

## Regulation of the Pituitary Somatotroph Cell by GHRH and Its Receptor

KELLY E. MAYO, TERESA MILLER, VENITA DEALMEIDA,  
PAUL GODFREY, JING ZHENG, AND SHANE R. CUNHA

*Department of Biochemistry, Molecular Biology & Cell Biology, Northwestern University,  
2153 North Campus Drive, Evanston, Illinois 60208*

### ABSTRACT

Hormones from the hypothalamus mediate interactions between the nervous and endocrine systems by controlling the activity of specific target cells in the anterior pituitary gland. The hypothalamic peptide, growth hormone-releasing hormone (GHRH), acts on pituitary somatotroph cells to stimulate their proliferation during development and to regulate their ability to produce and secrete growth hormone (GH). These actions are mediated by a recently identified receptor for GHRH that belongs to family B-III of the G protein-coupled receptor superfamily. The rat GHRH receptor is expressed predominantly in the pituitary gland and in somatotroph cells. To investigate this tissue- and cell-specific expression, the receptor gene has been cloned and characterized. The receptor gene promoter is selectively expressed in pituitary cells and is regulated by the pituitary-specific transcription factor Pit-1. There is a sexual dimorphism in GHRH receptor expression in the rat pituitary, suggesting regulation by gonadal steroids. In addition, glucocorticoids are potent positive regulators of GHRH receptor gene expression. Substantial evidence points to an important role for GHRH in regulating the proliferation and functional activity of the somatotroph cell. This is best observed in the dwarf *little* mouse, which harbors a mutation in the extracellular domain of the GHRH receptor that abolishes the receptor's hormone-binding and signaling properties, resulting in severe somatotroph hypoplasia. Complementary studies in transgenic mice overexpressing the ligand GHRH reveal corresponding somatotroph hyperplasia. Consistent with these observations, GHRH potently activates the MAP kinase pathway in pituitary somatotroph cells. To better understand the hormone-binding and signaling properties of the GHRH receptor, mutant and chimeric receptors have been analyzed to define domains important for GHRH interaction. The GHRH receptor signals predominantly through cAMP-dependent pathways; however, a variant form of the GHRH receptor with an insertion into the third intracellular domain, generated through alternative RNA processing, binds GHRH but fails to signal, suggesting potential modulation of receptor function at a post-transcriptional level. This chapter will integrate these basic investigations of GHRH and its receptor with current information on the involvement of the GHRH signaling system in human diseases of GH secretion and growth.

### I. Introduction

#### A. THE NEUROENDOCRINE GROWTH HORMONE AXIS

The anterior pituitary gland receives neuroendocrine signals, in the form of hypothalamic peptides, which act to stimulate or suppress the secretion of each

of the pituitary hormones, thus providing for integration between the brain, where sensory information is being processed, and the pituitary gland, where an appropriate endocrine response is subsequently generated. In the case of the growth hormone (GH)-secreting pituitary somatotroph cell, the predominant positive neuroendocrine signal is growth hormone-releasing hormone (GHRH). Its actions are antagonized by the suppressive effects of somatostatin (Tannenbaum and Ling, 1984; Devesa *et al.*, 1992). Growth hormone secreted from the anterior pituitary acts on diverse target tissues, where it can directly affect cell proliferation and differentiation. In addition, GH is a potent stimulus for the production of the somatomedins, or insulin-like growth factors, which ultimately mediate many of the effects of GH on cellular metabolism (Daughaday, 1995). As is the case with most endocrine cascades, hormones such as the somatomedins and GH are able to exert feedback effects, typically repressing the activity of the system. Figure 1 shows in schematic fashion this hormonal cascade controlling GH secretion and linear growth in vertebrate organisms.

A second class of molecules able to stimulate GH secretion is the synthetic growth hormone secretagogues (GHS), including the hexapeptide GHRP-6 (Bowers *et al.*, 1984) and several nonpeptide mimics of this synthetic hormone (Smith *et al.*, 1993, 1997). These compounds are believed to act both at the hypothalamic and pituitary level to impact GH secretion, as shown in Figure 1. A specific G protein-coupled receptor for these secretagogues recently was identified that is expressed in both the hypothalamus and pituitary (Pong *et al.*, 1996; McKee *et al.*, 1997). However, an endogenous ligand for the GHS receptor has yet to be identified.

## B. GROWTH HORMONE-RELEASING HORMONE

GHRH was initially isolated from pancreatic tumors that caused acromegaly, then later characterized from the hypothalamus (Guillemin *et al.*, 1982; Rivier *et al.*, 1982; Spiess *et al.*, 1983). GHRH is released from neurosecretory cells in the arcuate nuclei of the hypothalamus (Merchenthaler *et al.*, 1984; Sawchenko *et al.*, 1985). GHRH also is expressed in the placenta, where it may have paracrine functions or contribute to fetal growth (Suhr *et al.*, 1989; Margioris *et al.*, 1990), and in the gonads, where it may be an autocrine or paracrine regulator of steroidogenesis and granulosa or Sertoli cell function (Berry and Pescovitz, 1988; Bagnato *et al.*, 1992; Ciampani *et al.*, 1992). An important role for GHRH in linear growth is suggested by clinical studies with tumors that secrete GHRH (Frohman and Szabo, 1981; Thorner *et al.*, 1982; Frohman and Jansson, 1986) and by animal studies with transgenic mice that overexpress GHRH (Hammer *et al.*, 1985; Stefaneanu *et al.*, 1989). In both cases, GH hypersecretion, somatotroph hyperplasia, and inappropriate growth (acromegaly or gigantism) are observed.

