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Regulation of Prolactin Gene Transcription *In Vivo:* Interactions Between Estrogen, Pimozide, and α-Ergocryptine

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SUMMARY

A single injection of pimozide, a dopamine antagonist, rapidly stimulated prolactin (PRL) gene transcription in male rats, whereas an injection of α-ergocryptine, a dopamine agonist, rapidly inhibited PRL gene transcription. Pretreatment with cycloheximide blocked the induction of PRL gene transcription by pimozide but had no effect on the inhibition of transcription by ergocryptine. The interactions between ergocryptine and 16α-estradiol, an estrogen that stimulates PRL gene transcription through two independent mechanisms, were also examined. Pretreatment with ergocryptine had no effect on the ability of 16α-estradiol to stimulate PRL gene transcription through a mechanism that is most probably mediated directly by the anterior pituitary estrogen receptor. However, ergocryptine pretreatment did block the ability of 16α-estradiol to stimulate transcription through a second, indirect, mechanism. This ergot alkaloid also blocked the ability of pimozide to stimulate PRL gene transcription. Pretreatment with 16α-estradiol had no effect on the ability of ergocryptine to inhibit PRL gene transcription, indicating that this estrogen did not grossly alter the responsiveness of the anterior pituitary to the dopamine agonist. The similarities between the effects of 16α-estradiol, via the indirect mechanism, and pimozide on PRL gene transcription suggest that estrogen may stimulate PRL gene transcription *in vivo* in part by reducing the release of dopamine from hypothalamic neurons.

It has been shown that estrogen stimulates the transcription of the rat PRL gene (1, 2) and thereby increases the level of PRL mRNA (3–5) and the rate of PRL synthesis (6–9). More recently, it has been demonstrated that estrogen stimulates PRL gene transcription *in vivo* through at least two independent mechanisms (10–12). One of these stimulatory mechanisms appears to be mediated directly by the estrogen receptor of the anterior pituitary; following injection of 16α-estradiol, a short-acting estrogen, the rate of PRL gene transcription rapidly increased and subsequently declined in parallel with the level of occupied estrogen receptor (10, 11). Furthermore, the induction of this initial phase of stimulated PRL gene transcription was insensitive to pretreatment with inhibitors of pituitary protein synthesis (10, 11). An estrogen response element has been localized approximately 1.5 kilobases upstream from the transcription start site of the PRL gene (13, 14). Therefore, it is probable that the occupied estrogen receptor binds to this estrogen response element and enhances the transcription of the PRL gene. A second mechanism through which estrogen stimulates PRL gene transcription appears to be indirect; a second phase of stimulated PRL gene transcription was observed 6 hr following 16α-estradiol injection, and the induction of this second phase was blocked by pretreatment with cycloheximide (10, 11). We have suggested previously that this second phase of stimulated transcription might result from either an estrogen-induced alteration in the level of one or more hormonal regulators of PRL gene transcription, an alteration in the responsiveness of the lactotrophes to a second regulator or regulators, or a combination of both of these mechanisms (10, 11).

Many hormones and growth factors have been shown to regulate PRL gene expression (11). Dopamine agonists inhibited the transcription of the PRL gene both *in vitro* (15) and *in vivo* (16), whereas a dopamine antagonist stimulated this transcription *in vivo* (16). These inhibitory and stimulatory effects on transcription have been shown to be subsequently reflected in alterations in the level of PRL mRNA (17, 18) and the rate of PRL synthesis (17–19). Estrogen has also been shown to alter the responsiveness of cultured pituitary cells to dopamine agonists. The inhibitory effects of dopamine agonists on PRL secretion (20), the level of PRL mRNA (21), and the rate of PRL synthesis (21) were reduced in cultured pituitary cells that were treated with 17β-estradiol.

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ABBREVIATIONS: PRL, prolactin; HB, homogenization buffer; WB, wash buffer; SDS, sodium dodecyl sulfate.

In this study we have investigated *in vivo* the relationships between 16α-estradiol, α-ergocryptine (a dopamine agonist), and pimozide, (a dopamine antagonist) in the regulation of transcription of the rat PRL gene. The data presented illustrate that the induction of the second phase of stimulated PRL gene transcription by 16α-estradiol and the induction of PRL gene transcription by pimozide share many similarities. These data support the hypothesis that estrogen stimulates the transcription of the PRL gene *in vivo* in part by reducing the release of dopamine from hypothalamic neurons.

Materials and Methods

Treatment of animals. Male rats, 5 to 6 weeks of age, were obtained from the Holtzman Co. (Madison, WI) and were housed in our climate-controlled animal facilities under a 12-hr light (6 a.m. to 6 p.m.)/12-hr dark cycle. The animals were allowed continuous access to food and water and were housed in our facilities for 5 to 7 days before being used in any experiment. To lessen the possible effects of diurnal variation on the parameters being investigated, the animals were sacrificed between 2:30 and 4:30 p.m. Each treatment group contained 8–10 animals, due to the small size of the anterior pituitary and the small amount of PRL mRNA synthesized by isolated pituitary nuclei during the *in vitro* reaction.

Pimozide (McNeil Pharmaceuticals, Spring House, PA) was solubilized in 0.1 M tartaric acid or absolute ethanol and was injected subcutaneously at a dose of 0.63 mg/kg of body weight. α-Ergocryptine (Sigma, St. Louis, MO) was solubilized in absolute ethanol and administered subcutaneously at a dose of 4 mg/kg. Cycloheximide (Sigma) was prepared in phosphate-buffered saline and injected intraperitoneally at a dose of 3.33 mg/kg. 17β-Estradiol (Sigma) and 16α-estradiol (Parke-Davis, Ann Arbor, MI) were solubilized in sesame oil and administered intraperitoneally at a dose of 10 μg/animal. Control animals always received an equivalent volume of the various vehicles. The injection schedules are described in the figure legends.

