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The Proximal 350 bp of 5'-Flanking Sequence of the Human α-Subunit Glycoprotein Hormone Gene Functions in the Pituitary Gland, but Not the Placenta, in Transgenic Mice

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To understand better the minimal DNA sequence requirements for regulated expression of the human α-subunit glycoprotein hormone gene (Hα), two lines of transgenic mice were constructed that contained a fusion gene (Hα-350CAT) consisting of only 350 bp of 5'-flanking sequence of Hα linked to the bacterial gene encoding chloramphenicol acetyltransferase (CAT). CAT activity was detectable in pituitary, but not in brain, heart, kidney, liver, lung, pancreas, or spleen in transgenic mice. Gonadectomy increased ($p < 0.05$) CAT activity in the pituitaries of males (6.5 ± 1.4% conversion/μg protein; mean ± SEM) and females (14.5 ± 4.2) compared to intact males (1.2 ± 0.3) and females (6.7 ± 1.0). In addition, administration of a GnRH antagonist (antide; 60 μg/injection; one injection every other day) for 10 d to gonadectomized animals decreased ($p < 0.05$) CAT activity in males (3.5 ± 0.8) and females (2.9 ± 0.5) compared to gonadectomized animals that received saline. Antide also reduced ($p < 0.05$) serum concentrations of luteinizing hormone in gonadectomized males and females compared to gonadectomized animals that received saline. Surprisingly, CAT activity in the placenta of Hα-350CAT transgenic mice was not detectable (<3 SD above the mean of CAT activity in placenta from nontransgenic mice; $n = 77$). Thus, expression of the human α-subunit promoter in the placenta of transgenic mice appears to require DNA sequences upstream of the proximal 350 bp of 5'-flanking sequence, whereas the proximal 350 bp of the human α-subunit gene contains sufficient DNA sequence to target pituitary-specific expression and confer responsiveness to gonadal hormones and GnRH.

Key Words: Glycoprotein hormone; GnRH; estrogen; pituitary; placenta; transgenic mice.

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Introduction

Glycoprotein hormones are synthesized and secreted from specific cell types in the anterior pituitary gland of all mammals and in the placenta of primates and horses. In the anterior pituitary gland, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are produced in gonadotropes, whereas thyroid-stimulating hormone (TSH) is produced in thyrotropes. Chorionic gonadotropin (CG) is produced in syncytiotrophoblasts of the placenta in primates and horses. These glycoprotein hormones are composed of a common α-subunit and a hormone-specific β-subunit, which heterodimerize through noncovalent linkages to generate biologically active hormone (Pierce and Parsons, 1981).

In humans, α-subunit is the product of a single-copy gene that is expressed in gonadotropes, thyrotropes, and syncytiotrophoblasts (reviewed by Jameson and Hollenberg, 1993). Thus the promoter-regulatory region of the α-subunit gene must contain DNA sequences that direct expression of α-subunit to three distinct cell types. Using transient expression assays and human placental cell lines, a minimal placenta-specific enhancer consisting of two regulatory elements within the proximal 182 bp of 5'-flanking sequence of the human α-subunit gene was described (reviewed by Jameson and Hollenberg, 1993). The placenta-specific enhancer consists of a trophoblast-specific element (TSE; Delegeane et al., 1987; also referred to as an upstream-regulatory element or URE; Bokar et al., 1989) and tandemly repeated cyclic AMP response elements (CRE; Delegeane et al., 1987; Deutsch et al., 1987; Silver et al., 1987). Expression of the human α-subunit gene in placenta is further augmented by the presence of a junctional response element (JRE; Andersen et al., 1990) and a CCAAT box (Kennedy et al., 1990). Thus, expression of the human α-subunit gene in placenta requires a complex interaction of DNA sequences that bind unique DNA binding proteins.

