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Involvement of transforming growth factor α in the release of luteinizing hormone-releasing hormone from the developing female hypothalamus

(trophic interactions/neuropeptide release/median eminence/prostaglandin E_2)

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ABSTRACT **Little is known about the presence of trophic factors in the hypothalamus and the role they may play in regulating the functional development of hypothalamic neurons. We have investigated the ability of epidermal growth factor (EGF) and transforming growth factor α (TGF-α) to affect the release of luteinizing hormone-releasing hormone (LHRH), the neuropeptide that controls reproductive development. We have also determined whether the genes encoding EGF and TGF-α are expressed in the prepubertal female hypothalamus. Northern blot analysis of poly(A)$^+$ RNA utilizing a single-stranded EGF cDNA probe failed to reveal the presence of EGF mRNA in either the hypothalamus or the cerebral cortex at any age studied (fetal day 18 to postnatal day 36). In contrast, both a complementary RNA probe and a double-stranded TGF-α cDNA recognized in these regions a 4.5-kilobase (kb) mRNA species identical to TGF-α mRNA. The abundance of TGF-α mRNA was 3–4 times greater in the hypothalamus than in the cerebral cortex. Both EGF and TGF-α (2–100 ng/ml) elicited a dose-related increase in LHRH release from the median eminence of juvenile rats *in vitro*. They also enhanced prostaglandin E_2 (PGE_2) release. The transforming growth factors TGF-β_1 and -β_2 were ineffective. Only a high dose of basic fibroblast growth factor was able to increase LHRH and PGE_2 release. Blockade of the EGF receptor transduction mechanism with RG 50864, a selective inhibitor of EGF receptor tyrosine kinase activity, prevented the effect of both EGF and TGF-α on LHRH and PGE_2 release but failed to inhibit the stimulatory effect of PGE_2 on LHRH release. Inhibition of prostaglandin synthesis abolished the effect of TGF-α on LHRH, indicating that PGE_2 mediates TGF-α-induced LHRH release. The results indicate that the effect of EGF and TGF-α on LHRH release is mediated by the EGF/TGF-α receptor and suggest that TGF-α rather than EGF may be the physiological ligand for this interaction. Since in the central nervous system most EGF/TGF-α receptors are located on glial cells, the results also raise the possibility that—at the median eminence—TGF-α action may involve a glial–neuronal interaction, a mechanism by which the trophic factor first stimulates PGE_2 release from glial cells, and then PGE_2 elicits LHRH from the neuronal terminals.**

The mitogenic peptide epidermal growth factor (EGF) and its structural and functional homolog, transforming growth factor α (TGF-α), have been reported to be present in brain (1–4). It appears, however, that under physiological conditions the ability of the central nervous system to synthesize EGF is limited, as suggested by the exceedingly low EGF mRNA levels detected in several brain areas with a sensitive RNA protection assay (5). Although the brain has been shown to contain an EGF immunoreactive material (1), not all of this substance(s) may correspond to authentic EGF, as judged by the inability of a specific two-site immunoassay to detect the peptide (6) and by the electrophoretic behavior of the cross-reacting substance, which differs substantially from that of EGF (7). In contrast, recent reports have unambiguously demonstrated the presence of both TGF-α and its mRNA in mammalian brain (3, 4, 8, 9).

Although EGF has been shown to be mitogenic for glial cells (10) and to have neuronotrophic activity (11), little is known about the possible involvement of EGF and/or TGF-α in the functional and developmental regulation of specific neuronal populations. Whether they have a role to play in neuroendocrine processes is equally unknown. The present experiments were undertaken to determine whether EGF and/or TGF-α are produced in the developing female hypothalamus and to assess the potential ability of these factors to affect the release of luteinizing hormone-releasing hormone (LHRH), the neuropeptide that controls sexual development. We have selected this line of inquiry because (*i*) the hypothalamus is central to neuroendocrine regulation, (*ii*) EGF may be able to affect the release of LHRH (12), and (*iii*) the morphological and functional development of the LHRH neuronal network that leads to the initiation of mammalian puberty may involve the participation of trophic factors of brain origin. A partial report of these results has appeared (9).