Preparation of nuclei from cells of the anterior pituitary. The animals were killed by decapitation, and the anterior pituitary glands were quickly removed and placed into ice-cold HB [10 mM Tris (Sigma), 24 mM KCl, 10 mM $MgCl_2$, 1.0 mM dithioerythritol (Calbiochem, La Jolla, CA), pH 7.9]. The pituitaries were rinsed with HB and disrupted, with 10 strokes of a motor-driven Potter-Elvehjem homogenizer, into 1.0 ml of HB containing 0.25% Nonidet P-40, (Bethesda Research Laboratories, Bethesda, MD). The homogenizer was rinsed with an additional 0.5 ml of this buffer, which was subsequently combined with the initial 1.0-ml aliquot. The resulting cell nuclei were pelleted (5000 $\times$ *g* at 4° for 10 min) through a 2.0-ml cushion of HB containing 0.8 M sucrose (Bethesda Research Laboratories). The pellet was resuspended in HB containing 0.25% Nonidet P-40, and the nuclei were repelleted (7000 $\times$ *g* at 4° for 45 min) through 2.0-ml cushions of HB containing 1.9 M sucrose. The final pellet was resuspended in storage buffer [50 mM Tris, 5 mM $MgCl_2$, 25% glycerol (Sigma), 0.1 mM EDTA (Sigma), 1.0 mM dithioerythritol, pH 7.9]. Nuclei that were essentially free of contaminating whole cells were observed upon examination by phase-contrast microscopy. Freshly prepared nuclei were used in all experiments.

In vitro synthesis of RNA by isolated nuclei. One hundred microliters of freshly prepared pituitary nuclei in storage buffer were added to 100 μl of incubation buffer [83 mM Tris, 150 mM $(NH_4)_2SO_4$, 15 mM NaF, 1.5 mM ATP, 1.5 mM GTP, 1.5 mM CTP (all nucleotide triphosphates obtained from Calbiochem), 2.0 mM phosphoenolpyruvate (Calbiochem), 50 μg/ml pyruvate kinase (Boehringer Mannheim, Indianapolis, IN), 1.0 mM $MnCl_2$, pH 7.9] and 50 μl of 83 mM Tris containing 250 μ Ci of [α-^{32}P]UTP (Amersham, Arlington Heights, IL). The samples were incubated at 30° for 20 min. We have previously shown that, under these conditions, the RNAs for PRL and growth hormone are synthesized asymmetrically from a DNA template by RNA polymerase II (2).

Purification of RNA. One hundred micrograms of *Escherichia coli* tRNA (Sigma) were added to each sample to serve as carrier. DNAse free of contaminating RNAse (Worthington, Freehold, NJ) was added to a concentration of 50 μg/ml, and the samples were incubated at 37° for 30 min. Following incubation, 0.1 volume of 10× SET [100 mM Tris, 50 mM EDTA, 10% SDS (Sigma), pH 7.4] was added, followed by proteinase K (Boehringer Mannheim) to a concentration of 100 μg/ml. The samples were incubated at 45° for 30 min. Four volumes of guanidine buffer [20 mM Tris, 1.0 mM EDTA, 1.0% *N*-lauroyl sarcosine (Sigma), 7.0 M guanidine HCl (Sigma), pH 7.4] were added, and the samples were incubated at 60° for 15 min. The RNA was purified by centrifugation through 1.5-ml cushions of CsCl [50 mM Tris, 2.0 mM EDTA, 6.2 M CsCl (Bethesda Research Laboratories), pH 7.4] at 33,000 rpm and 20° for 18 hr in an SW-50.1 rotor (Beckman Instruments, Palo Alto, CA). The RNA pellets were solubilized in sterile H_2O and extracted once with 2 volumes of phenol/chloroform (50:50, V/V) and once with 2 volumes of chloroform alone. One-tenth volume of 3.0 M sodium acetate (pH 5.2) was added, followed by 3 volumes of 100% ethanol. The RNA was precipitated at −20° and pelleted by centrifugation at 12,000 $\times$ *g* and 4° for 15 min. The pellets were dried under vacuum.

Quantification of PRL RNA sequences. The RNA pellets from above were solubilized in hybridization buffer [20 mM $NaPO_4$, 0.75 M NaCl, 75 mM sodium citrate, 0.2% SDS, 50% formamide (Kodak, Rochester, NY), 1.0 mg/ml *E. coli* tRNA, 0.3 mg/ml Ficoll 400 (Pharmacia, Piscataway, NJ), 0.5 mg/ml poly(A) (PL Biochemicals, Milwaukee, WI), 0.02% bovine serum albumin (Sigma), pH 7.4]. Following preincubation at 65% for 15 min, 100 μl aliquots of the above RNA solutions, containing 0.5 $\times$ 10^6 to 10 $\times$ 10^6 cpm of ^{32}P-labeled RNA, were added to tubes containing nitrocellulose (BA-85, 0.45-μm; Schleicher & Schuell, Inc., Keene, NH) disks onto which PRL cDNA, growth hormone cDNA, or pBR322 DNA had been immobilized (2). To serve as an internal hybridization standard, approximately 1000 cpm of ^{3}H-labeled PRL cRNA, synthesized from PRL cDNA with *E. coli* RNA polymerase (provided courtesy of Dr. Richard Burgess, University of Wisconsin), was also added. These samples were incubated at 45° for 40 to 48 hr. Each RNA sample was assayed in triplicate. Following incubation, the nitrocellulose disks were removed and washed twice in 2.0 ml of WB, (10 mM Tris, 0.3 M NaCl, 2.0 mM EDTA, pH 7.4) containing 0.1% SDS, at 45° for 30 min, and twice with 2.0 ml of WB at 45° for 30 min. The disks were next washed with 0.5 ml of WB, containing RNAse A (Sigma) at a concentration of 50 μg/ml, at 37° for 30 min. Finally, the disks were washed twice with 2.0 ml of WB containing 0.1% SDS at 65° for 30 min, and twice with 2.0 ml of WB at 45° for 30 min. Specifically bound RNA was eluted into 0.1 ml of 40 mM NaOH at 80° for 20 min, and the samples were neutralized with 0.04 ml of 0.1 M HCl. Four milliliters of Aquasol (New England Nuclear, Boston, MA) were added, and radioactivity was measured in a Rack-Beta liquid scintillation counter (LKB, Gaithersburg, MD) adjusted to quantify ^{32}P and ^{3}H with no crossover. Counting efficiencies were approximately 20% for ^{3}H and approximately 70% for ^{32}P. Each sample was counted for 60–90 min to minimize counting error. The data are expressed as ppm and were calculated by subtracting the cpm nonspecifically bound to the disk containing pBR322 DNA from the cpm bound to the disk containing the PRL cDNA, correcting this difference for the observed efficiency of hybridization, and dividing this number by the amount (in millions of cpm) of ^{32}P-labeled RNA included in each assay. Under the conditions described above, nonspecific binding of ^{32}P-labeled RNA to the disk containing pBR322 DNA was usually less than 50 cpm, the efficiency of hybridization averaged 20%, and no hybridization of ^{3}H-labeled PRL cRNA to an immobilized growth hormone (an evolutionarily related gene) cDNA probe was observed.