Recently, transient expression assays were used with primary cultures of pituitary cells or an immortalized cell line of gonadotrope origin (mouse αT3-1 cells; Windle et

al., 1990) to study mechanisms that direct pituitary-specific expression and hormonal regulation of the human α-subunit gene (reviewed by Hamernik, 1995). Expression of the human α-subunit gene in αT3-1 cells involves the gonadotrope-specific element (GSE; Horn et al., 1992), an α-activator sequence (αACT; Steger et al., 1991), two E boxes (αEB1 and αEB2; Jackson et al., 1995), the CRE, the pituitary glycoprotein hormone basal element (PGBE), and two α-basal elements (αBE1 and αBE2; Heckert et al., 1995). Additional DNA sequences and DNA binding proteins are required for expression of the α-subunit gene in thyrotropes (Ocran et al., 1990; Sarapura et al., 1990; Kendall et al., 1994). Each of these DNA sequences binds a unique DNA binding protein to regulate transcription of the α-subunit gene. Thus, expression of the α-subunit gene in the pituitary requires a complex interaction of DNA sequences that are unique for pituitary-specific expression and some DNA sequences that are shared with the placenta-specific enhancer.

In addition to DNA sequences that direct appropriate cell-specific expression, the α-subunit gene also contains specific DNA sequences that confer hormone responsiveness. A thyroid hormone responsive element (TRE) is located near the transcription initiation site in the human α-subunit gene and, when bound by thyroid hormone receptor complex, transcription of α-subunit is repressed by a mechanism that prevents binding of RNA polymerase II to the TATA box (Chatterjee et al., 1989). Although the estrogen receptor complex failed to bind directly to the human α-subunit gene (Keri et al., 1991), the androgen receptor complex bound with high affinity to the human α-subunit promoter and inhibited transcription after transient transfection in αT3-1 cells (Clay et al., 1993). GnRH responsive regions in the human (Kay and Jameson, 1992) and mouse (Schoderbek et al., 1993) α-subunit promoter, which confer activation by mitogen-activated protein kinase (Roberson et al., 1995), have also been identified.

Although transient expression systems have generated a working model for placenta- or pituitary-specific expression, use of this in vitro system can be problematic. For example, results obtained from in vitro transient expression assays are not always consistent with results obtained from in vivo studies with transgenic mice (Hammer et al., 1987; Pinkert et al., 1987; Swift et al., 1989; Morello et al., 1993). The transgenic mouse model is a powerful physiological system that allows identification of transcriptional regulatory nucleotides required for cell-specific expression, as well as hormonal, temporal, and spatial regulation (Nilson et al., 1995). Using transgenic mice, Bokar et al. (1989) demonstrated that the proximal ~1500 bp of 5'-flanking sequence of the human α-subunit gene directed expression to the pituitary gland and the placenta. Subsequently, we reported that the proximal ~1500 bp of human, or 315 bp of bovine, α-subunit 5'-flanking sequence directed expression specifically to gonadotropes, but not thyrotropes (Kendall et al., 1991; Hamernik et al., 1992), and conferred responsiveness to estradiol (Keri et al., 1991), androgens (Clay et al., 1993), and GnRH (Hamernik et al., 1992). Additional studies indicated that a truncated human α-subunit promoter consisting of only 152 or 168 bp of 5'-flanking sequence was insufficient to direct expression to the pituitary gland in transgenic mice (Horn et al., 1992). Thus, our objective was to determine if a minimal promoter that contained only 350 bp of 5'-flanking sequence of the human α-subunit gene would function in the pituitary gland and in the placenta of transgenic mice. The proximal 350 bp of 5'-flanking sequence of the human α-subunit gene contains all DNA sequences needed for expression of α-subunit in gonadotropes and syncytiotrophoblasts in vitro; thus, we hypothesized that this truncated promoter would also function appropriately in the pituitary gland and in the placenta of transgenic mice.

Results

Expression of the 350-bp Human α-Subunit Promoter in the Pituitary Gland of Transgenic Mice

To determine if DNA sequences needed for pituitary-specific expression are contained within the proximal 350 bp of 5'-flanking sequence of the human α-subunit glycoprotein hormone gene, three lines of transgenic mice were constructed that contained pHα-350CAT. Founder animals were mated with nontransgenic mice (CF1) to establish new lines of mice. A variety of tissues were collected from founders and progeny (n = 3 animals/line) and analyzed for expression of the chloramphenicol acetyltransferase (CAT) reporter gene. Representative tissue surveys of CAT activity conducted with progeny from founder #35 and #161 are shown in Fig. 1. High levels of CAT activity were present in the pituitary gland, but CAT activity was not detectable in the brain, liver, lung, heart, spleen, kidney, gonad, or pancreas in transgenic mice from founder #35 or #161. Founder #157 and subsequent progeny (n = 37) failed to express CAT activity in pituitary (data not shown) and were not used for further studies involving hormonal regulation of pituitary-specific expression. Basal CAT activity in the pituitary gland was approximately 10-fold higher in mice from founder #161 compared to basal pituitary CAT activity in progeny from founder #35.