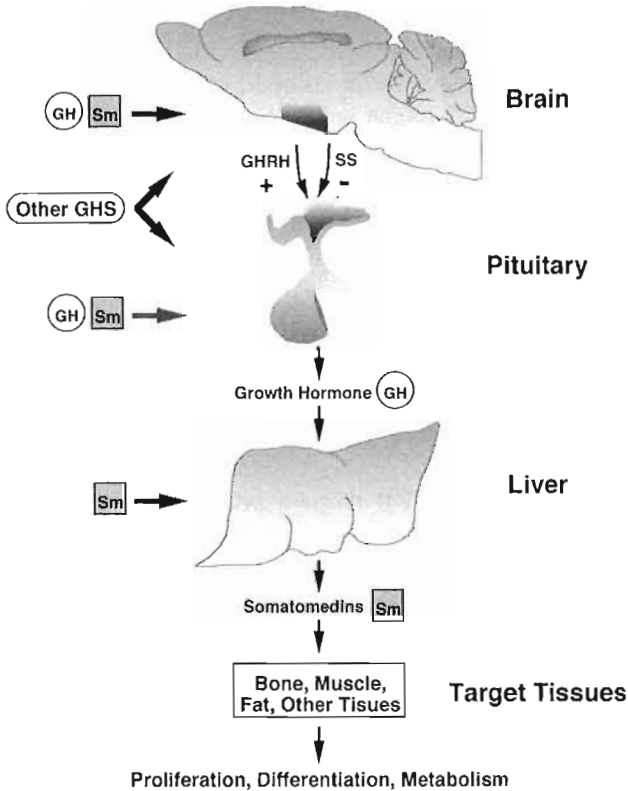


FIG. 1. Neuroendocrine regulation of the growth hormone (GH) axis. GHRH and somatostatin from the hypothalamus are the predominant stimulatory and inhibitory factors, respectively, for pituitary GH synthesis and secretion. In addition to its direct effects on target tissues, many of the effects of GH are mediated by somatomedins produced in the liver. Growth hormone and somatomedins are, in turn, important mediators of negative feedback regulation. Other growth hormone secretagogues (GHS) indicate the potential for novel GHS that act on a receptor expressed in hypothalamus and pituitary.

GHRH is a peptide hormone of 42–44 amino acids, depending on species, that is processed from a larger precursor protein of 103–108 amino acids (Gubler *et al.*, 1983; Mayo *et al.*, 1983, 1985; Frohman *et al.*, 1989; Suhr *et al.*, 1989). The peptide is C-terminally amidated in many species but not in rodents. GHRH is structurally related to a large family of peptide hormones, including secretin, glucagon, glucagon-like peptide-1 (GLP-1), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), peptide with histidine as N-terminus and isoleucine as C-terminus (PHI), and gastric inhibitory peptide (GIP) (Campbell and Scanes, 1992).

### C. THE GHRH RECEPTOR

Substantial biochemical evidence suggests that GHRH acts through a G protein-coupled receptor to increase levels of the cellular second messenger cAMP in the somatotroph cell (Bilezikjian and Vale, 1983; Labrie *et al.*, 1983). Genetic studies supporting this notion include the findings that 1) many GH-secreting pituitary tumors harbor activating mutations in the  $G_{sa}$  protein (Vallar *et al.*, 1987; Landis *et al.*, 1989); 2) somatotroph hyperplasia and GH hypersecretion result from pituitary expression of a cholera toxin transgene in transgenic mice (Burton *et al.*, 1991); and 3) somatotroph hypoplasia and GH deficiency result from the pituitary expression of a dominant-negative mutant form of the cAMP-responsive transcription factor CREB in transgenic mice (Struthers *et al.*, 1991).

Based on these observations, several groups identified novel G protein-coupled receptor cDNAs from human, rat, mouse, and pig encoding 423 residue proteins that have the structural and functional characteristics expected of a GHRH receptor (Lin *et al.*, 1992; Mayo, 1992; Gaylinn *et al.*, 1993; Hsiung *et al.*, 1993). The predicted GHRH receptor protein 1) has the seven potential membrane-spanning motifs of a G protein-coupled receptor, 2) is homologous to the receptors for peptides related to GHRH, 3) is of the size expected from GHRH photoaffinity cross-linking studies, and 4) is expressed predominantly in the anterior pituitary gland. Most importantly, when the GHRH receptor protein is expressed in transfected cells, those cells acquire the ability to bind GHRH with high affinity and selectivity and to respond to GHRH to increase intracellular levels of the second messenger cAMP. The general structure of the GHRH receptor protein is shown in Figure 2A, which also illustrates several key features to be discussed in subsequent sections. Figure 2B is a phylogenetic tree based on sequence identity for representative receptor sequences of family B of the G protein-coupled receptor superfamily, showing the relatedness between the GHRH receptor and receptors for GHRH-related peptides. A second branch of this family includes receptors for peptides that are not related in sequence to GHRH, including the parathyroid hormone (PTH), calcitonin, and corticotropin-releasing hormone (CRF) receptors (Segre and Goldring, 1993).