Results

A single injection of pimozide rapidly stimulated the transcription of the rat PRL gene (Fig. 1). In this experiment, PRL

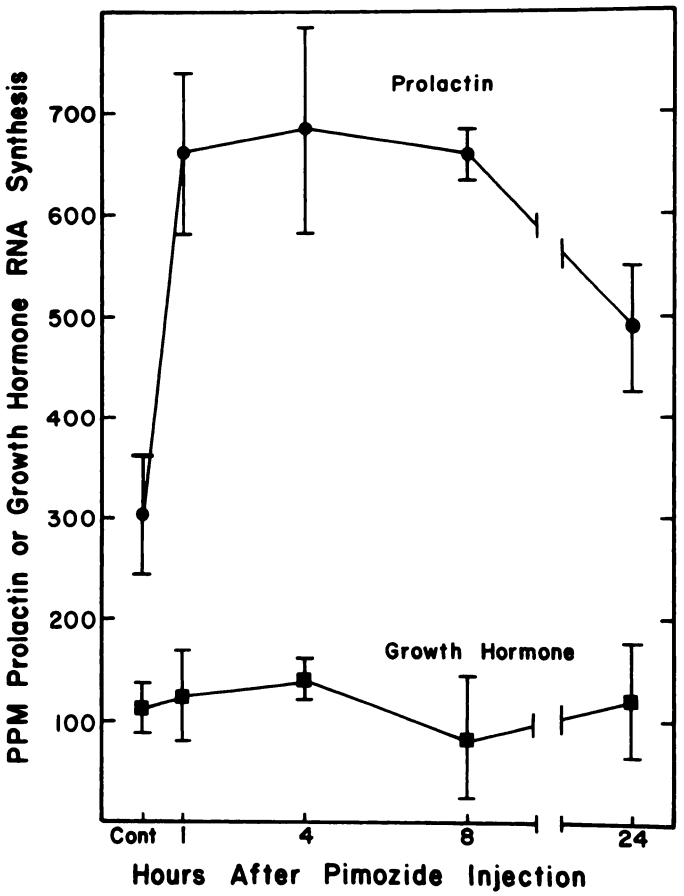

Fig. 1. Effect of pimozide on transcription of the PRL and growth hormone genes. Pimozide (0.63 mg/kg) was injected subcutaneously at the indicated times before killing. Control animals received the tartaric acid vehicle. The animals were killed, anterior pituitary nuclei were prepared, and transcription was assayed as described in Materials and Methods. Each *point* represents the mean ± the standard error (three determinations) for the assay of RNA from a single *in vitro* reaction containing nuclei prepared from 8–10 animals.