The 350-bp Human a-Subunit Promoter Is Not Expressed in the Placenta of Transgenic Mice

To determine if DNA sequences needed for placenta-specific expression are contained within the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene, male Hα-350CAT transgenic mice were mated to CF1 females. Surprisingly, CAT activity was not detected in the placentas of mice containing the Hα-350CAT transgene (Fig. 2). Five or six litters consisting of 31 transgenic pups from founder #35, 46 transgenic pups from

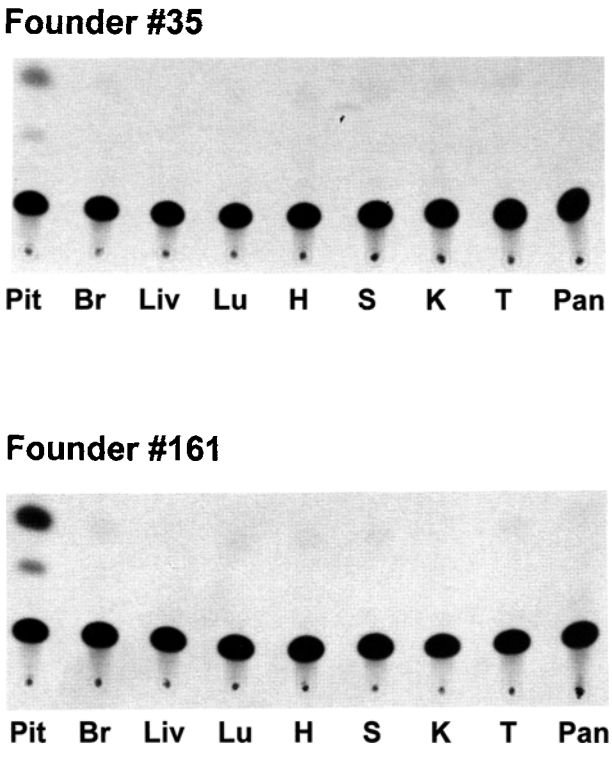

Fig. 1. The proximal 350 bp of 5'-flanking sequence of the human α-subunit gene directs expression of the CAT reporter gene to the pituitary gland (Pit), but not the brain (Br), liver (Liv), lung (Lu), heart (H), spleen (S), kidney (K), testes (T), or pancreas (Pan) of transgenic mice. A representative CAT assay of tissues collected from two lines of Hα-350CAT transgenic mice (Founder #35, top panel; Founder #161, bottom panel) is shown.

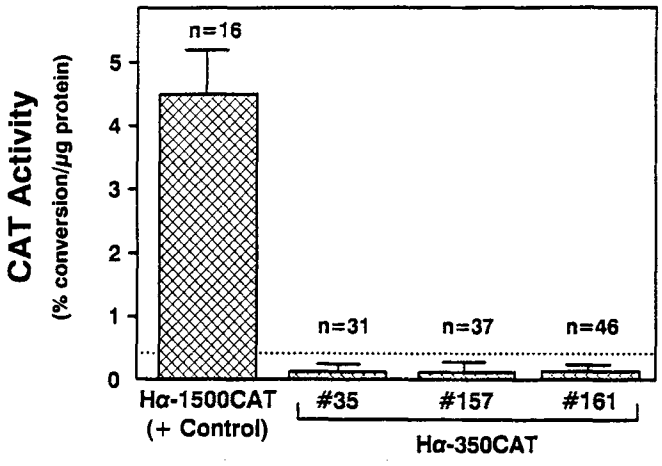

Fig. 2. The proximal 350 bp of 5'-flanking sequence of the human α-subunit gene fails to target expression to the placenta of transgenic mice. The horizontal dashed line represents background CAT activity (mean ± 3 SD or 0.37% conversion/μg protein) in placentas from nontransgenic mice. Placentas from Hα-1500CAT transgenic mice (Bokar et al., 1989) were included as positive controls (+ control). CAT activity was not detectable (<0.37 % conversion/μg protein) in placentas (n = 114) obtained from three independent lines of Hα-350CAT transgenic mice (Founders #35, #157, and #161).

