# Effects of Estrogen and Epidermal Growth Factor on Prolactin and Pit-1 mRNA in $GH_3$ Cells (43526)

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*Abstract.* **The effects of epidermal growth factor (EGF) and 17β-estradiol ($E_2$) on the expression of prolactin (PRL), the transcription factor Pit-1/GHF-1 (Pit-1), and on dopamine $D_2$ receptor mRNA in $GH_3$ cells were analyzed by immunocytochemistry, *in situ* hybridization, and Northern analysis in a defined serum-free cell culture medium. Radioimmunoassay was used to determine PRL secretion. Both EGF and $E_2$ stimulated PRL mRNA and PRL secretion, although the effects of EGF were more rapid than those of $E_2$. Pit-1 mRNA levels were not significantly changed in spite of the 2- to 8-fold increases in PRL mRNA levels and significant increases in PRL secretion. Analysis of dopamine D2 receptor mRNA by *in situ* hybridization and Northern hybridization detected expression of dopamine receptor in $GH_3$ cells, but the receptor mRNA levels were not modified by EGF or $E_2$ treatment in complete serum or in serum-free media. These observations suggest that EGF and $E_2$ modulate PRL mRNA levels and PRL secretion significantly *in vitro*, while the mRNA levels of Pit-1 do not change significantly in $GH_3$ cells cultured in a defined culture medium.** [P.S.E.B.M. 1993, Vol 202]

Prolactin (PRL) gene expression in pituitary cells is regulated by various hormones and growth factors. Estrogens have been shown to stimulate PRL gene expression in normal and neoplastic pituitary cells both *in vivo* and *in vitro* (1–6). Whereas epidermal growth factor (EGF) has a stimulatory effect on PRL gene expression *in vitro* (7, 8), other hormones, such as dopamine, inhibit PRL gene expression in normal pituitary cells. However, pituitary tumors such as the $GH_3$ cell line are unresponsive to dopaminergic stimulation (9, 10). Recent studies have suggested that EGF can regulate dopamine receptor expression in $GH_3$ cells, leading to increased responsiveness to dopamine after *in vitro* treatment (11). This latter observation suggests that a growth factor such as EGF could modulate differentiation in a pituitary cell line as indicated by the ability of the $GH_3$ cells to respond to the PRL inhibitory hormone dopamine.

The isolation and physiologic characterization of the pituitary Pit-1/GHF-1 (Pit-1) transcription factor has provided some insights into the regulation of PRL and GH secretion by a transcriptional regulator that participates in the determination of pituitary-specific cell development and differentiation (12–16). A recent study has suggested that Pit-1 is needed not only for establishing and maintaining a differentiated phenotype, but is also important for cell proliferation in neoplastic pituitary cells (17). However, detailed analysis of Pit-1 mRNA expression *in vitro* has not been reported previously.

Because of the complexity of various factors in regulating PRL gene expression, the role of serum factors in influencing these interactions is not completely understood (18–20). Several workers have shown that serum contains factors that inhibit estrogen-sensitive functions in a number of cell types, including pituitary cells (18–20). The use of a serum-free defined medium can help to elucidate the role of specific hormones and growth factors in the regulation of pituitary cells. We performed experiments to analyze the effects of 17β-estradiol ($E_2$) and EGF in regulating the expression of PRL, Pit-1, and dopamine $D_2$ receptor genes in $GH_3$ cells in serum-free media in order to better understand the direct role of these interactions without the influence of unknown factors in serum. Pit-1 transcription factor expression was also analyzed to learn more

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about the regulation of this factor by $E_2$ and EGF, which are known to stimulate PRL mRNA.

## Materials and Methods

**Materials.** The GH3 cell clone derived from the rat MtT/W5 pituitary tumor (21, 22) was obtained from the American Type Culture Collection (Rockville, MD); epidermal cell growth factor from mouse submaxillary gland, 17$\beta$-estradiol, and 2-bromoergocryptine (BCR) were from Sigma Chemical Co. (St. Louis, MO); Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin-fungizone stock solution, horse serum, and fetal calf serum were purchased from Gibco Laboratory (Grand Island, NY).

**Cell Culture.** The GH3 cell line was grown in DMEM supplemented with 15% horse serum, 2.5% fetal calf serum, 5 $\mu$g/ml of insulin, and 1% antibiotics (100 units/ml of penicillin, 100 $\mu$g/ml of streptomycin, and 0.25 $\mu$g/ml of fungizone).