MATERIALS AND METHODS

Animals. Sprague–Dawley rats were purchased from Bantin and Kingman (Fremont, CA). They were housed in a room with controlled temperature (23–25°C) and a 14/10-h light/dark cycle (lights on from 0500 to 1900). Ad libitum access to tap water and pelleted food was provided.

Tissue Dissection. The medial basal hypothalamus (MBH) was dissected by two lateral cuts along the hypothalamic sulci—one caudal cut in front of the mammillary bodies and one rostral cut immediately behind the optic chiasm. The preoptic area (POA) was dissected by a transverse cut immediately behind the optic chiasm and two oblique cuts initiated at the lateral edges of the optic chiasm and converging to a point rostral to the decussation of the optic nerves. The depth of MBH and POA fragments was ≈1 mm.

RNA Preparation and Blotting. Total RNA was prepared by the guanidinium isothiocyanate/acid phenol method (13) as described (14). Poly(A)$^+$ RNA was isolated as reported (14, 15). RNA samples were size-fractionated in 1.1% agarose gels (16, 17), transferred to nitrocellulose membranes (Schlei-

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Abbreviations: TGF-α, TGF-β_1, and TGF-β_2, transforming growth factors α, β_1, and β_2; LHRH, luteinizing hormone-releasing hormone; PGE_2, prostaglandin E_2; EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; ME, median eminence; cRNA, complementary RNA; MBH, medial basal hypothalamus; POA, preoptic area.

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cher & Schuell) by capillary blotting (18), and the RNA was fixed by baking.

Probes. A 574-base-pair (bp) *Pst* I–*Pvu* II DNA fragment derived from the EGF cDNA pmegf-10 (ref. 19; provided by M. Selby and W. J. Rutter, University of California, San Francisco) was forced-cloned into the *Pst* I–*Sma* I sites of the phage M13mp9 and used to synthesize a single-stranded ^{32}P-labeled cDNA probe with a specific activity of 5×10^8 cpm/μg as described (14).

A double-stranded cDNA and a complementary RNA (cRNA) were used to detect TGF-α mRNA. The former, which is complementary to the 5′ end of rat TGF-α mRNA (ref. 20; provided by Timothy Rose, Oncogene, Seattle, WA), consisted of a 400-bp *Eco*RI–*Bgl* II DNA fragment cloned into the *Eco*RI–*Bam*HI sites of the plasmid pSP64. The fragment was isolated by *Eco*RI–*Hin*dIII digestion and was labeled with [^{32}P]dCTP by the random-primer method (21) to a specific activity of $6–8 \times 10^8$ cpm/μg of DNA. Linearization of pSP64-TGF-α cDNA with *Sac* II provided a template that was used to transcribe a 238-bp antisense RNA by using SP6 polymerase. The transcription procedure used, which routinely results in specific activities of 10^9 cpm/μg of RNA, has been described (14).

In one experiment the relative abundance of TGF-α mRNA in the juvenile hypothalamus was compared with that of the cerebral cortex. The levels found were normalized by using cyclophilin mRNA as a reference. Cyclophilin mRNA is constitutively expressed in brain (22) and was detected by hybridization of the blots to a 700-bp *Bam*HI-*Pst* I fragment of the cDNA p1B15 (23), labeled with [^{32}P]dCTP by the random-primer method to a specific activity of 5×10^8 cpm/μg.