founder #161, and 37 transgenic pups from founder #157 were analyzed. The CAT activity in placentas collected from nontransgenic mice was 0.16 ± 0.07% conversion/μg protein (mean ± SD); thus for placentas from transgenic mice to be positive for CAT expression, CAT activity had to be >0.37 (0.16 + 3 × 0.07)% conversion/μg protein. In placentas from transgenic mice, CAT activity was below 0.25% conversion/μg protein indicating that expression of the Hα-350CAT transgene was nondetectable. In agreement with previous findings (Bokar et al., 1989), the Hα-1500CAT transgene exhibited high levels of CAT expression in the placenta of transgenic mice (Fig. 2).

Gonadal Hormones and GnRH Regulate Expression of the 330-bp Hα-Subunit Promoter in the Pituitary Gland of Transgenic Mice

Subsequent studies were conducted to determine if the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene contained DNA sequences necessary for hormone responsiveness. Effects of gonadectomy and administration of a GnRH antagonist were examined in male or female Hα-350CAT transgenic mice from founders #35 and #161. Removal of gonadal hormones increased ($p < 0.05$) CAT activity in the pituitary gland of male or female mice from founder #35 or #161 compared to that measured in the pituitary gland of gonad-intact male or female transgenic mice (Fig. 3). Administration of antide, a GnRH antagonist, reduced ($p < 0.05$) CAT activity in the pituitary gland compared to that measured in the pituitary gland of gonadectomized transgenic mice (Fig. 3). Interestingly, basal CAT activity was approximately twofold higher in the pituitary of intact female mice compared to that measured in the pituitary gland of intact male mice.

Serum concentrations of LH were measured at the time of tissue collection to determine effectiveness of the antide treatment. There were no differences ($p > 0.05$) in serum concentrations of LH between the two lines of Hα-350CAT transgenic mice; thus, LH data from the two lines of transgenic mice were pooled within treatment. Administration of antide to gonadectomized male or female transgenic mice reduced ($p < 0.05$) serum concentrations of LH compared to those measured in gonadectomized transgenic mice (Fig. 4).

Discussion

Our initial studies with transgenic mice demonstrated that the proximal ~1500 bp of 5'-flanking sequence of the human α subunit gene contained aufficient information to direct expression specifically to the placenta and to gonadotropes (but not thyrotropes) within the pituitary gland of transgenic mice (Bokar et al., 1989; Hamernik et al., 1992). In this article, we describe further studies with transgenic mice containing a truncated promoter consisting of only 350 bp of 5'-flanking sequence of the human α-sub-