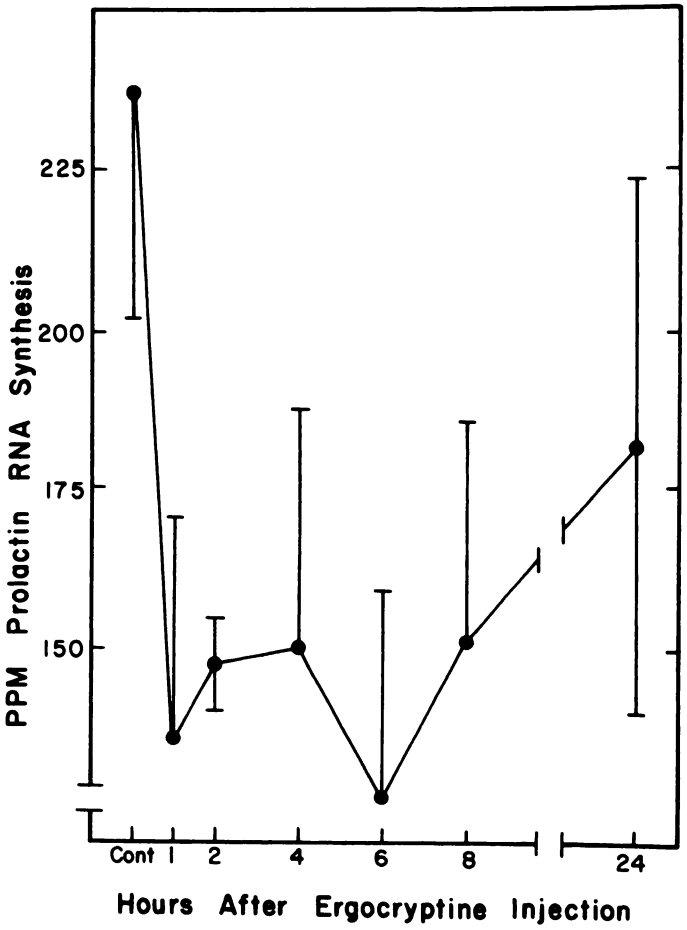

Fig. 2. Inhibitory effect of ergocryptine on PRL gene transcription. Ergocryptine (4 mg/kg) was injected subcutaneously at the indicated times before killing. Control animals received the ethanol vehicle. The animals were killed, anterior pituitary nuclei were prepared, and transcription was assayed as described in Materials and Methods. Each *point* represents the mean ± the standard error (three determinations) for the assay of RNA from a single *in vitro* reaction containing nuclei prepared from 8–10 animals.

gene transcription was stimulated approximately 2-fold within 1 hr of pimozide injection and remained stimulated for at least 24 hr following injection. This experiment is representative of a total of six experiments in which the effects of pimozide were examined at various time points. Average stimulations of 1.7-fold were observed at 1 hr, 2.8-fold at 2 and 3 hr, 2.0-fold at 4 and 8 hr, and 1.6-fold at 24 hr (data not shown). In one of these experiments, PRL gene transcription was stimulated 1.6- ± 0.3-fold within 30 min of pimozide injection (data not shown). Pimozide had no effect on the transcription of the evolutionarily related growth hormone gene over a 24-hr time course (Fig. 1).

Ergocryptine rapidly inhibited PRL gene transcription (Fig. 2). This experiment is representative of a total of five experiments in which the effects of ergocryptine were examined at various time points. In these experiments, the transcription of the PRL gene was consistently inhibited by approximately 50% at the 1- through 8-hr time points (data not shown). The 24-hr time point was examined only in the illustrated experiment. Ergocryptine treatment had no effect on the synthesis of total RNA by the isolated pituitary nuclei, suggesting that the inhibition of PRL gene transcription by ergocryptine is not due to general effects on either RNA polymerase II or associated transcription components (data not shown).

We next examined the effects of cycloheximide on the induction of PRL gene transcription by pimozide and the inhibition of transcription by ergocryptine. In these experiments, transcription was examined 1 hr after injection of pimozide or ergocryptine. In animals pretreated with the phosphate-buffered saline vehicle, pimozide stimulated transcription of the PRL gene 1.7-fold, whereas ergocryptine inhibited transcription by 60% (Fig. 3). Cycloheximide, administered 1 hr before injection of pimozide or ergocryptine, blocked the induction of PRL gene transcription by pimozide, whereas the inhibitory effect of ergocryptine was still observed, although perhaps somewhat reduced (Fig. 3). Similar results were observed in another experiment (data not shown). When administered through this regimen, cycloheximide inhibited pituitary protein synthesis by greater than 85% (2, 10).

The interactions between ergocryptine and 16α-estradiol in the regulation of PRL gene transcription were investigated. Transcription was examined at two time points, relative to injection of 16α-estradiol, 1 hr, at which PRL gene transcription would be mediated directly by the occupied estrogen receptor, and 6 hr, at which transcription would be mediated by one or more indirect mechanisms (10, 11). When ergocryptine was

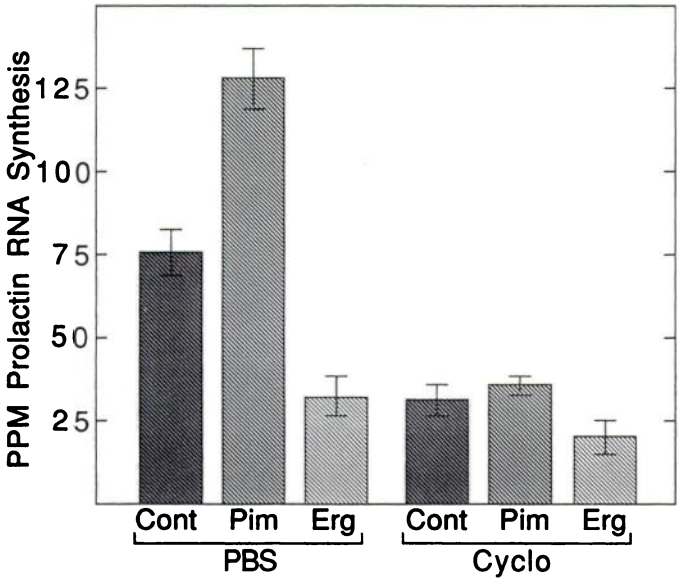

Fig. 3. Effects of cycloheximide pretreatment on the stimulation of PRL gene transcription by pimozide and the inhibition of PRL gene transcription by ergocryptine. Animals were pretreated with either the phosphate-buffered saline (PBS) vehicle or cycloheximide (3.33 mg/kg) (*Cyclo*) 1 hr before the injection of pimozide (0.63 mg/kg) (*Pim*), ergocryptine (4 mg/kg) (*Erg*), or the ethanol vehicle, (*Cont*). One hour after the injection of the dopamine antagonist or agonist, the animals were killed, anterior pituitary nuclei were prepared, and transcription was assayed as described in Materials and Methods. Each *bar* represents the mean ± the range for the assay in triplicate of RNA from duplicate *in vitro* reactions, each containing nuclei prepared from 8–10 animals.