### D. THE PITUITARY SOMATOTROPH CELL

Substantial progress has been made in understanding the early developmental events leading to pituitary organogenesis, largely through genetic approaches in mice (Andersen and Rosenfeld, 1994; Rosenfeld *et al.*, 1996; Watkins-Chow and Camper, 1998). Development of the anterior pituitary gland appears to involve a complex series of inductive events in which BMP4 from the ventral diencephalon establishes a zone of oral ectoderm from which expression of Sonic hedgehog is restricted, forming Rathke's pouch. A BMP2 signaling gradient subsequently arises from Rathke's pouch. The interaction of this ventral gradient with an

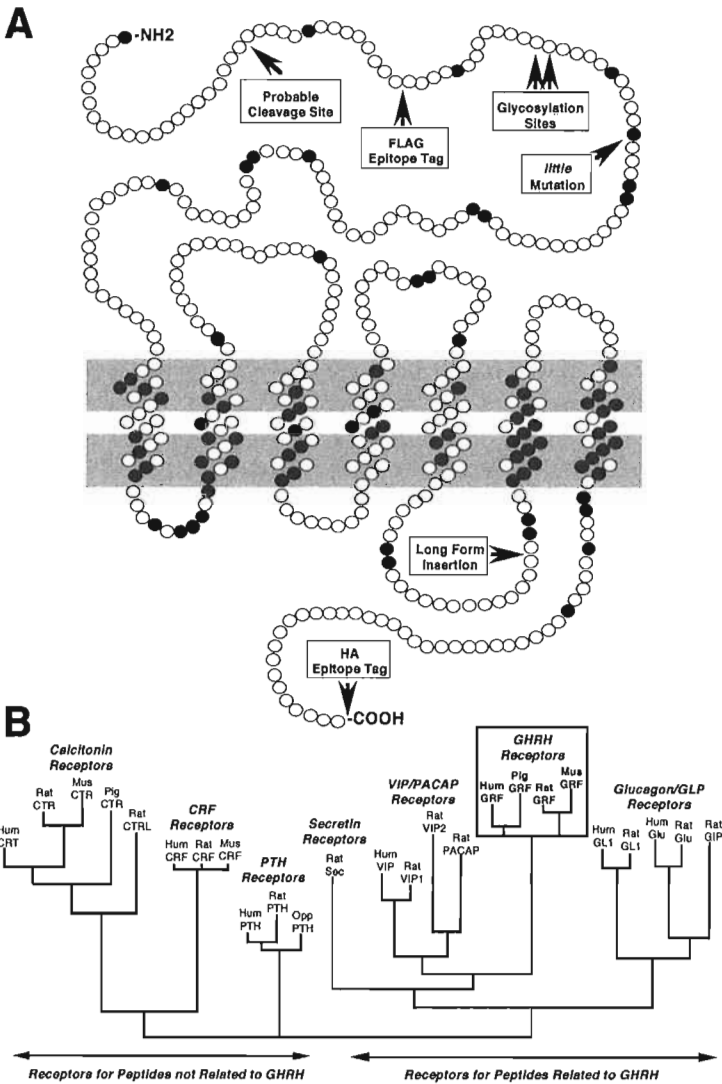


FIG. 2. Schematic structure of the rat GHRH receptor. Panel A points out key features, including the signal sequence cleavage site, glycosylation sites, a 41-amino-acid insertion in an alternatively spliced receptor isoform, aspartic acid residue 60 (mutated in the dwarf *little* mouse), and the locations of FLAG and hemagglutinin (HA) epitope tags introduced to facilitate receptor protein detection. Shaded residues are conserved in the related rat secretin, glucagon, VIP, GLP-1, and PACAP receptors. Panel B shows a phylogenetic tree based on sequence identity for representative receptor sequences of family B of the G protein-coupled receptor superfamily. The GHRH receptors are shown in the shaded rectangle. [Adapted from the G protein-coupled receptor (GPCR) database (<http://swift.embl-heidelberg.de/7tm>).]

opposing dorsal gradient of FGF8 is proposed to pattern the future pituitary and initiate the expression of transcription factors required for the specification of the five distinct, hormone-secreting cell types (Takuma *et al.*, 1998; Treier *et al.*, 1998). A large number of homeobox transcription factors—including Lhx3, Lhx4, Titf1, Pitx1, and Rpx—participate in these early developmental events of pituitary gland formation (Watkins-Chow and Camper, 1998). Two of these transcriptional regulatory proteins, Prop-1 and Pit-1, are critical for specifying the thyrotroph, lactotroph, and somatotroph lineages. Mutation of these genes in mouse or human results in a failure of these cell lineages to form (Li *et al.*, 1990; Sornson *et al.*, 1996).

In many respects, less is known about the factors that result in further specialization of cells in the Prop-1/Pit-1 lineage. Pit-1-dependent thyrotroph and somatotroph/lactotroph precursor populations diverge by embryonic day 16 in the mouse (Andersen and Rosenfeld, 1994). Subsequently, this somatotroph/lactotroph precursor cell gives rise to fully differentiated somatotrophs and to a somatolactotroph population that eventually gives rise to lactotrophs, which appear largely after birth (Andersen and Rosenfeld, 1994). All of these cells express Pit-1; therefore, additional factors (or the absence of factors) are clearly required to specify the somatotroph cell. Acquisition of the GHRH receptor defines the fully differentiated somatotroph phenotype, allowing these cells to be subsequently expanded under the trophic influence of GHRH.