Hybridization. Hybridization and washing of the blotted membranes was carried out as reported for single- (14) and double-stranded cDNAs (14, 17) and for cRNA probes (14). In brief, hybridization to cDNA probes was carried out for 16–18 h at 65°C in a 15-ml solution containing 1×10^6 cpm of ^{32}P-labeled probe per ml, 10× Denhardt's solution (0.2% Ficoll/0.2% polyvinylpyrrolidone/0.2% bovine serum albumin) 4× SSC (0.06 M sodium citrate/0.6 M sodium chloride, pH 7.0), poly(adenylic acid) at 10 μg/ml, denatured salmon sperm DNA at 100 μg/ml, 1 mM EDTA, and 1% SDS. Prehybridization was for 4–5 h in the same medium without the labeled probe. After hybridization the membranes were washed in 2× SSC/1% SDS for 1 h at room temperature, once in 0.1× SSC/0.1% SDS for 30 min at room temperature, and once in 0.1× SSC/1% SDS at 60°C for 30 min. Hybridization to TGF-α cRNA was for 18 h at 60°C in a 15-ml volume containing 1×10^6 cpm of ^{32}P-labeled TGF-α cRNA per ml, 50% formamide, 7.5× Denhardt's solution, 4× SSC, 1 mM EDTA, 100 μg of denatured salmon sperm DNA per ml, and 0.1% SDS. Prehybridization was for 4–5 h. After hybridization the membranes were washed five times at 65°C in 0.1× SSC/0.1% SDS, 30 min each time. After washes, the membranes were exposed to Kodak XAR-5 film at −70°C with two DuPont Cronex intensifying screens.

Median Eminence (ME) Incubations. MEs from 28-day-old female rats were microdissected and incubated *in vitro* at 37°C in a Krebs–Ringer bicarbonate buffer (pH 7.4) containing 4.5 mg of D-dextrose per ml as described (24). In all experiments the MEs were preincubated for 15 min, then for 30 min after a change of medium, and finally for 2 h with the growth factors of interest. At the end of this period, the medium was removed and assayed for LHRH and PGE_2 as reported (24). The LHRH assay utilizes ^{125}I-labeled LHRH and the polyclonal antibody HFU 60 (produced by H.F.U.) at a 1:25,000 dilution; the assay detects as little as 0.4 pg of LHRH per tube. The PGE_2 assay uses [^{3}H]PGE_2 (DuPont/NEN) as the ligand and the polyclonal antibody SC10-11/23 (provided by W. B. Campbell, Department of Pharmacology, University of Texas Health Science Center, Dallas) at a 1:8000 dilution. The assay detects 3.6–7.5 pg of PGE_2 per tube. All substances tested for LHRH and PGE_2 release were routinely assayed in each experiment and found not to interfere with either the PGE_2 or LHRH assays.

EGF (culture grade, 95% pure) was purchased from Collaborative Research; Recombinant TGF-α, TGF-β_1, and TGF-β_2 were the gifts of D. Twardzik (Oncogene); basic fibroblast growth factor (bFGF) purified from bovine pituitary glands was a gift from D. Gospodarowicz (Cancer Research Institute, San Francisco), and human recombinant bFGF was a gift from G. Shipley (Oregon Health Sciences University, Portland). The compound RG 50864 (provided by A. B. Schreiber, Rorer Central Research, King of Prussia, PA) was used to block the EGF receptor. RG 50864 belongs to a family of compounds that selectively blocks tyrosine kinase activity of the EGF receptor (25). The inhibitor was initially dissolved in ethanol and then diluted in the incubation medium to a final concentration of 60 μM, which was present throughout the entire incubation period. This concentration selectively blocks EGF receptor tyrosine kinase in cultured cells (26). To further ensure the specificity of the inhibitory effect of RG 50864 on EGF- and TGF-α-induced LHRH and PGE_2 release, experiments were carried out to determine if the inhibitor would block PGE_2-induced LHRH release. PGE_2 is thought to act directly on (or within) LHRH neurons to stimulate LHRH release (27). In one experiment, the MEs were exposed to RG 50864 for 45 min and then to PGE_2 for 30 min, still in the presence of the inhibitor. In the second experiment, the MEs were incubated with RG 50864 for 165 min—i.e., 15-min preincubation—followed by 30 min of additional preincubation and 2 h of incubation proper, to have the same conditions used when testing the effect of the inhibitor on EGF/TGF-α action. PGE_2 was added during the last 30 min of incubation.

To determine if the effect of EGF/TGF-α on LHRH release is mediated by prostaglandins, cyclooxygenase activity was inhibited by indomethacin (Merck Sharpe & Dohme). The blocker, which was present throughout the entire incubation period, was initially dissolved in 0.1 M sodium phosphate buffer (pH 8.0) and then in incubation medium to its final concentration.

