Estrogen Stimulates both Prolactin and Growth Hormone mRNAs Expression in the MtT/F4 Transplantable Pituitary Tumor (42989)

LONG JIN, JIANGYUE SONG, AND RICARDO V. LLOYD
Department of Pathology, University of Michigan Medical Center, Ann Arbor, Michigan 48109

Abstract. **The effects of 17β-estradiol (E_2) on MtT/F4 pituitary tumor growth and on prolactin (PRL) and growth hormone mRNA expression were analyzed in F344 female rats. E_2 (10 mg) stimulated pituitary PRL cell hyperplasia and PRL mRNA, but inhibited growth of the transplantable tumors. The expression of both PRL and growth hormone mRNA levels was increased in the MtT/F4 tumors. The effects of E_2 on increasing PRL mRNA levels were more marked in the pituitary compared with the tumors. These results indicate that estrogens stimulate proliferation and PRL expression in the pituitary while inhibiting cell proliferation in the MtT/F4 tumor. E_2 also stimulated both growth hormone and PRL mRNA expression in the MtT/F4 transplantable tumor.**

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Estrogens are known to cause anterior pituitary prolactin (PRL) cell hyperplasia and pituitary tumors in rats (1–5). Recent studies of transplantable pituitary tumors such as the MtT/W15 (6) and the MtT/F4 (7, 8) have indicated that estrogens inhibit the growth of these pituitary tumors. Our recent studies of the MtT/W15 tumors indicated that estrogens inhibited tumor growth, and produced an increase in the expression of growth hormone (GH) protein and messenger RNA (mRNA) while decreasing the expression of PRL protein and mRNA both *in vivo* and *in vitro* (9–12).

In this report we analyzed the effects of chronic estrogen treatment on tumor growth and on GH and PRL mRNA and protein expression in the MtT/F4 pituitary tumors to determine whether a similar modulation of hormone gene expression and inhibition of tumor growth are present in this transplantable pituitary tumor.

Materials and Methods

Animals. Forty-day-old Fischer 344 female rats (Harlan Sprague-Dawley, Indianapolis, IN) were implanted with a 2-mm^3 portion of transplantable MtT/F4 tumor in the right flank. A silastic tube with 10 mg of 17 β-estradiol (E_2) was placed sc in one group of animals. The animals were sacrificed 4 weeks after the tumors became palpable (2 months after implantation for control groups, 3 months after implantation for the E_2-treated group). The tumors and pituitaries were excised, weighed, and used for immunochemical *in situ* hybridization and Northern hybridization studies.

Immunochemistry and Hybridization Studies. Immunochemistry with antibodies against rat PRL and rat GH obtained from the National Pituitary Agency and against ACTH (Dako, Carpinteria CA) was done with the avidin-biotin peroxidase system as described previously (9–12).

In situ hybridization with synthetic oligonucleotide probes against rGH and rPRL labeled with sulfur-35 was used with frozen tissue sections fixed in paraformaldehyde as described previously (11). Aliquots of these tissues were used to extract the RNA by cesium chloride gradient ultracentrifugation. Northern hybridization studies were performed with the same oligonucleotides used for *in situ* hybridization labeled with phosphorus-32 as described previously (11, 12). The same blots were used to probe for rPRL and rGH with washing in between experiments as reported previously (11, 12).

Quantification of Data. The results of immunocytochemistry staining were analyzed by counting 1000 cells/slide to determine the percentage of PRL and GH cells. *In situ* hybridization data were quantified by grain counting of 100 cells/animal from random photographs which were magnified ×1100 and the results were expressed as the mean grain count (MGC):

$$\text{MGC} = (\text{number of silver grains/number of nuclei}) \times (\text{weeks of exposure}).$$

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The *in situ* hybridization slides were usually exposed for 1 week before developing. The background hybridization seen in liver tissue was subtracted from the pituitary tissue samples. Northern hybridization data were analyzed by measuring the optical density of the autoradiograms with a LKB ultrascan XL from Pharmacia LKB Biotechnology, Inc. and the results were expressed relative to the control autoradiogram.