The following sections of this chapter consider the regulation and actions of GHRH and its receptor, within the framework of the development and differentiation of the pituitary somatotroph cell. Section II considers the tissue- and cell-specific expression of the GHRH receptor gene, a critical early event in establishment of the differentiated somatotroph phenotype. Section III discusses the regulation of GHRH receptor synthesis and increased responsiveness to GHRH, focusing on the role of the steroid hormones in this process. Section IV presents evidence from genetic models for an important role of GHRH in the proliferation of the somatotroph cell during pituitary development. Section V considers functional aspects of hormone binding and signal transduction by the GHRH receptor, leading to enhanced GH synthesis and secretion in the mature somatotroph cell. Finally, the summary section VI will attempt to integrate this information and discuss relevance of these basic investigations for understanding human diseases impacting the GH axis.

## II. Expression of the GHRH Receptor

### A. PITUITARY-SPECIFIC EXPRESSION

The GHRH receptor is expressed predominantly in the pituitary gland, with lesser expression in several other tissues. Figure 3A in an example of a reverse transcription-polymerase chain reaction (RT-PCR) assay used to detect GHRH

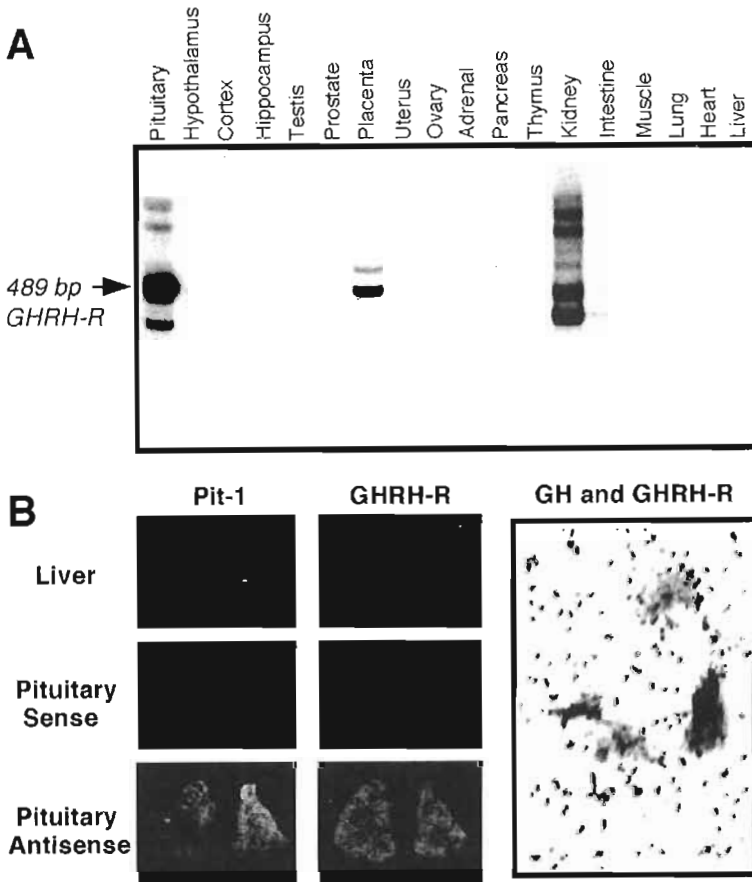


FIG. 3. Expression of the GHRH receptor mRNA in rat tissues and in the pituitary. (A) An RT-PCR assay of receptor mRNA expression in the indicated rat tissues. The expected 489-bp PCR product is indicated by the arrow. (B) *In situ* hybridization localization of the GHRH receptor mRNA and a Pit-1 mRNA control to the rat anterior pituitary gland. The right portion of panel B is a double-label, *in situ* hybridization using digoxigenin-growth hormone and  $^{35}\text{S}$ -GHRH-R probes, showing colocalization to several pituitary somatotroph cells. [Panel B adapted with permission from Mayo, K.E. *Mol. Endocrinol.* 6, 1734-1744, 1992. Copyright © 1992 The Endocrine Society.]

receptor mRNA in a large number of rat tissues. The receptor mRNA is highly expressed in the pituitary, as expected. In addition, receptor RNA transcripts are found in the placenta, a tissue that also expresses the ligand GHRH (Suhr *et al.*, 1989; Margioris *et al.*, 1990). The most surprising finding is the strong RNA signal seen in the kidney, also observed by others (Matsubara *et al.*, 1995). Our preliminary analysis indicates that this kidney transcript differs from the pituitary

transcript near the 5' end of the mRNA and that it is unlikely to encode a functional GHRH receptor protein. There is little expression of the GHRH receptor mRNA in other tissues, although, at higher amplification cycles, the RNA is detected at low levels in a broad range of tissues (Matsubara *et al.*, 1995).

Within the pituitary gland, the GHRH receptor is co-expressed with the pituitary-specific transcription factor Pit-1 in the anterior lobe, as shown using *in situ* hybridization in Figure 3B. The receptor transcript can be co-localized to GH-expressing somatotroph cells using double-label, *in situ* hybridization approaches, although the sensitivity of this technique is probably not sufficient to exclude expression of the receptor in other pituitary cell types. Expression of the GHRH receptor in the pituitary is developmentally regulated; the transcript is first detected on embryonic day 16.5 in the mouse (Lin *et al.*, 1992), peaks in the late embryonic period, and then declines before increasing again with the onset of puberty (Korytko *et al.*, 1996). GHRH-binding sites and GHRH receptor mRNA decrease in aged rats in association with the decline in GH (Abribat *et al.*, 1991; Girard *et al.*, 1999).