Statistics. Differences in LHRH and PGE_2 release were analyzed by a one-way analysis of variance and the Student–Newman-Keul multiple comparison test.

RESULTS

Expression of EGF and TGF-α mRNAs in the Developing Female Hypothalamus. Hypothalami from animals at different developmental phases (fetal day 18, postnatal days 3, 12, 25, and 36) were analyzed for EGF mRNA expression with a single-stranded cDNA probe. At no age was EGF mRNA detected. Fig. 1*A* shows that EGF mRNA was readily detected in rat kidney and mouse submaxillary gland but not in the cerebral cortex or the MBH, even when substantial amounts of either poly(A)$^+$ RNA (15 μg) or poly(A)$^-$ RNA (25 μg) were used. In contrast, TGF-α mRNA was detected in both the MBH and POA with either a double-stranded cDNA (Fig. 1*B*) or an antisense RNA probe (Fig. 1*C*). Both probes detected a greater TGF-α mRNA abundance in neonatal (day 3) POA than in fetal (day 18) MBH. Although this may reflect a regional difference, it may be more related to age. We have found in other experiments that TGF-α mRNA levels increase during postnatal development in both regions (Y.Y.M. and S.R.O., unpublished data).

Since the effect of EGF and TGF-α on LHRH release was routinely examined in juvenile 28-day-old rats, an additional experiment was carried out to define the presence of TGF-α mRNA in the hypothalamus of animals of this age. Fig. 2

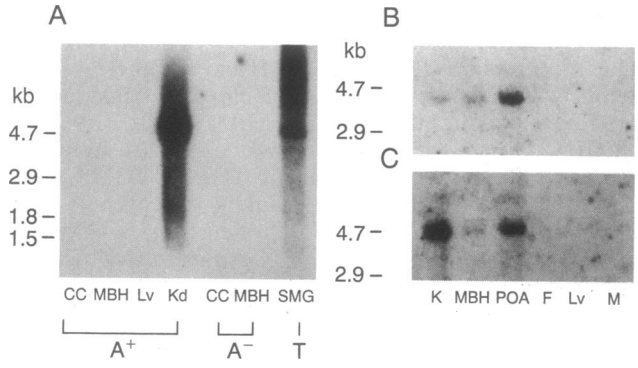

FIG. 1. (*A*) Absence of detectable levels of EGF mRNA in the hypothalamus (lanes MBH) and cerebral cortex (lanes CC) of juvenile female rats as determined by RNA blot hybridization of poly(A)$^+$ RNA (15 μg; lanes A$^+$) and poly(A)$^-$ RNA (25 μg; lanes A$^-$) to a ^{32}P-labeled single-stranded mouse EGF cDNA probe. Lane T contained total RNA at 1 μg. EGF mRNA is not expressed in rat liver (lane Lv) but is highly expressed in rat kidney (lane Kd) and mouse submaxillary gland (lane SMG). (*B*) Detection of TGF-α mRNA in the hypothalamus and POA of feto-neonatal female rats by RNA blot hybridization to a double-stranded rat TGF-α cDNA probe. All lanes have 5 μg of poly(A)$^+$ RNA. Lanes: K, human keratinocytes; MBH, MBH from 18-day-old rat fetuses; POA, POA from 3-day-old neonatal rats; F, human fibroblasts; Lv, rat liver; M, rat muscle. (*C*) The same blot depicted in *B* but hybridized to a rat TGF-α cRNA. kb, Kilobases.

shows that TGF-α mRNA is much more abundant in the hypothalamus (both the MBH and POA) than in the cerebral cortex. No expression was found in human fibroblasts, rat kidney, or rat liver. Unexpectedly, the TGF-α cRNA probe hybridized strongly to a 1.5- to 1.6-kb mRNA species in muscle, as recently reported by others in breast cell lines (28, 29). Cyclophilin mRNA levels were found to be equally elevated in the three brain areas studied but low and variable in the other tissues examined, in agreement with a previous report (22).