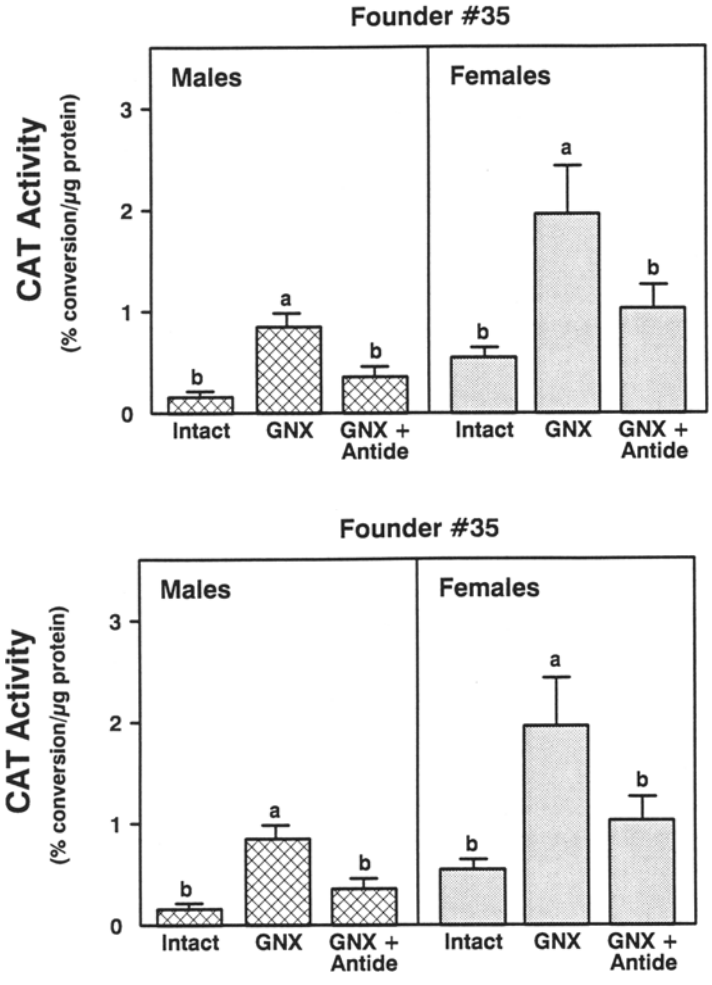

Fig. 3. The proximal 350 bp of 5'-flanking sequence of the human α-subunit gene contains sufficient information to confer responsiveness to gonadal hormones and GnRH in the pituitary of transgenic mice. Hormonal regulation studies were conducted with two lines of Hα-350CAT transgenic mice, which originated from founder #35 (top panel) or founder #161 (bottom panel). Values shown are the mean ± SEM of 6–13 animals/group. Within each founder animal, vertical bars with different superscripts are different ($p < 0.05$).

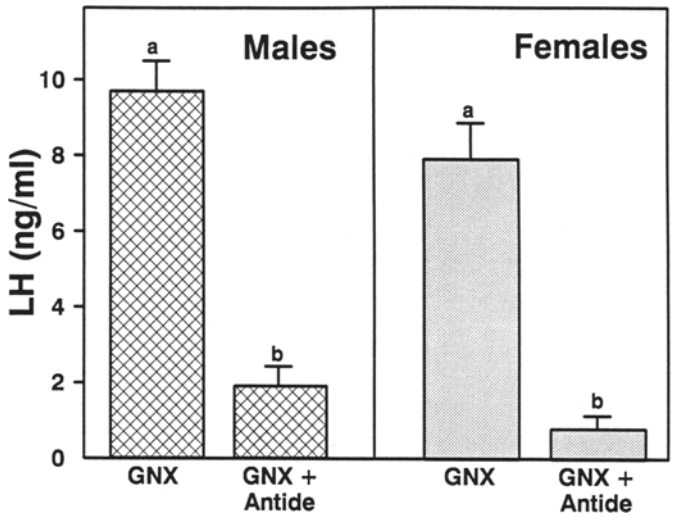

Fig. 4. Antide, a GnRH antagonist, decreased serum concentrations of LH in Hα-350CAT transgenic mice. Values shown are the mean + SEM of 16–24 animals/group. Within gender, vertical bars with different superscripts are different ($p < 0.05$).

unit gene. Whereas the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene contained sufficient information to direct expression to gonadotropes in transgenic mice, this minimal promoter-regulatory region failed to direct expression to the placenta of transgenic mice. From these results, we conclude that placenta-specific expression of the human α-subunit gene in transgenic mice requires DNA sequences in addition to the proximal 350 bp of 5'-flanking sequence.

Lack of expression of the 350 bp human α-subunit promoter-regulatory region in the placenta of transgenic mice was surprising. Numerous earlier studies (reviewed by Jameson and Hollenberg, 1993) used in vitro transient transfection systems with human placental cell lines to define a minimal placenta-specific enhancer consisting of only 182 bp of 5'-flanking sequence of the human α-subunit gene. This minimal placenta-specific enhancer contains DNA sequences important for trophoblast-specific expression (Delegeane et al., 1987; Bokar et al., 1989), cAMP responsiveness (Delegeane et al., 1987; Deutsch et al., 1987; Silver et al., 1987), a junctional regulatory element (Andersen et al., 1990), and a CCAAT box (Kennedy et al., 1990). Thus, we expected to see expression of the 350-bp human α-subunit promoter in the placenta of transgenic mice. One possible explanation for the discrepancy between our studies with transgenic mice and previous studies with in vitro transient transfection assays is that correct temporal and spatial expression of foreign DNA in transgenic mice requires more stringent DNA sequence requirements (Nilson et al., 1995). In support of this argument, studies with several other genes have revealed different DNA sequence requirements for cell-specific expression in cell lines compared to those required for expression in transgenic mice (Hammer et al., 1987; Pinkert et al., 1987; Swift et al., 1989; Morello et al., 1993). Thus, additional studies with transgenic mice containing varying amounts of 5'-flanking sequence of the human α-subunit gene are required to define further the DNA sequences that direct expression of the human α-subunit gene to the placenta of transgenic mice.