## B. THE GHRH RECEPTOR GENE

To investigate the gene regulatory events important for expression of the GHRH receptor in the pituitary gland, the rat GHRH receptor gene was cloned and characterized (Miller *et al.*, 1999). Figure 4A shows the structure of the gene, which is composed of 14 exons and spans approximately 15 kilobases of genomic DNA. Exon 11 is an alternatively spliced exon that encodes an insertion in the third cytoplasmic loop of the receptor that was identified during the initial cloning of the cDNA (Mayo, 1992). As shown in Figure 4C, there is no apparent correspondence between exons of the gene and structural features of the protein (such as the membrane-spanning domains). The exons are small, averaging about 100 bp in size, while the introns vary from 111 bp to more than 2 kb. Overall, the gene has a structural organization similar to the genes for other G protein-coupled receptors in family B-III (Lin *et al.*, 1993; Yamada *et al.*, 1995; Chatterjee *et al.*, 1997; Pei, 1997). Family B-III has the most complex gene organization reported among the G protein-coupled receptors. A partial structure of the 5' half of the human GHRH receptor gene has recently been reported (Petersenn *et al.*, 1998) that displays a similar organization to that of the rat gene.

Four transcriptional start sites utilized in the pituitary gland were mapped between 84 and 286 bp upstream of the translational initiation codon. This region does not contain consensus "CCAT" or "TATA" sequences. The 5' flanking region of the gene was sequenced; Figure 4B shows a select number of the potential transcription factor-binding sites identified in the putative promoter. Of particular interest is the presence of several potential binding sites for the pituitary-

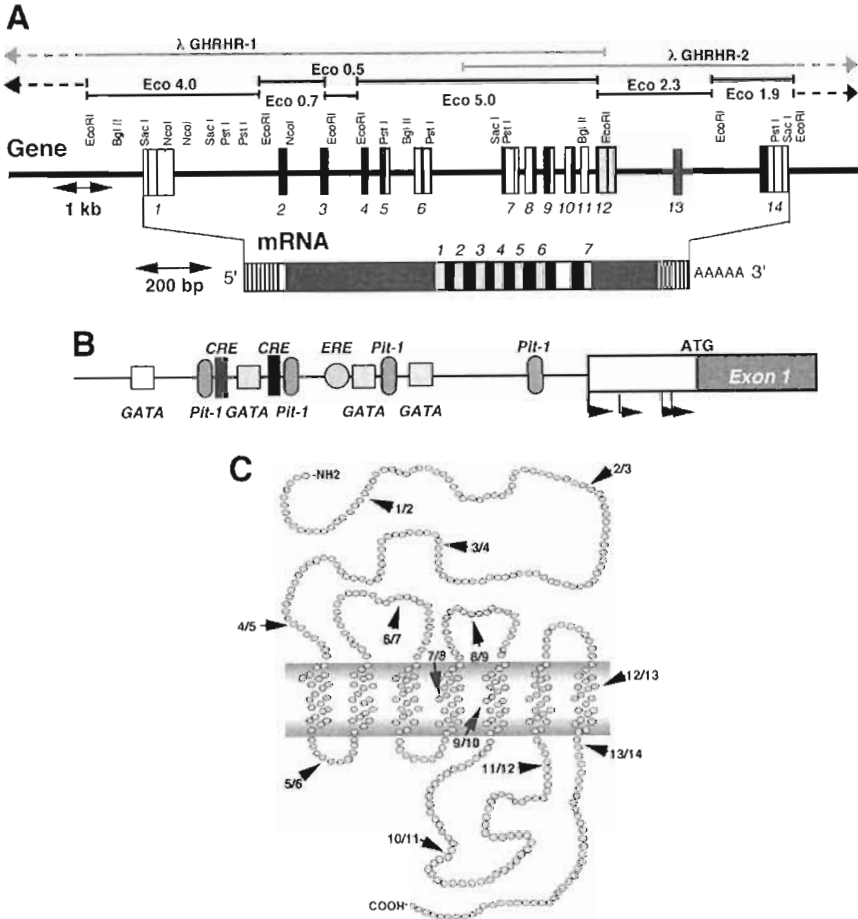


FIG. 4. Structure of the rat GHRH receptor gene and promoter. Panel A shows overlapping phage lambda clones and *EcoRI* subclones, the arrangement of the 14 exons within genomic DNA, and the structure of the mRNA, with the regions corresponding to the seven transmembrane domains numbered. Exon 11 (shown in white) is alternatively spliced. Panel B shows transcription start sites mapped by primer extension of pituitary RNA (indicated by arrows). Selected transcription factor binding consensus sites in the 5' flanking region are shown (there are also numerous GRE half sites). Panel C is a schematic of the rat GHRH receptor protein showing the location and distribution of the exon-intron boundaries (indicated with arrows and the corresponding junctional exon numbers). [Adapted with permission from Miller, T.L., Godfrey, P.A., DeAlmeida, V.I., and Mayo, K.E. *Endocrinology* 140, 4152-4165, 1999. Copyright © 1999 The Endocrine Society.]

specific transcription factor Pit-1, which is required for establishment of the somatotroph lineage (Li *et al.*, 1990; Radovick *et al.*, 1992).