Before starting the experiments, the cells were treated with 0.25% trypsin, washed, and then plated in 35-mm plates at a concentration of $1 \times 10^5$ cells/plate and grown for 72 hr in DMEM supplemented with 15% horse serum, 2.5% fetal calf serum, 5 $\mu$g/ml of insulin, and 1% antibiotics. Three days later, the cells were incubated in serum-free DMEM without phenol red supplemented with 1× ITS+ (insulin, 6.25 $\mu$g/ml; transferrin, 6.25 $\mu$g/ml, selenium, 6.25 ng/ml; bovine serum albumin, 1.25 mg/ml; and linoleic acid, 5.35 $\mu$g/ml, from Collaborative Research, Bedford, MA). Additional supplements included 1.96 ng/ml of dexamethasone, 19.53 pg/ml of triiodothyronine, and 1% antibiotics. In some experiments, cells were treated with 100 ng/ml of EGF or $10^{-7}$ *M* $E_2$. Preliminary experiments examined 1 ng/ml, 10 ng/ml, and 100 ng/ml of EGF, and 100 ng/ml had the greatest effect on the $GH_3$ cells without affecting cell viability. The concentrations of $E_2$ were derived from previous experiments (23, 24). The medium was changed every other day. The conditioned medium was used for radioimmunoassay of prolactin on Days 1 and 7. Cells were harvested on Days 1, 3, and 7. Aliquots of cells were used to make cytospins for immunocytochemistry (ICC) and for *in situ* hybridization (ISH). The remaining cells were used for RNA extraction. In other experiments, to study the inhibition of BCR on PRL production, cells were treated with EGF or $E_2$ for 3 days and then the cells were treated with $10^{-6}$ *M* BCR for 24 hr. The medium was collected for radioimmunoassay of PRL, and the cells were harvested and used for ICC or ISH. In other experiments, after treatment with EGF or $E_2$ for 4 days, total RNA was extracted and hybridization analyses were performed for dopamine $D_2$ receptors.

**Immunocytochemistry and *In Situ* Hybridization.** ICC with antibody against the rat PRL obtained from the National Pituitary Agency was done with the avidin-biotin peroxidase system, as described previously (23–25). The results of ICC staining were analyzed by counting 500 cells/slide to determine the absolute positive percentage of PRL cells. The ISH procedure was done as described previously (23–25). Dopamine $D_2$ receptor oligonucleotide probe was purchased from NEN Research Products (Boston, MA). Antisense and sense *pit*-1 oligonucleotide probes were synthesized according to the published *pit*-1 cDNA sequence (15). The *pit*-1 antisense and sense probes had the following sequences 5′GAG CCG TGG ACA GCA, GCC, AGC, GCT, TCG, CCC AC-3′ and 5′ CTC TGA GAA TGC ACC ACA GTG CCG CTG AGT GTC T-3′ at nucleotides 510–541 and 157–184 of the mouse cDNA, respectively. All the probes were labeled with $^{35}$S-dATP using terminal deoxynucleotidyltransferase labeling followed by purification on a 12% polyacrylamide gel (26). For ISH, $2 \sim 3 \times 10^6$ cpm/slide were used. Positive cells had five or more silver grains per cell. The results were expressed as the percentage of PRL cells. Controls for ISH included pretreatment of the cells with RNAse A (100 $\mu$g/ml) for 60 min at 37°C before hybridization and using a sense oligonucleotide probe for Pit-1 in addition to the antisense probe.

**RNA Extraction and Northern Blot Analysis.** Total RNA was extracted from the cells by the method of single-step thiocyanate-phenol-chloroform extraction described previously by Chomczynski and Sacchi (27). Equal amounts of total RNA (40 $\mu$g) were electrophoresed on a 1% agarose gel and then transferred to nylon filters (26). Blots were baked for 30 min at 80° in a vacuum oven and exposed to UV light for 1.5 min. The blots were then prehybridized, and hybridized at 55°C with rat PRL cDNA probe that was labeled with $^{32}$P-dATP with the random prime method (28). After washing, the filters were exposed to x-ray film for 1 to 2 days at −70°C. The radioactivity was washed off and the same blots were rehybridized with rat Pit-1 cDNA probe and finally hybridized with a $\beta$-actin oligonucleotide probe. Poly $A^+$ RNA was prepared by purification on oligo deoxythymidine-cellulose from control and EGF-treated cells (26) (Pharmacia, Piscataway, NJ). Equal amounts of mRNA (4–8 $\mu$g) were run on a 1% agarose gel and transferred to the nylon filter and the blot was in turn hybridized with dopamine D2 receptor oligonucleotide and cDNA probes. The density of each band on the filters was measured by laser densitometry and the results were expressed as the percentage of the control.

**cDNA Probes.** Mouse Pit-1 cDNA cloned into Bluescript pK5 was kindly provided by Dr. Sally Camper (University of Iowa, Iowa City, IA) (15). A 672-bp *pit*-1 cDNA (from aa 47 to aa 269) was cloned into *Hind*III and *Bam*HI restriction sites. A 2.4-kb cDNA probe for rat dopamine D2 receptor was provided by Dr. O. Civelli (University of Iowa) (29). The

probe cloned into *Eco*RI restriction site of pGEM Blue covered the full-length coding sequence of the dopamine D2 receptor. PRL cDNA probe from Dr. R. Maurer (University of Iowa) was used as described previously (30).