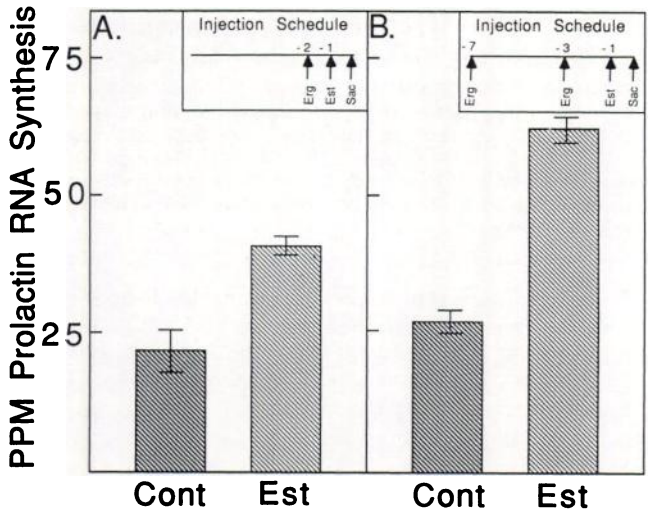

Fig. 4. Effect of ergocryptine pretreatment on the induction of the initial phase of stimulated PRL gene transcription by 16α-estradiol. A, Ergocryptine (4 mg/kg) (*Erg*) was administered as a single injection 1 hr before 16α-estradiol (10 μg) (*Est*) or its vehicle (*Cont*). B, Ergocryptine (4 mg/kg) was administered in two injections, 6 and 2 hr before 16α-estradiol. These injection schedules are illustrated in their respective panels. In both experiments, the animals were killed 1 hr after 16α-estradiol treatment and PRL gene transcription was assayed as described in Materials and Methods. Each *bar* represents the mean ± the standard error (three determinations) for the assay of RNA from a single *in vitro* reaction containing nuclei from 8–10 animals. *Sac*, sacrifice.

injected 1 hr before 16α-estradiol and transcription was examined 1 hr after injection of this estrogen, transcription of the PRL gene was stimulated 1.8-fold (Fig. 4A), a level of stimulation comparable to that observed 1 hr after the injection of

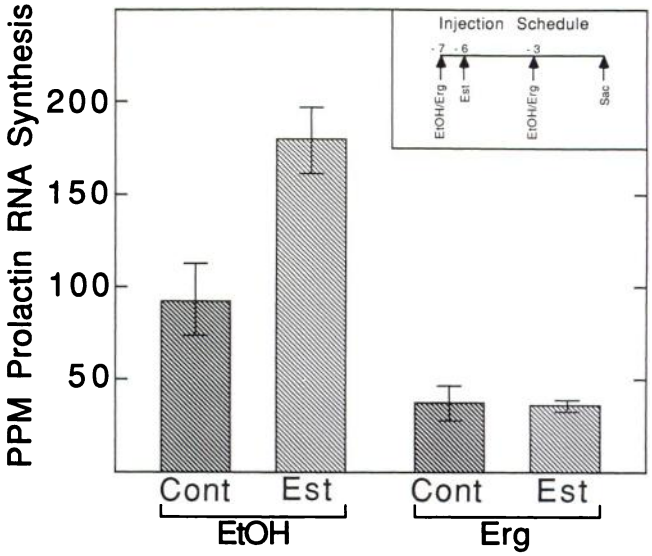

Fig. 5. Effect of ergocryptine pretreatment on the induction of the second phase of stimulated PRL gene transcription by 16α-estradiol. The ethanol vehicle (*EtOH*) or ergocryptine (4 mg/kg) (*Erg*) was administered through a regimen identical to that used in the experiment illustrated in Fig. 4B. Sesame oil or 16α-estradiol (10 μg) (*Est*) was injected 6 hr before the time the animals were killed (*Sac*). This injection schedule is illustrated in the figure *inset*. After killing, anterior pituitary nuclei were prepared and transcription was assayed as described in Materials and Methods. Each *bar* represents the mean ± the range for the assay in triplicate of RNA from duplicate *in vitro* reactions, each containing nuclei prepared from 8–10 animals.

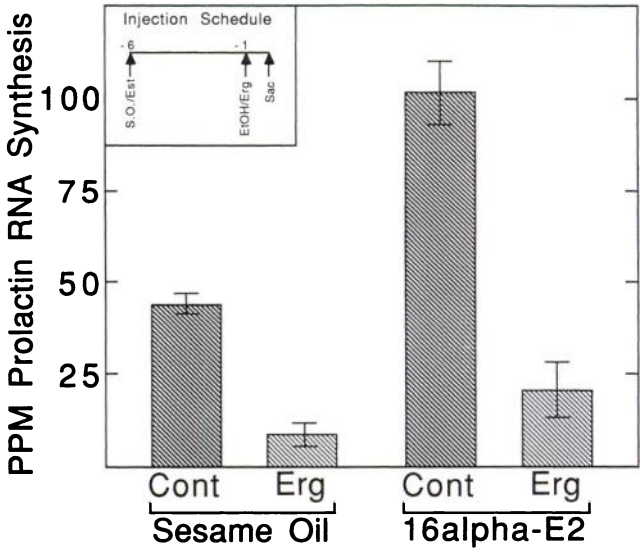

Fig. 6. Effect of 16α-estradiol pretreatment on the inhibition of PRL gene transcription by ergocryptine. Sesame oil (*S.O.*) or 16α-estradiol (10 μg) (16alpha-E2) was injected 5 hr before the injection of ergocryptine (4 mg/kg) (*Erg*) or its ethanol vehicle (*Cont*). This schedule is illustrated in the figure *inset*. One hour after ethanol/ergocryptine injection, the animals were killed (*Sac*) and PRL gene transcription was assayed as described in Materials and Methods. Each *bar* represents the mean ± range for the assay in triplicate of RNA from duplicate *in vitro* reactions, each containing nuclei prepared from 8–10 animals.