The 5' flanking region of the GHRH receptor gene directs regulated transcription in transfected pituitary cells. As shown in Figure 5A, various 5' restriction fragments were fused to a luciferase reporter gene. When these constructs were tested in the GH-secreting pituitary tumor cell line GH3 (Figure 5B), the largest constructs had considerable basal promoter activity. Basal activity of the promoter progressively decreased as the 5' flanking sequences were truncated. There are several potential binding sites for Pit-1 in the GHRH receptor gene promoter. When GH3 cells, which express endogenous Pit-1, are co-transfected with a Pit-1 expression construct, a two-fold stimulation of promoter activity was observed. In COS7 cells, which do not express endogenous Pit-1, the GHRH receptor promoter was inactive (Figure 5C). However, there was a robust activation of the promoter when Pit-1 was co-transfected into these cells. Pit-1 responsiveness in both GH3 and COS7 cells diminishes with progressive deletion of the promoter, although all constructs except the shortest one are Pit-1 responsive. Co-transfection of a Pit-1 construct with a deletion in the homeodomain (Pit-1q), which is required for DNA binding, did not stimulate promoter activity. Pit-1 also has been observed to transactivate the human and mouse GHRH receptor genes (Lin *et al.*, 1992; Petersenn *et al.*, 1998) and appears to be an important determinant of the pituitary-specific expression of this gene.

### III. Regulation of GHRH Receptor Expression

#### A. REGULATION BY GLUCOCORTICOIDS

Glucocorticoid hormones have complex and paradoxical effects in the GH axis. While they are generally growth suppressive, they act directly on the pituitary to stimulate GH gene expression (Evans *et al.*, 1982; Moore *et al.*, 1985) and augment basal and GHRH-induced GH release (Vale *et al.*, 1983; Wehrenberg *et al.*, 1983). Glucocorticoids are also emerging as important regulators of somatotroph function. Glucocorticoids stimulate somatotroph differentiation in the fetal rat pituitary *in vivo* and in pituitary explants *in vitro* (Hemming *et al.*, 1984; Nogami and Tachibana, 1993; Nogami *et al.*, 1995). Administration of glucocorticoids during late gestation advances the timing of somatotroph development, whereas administration of a glucocorticoid synthesis inhibitor delays or suppresses somatotroph development (Nogami and Tachibana, 1993). Serum corticosterone concentrations in the rat fetus are transiently elevated during days 17–21 of gestation, with a peak at day 19, coincident with the time when somatotrophs are detected in the rat pituitary (Dupouy *et al.*, 1974; Bourdoursque *et al.*, 1988). Glucocorticoids also can stimulate transdifferentiation from a lactotroph to somatotroph phenotype in cell culture (Kineman *et al.*, 1992).

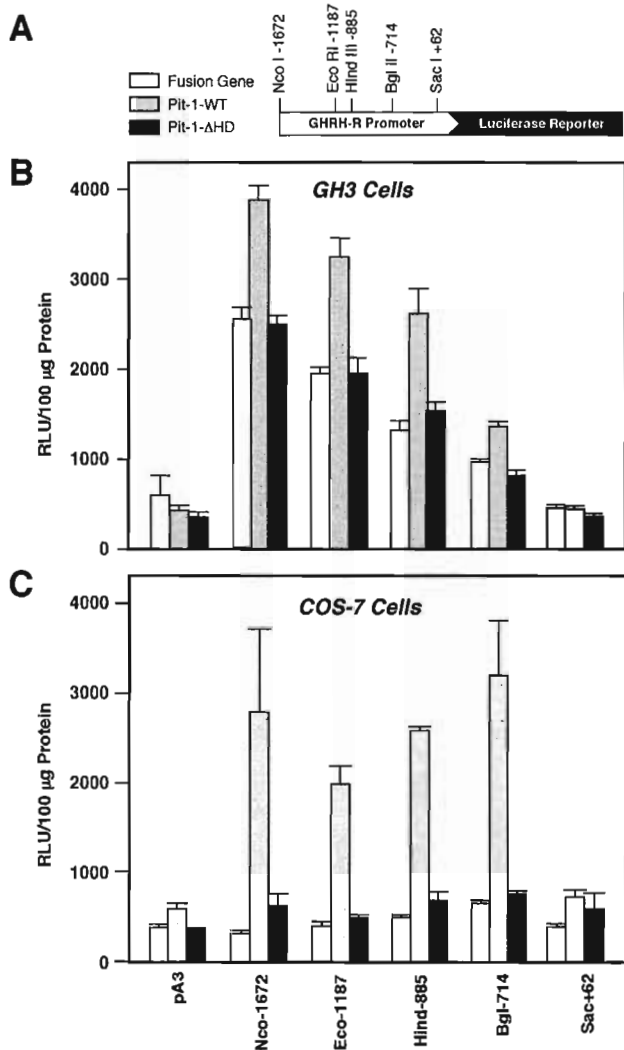


FIG. 5. Expression of the GHRH receptor promoter in transfected cells. (A) A map of the GHRH receptor promoter-luciferase reporter fusion gene showing the locations of the restriction enzyme sites used to generate the deletion constructs (Nco-1672, Eco-1187, Hind-885, Bgl-714, and Sac+62 5' GHRH receptor sequences fused to a pA3-luciferase vector). These constructs were transfected into cells either alone or together with a Pit-1 expression construct (Pit-1-WT) or a mutant version of Pit-1 lacking a portion of the homeodomain (Pit-1- $\Delta$ HD). (B) Luciferase activity assays in lysates from transfected rat pituitary GH3 cells. (C) An identical experiment performed in monkey kidney COS-7 cells. [Adapted with permission from Miller, T.L., Godfrey, P.A., DeAlmeida, V.I., and Mayo, K.E. *Endocrinology* **140**, 4152-4165, 1999. Copyright © 1999 The Endocrine Society.]