Statistical analysis was done with the Student's *t* test.

Results

Tumors in the control rats became palpabel 3 weeks after implantation and there was a latent period of approximately 2 months before the tumors were palpable in the E_2-treated rats. The pituitary glands were markedly enlarged after E_2-treated (Table I). The enlarged adrenal glands in both groups indicated that the tumors were also secreting ACTH. The adrenal weights were generally related to tumor size, suggesting production of more ACTH by the larger tumors.

Immunochemical studies showed a 2-fold increase in PRL cells and a relative decrease in the GH cells in the pituitary (Table II). Tumors from E_2-treated rats also showed an increase in the percentage of immunoreactive PRL and GH cells. Immunoreactive ACTH was present in the tumors from control and E_2-treated rats. There was no significant difference in the percentage of ACTH cells in these two groups (data not shown).

Analysis of the pituitary by *in situ* hybridization revealed a significant increase in the PRL mRNA reflected by MGC while there was a relative decrease in the GH mRNA (Figs. 1 and 2). The MtT/F4 tumors had a significant increase in both PRL and GH mRNA (Table II and Figs. 3 and 4).

Controls for *in situ* hybridization included hybrid-

Table I. Body and Organ Weights of Control and Estrogen-Treated Rats with MtT/F4 Tumors[a]

Group	Body weight (g)	Tumor weight (g)	Pituitary weight (mg)	Adrenal weight (mg)
Control	156 ± 6	7.6 ± 1.3	6.5 ± 0.5	308 ± 90
17β-estradiol	168 ± 13	3.6 ± 2*	110 ± 13***	203 ± 110

[a] Data are expressed as mean ± SE for $n = 4$ rats/group.
* $P < 0.05$, *** $P < 0.001$.

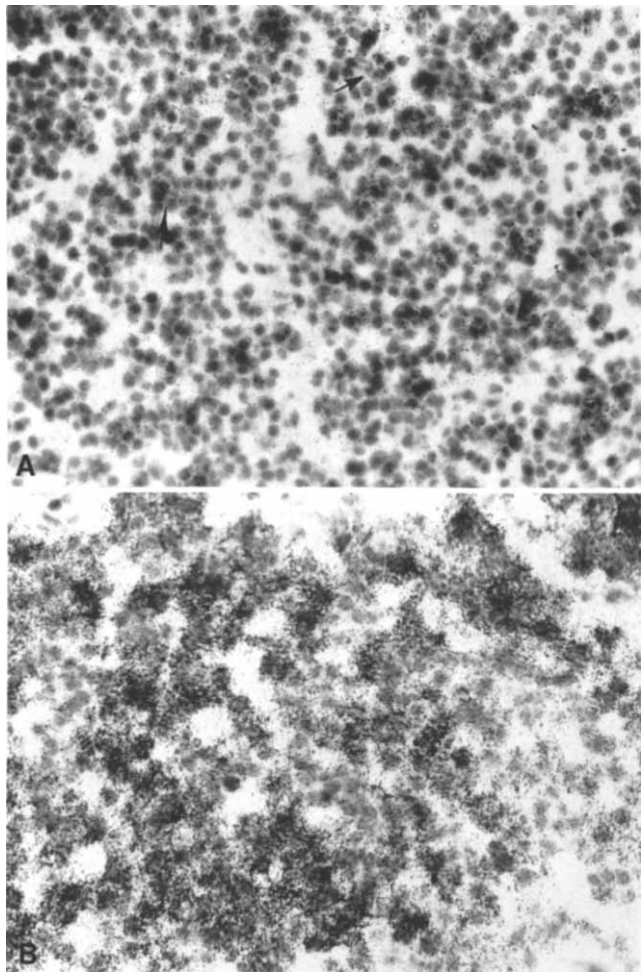

Figure 1. *In situ* hybridization with an ^{35}S-labeled rPRL oligonucleotide probe. The normal pituitary (A) shows a positive hybridization signal in some cells (arrows). After E_2 treatment (B) there is marked PRL cell hyperplasia and a significant increase in PRL mRNA expression (original magnification ×300).