16α-estradiol alone (10, 11). A similar level of stimulation was observed 1 hr after injection of 16α-estradiol into rats that were pretreated with ergocryptine 6 hr before and again 2 hr before injection of this estrogen (Fig. 4B). These data illustrate that ergocryptine, when administered through two different regi-

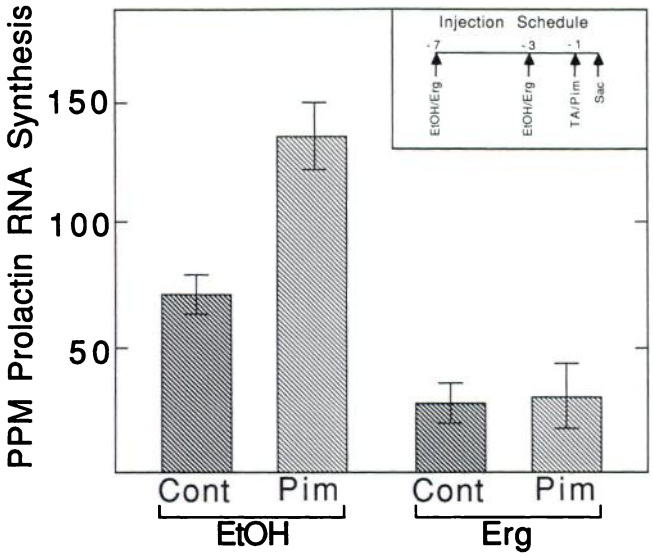

Fig. 7. Effect of ergocryptine pretreatment on the induction of PRL gene transcription by pimozide. The ethanol vehicle (*EtOH*) or ergocryptine (4 mg/kg) (*Erg*) was injected 6 hr and again 2 hr before the injection of tartaric acid (*TA*) or pimozide (0.63 mg/kg) (*Pim*). This injection schedule is illustrated in the figure *inset*. One hour after tartaric acid/pimozide injection, the animals were killed (*Sac*), anterior pituitary nuclei were prepared, and transcription was assayed as described in Materials and Methods. Each *bar* represents the mean ± the standard error (three determinations) for the assay of RNA from a single *in vitro* reaction containing nuclei from 8–10 animals. *Cont*, control.

mens, did not block the ability of 16α-estradiol to stimulate PRL gene transcription through the direct mechanism. Ergocryptine did block the induction of PRL gene transcription by 16α-estradiol through the indirect mechanism, however. No induction of PRL gene transcription was observed 6 hr after injection of 16α-estradiol in animals that received two injections of ergocryptine, whereas the usual 2.0-fold stimulation of transcription was observed 6 hr after injection of 16α-estradiol in animals that were treated with the ethanol vehicle (Fig. 5). Ergocryptine treatment resulted in a 60% inhibition of PRL gene transcription in animals that received the sesame oil vehicle (Fig. 5), an observation that was consistent with the data presented in Fig. 2.

We also investigated the effect of pretreatment with 16α-estradiol on the ability of ergocryptine to inhibit PRL gene transcription. Pretreatment with this estrogen had no apparent effect on the ability of ergocryptine to inhibit transcription. Ergocryptine inhibited PRL gene transcription by 70 to 80% in animals pretreated with either the sesame oil vehicle or 16α-estradiol (Fig. 6). In this experiment, PRL gene transcription was stimulated 2.2-fold by 16α-estradiol in the rats that also received an injection of the ethanol vehicle, a level of stimulation that is consistent with previous observations (10, 11).

Finally, we examined the interaction between ergocryptine and pimozide in the regulation of PRL gene transcription. Pimozide stimulated transcription of the PRL gene 1.9-fold in animals treated with the ethanol vehicle, whereas no stimulation by pimozide was observed in animals pretreated with ergocryptine (Fig. 7). Consistent with previous experiments, ergocryptine inhibited PRL gene transcription by approximately 60% in animals treated with the tartaric acid vehicle.

Discussion

At least two classes of dopamine receptors can be distinguished by their biochemical properties (22). One class of dopamine receptor, the D2 receptor, has been shown to be negatively coupled to adenylate cyclase in the anterior pituitary of both male and female rats (23). The apparent affinities of 11 dopamine antagonists for the D2 receptor of the rat anterior pituitary have been shown to be highly correlated with the abilities of these antagonists to reverse the inhibition of PRL secretion by dopamine (23, 24). These and other data discussed below strongly suggest that dopamine regulates PRL gene expression by acting through the D2 receptor.

Pimozide, a dopamine antagonist, has been shown to interact specifically and with high affinity with the D2 receptor of the anterior pituitary (23, 24). Low concentrations of pimozide blocked the dopamine-mediated inhibition of PRL secretion by cultured pituitaries, whereas pimozide alone at low concentrations had no effect (25). Higher concentrations of pimozide inhibited PRL secretion from cultured pituitaries (25) and inhibited the secretion of both PRL and growth hormone from cultured pituitary cells (26). These effects of pimozide at higher concentrations were probably due to its association with and inactivation of the calcium-calmodulin complex (27), which has been shown to play an integral role in the regulation of PRL gene expression (28). The dose of pimozide used in this study has been shown to increase the level of PRL mRNA (3) and the rate of PRL synthesis (8). In this study we have shown that a single injection of pimozide stimulates transcription of the rat PRL gene for at least 24 hr, without significantly affecting transcription of the closely related growth hormone gene. Therefore, pimozide administered to rats at this dose appeared to act predominantly, if not exclusively, as a dopamine antagonist. It is not clear from these data whether the prolonged stimulatory effect of pimozide was due to a continuous blockage of D2 receptors or due to a stable metabolic effect induced by transient blockage of this receptor.