Effect of Growth Factors on LHRH and PGE$_2$ Release from the ME. EGF and TGF-α were equally effective in stimulating LHRH release from the ME of juvenile 28-day-old female rats (Fig. 3). The first significant increase was seen at the 10

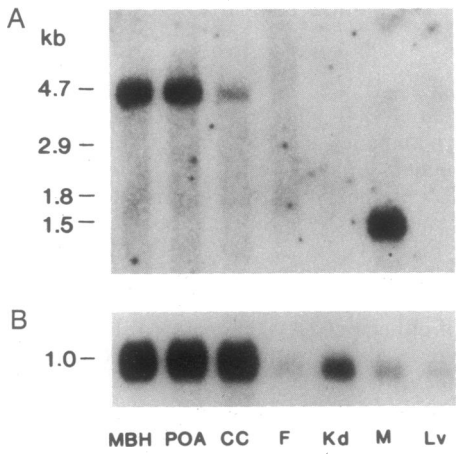

FIG. 2. (*A*) Detection of TGF-α mRNA in the hypothalamus of 28-day-old juvenile female rats by RNA blot hybridization to a rat TGF-α cRNA. (*B*) Levels of cyclophilin mRNA in the tissues depicted in *A*. Cyclophilin mRNA is constitutively expressed in brain and other tissues at different levels. All lanes were loaded with 5 μg of poly(A)$^+$ RNA. CC, cerebral cortex; F, human fibroblasts; Kd, kidney; M, muscle; Lv, liver. Notice the greater abundance of TGF-α mRNA in hypothalamic tissues than in the cerebral cortex, and the presence of a 1.5-kb mRNA species in muscle.

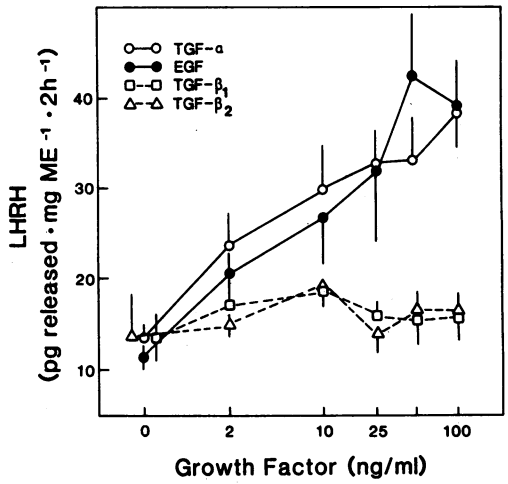

FIG. 3. Stimulation of LHRH release from ME fragments of 28-day-old juvenile female rats *in vitro* by EGF and TGF-α. Each point is the mean of four to eight individual MEs. Vertical lines represent the SEM.

ng/ml dose. Neither TGF-β_1 nor TGF-β_2, used as controls, was able to alter LHRH release. bFGF increased LHRH release only when used at a pharmacological dose (Fig. 4). This effect, however, could not be attributed to contaminants in the purified bFGF preparation used because it also occurred when recombinant bFGF was used. Both EGF and TGF-α stimulated PGE$_2$ release (Fig. 5), but a dose-related response was not observed, likely because only the outflow of prostaglandins into the incubation medium was measured. As in the case of LHRH, TGF-β_1 and -β_2 were ineffective, and bFGF stimulated PGE$_2$ formation only at the highest dose tested.

Effect of EGF Receptor Blockade on EGF and TGF-α-Induced LHRH and PGE$_2$ Release. RG 50864 decreased basal LHRH release and abolished both EGF- and TGF-α-induced LHRH release (Fig. 6 *Upper*). Likewise, the inhibitor eliminated the PGE$_2$ response to TGF-α [basal PGE$_2$ release (control; n = 5) = 53 ± 7 pg of PGE$_2$/mg of ME per 30 min; TGF-α-treated (100 ng/ml, n = 7) = 180 ± 27 pg of PGE$_2$/mg of ME per 30 min; TGF-α + RG 50864 (60 μM, n = 7) = 51 ± 8 pg of PGE$_2$/ mg of ME per 30 min]. In contrast, RG 50864 failed to affect the LHRH response to PGE$_2$, which was examined in two different experimental situations—namely, after short (45 min) and long (2 h and 45 min) exposures to the inhibitor (Fig. 6 *Lower*).