Plasmids were amplified in large-scale bacterial culture, prepared by alkaline lysis method, and purified by ultracentrifugation in cesium chloride-ethidium bromide gradient. Inserts were purified by electrophoresis in 1% low-melting agarose gel (26).

**Other Methods.** Radioimmunoassay for PRL was performed as described previously (25). Statistical analysis was done by Student's *t* test.

## Results

**Effects of $E_2$ and EGF on PRL.** $GH_3$ cells cultured in a defined medium proliferated rapidly and there was a 10- to 15-fold increase in the number of cells in control dishes during the 7 days of culture. ICC staining and ISH analyses revealed a decrease in the percentages of PRL-positive cells in the control dishes during the culture period (Table I). Treatment with EGF and $E_2$ led to significant increases in the percentage of $GH_3$ cells expressing PRL hormone on Days 1, 3, and 7 for EGF and $E_2$. $E_2$ treatment did not lead to a significant increase in the percentage of cells expressing PRL mRNA on Day 1, while the percentages of these cells were increased on Days 3 and 7. EGF increased the percentage of cells expressing PRL mRNA on Days 1, 3, and 7 (Fig. 1). ISH was usually more sensitive than ICC in detecting PRL-positive cells (Table I).

Northern hybridization analysis revealed that the effects of EGF on PRL mRNA were more rapid than those of $E_2$, with a 3-fold increase by Day 1. The effects of EGF and $E_2$ on increasing PRL mRNA were 6- to 8-fold above control levels after 7 days in culture (Fig. 2).

$GH_3$ cells secreted large amounts of PRL into the medium and the rate of secretion decreased with the duration of culture in the defined culture medium

**Table I.** Regulation of PRL Expression in $GH_3$ Cells by EGF and $E_2$

| Treatment[b] | Percentage of PRL in $GH_3$ cells[a] | | | | | |
|---|---|---|---|---|---|---|
| | Day 1 | | Day 3 | | Day 7 | |
| | ICC | ISH | ICC | ISH | ICC | ISH |
| Control | 14 ± 3 | 30 ± 1 | 9 ± 2 | 25 ± 1 | 5 ± 2 | 15 ± 1 |
| EGF | 32 ± 3[c] | 49 ± 2[d] | 30 ± 3[d] | 34 ± 2[e] | 16 ± 3[c] | 27 ± 1[d] |
| $E_2$ | 29 ± 3[e] | 34 ± 2 | 26 ± 1[d] | 30 ± 2[e] | 21 ± 2[d] | 27 ± 1[d] |

[a] The data for $GH_3$ are absolute percentages of PRL cells.
[b] Mean ± SE for 10 slides were counted for each group from five different experiments.
[c] $P < 0.005$ compared with control-cultured cells.
[d] $P < 0.001$ compared with control-cultured cells.
[e] $P < 0.05$ compared with control-cultured cells.