Ergocryptine and related ergot alkaloids are potent dopamine agonists. Ergocryptine has been shown to interact with the D2 receptor of the anterior pituitary with an affinity greater than that for dopamine (24). Upon treatment of cultured rat pituitary cells, ergocryptine inhibited PRL secretion (24) and rapidly inhibited PRL gene transcription (15). The inhibition of PRL gene transcription by ergocryptine was reversed by monobutyryl cyclic adenosine monophosphate (15), suggesting that this dopamine agonist functions through the D2 receptor. The semisynthetic derivatives of ergocryptine, dihydroergocryptine and bromocriptine, have been shown to inhibit PRL secretion from cultured pituitary cells (24, 26) and in intact male rats (18), respectively. Bromocriptine has also been shown to decrease the level of PRL mRNA and the rate of PRL synthesis in intact male rats, without having an effect on either the level of growth hormone mRNA or the rate of growth hormone synthesis (18). Similar effects of bromocriptine on PRL and growth hormone mRNA have been observed in primary cultures of rat anterior pituitary cells (29). In this study, ergocryptine inhibited PRL gene transcription in male rats by approximately 50% within 1 hr of injection, and inhibition of transcription was observed for at least 24 hr. Again, it is not clear at this time whether the prolonged inhibitory effect of ergocryptine was due to a continuous occupancy of D2 receptors or due to a stable metabolic effect induced by transient occupancy of this receptor.

We have previously demonstrated that estrogen stimulates PRL gene transcription *in vivo* through at least two independ-

ent mechanisms (10–12). In male rats treated with 16α-estradiol, these mechanisms resulted in two temporally distinct phases of stimulated PRL gene transcription (10, 11). We have presented the hypothesis that the second phase of stimulated transcription is at least partly due to a reduction in dopaminergic stimulation of the anterior pituitary (11). The following observations from this study and previous studies (10, 11) support this hypothesis: 1) the magnitude and duration of the stimulation of PRL gene transcription by pimozide resembled the second phase of stimulated transcription observed in response to 16α-estradiol, 2) neither 16α-estradiol nor pimozide altered the transcription of the growth hormone gene, 3) cycloheximide pretreatment blocked both the induction of PRL gene transcription by pimozide and the induction of the second phase of transcription by 16α-estradiol, and 4) pretreatment with ergocryptine blocked the induction of PRL gene transcription by pimozide and blocked the induction of the second phase of transcription by 16α-estradiol.

PRL gene expression is regulated by many hormones and growth factors (11). Many of these regulatory factors alter intracellular concentrations of calcium and/or cyclic adenosine monophosphate, both of which have been shown to affect PRL gene transcription (11, 28, 30). Therefore, it is possible that manipulation of one regulatory system (by pretreatment with ergocryptine, for example) might alter regulation through other systems as well. It is also possible that the inhibitory effect of ergocryptine on PRL gene transcription is simply dominant over the stimulatory effect of 16α-estradiol, acting through the indirect mechanism, and pimozide, acting through the blockade of the D2 dopamine receptor. We note, therefore, that although the data from this study are consistent with the hypothesis that estrogen indirectly stimulates PRL gene transcription by reducing dopaminergic stimulation, they do not prove it.

Physiological concentrations of estrogen have been shown to decrease the ability of dopamine agonists to inhibit PRL secretion (20, 31) and PRL synthesis (21) by cultured anterior pituitary cells. In this study, a single injection of 16α-estradiol administered 5 hr before ergocryptine did not block the ability of this dopamine agonist to inhibit transcription of the PRL gene. From these data we can only conclude that this estrogen, administered as in our previous studies, did not totally block the responsiveness of the anterior pituitary to dopaminergic stimuli. We note that this study, in which the response to a single dose of ergocryptine was examined, was not designed to detect subtle changes in responsiveness to this dopamine agonist.

The dopamine that functions in the regulation of PRL gene expression arises primarily from the tuberoinfundibular neurons of the mediobasal hypothalamus (32). The release of dopamine from these neurons into the hypophysial portal blood, in which it is transported to the anterior pituitary, is regulated by estrogen (33, 34). To our knowledge, a study in which the release of dopamine from these neurons is examined in the initial 6 hr following estrogen injection has not been conducted. Confirmation of the hypothesis that estrogen indirectly stimulates PRL gene transcription by reducing the release of dopamine from the hypothalamus will require such a direct examination.