Expression of the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene in the pituitary gland of transgenic mice confirmed previous results obtained with in vitro transient transfection assays in mouse αT3-1 cells of gonadotrope origin. This truncated promoter-regulatory region of the human α-subunit gene contains DNA sequences (i.e., the GSE), which direct expression specifically to the gonadotrope-stem cell line, αT3-1 cells (and presumably gonadotropes; Horn et al., 1992), as well as DNA sequences that comprise the composite regulatory element (i.e., αBE1

and αBE2) needed for expression of α-subunit in gonadotropes (Heckert et al., 1995). A previous report (Horn et al., 1992) demonstrated that 152 or 168 bp of 5'-flanking sequence of the human α-subunit promoter were insufficient to direct expression of the human α-subunit gene to the pituitary gland of transgenic mice. Thus, we suggest that the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene contains the minimal amount of DNA sequence information to generate correct temporal and spatial expression of the α-subunit gene in the pituitary gland of transgenic mice.

We did not attempt to determine if the Hα-350CAT transgene was expressed in thyrotropes in transgenic mice. In previous studies (Windle et al., 1990; Kendall et al., 1991; Hamernik et al., 1992), the proximal ~1500 bp of 5'-flanking sequence of the human α-subunit gene did not direct expression to thyrotropes in the pituitary gland of transgenic mice. Thus, we expected that a truncated human α-subunit promoter would also fail to direct expression in thyrotropes in transgenic mice.

Interestingly, there was an approximate 10-fold difference in basal expression of the Hα-350CAT transgene between the two lines of transgenic mice. These observations are consistent with others (Palmiter et al., 1982; Swift et al., 1989; Fallest et al., 1995) and may result from integration of the transgene in the mouse genome at different locations, which may influence basal expression. Although differences in basal expression of the transgene were observed between the two lines of transgenic mice, hormonal manipulation in both lines of mice resulted in relatively similar changes in transgene expression. Thus, changes in transgene expression were regulated by specific hormones and were not owing to positional effects of the transgene in the mouse genome. In addition, gonad-intact female mice had two- to fivefold higher levels of basal expression from the Hα-350CAT transgene in the pituitary gland compared to that measured in the pituitary gland of gonad-intact male mice. Reasons for the gender difference in expression of the Hα-350CAT transgene in the pituitary gland of transgenic mice are not known, but may be owing to direct transcriptional repressive actions of androgen at the α-subunit promoter (Clay et al., 1993). Gender differences in basal expression have also been reported in transgenic mice harboring the proximal 2 kb of 5'-flanking sequence of the rat LHβ-subunit gene (Fallest et al., 1995).

In addition to containing sufficient information to direct pituitary-specific expression, the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene also contains sufficient information to confer responsiveness to gonadal hormones and GnRH. In agreement with previous studies (Keri et al., 1991; Clay et al., 1993), gonadectomy of Hα-350CAT transgenic mice resulted in elevated amounts of CAT activity compared to that measured in gonad-intact animals. Although the human α-subunit gene lacks a high-affinity binding site for the estradiol receptor complex (Keri et al., 1991), a high-affinity binding site for the androgen receptor complex is found at approximately 101 bp upstream from the transcription initiation site in the human α-subunit gene (Clay et al., 1993). Thus, removal of gonadal androgens in male mice may directly relieve transcriptional repression of the human α-subunit gene. In addition, removal of gonadal steroids leads to increased secretion of GnRH, which may then lead to increased expression of the α-subunit promoter. Our studies with gonadectomized mice cannot alone determine if expression of the 350 bp human α-subunit promoter is regulated by direct effects of gonadal steroids or GnRH.