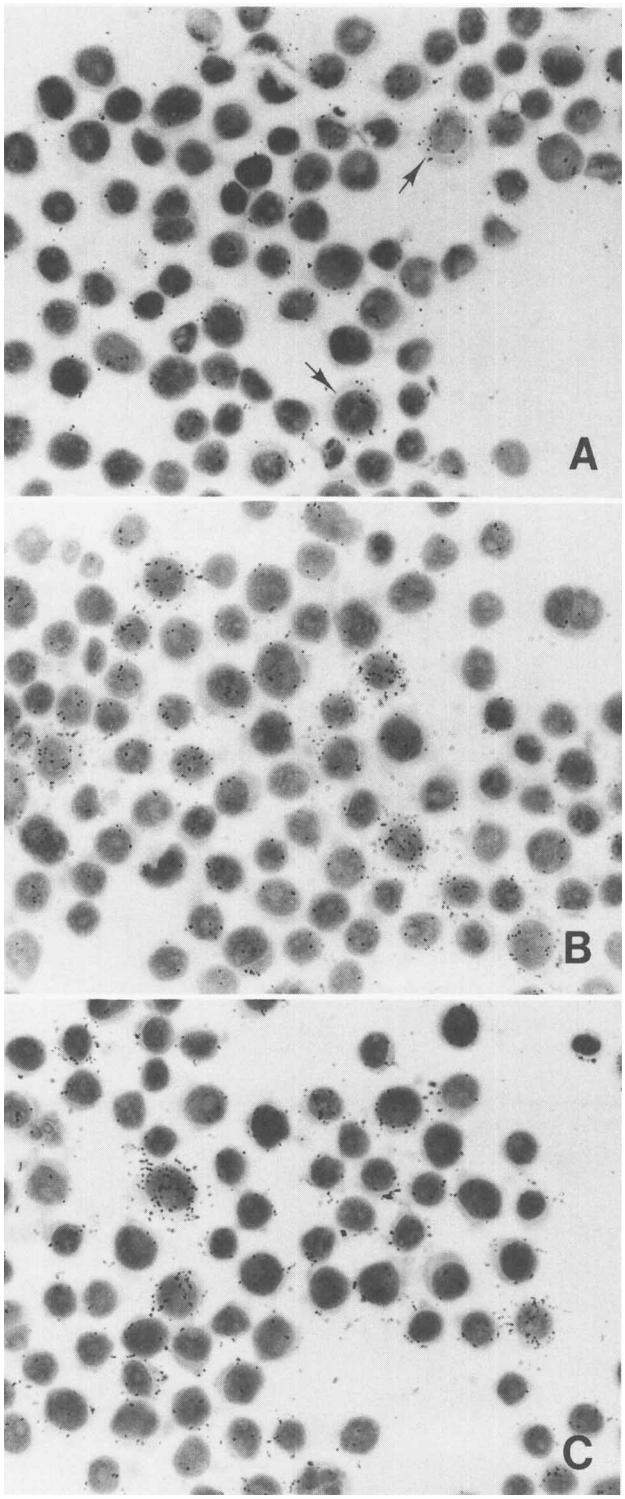


**Figure 1.** *In situ* hybridization analysis of PRL gene expression in $GH_3$ tumor cells cultured in a defined medium for 7 days. The (A) control sections show a few positive cells (arrows), whereas (B) EGF and (C) $E_2$ treatment led to increased numbers of silver grains per cell and increased numbers of positive cells for PRL mRNA (×400).

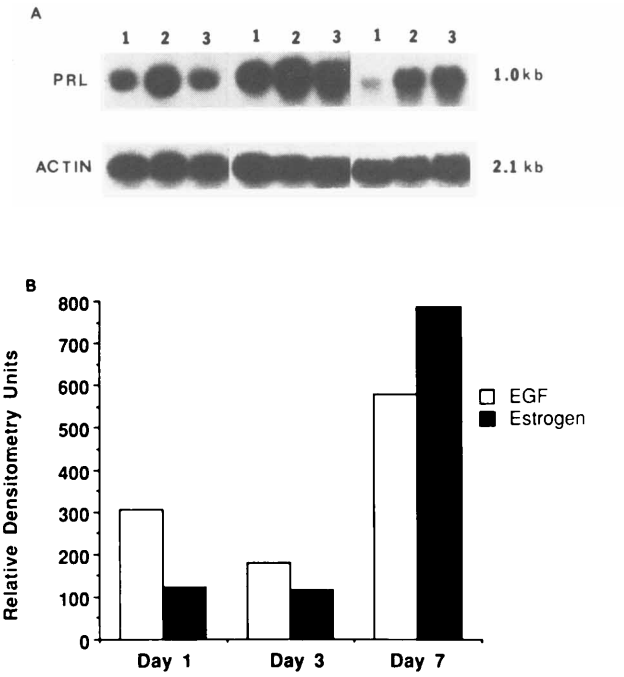


**Figure 2.** Northern hybridization analysis of PRL mRNA expression in cultured $GH_3$ cells. Upper panel: mRNA transcripts of 1.0 kb for PRL and 2.1 kb for actin were detected in $GH_3$ cells. Lane 1, control; Lane 2, EGF; and Lane 3, $E_2$ treatment. Total RNA was extracted and analyzed by electrophoresis on a formaldehyde agarose gel and transferred to nylon membrane. Forty micrograms of total RNA per lane were used. Equal loading of RNA was checked by hybridization with actin. Lower panel: the bands were quantified by densitometric analysis and expressed relative to the control.