Prior to the identification of the GHRH receptor, it had been shown that glucocorticoids could increase GHRH-binding sites in the rat pituitary and enhance pituitary responsiveness to GHRH (Seifert *et al.*, 1985a, 1985b). Following the cloning of the receptor, several groups reported that glucocorticoids stimulate GHRH receptor gene expression both *in vivo* and *in vitro* (Lam *et al.*, 1996; Korytko and Cuttler, 1997; Miller and Mayo, 1997). An example of this is shown in Figure 6. As shown in Figure 6A, there is a five-fold increase in pituitary GHRH receptor mRNA in the rats treated *in vivo* with corticosterone, compared to those that were adrenalectomized. Figure 6 also shows the ability of the synthetic glucocorticoid dexamethasone to regulate receptor gene expression in pri-

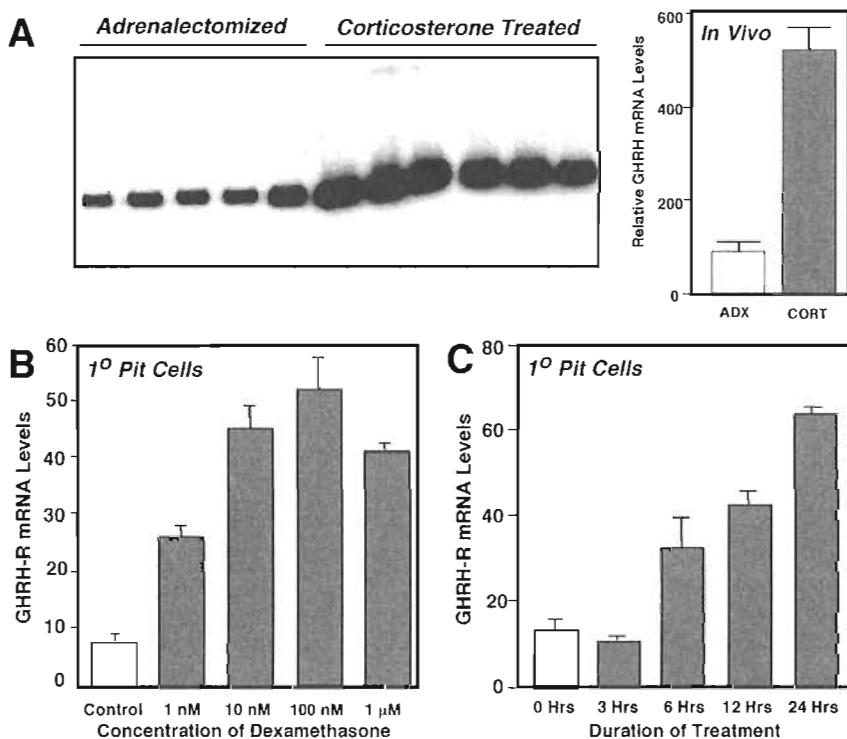


FIG. 6. Regulation of GHRH receptor mRNA by glucocorticoids *in vivo* and *in vitro*. Panel A shows an RT-PCR gel and its quantification of pituitary RNA from adrenalectomized versus corticosterone treated rats. The lower two panels show GHRH receptor mRNA levels in primary cultured pituitary cells treated with dexamethasone at varying doses (B) or for varying times (C). For quantification, all RNA measurements are normalized to levels of ribosomal protein L19 mRNA. [Adapted with permission from Miller, T.L., and Mayo, K.E. *Endocrinology* **138**, 2458–2465, 1997. Copyright © 1997 The Endocrine Society.]

mary rat pituitary cell cultures *in vitro*. There is a dose-dependent (Figure 6B) and time-dependent (Figure 6C) increase in GHRH receptor mRNA to about five-fold of control values 24 hours following dexamethasone addition. This is likely to be a direct transcriptional response to the steroid hormone in that the mRNA induction is completely blocked by the transcription inhibitor actinomycin D (Miller and Mayo, 1997). GHRH receptor mRNA is also positively regulated by glucocorticoids in the MtT/S pituitary tumor cell line, which expresses the endogenous GHRH receptor gene (Miller *et al.*, 1999).

## B. REGULATION BY GONADAL STEROIDS

A hallmark of GH secretion in the rat is its sexually dimorphic pattern (Terry *et al.*, 1977; Gonzalez and Jolin, 1981). The adult male secretory pattern is characterized by high-amplitude GH pulses at 3- to 4-hour intervals, while the female pattern is characterized by infrequent low amplitude pulses and higher basal secretion (Gatford *et al.*, 1998). While gonadal steroids can modify the pattern of GH secretion in the adult, androgens exert their most significant actions to establish a male pattern of GH secretion during the neonatal period (Jansson and Frohman, 1987). It is thought that a positive action of androgen, rather than a repression by estrogen, is dominant in establishing the sexual dimorphism, in that testicular feminized rats, which are androgen resistant, have GH secretory patterns that resemble females or neonatally castrated males (Millard *et al.*, 1986). Gonadal steroids are likely to act at many levels to modify the pattern of GH secretion. For example, androgens directly increase GHRH gene expression in the arcuate nucleus of the hypothalamus (Zeitler *et al.*, 1990).