Table II. Results of Immunochemical and *In Situ* Hybridization Analyses of Pituitary Tumors in Rats with MtT/F4 Tumors[a]

Group	Pituitary				Tumor			
	ICC[b] (% cells)		ISH (MGC)		ICC (% cells)		ISH (MGC)	
	PRL	GH	PRL	GH	PRL	GH	PRL	GH
Control	32 ± 1	39 ± 1	7.6 ± 0.8	15.6 ± 0.7	5 ± 1	4 ± 0.5	5.6 ± 0.7	6.9 ± 0.1
17β-estradiol	62 ± 2***	7 ± 0.4***	60 ± 5***	7.9 ± 0.7***	23 ± 2***	14 ± 3*	12.5 ± 2*	21.7 ± 4**

[a] Data are expressed as mean ± SE for n = 4. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control. The distribution of immunoreactive cells was determined by counting 1000 cells after immunochemical staining for PRL or GH and the results were expressed as the percentage of the total cells.
[b] ICC, immunocytochemistry; ISH, *in situ* hybridization.

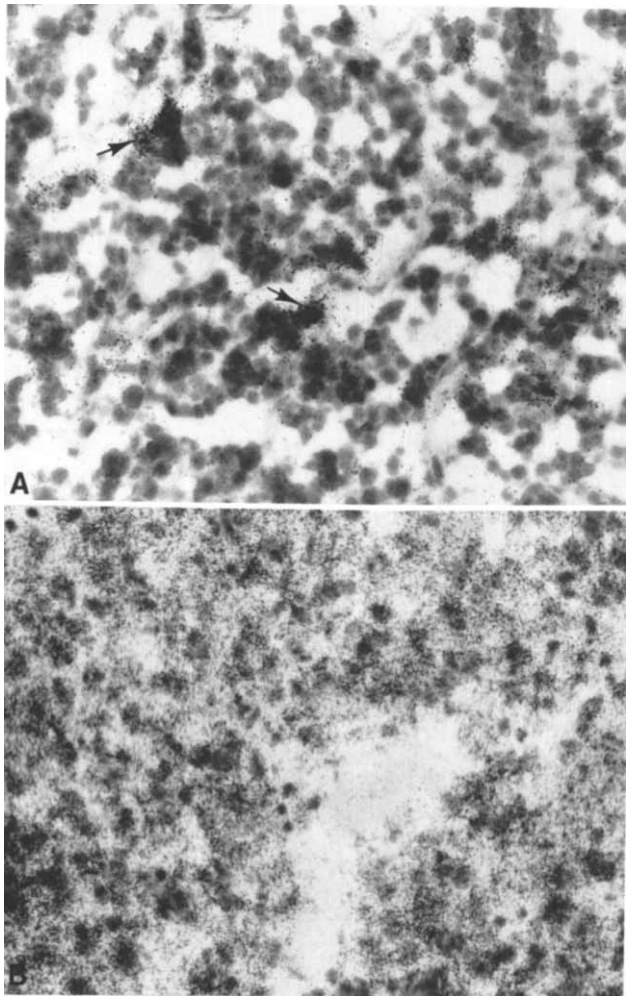

Figure 2. MtT/F4 tumor showing focal cells expressing rPRL mRNA (A). After E_2 treatment there was an increase in the number of cells expressing rPRL mRNA (B) (original magnification ×300).

ization in rat liver tissue with ^{35}S-rat GH and ^{35}S-rat PRL probes which produced no hybridization signal above background. Pretreatment of the tissue with RNAse (100 mg/ml for 60 min at 37°C) before *in situ* hybridization reduced the hybridization signal to background levels (data not shown).

