the estrous cycle, with the highest activity being at estrus (Takahashi *et al.,* 1984; Oishi *et al.,* 1993). This high proliferation of mammotrophs depends upon ovarian estrogen, since ovariectomy decreases the high levels of mitotic activity observed at estrus (Takahashi *et al.,* 1984). As TGF-α expression is stimulated by estrogen, the high proliferation rate of mammotrophs at estrus may be produced by the enhanced TGF-α release induced by high estrogen titers during a proestrous day. Accordingly, it is necessary to study a change in TGF-α gene expression during the estrous cycle in female mice.

We have recently reported detecting EGF-R mRNA in the mouse pituitary (Honda *et al.,* 2000). Fan and Childs (1995) have also found that all subsets of rat pituitary cells express EGF-Rs. EGF-R expression changes with various conditions such as stress and the estrous cycle (Fan and Childs, 1995; Armstrong and Childs, 1997; Armstrong and Childs, 1997). Moreover, estrogen stimulates EGF-R levels in the rat uterus (Mukku and Stancel,1985). Similarly, estrogen treatment with E2 increases both EGF-R mRNA (Oomizu *et al*., 2000) and TGF-α mRNA levels in the mouse pituitary. Thus, estrogen appears to stimulate pituitary growth at the level of both TGF-α and EGF receptor production.

Roh *et al*. (2001) has shown that EGF-R signaling is required for the differentiation of pituitary cells only in early pituitary organogenesis. The overexpression of human TGF-α induced in transgenic mice accelerates the development of pituitary mammotrophic adenoma (McAndrew *et al.,* 1995). In pituitary tumor cells, TGF-α affects cell proliferation in either a stimulatory or inhibitory manner (Ramsdell, 1991; Finley and Ramsdell, 1994). Thus, TGF-α is involved in the growth of normal and tumor pituitary cells. However, it remains to be determined whether factors such as age and sex affect the TGF-α-induced proliferation of pituitary cells in mice.

In conclusion, we have shown in the present study that estrogen acts on somatotrophs to stimulate TGF-α synthesis, and that this TGF-α acts on pituitary cells to stimulate DNA replication. It is known that TGF-α stimulates the proliferation of mammotrophs (Oomizu *et al*., 2000). Therefore, these findings suggest that estrogen induced growth of mammotrophs is governed by at least the presence of TGF-α-producing somatotrophs.

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