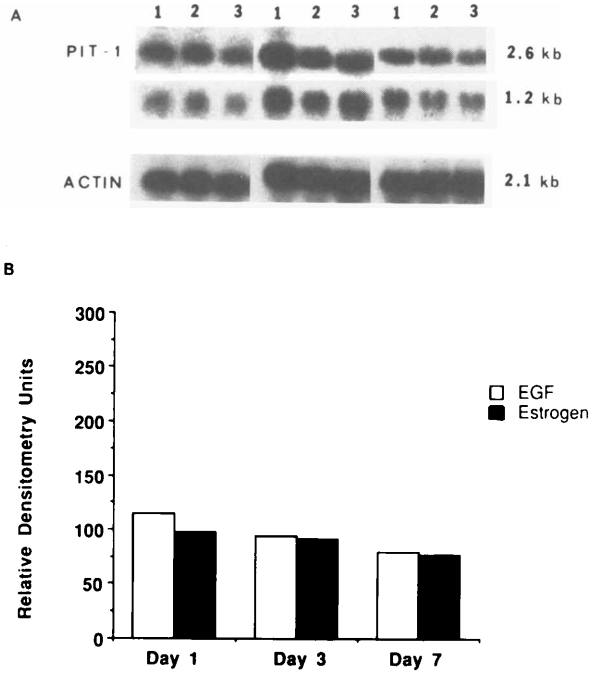


**Figure 3.** Northern hybridization analysis of Pit-1 mRNA expression in $GH_3$ cells. Upper panel: principal mRNA transcripts of 2.6 kb and 1.2 kb were detected in $GH_3$ cells. Forty micrograms of RNA per lane were used. Lane 1, control; Lane 2, EGF; and Lane 3, $E_2$-treated cells. Equal loading of each lane was checked with actin. Lower panel: densitometric analysis of the bands showed no significant change in Pit-1 mRNA levels after EGF and $E_2$ treatment relative to the controls.

**Table II.** Secretion of PRL from $GH_3$ Cells by EGF and $E_2$[a]

| Treatment | PRL release ($\mu$g/ml) | |
|---|---|---|
| | Day 1 | Day 7 |
| Control | 1.69 ± 0.35 | 0.47 ± 0.11 |
| EGF | 4.04 ± 0.90[b] | 1.04 ± 0.21[b] |
| $E_2$ | 2.83 ± 0.78 | 1.72 ± 0.44[b] |

[a] Values are mean ± SE from four experiments performed in duplicate.
[b] $P < 0.05$.

(Table II). EGF caused a significant secretion of PRL on Day 1, while both EGF and $E_2$ led to significant increases in PRL secretion on Day 7. Estrogen had a greater effect on PRL secretion on Day 7 compared with EGF. The combined use of EGF and $E_2$ did not have a synergistic effect on PRL mRNA levels or on PRL secretion (data not shown).

**Pit-1 mRNA Expression.** Hybridization studies of Pit-1 mRNA showed two major mRNA transcripts of 2.6 kb and 1.2 kb and a less intense 4.5-kb transcript. Densitometric analysis did not show any significant changes in the 2.6-kb transcript after treatment with EGF or $E_2$ on Days 1, 3, and 7 (Fig. 3), in spite of the 2- to 8-fold increase in PRL mRNA present after EGF and $E_2$ treatment. ISH revealed diffuse labeling of most $GH_3$ cells with the Pit-1 oligonucleotide probe, while the sense control had only a background hybridization signal (Fig. 4).

**Dopamine $D_2$ Receptor and Bromocriptine Treatment.** Northern hybridization analysis of dopamine D2 receptor mRNA in $GH_3$ cell line revealed a single 2.5-kb band (Fig. 5). The amount of the mRNA transcript did not change after EGF treatment for 4 days. Dopamine $D_2$ receptor was also detected by ISH in $GH_3$ cells. ISH analysis revealed diffuse labeling of most $GH_3$ cells for dopamine receptors. The hybridization signal was abolished by prior RNAse treatment (Fig. 6).

In order to determine whether EGF treatment led to a functional dopamine receptor in $GH_3$ tumor cells, the cells were treated with EGF or $E_2$ in serum-free medium and then treated with BCR for 24 hr. When the medium was assayed for PRL, there was no significant inhibition of PRL secretion by BCR treatment (Table III). ICC staining for PRL did not show any significant change in the percentage of PRL-positive cells (Table III). In another set of experiments, the effects of serum-free and complete media were compared with respect to EGF treatment followed by BCR treatment. There was no significant inhibition of PRL secretion by BCR treatment in serum-free or complete media (Table IV). ICC staining did not show any sig-