The ability of GnRH to increase amounts of gonadotropin subunit mRNA and transcription of the α-subunit gene has been well established (Hamernik and Nett, 1988; Lalloz et al., 1988; Shupnik, 1990; Haisenleder et al., 1991; Hamernik, 1995). In addition, we have previously demonstrated that GnRH stimulated expression of the proximal ~1500 bp of human, or 315 bp of bovine, α-subunit 5'-flanking sequence in ovariectomized transgenic mice that were treated with GnRH every other hour for 7 d (Hamernik et al., 1992). To determine further if GnRH regulates expression of the proximal 350 bp of 5'-flanking sequence of the human α-subunit promoter in transgenic mice, we conducted studies with antide, a potent GnRH antagonist. In previous studies, antide decreased expression of the bovine (Keri et al., 1994) or rat (Fallest et al., 1995) LHβ subunit promoters in transgenic mice and required a less labor-intensive experimental approach than pulsatile injection of GnRH. In the current study, serum concentrations of LH at the end of antide treatment were measured to confirm that administration of antide was effective in decreasing expression of the endogenous mouse gonadotropin subunit genes. As expected, treatment of gonadectomized animals with antide decreased serum concentrations of LH by 80–90% compared to those values measured in vehicle-treated gonadectomized animals. When gonadectomized male or female mice containing the Hα-350CAT transgene were treated with antide, CAT activity in the pituitary was reduced to values that were similar to those of gonad-intact mice, indicating that expression of the Hα-350CAT promoter in transgenic mice requires GnRH.

Our ability to localize a GnRH-responsive region to the proximal 350 bp of the human α-subunit promoter in transgenic mice is consistent with studies employing transient transfection of the human α-subunit promoter-regulatory region into primary cultures of rat pituitary cells and treatment with a GnRH agonist. Recently, Kay and Jameson (1992) used these in vitro procedures to identify a region of GnRH responsiveness consisting of –346 to –244 bp of 5'-flanking sequence of the human α-subunit gene. Thus, we conclude that the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene contains sufficient DNA sequences to respond to GnRH. Because gonadotropes

are the only cell type in the pituitary gland that contain receptors for GnRH (Huckle and Conn, 1988), these data provide indirect evidence that the proximal 350 bp of the human α-subunit promoter directs expression specifically to gonadotropes in transgenic mice.

In summary, we have used transgenic mice to demonstrate that the proximal 350 bp of 5'-flanking sequence of the human α-subunit gene contains sufficient DNA sequences to target expression to the pituitary gland and confer responsiveness to gonadal steroids and GnRH. Our results obtained with transgenic mice harboring a truncated human α-subunit promoter support the current model describing DNA sequence requirements for gonadotrope-specific expression and hormonal regulation. Although the proximal 350 bp of 5'-flanking sequence of the human α-subunit promoter contains sufficient information to function in human placenta cell lines in vitro, this region of DNA did not target expression to the placenta of transgenic mice. Thus, it appears that sequences upstream of –350 are required for expression of the human α-subunit gene in the placenta of transgenic mice.

Materials and Methods

Construction of Hα-330CAT

A truncated promoter-regulatory fragment of the human α-subunit glycoprotein hormone gene containing only 350 bp of 5'-flanking sequence was obtained by PCR using the plasmid pBSKHα-1500CAT as template. The sense primer contained the DNA sequence: 5'-TAAATATCA GGTACT-3' corresponding to nucleotides –350/–336 of human α-subunit 5'-flanking sequence and contained a *Hin*dIII restriction enzyme site at the 5'-end to facilitate cloning of the PCR product. The antisense primer contained the DNA sequence: 5'-CTCCATTTTAGCTTCCTTAGC TCC-3' corresponding to the 5'-end of the CAT gene. The PCR product contained a *Hin*dIII site in the multiple cloning site of pBSKHα-1500CAT between the human α-promoter and the CAT reporter gene. Thus, the PCR product was digested with *Hin*dIII, gel-purified, and subcloned into the *Hin*dIII site of pBSKCAT to generate pBSKHα-350CAT. The nucleotide sequence of pBSKHα-350CAT was verified by dideoxysequencing (Sequenase, United States Biochemical, Cleveland, OH).