Northern hybridization studies showed that the pituitary had a 14-fold increase in PRL mRNA while the GH mRNA showed a relative decrease after E_2 treatment probably secondary to PRL cell hyperplasia (Fig. 4). Rat PRL and rGH mRNA migrated as a single species at approximately 1 Kb. Interestingly, the pituitary levels of PRL mRNA were 8-fold higher in a normal pituitary from F344 rats without a tumor, compared with pituitary PRL mRNA levels from tumor-bearing rats. The pituitary GH mRNA levels were similar to the pituitary of a rat without a tumor and rats with tumors from these groups (Fig. 5). The MtT/F4 tumors showed a 2-fold increase in PRL mRNA and a larger increase in GH mRNA after 3 months of E_2 treatment (Fig. 5).

Discussion

Estrogens are known to have a regulatory effect on the normal rat pituitary and on transplantable pituitary tumors. In this study we showed that E_2 inhibited the growth of the MtT/F4 tumors and modified the levels of PRL and GH mRNA expression. Our recent studies of the MtT/W15 tumor showed that E_2 inhibited tumor growth while stimulating GH mRNA levels and inhibiting PRL mRNA levels (11). The MtT/F4 tumors also showed stimulation of GH mRNA and protein after E_2 treatment in the present experiments. However, in contrast to the MtT/W15 tumor, PRL gene expression was also stimulated. Previous studies by Trouillas *et al.* (8) found an increase in immunoreactive PRL in MtT/F4 tumors after 17β-estradiol treatment. The present studies have expanded on these observations by showing that the mRNA for PRL and GH are also increased after treatment with 17β-estradiol. These observed changes in mRNA levels due to alterations in GH and PRL mRNAs could occur at the level of transcription

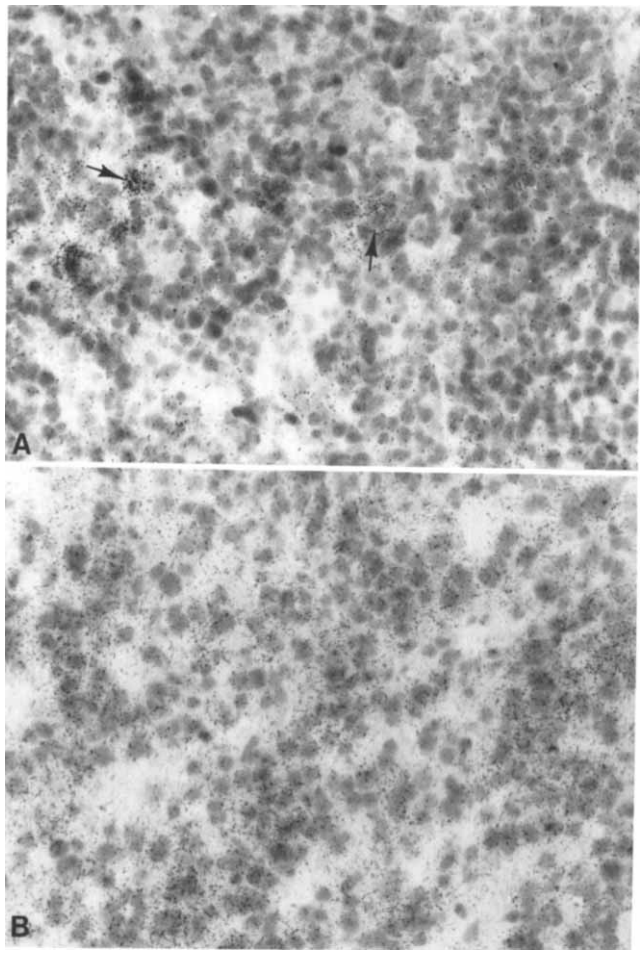

Figure 3. MtT/F4 tumor showing focal expression of rGH mRNA (arrows) (A). After E_2 treatment there was a significant increase in the number of cells expressing rGH mRNA (B) (original magnification ×300).