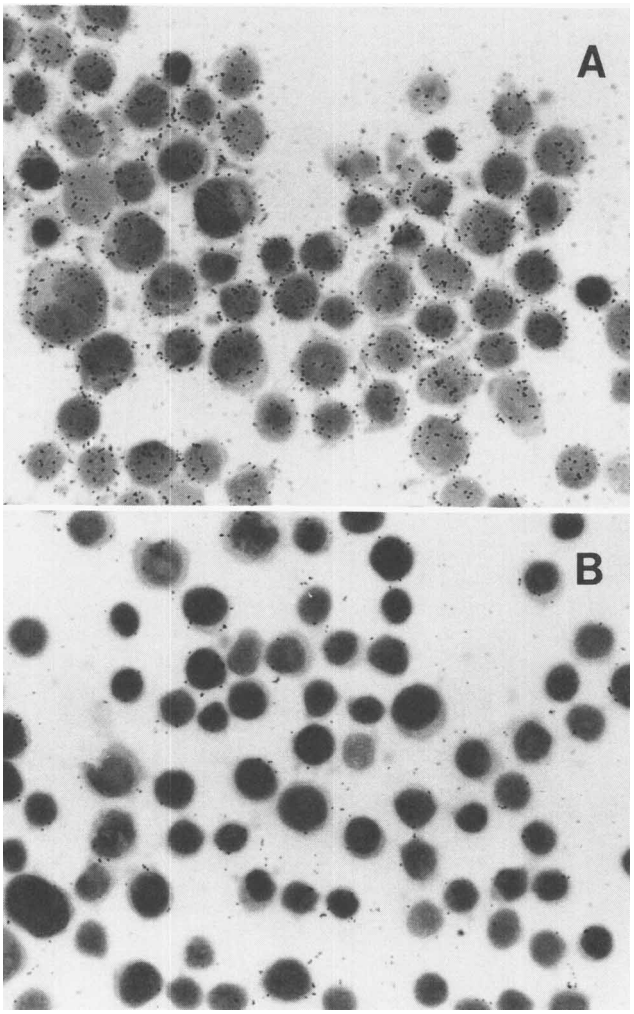


**Figure 4.** *In situ* hybridization showing Pit-1 mRNA expression in $E_2$-treated $GH_3$ cells. (A) Most of the cells show a positive hybridization signal. (B) The sense control probe did not produce a positive hybridization signal (×400).

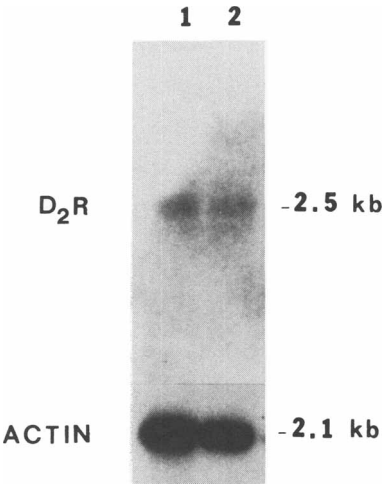


**Figure 5.** Northern hybridization analysis of dopamine $D_2$ receptor in $GH_3$ cells. Each lane contains 4.8 $\mu$g of polyA + RNA. Lane 1, control, and Lane 2, EGF-treated cells. Actin was used to check for equal loading in each lane. There was no increase in the D2 receptor mRNA after EGF treatment.

nificant change in the percentage of PRL positive cells (Table IV).

## Discussion

Most analyses of estrogen regulation of PRL gene expression *in vitro* have utilized complete serum or serum treated with dextran-coated charcoal (stripped serum). Recent studies have shown that stripped serum can decrease PRL mRNA after 1 week in culture (19). Other studies have shown that serum inhibits proliferation in many cultured cells, including pituitary cells (18). Other workers have used serum-free medium in studies of pituitary cell lines to select pituitary cell populations with specific nutritional requirements (31, 32). Riss *et al.* (32) found that $GH_4C_1$ and $GH_3$ cell lines required supraphysiologic concentrations of triiodothyronine initially in serum-free medium, but that the thyroid hormone requirement could be reduced in serial passages. We used a serum-free defined medium to characterize the effects of EGF and $E_2$ on PRL gene regulation in the $GH_3$ cell line. Our results show that EGF has a more rapid effect on regulating PRL synthesis and secretion, while $E_2$ has a slower and more sustained effect on regulating PRL mRNA levels. The immunocytochemical, ISH, Northern hybridization, and secretion data were in good agreement which indicates that these effects were mediated at the level of transcription, hormone storage, and PRL secretion. The effects of EGF on the stimulation of PRL mRNA levels in $GH_4$ cells are rapid, with a maximum effect observed in less than 18 hr (8). EGF was found to stimulate the transcription of the PRL gene by DNA sequences present in the 5′-portion of the prolactin gene (7, 8). EGF also decreased the rate of cell proliferation while stimulating PRL synthesis and inhibiting GH synthesis in the $GH_4C_1$ cell line (33, 34). Various studies have shown that estrogen also regulates the synthesis of EGF in mouse uterine tissues (35, 36). In the pituitary, the effects of estrogen on cell proliferation and on PRL expression have been shown to occur by independent mechanisms (19, 20). The present studies indicate that in our defined cell culture system, which did not contain serum, both estrogen and EGF have a stimulatory effect on PRL mRNA and PRL secretion, supporting a direct effect of these factors on the regulation of the PRL gene. Estrogen has also been shown to exert a regulatory effect on PRL gene methylation in various pituitary transplantable tumors and in normal pituitary cells, indicating a direct effect of $E_2$ on the PRL gene (30).