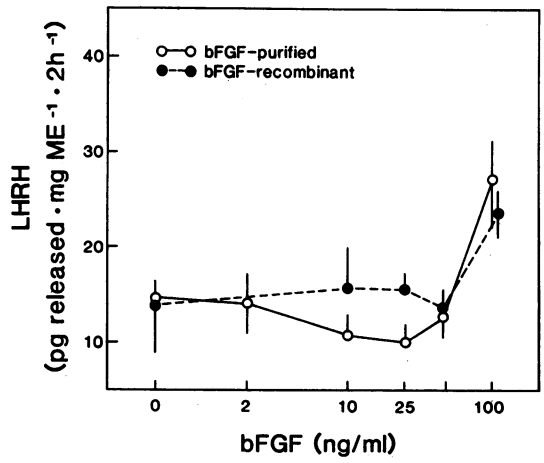

FIG. 4. Effect of bFGF, either purified from pituitary glands or recombinant, on LHRH release from the ME of 28-day-old female rats. Each point is the mean of four to eight individual MEs.

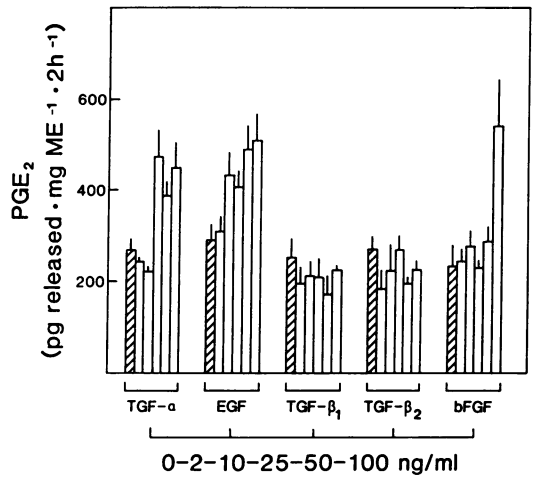

FIG. 5. Effect of different growth factors on PGE_2 formation from the ME of 28-day-old juvenile female rats *in vitro*. Hatched bars, basal release; open bars, release after growth-factor treatment. Each bar represents the mean ± SEM (vertical lines) of four to eight individual MEs.

Effect of Blockade of Prostaglandin Synthesis on TGF-α-Induced LHRH Release. *In vitro* exposure of MEs to indomethacin to block cyclooxygenase activity and, hence, PGE_2 synthesis eliminated the effect of TGF-α on LHRH release (Fig. 7).

DISCUSSION

Early studies have shown that the actions of EGF upon brain cells include stimulation of astrocyte proliferation (10) and promotion of cortical neuron survival and differentiation in culture (11). Nevertheless, the inability to detect authentic EGF and the very low levels of EGF mRNA found throughout the brain (5, 7) suggest that *in vivo* EGF actions may be shared by another, more abundant endogenous constituent of the brain. A likely candidate for this role is TGF-α, which shares 35% sequence homology with EGF, interacts with the same receptor (30), and is produced by brain cells (3, 4, 8). Although some evidence already exists supporting this concept (31), little (if anything) is known regarding the ability of EGF and/or TGF-α to affect mature, differentiated functions of specific neuronal subpopulations. Whether they participate in the regulation of central neuroendocrine functions is equally unknown.

The present study shows that EGF and TGF-α can act at the ME of the hypothalamus to elicit LHRH release. The detection of TGF-α mRNA in the developing hypothalamus, in contrast to the absence (or very low levels) of EGF mRNA found in this area, strongly suggests that TGF-α (and not EGF) is the peptide physiologically involved in LHRH secretion. Our failure to detect EGF mRNA in the hypothalamus should not be construed as a demonstration that EGF mRNA is not expressed in this area. Perhaps the mRNA levels are extremely low or their expression is limited to discrete cell groups. Solution hybridization assays and hybridization histochemistry may clarify this issue.