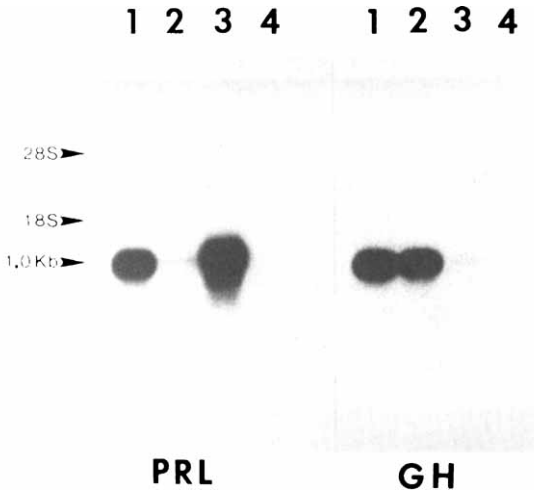

Figure 4. Northern blot hybridization with ^{32}P-labeled oligonucleotide probes for rPRL and rGH. Lane 1 represents pituitary RNA from a rat without a transplantable tumor while lane 2 shows pituitary RNA from a rat with a transplantable tumor. Lane 3 represents pituitary RNA from a rat with a transplantable tumor treated with E_2. Lane 4 shows rat liver RNA used as a control. 10 micrograms of total RNA were used in each lane. The rPRL mRNA in Lane 2 is markedly suppressed. E_2 treatment led to an 14-fold increase in pituitary PRL mRNA and a relative decrease in GH mRNA in the rat without a transplantable tumor.

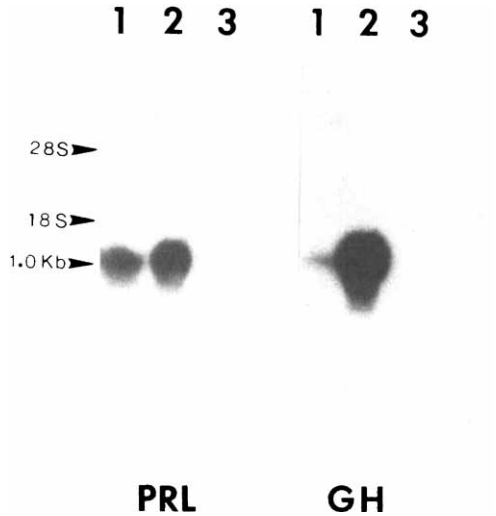

Figure 5. Northern hybridization study of MtT/F4 tumors. Lane 1 represents tumor from an untreated rat while Lane 2 shows a tumor from a rat treated with E_2. Lane 3 represents rat liver RNA. Thirty micrograms of total RNA was used in each lane. There was a 2-fold increase in rPRL mRNA and a 26-fold increase in rGH mRNA after E_2 treatment.

or may be related to modifications in the half lives of the mRNAs (14–17).

The pituitary of rats treated with E_2 underwent PRL cell hyperplasia with an increase in PRL mRNA as previously reported (1–6). Interestingly, the levels of PRL mRNA in the pituitaries of tumor-bearing animals were lower than levels from rats without tumors. This observation suggests that the high circulating levels of PRL from the MtT/F4 tumor may have inhibited pituitary PRL mRNA synthesis. Other workers have observed that pituitary GH mRNA from rats with MtT/W15 was suppressed and that this suppression was related to the length of time the animals had the transplantable tumor (13). The GH mRNA levels in our experiments were not suppressed probably due to the relatively low levels of GH secretion by MtT/F4 tumors and the possible antagonistic effects of ACTH and glucocorticoids on GH in these rats (18, 19).

The mechanisms by which estrogens increase expression of PRL and GH mRNAs in the MtT/F4 tumor are unknown. Possible mechanisms of increased gene expression might involve (i) changes in the pattern of methylation of the PRL and GH genes (20, 21), (ii) stimulation of specific transcription factors such as Pit-1/GHF-1 which may regulate expression of GH and PRL specifically in these tumors (22, 23), or (iii) changes in the estrogen receptor protein and mRNA levels which could lead to modifications of PRL and GH protein and mRNA expression and inhibition of tumor growth. The elucidation of the possible mechanisms involved must await further experimental work. Current work in our laboratory is directed at exploring these possible mechanisms. The experimental evidence to date indicates that there is a paradoxical effect of estrogens in stimulating pituitary PRL cell proliferation while inhibiting the proliferation of transplantable tumors such as MtT/W15 and MtT/F4.

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