Although the transcription factor Pit-1 has been shown to be essential for specific pituitary cell development (13–17) and may play a role in pituitary cell proliferation (17), our *in vitro* studies indicated that Pit-

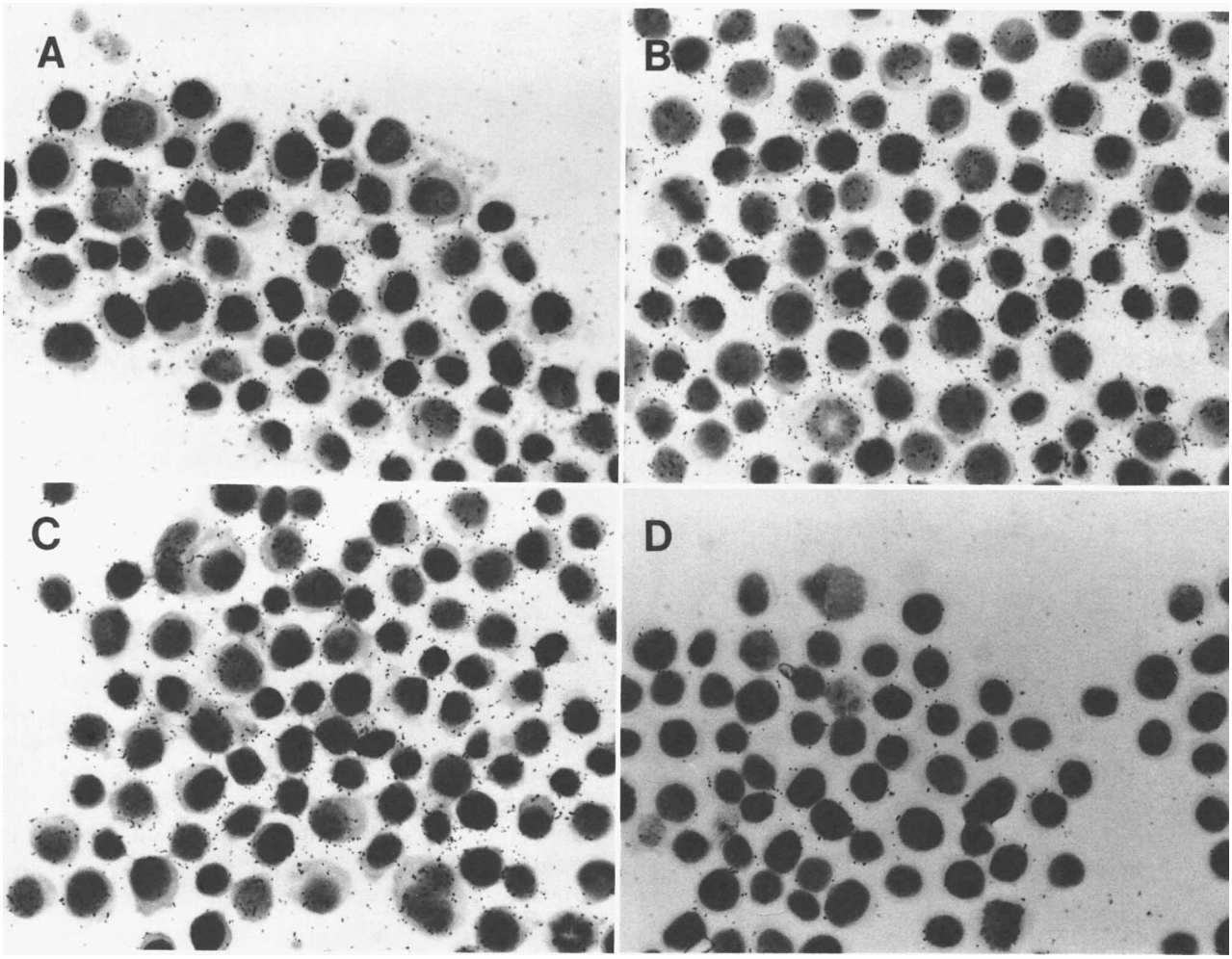


**Figure 6.** *In situ* hybridization showing dopamine $D_2$ receptor in $GH_3$ cells. There is diffuse labeling in all of the cells. The (A) control section had similar numbers of silver grains per cells as (B) EGF- and (C) $E_2$-treated cells. (D) Ribonuclease A treatment before hybridization eliminated the positive hybridization signal (×400).