Stimulation of LHRH release by TGF-α appears to involve activation of EGF receptors, as indicated by the ability of RG 50864 to block both TGF-α and EGF-induced LHRH release. RG 50864 belongs to a family of arylidene derivatives recently described to inhibit the tyrosine kinase activity of the EGF receptor at concentrations 1/100th to 1/700th the concentration needed to block tyrosine kinase of the insulin receptor (25). Like insulin, EGFs mechanism of action involves both autophosphorylation of its receptor and tyrosine phosphorylation of a variety of cellular target proteins (32).

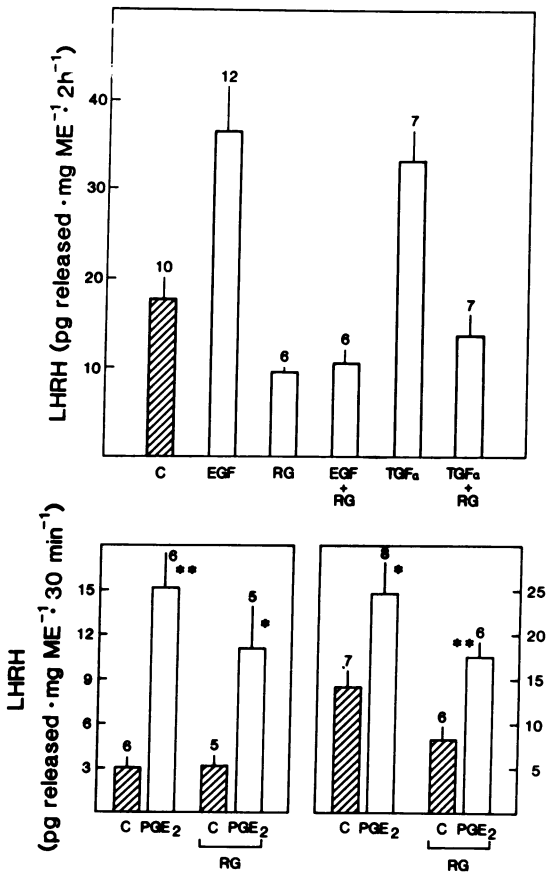

FIG. 6. (*Upper*) Effect of RG 50864 (RG), an inhibitor of the EGF receptor tyrosine kinase, on EGF and TGF-α (TGFα)-induced LHRH release from the ME of juvenile female rats *in vitro*. Each growth factor was tested at the concentration of 100 ng/ml; RG 50864 was used at 60 μM. (*Lower*) Failure of RG 50864 to block the stimulatory effect of PGE_2 on LHRH release from the ME. (*Lower left*) MEs were preincubated for 45 min with the inhibitor, followed by a 30-min incubation with PGE_2 in the presence of the inhibitor. (*Lower right*) MEs were treated with the inhibitor as in the case of the growth factors (i.e., for 2 h and 45 min), and the prostaglandin was added during the last 30 min of incubation. Hatched bars, basal release (control); open bars, release after growth-factor treatment. Numbers above bars are the number of MEs per group. Vertical lines show the SEM. *, $P < 0.02$; **, $P < 0.01$ vs. basal release (C).

An intact tyrosine kinase activity is essential for the biochemical effects of EGF to occur (33). The dose of RG 50864 used has been recently shown to inhibit EGF-stimulated [^{3}H]thymidine incorporation, receptor autophosphorylation, tyrosine phosphorylation of cellular substrates, and proliferation of cells in culture (26). Since this study showed that cells exposed for several days to 40–80 μM RG 50864 exhibited inhibition of EGF-independent proliferation, we performed additional experiments in which LHRH was stimulated with PGE_2 in the presence of RG 50864 to assess the specificity of the blocker in our short-term incubation system. RG 50864 failed to affect the stimulatory effect of PGE_2 on LHRH release, thus arguing against a general (or toxic) nonspecific effect of the inhibitor when antagonizing EGF/TGF-α-induced LHRH release. PGE_2 is an obligatory component of norepinephrine-induced LHRH secretion, which appears to act directly on (or within) LHRH neurons to enhance the release of the neuropeptide (27).