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References

1. Maurer, R. A. Estradiol regulates the transcription of the prolactin gene. *J. Biol. Chem.* **257**:2133–2136 (1982).
2. Shull, J. D., and J. Gorski. Estrogen stimulates prolactin gene transcription by a mechanism independent of pituitary protein synthesis. *Endocrinology* **114**:1550–1557 (1984).
3. Stone, R. T., R. A. Maurer, and J. Gorski. Effect of estradiol 17-β on preprolactin messenger ribonucleic acid activity in the rat pituitary gland. *Biochemistry* **16**:4915–4921 (1977).
4. Ryan, R., M. A. Shupnik, and J. Gorski. Effect of estrogen on preprolactin messenger ribonucleic acid sequences. *Biochemistry* **18**:2044–2048 (1977).
5. Seo, H., S. Refetoff, G. Vassart, and H. Brocas. Comparison of primary and secondary stimulation of male rats by estradiol in terms of prolactin synthesis and mRNA accumulation in the pituitary. *Proc. Natl. Acad. Sci. USA* **76**:824–828 (1979).
6. MacLeod, R. M., A. Abad, and L. L. Eidson. *In vivo* effect of sex hormones on the *in vitro* synthesis of prolactin and growth hormone in normal and pituitary tumor-bearing rats. *Endocrinology* **84**:1475–1483 (1969).
7. Yamamoto, K., K. Kasai, and T. Ieiri. Control of pituitary functions of synthesis and release of prolactin and growth hormone by gonadal steroids in male and female rats. *Jpn. J. Physiol.* **25**:645–658 (1975).
8. Maurer, R. A., and J. Gorski. Effect of estradiol-17β and pimozide on prolactin synthesis in male and female rats. *Endocrinology* **101**:76–84 (1977).
9. Lieberman, M. E., R. A. Maurer, and J. Gorski. Estrogen control of prolactin synthesis *in vitro*. *Proc. Natl. Acad. Sci. USA* **75**:5946–5949 (1978).
10. Shull, J. D., and J. Gorski. Estrogen regulates the transcription of the rat prolactin gene *in vivo* through at least two independent mechanisms. *Endocrinology* **116**:2456–2462 (1985).
11. Shull, J. D., and J. Gorski. The hormonal regulation of prolactin gene expression: an examination of mechanisms controlling prolactin synthesis and the possible relationship of estrogen to these mechanisms. *Vitam. Horm.* **43**:197–249 (1986).
12. Shull, J. D., and J. Gorski. Estrogen regulation of prolactin gene transcription *in vivo*: paradoxical effects of 17β-estradiol dose. *Endocrinology* **124**:279–285 (1989).
13. Maurer, R. A., and A. C. Notides. Identification of an estrogen-responsive element from the 5′-flanking region of the rat prolactin gene. *Mol. Cell. Biol.* **7**:4247–4254 (1987).
14. Waterman, M. L., S. Adler, C. Nelson, G. L. Greene, R. M. Evans, and M. G. Rosenfeld. A single domain of the estrogen receptor confers deoxyribonucleic acid binding and transcriptional activation of the rat prolactin gene. *Mol. Endocrinol.* **2**:14–21 (1988).
15. Maurer, R. A. Transcriptional regulation of the prolactin gene by ergocryptine and cyclic AMP. *Nature (Lond.)* **294**:94–97 (1981).
16. Shull, J. D., S. R. Mellon, and J. Gorski. Regulation of prolactin gene transcription *in vivo*: roles of estrogen and dopamine. *7th Int. Congr. Endocrinol.* 2140 (1984).
17. Maurer, R. A. Dopaminergic inhibition of prolactin synthesis and prolactin messenger RNA accumulation in cultured pituitary cells. *J. Biol. Chem.* **255**:8092–8097 (1980).
18. Brocas, H., A. V. Coevorden, H. Seo, S. Refetoff, and G. Vassart. Dopaminergic control of prolactin mRNA accumulation in the pituitary of the male rat. *Mol. Cell. Endocrinol.* **22**:25–30 (1981).
19. Hill, M. K., R. M. MacLeod, and P. Orcutt. Dibutyryl cyclic AMP, adenosine, and guanosine blockage of the dopamine, ergocryptine and apomorphine inhibition of prolactin release *in vitro*. *Endocrinology* **99**:1612–1617 (1976).
20. Raymond, V., M. Beaulieu, F. Labrie, and J. Boissier. Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. *Science (Wash. D. C.)* **200**:1173–1175 (1978).
21. Maurer, R. A. Relationship between estradiol, ergocryptine, and thyroid hormone: effects on prolactin synthesis and prolactin messenger ribonucleic acid levels. *Endocrinology* **110**:1515–1520 (1982).
22. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature (Lond.)* **277**:93–96 (1979).
23. Enjalbert, A., and J. Bockert. Pharmacological characterization of the D2 dopamine receptor negatively coupled with adenylate cyclase in rat anterior pituitary. *Mol. Pharmacol.* **23**:576–584 (1983).
24. Caron, M. G., M. Beaulieu, V. Raymond, B. Gayne, J. Drouin, R. J. Lefkowitz, and F. Labrie. Dopaminergic receptors in the anterior pituitary gland. *J. Biol. Chem.* **253**:2244–2253 (1978).
25. MacLeod, R. M., and S. W. J. Lamberts. The biphasic regulation of prolactin secretion by dopamine agonists-antagonists. *Endocrinology* **103**:200–203 (1978).
26. West, B., and P. S. Dannies. Antipsychotic drugs inhibit prolactin release from rat anterior pituitary cells in culture by a mechanism not involving the dopamine receptor. *Endocrinology* **104**:877–880 (1979).
27. Levin, R. M., and B. Weiss. Selective binding of antipsychotics and other psychoactive agents to the calcium-dependent activator of cyclic nucleotide phosphodiesterase. *J. Pharmacol. Exp. Ther.* **208**:454–459 (1979).
28. Bancroft, C., G. G. Gick, M. E. Johnson, and B. A. White. Regulation of growth hormone and prolactin gene expression by hormones and calcium. *Biochem. Actions Horm.* **12**:173–213 (1985).
29. Wood, G. F., K. Docherty, D. B. Ramsden, and M. C. Sheppard. A comparison of the effects of bromocriptine and somatostatin on growth hormone gene

expression in the rat anterior pituitary gland *in vitro*. *Mol. Cell. Endocrinol.* **52**:257–261 (1987).
30. Murdoch, G. H., M. G. Rosenfeld, and R. M. Evans. Eukaryotic transcriptional regulation and chromatin-associated protein phosphorylation by cyclic AMP. *Science (Wash. D. C.)* **218**:1315–1317 (1982).
31. Giguere, V., H. Meunier, R. Veilleux, and F. Labrie. Direct effects of sex steroids on prolactin release at the anterior pituitary level: interactions with dopamine, thyrotropin-releasing hormone, and isobutylmethylxanthine. *Endocrinology* **111**:857–862 (1982).
32. Moore, K. E. Interactions between prolactin and dopaminergic neurons. *Biol. Reprod.* **36**:47–58 (1987).
33. Wise, P. M., N. Rance, and C. A. Barraclough. Effects of estradiol and progesterone on catecholamine turnover rates in discrete hypothalamic regions in ovariectomized rats. *Endocrinology* **108**:2186–2193 (1981).
34. Cramer, O. M., C. R. Parker, Jr., and J. C. Porter. Estrogen inhibition of dopamine release into hypophysial portal blood. *Endocrinology* **104**:419–422 (1979).

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