**Table III.** Effects of BCR, EGF, and $E_2$ on the Secretion of PRL and on Immunoreactive PRL in $GH_3$ Cells[a]

| Treatment | PRL secreted (μg/ml) | ICC percentage PRL |
|---|---|---|
| Control | 1.25 ± 0.07 | 12 ± 1 |
| Control + BCR | 1.18 ± 0.10 | 13 ± 1 |
| EGF | 5.01 ± 0.71 | 26 ± 3 |
| EGF + BCR | 5.71 ± 0.67 | 31 ± 1 |
| $E_2$ | 5.27 ± 1.69 | 30 ± 2 |
| $E_2$ + BCR | 6.53 ± 1.25 | 32 ± 3 |

[a] Values are mean ± SE from three experiments performed in triplicate for PRL secretion and three experiments performed in duplicate for ICC analysis. Statistical comparison of control versus control + BCR, EGF versus EGF + BCR, or $E_2$ versus $E_2$ + BCR revealed no statistical differences.

**Table IV.** Effects of BCR and EGF on the secretion of PRL and on Immunoreactive PRL in $GH_3$ Cells in Serum-Free and Complete Medium[a]

| Treatment | PRL secreted (μg/ml) | ICC percentage PRL |
|---|---|---|
| Serum-free medium | | |
| Control | 3.68 ± 0.66 | 13 ± 0.4 |
| Control + BCR | 4.99 ± 2.35 | 13 ± 0.5 |
| EGF | 10.82 ± 5.01 | 33 ± 2 |
| EGF + BCR | 8.95 ± 3.74 | 33 ± 2 |
| Complete medium | | |
| Control | 4.35 ± 1.58 | 17 ± 0.5 |
| Control + BCR | 5.02 ± 0.89 | 16 ± 2 |
| EGF | 13.51 ± 5.44 | 31 ± 2 |
| EGF + BCR | 10.90 ± 3.79 | 35 ± 1 |

[a] Values are mean ± SE from two experiments performed in triplicate for PRL secretion and two experiments performed in triplicate for ICC analysis. Statistical comparison of control vs control + BCR and EGF vs EGF + BCR showed no significant differences.

1 mRNA levels were relatively unchanged by both EGF and $E_2$ in spite of a 2- to 8-fold change in PRL gene expression. Because Pit-1 protein, which is known to bind various regions of the 5′-proximal promoter region of the PRL and GH genes, was not analyzed directly in these studies, it is possible that the levels of this protein change more rapidly in modulating PRL gene expression while the mRNA is more stable. Ingraham *et al.* (12) reported a decrease in Pit-1 mRNA transcripts in estrogen-treated rat pituitary tissues. However, the duration of treatment and the concentration of $E_2$ used was not specified in these reports. We have also found a decrease in Pit-1 mRNA transcripts in normal and transplantable pituitary cells *in vivo* (16), so other factors may be contributing to the regulation of Pit-1 mRNA levels that were not present in our defined cell culture system.

Dopamine has an important role in the regulation of pituitary functions; however, the $GH_3$ cell line does not have functional dopamine receptors (9). Our finding that the dopamine D2 receptor mRNA is expressed in $GH_3$ cells does not explain the lack of functional dopamine D2 receptor in these cells, since the mRNA transcript was similar to that of normal pituitary cells in this study and in a recent report (10). Some investigators reported that EGF may also induce functional expression of dopamine receptors in the $GH_3$ cell line (11). We used the dopamine agonist BCR, which inhibits pituitary PRL secretion, to determine whether this drug would inhibit functional dopamine receptors in the $GH_3$ cell line after treatment with EGF *in vitro*. We had observed previously that in normal pituitary and in the MtT/W15, transplantable tumor estrogen down-regulated dopamine receptor protein, although the dopamine receptor in MtT/W15 was also not functional (37). After treatment with EGF for 4 days, BCR did not have an inhibitory effect on PRL secretion as measured by radioimmunoassay, which suggests no changes in the functional status of the dopamine D2 receptors. Our study also indicates that EGF treatment did not alter the amounts of dopamine receptor mRNA transcripts when analyzed by Northern and *in situ* hybridization analyses, in spite of a rapid increase in PRL mRNA after EGF treatment. The difference between our study and that of effect of Missale *et al.* (11), who reported that EGF elicited major effects in the functional expression of dopamine receptors in the $GH_3$ cell line, may be related to the analysis of a different parameter to determine functional dopamine receptor, since they measured the biochemical level of the receptor and used quinpirole to inhibit the receptor, or the differences may be related to the use of horse and fetal calf serum in the culture media, or possibly to the specific $GH_3$ cell line used. Additional studies with EGF and other growth factors and hormones in defined cell culture medium will be needed to further analyze the role of EGF in regulating dopamine D2 receptors in $GH_3$ cells.

In summary, we have used a defined serum-free cell culture system to analyze the effects of $E_2$ and EGF on PRL, Pit-1, and dopamine mRNA in $GH_3$ cell lines. Our results indicate that $E_2$ and EGF can influence PRL mRNA directly in the absence of serum factors and that Pit-1 mRNA levels remain unchanged in spite of marked changes in PRL mRNA levels.

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