Generation of Hα-350CAT Transgenic Mice

The plasmid pBSKHα-350CAT was digested with *Cla*I and *Bam*HI and electrophoresed through an agarose gel to purify the Hα-350CAT insert from the pBSK vector. Linearized Hα-350CAT insert was microinjected into the male pronuclei of fertilized mouse eggs obtained from C57/BL6/SJL mice (Jackson Labs; Bar Harbor, ME) as described by Wagner et al. (1981). Injected eggs were transferred to the oviducts of pseudopregnant female mice and allowed to develop to term. After birth and subsequent weaning, mice were analyzed for the presence of the Hα-350CAT transgene by hybridizing mouse genomic DNA obtained from tail sections with a radioactive CAT probe on slot blots (Bokar et al., 1989). Three founder animals were obtained and each contained the Hα-350CAT transgene at a single site of integration in the mouse genome (determined by progeny testing and Southern blotting). In addition, the site of integration was different for each founder animal (data not shown). To estimate the number of copies of the transgene integrated in each line of transgenic mice, varying amounts of pBSKHα-350CAT were mixed with genomic DNA from a nontransgenic mouse and analyzed by Southern blotting (Kendall et al., 1994). Each line of transgenic mice contained 10–100 copies of the transgene (data not shown). One founder animal (#157) did not express the transgene in pituitary and was not used for further studies involving hormonal regulation. Two founder animals (#35 and #161) were mated to nontransgenic mice (CF 1; Sasco, Omaha, NE) to establish new lines of transgenic mice for subsequent studies of tissue-specific expression and hormonal regulation. All procedures for generation of transgenic mice and physiological studies with transgenic mice were approved by the Institutional Animal Care and Use Committee at the University of Nebraska.

Tissue-Specific Expression Hα-350CAT

Tissue-specific expression was investigated in founder animals and in mice from the third generation of offspring (n = 3 animals/line). Tissues (~100 mg) were collected from adult male transgenic mice or adult CF1 mice and homogenized in 200 μL 0.25*M* Tris-HCl (pH 7.8) and 0.5 m*M* phenylmethylsulfonylfluoride. Cell lysates were prepared for analysis of protein (Bio-Rad Hercules, CA; Bradford, 1976) and CAT activity (Bokar et al., 1989). Approximately 50 μg of protein were assayed for CAT activity (Gorman et al., 1982).

To determine if the Hα-350CAT transgene was expressed in placenta, male transgenic mice were mated to female CF1 mice. Pups and placentas were collected on day 17–19 of pregnancy. To identify transgenic fetuses, genomic DNA was isolated from each pup and analyzed by slot-blot hybridization to a radioactive CAT probe. Cell lysates were prepared from individual placentas, and CAT activity was measured as described above. Placentas were also collected from Hα-1500CAT transgenic mice to serve as positive controls (Bokar et al., 1989).

Hormonal Regulation of Hα-350CAT

Hormonal regulation studies were conducted with mice from the fourth and fifth generation of offspring. Sexually mature mice were anesthetized with avertin (1 cc 2.5% avertin/5 g body wt; Hogan et al., 1986) and gonadectomized. Immediately after gonadectomy, approximately one-half of the mice received a GnRH antagonist (antide; Sigma, St. Louis, MO) to occupy endogenous GnRH receptors and block endogenous GnRH from stimulating gonadotropes. Antide was administered via ip injections (60 μg/300 μL; one injection every 48 h) as described by Keri et al.

(1994). Control mice received similar injections of vehicle (20% [vol/vol] propylene glycol in saline). Pituitaries were collected 10 d after gonadectomy and from sexually mature gonad-intact male or female mice. Cell lysates were prepared and analyzed for CAT activity as described above. Trunk blood was collected at tissue collection to measure serum concentrations of LH by radioimmunoassay (Niswender et al., 1968).

Statistical Analyses

Tissues were considered positive for transgene expression if CAT activity was >3 SD above the mean of CAT activity in tissues collected from nontransgenic mice (Bokar et al., 1989). Only animals that were positive for CAT expression were used for further analysis. Serum concentrations of LH and CAT activity in pituitaries were analyzed by one-way analysis of variance, and treatment means were separated by Duncan's New Multiple Range Test (Steel and Torrie, 1980).

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