At this juncture it must be pointed out that long exposure to RG 50864 can inhibit DNA synthesis induced by platelet-derived growth factor (PDGF) in cultured cells (26). Our experiments do not rule out the possibility that the inhibitory

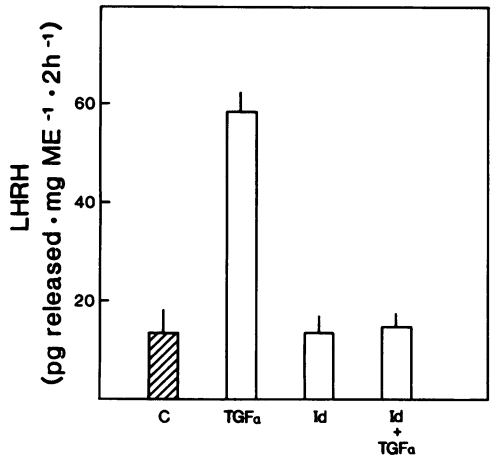

FIG. 7. Effect of blockade of prostaglandin synthesis with indomethacin (Id, 50 μM) on TGF-α (TGFα)-induced LHRH release from the ME of juvenile female rats. TGF-α was used at a concentration of 100 ng/ml. Each bar represents the mean ± SEM (vertical lines) of three to six individual MEs. C, basal release.

effect of RG 50864 on growth factor-induced LHRH release involves blockade of PDGF receptors.

A clue as to the mechanism by which EGF/TGF-α elicit LHRH release is provided by the findings that both peptides enhanced PGE_2 formation, and that blockade of prostaglandin synthesis completely obliterated the TGF-α effect. TGF-α may act directly on LHRH nerve terminals to stimulate PGE_2 and, hence, LHRH release in a genomic-independent manner. This possibility would be in keeping with our current understanding of PGE_2 action on LHRH release, according to which the prostaglandin is an intracellular messenger that acts within LHRH neurons to stimulate their secretory activity (27). However, the 2-h lag time needed for the TGF-α/EGF effect to become evident argues in favor of an indirect effect that may be mediated by cellular elements of the ME associated with LHRH nerve terminals. That these cells may be glia is suggested by the observations that most EGF receptors are found in glial cells (10, 34) and that astrocytes produce PGE_2 and other eicosanoids in response to neurotransmitter or neuropeptide stimulation (35, 36). Recently, a subpopulation of astrocytes has been shown to synthesize TGF-α, as evidenced by their immunoreactivity to TGF-α precursor antibodies (4). Moreover, we have found that hypothalamic lesions that result in precocious puberty cause activation of TGF-α gene expression to reactive astrocytes proliferating around the lesion (37). Infusion of RG 50864 to lesioned rats prevented the acceleration of sexual development, suggesting that the stimulatory effect of the lesion on puberty is mediated by TGF-α interacting with EGF/TGF-α receptors. Since TGF-α acts through both paracrine- and autocrine-mediated mechanisms (30, 38), it is plausible that the effect of the trophic factor on LHRH release may involve a two-step mechanism: an autocrine effect on glial cells/tanycytes to stimulate PGE_2 production and a subsequent effect of the prostaglandin on LHRH nerve terminals to elicit LHRH release. Although it is clear that further investigation will be required to validate this hypothesis, the close anatomical association of tanycytes and glial cells with LHRH nerve terminals in the ME (39) supports the feasibility of such interaction.

Taken altogether, the results provide evidence for an involvement of TGF-α in the developmental regulation of LHRH secretion. They also indicate that the mechanism by which TGF-α stimulates LHRH release involves interaction with EGF/TGF-α-like receptors and production of PGE_2 and raise the intriguing possibility that the PGE_2 intermediate is, at least in part, produced by glial elements of the ME.

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