These findings suggest that sexually dimorphic expression of the GHRH receptor might contribute to differential pituitary responsiveness to GHRH and to sexually dimorphic GH secretory patterns. As shown in Figures 7A and B, using two different mRNA detection approaches, expression of the GHRH receptor mRNA is significantly higher in the adult male than in the age-matched female pituitary gland. Despite this observation, removal and replacement of gonadal steroids appear to have only minimal effects on receptor gene expression in adult animals. Figure 7C shows that castration did not result in a decrease in pituitary GHRH receptor mRNA levels, although testosterone replacement of these castrated rats did increase receptor mRNA levels by nearly two-fold. Figure 7D shows that there is no change in pituitary GHRH receptor mRNA levels in response to ovariectomy or estrogen replacement in adult female rats. It is possible that the effects of gonadal steroids are manifest predominantly during the perinatal period, when the sexual dimorphism of the GH axis is being established, and that minimal regulation of receptor expression occurs in the adult pituitary gland. Others have observed a sexual dimorphism in pituitary GHRH receptor gene expression (Ono *et al.*, 1995). Interestingly, estrogen is reported to directly sup-

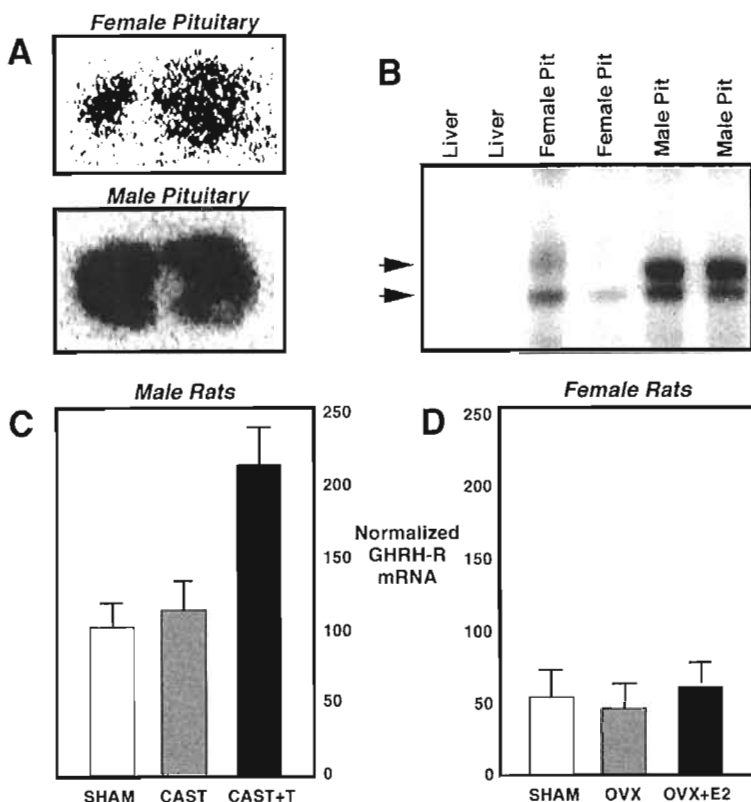


FIG. 7. Sexually dimorphic expression of the GHRH receptor mRNA. (A) An *in situ* hybridization to age-matched male or female rat pituitaries using an  $^{35}\text{S}$ -labeled antisense GHRH-R riboprobe. (B) An RNase protection assay using RNAs from male liver and female or male rat pituitaries. The arrows indicate two protected GHRH-R RNA species. The lower panels are quantifications of RT-PCR experiments using pituitary RNAs from male rats that were castrated and replaced with testosterone (C) or female rats that were ovariectomized and replaced with estrogen (D).

press activity of the human GHRH receptor promoter in transfected cells (Petersen *et al.*, 1998). A consensus estrogen-response element is found within the rat GHRH receptor gene promoter.

#### IV. GHRH and Somatotroph Cell Proliferation

##### A. PITUITARY HYPOPLASIA IN MICE LACKING A FUNCTIONAL GHRH RECEPTOR

During the perinatal period, a small number of GHRH-responsive somatotroph cells found within the anterolateral portion of the pituitary are proposed to

undergo a profound expansion to populate the entire pituitary gland (Lin *et al.*, 1993). There is strong evidence that this process requires the trophic actions of GHRH. Mutation of the GHRH receptor in the dwarf *little* mouse results in a failure of this proliferative event and a severe somatotroph hypoplasia (Godfrey *et al.*, 1993; Lin *et al.*, 1993). Similarly, mutation of the *Gsh-1* homeobox gene in mice results in a loss of GHRH expression in the hypothalamus and, as a consequence, the mutant mice exhibit a severe pituitary hypoplasia and extreme dwarfism (Li *et al.*, 1996).

The *little* dwarf mouse (Eicher and Beamer, 1976) has been used as an animal model for human isolated GH deficiency. Circulating GH levels and GH mRNA are substantially reduced in these mice, there are fewer pituitary somatotroph cells, and the cells that remain are sparsely granulated (Eicher and Beamer, 1976; Cheng *et al.*, 1983; Wilson *et al.*, 1988). The mutation, located on mouse chromosome 6, is recessive; homozygous mutant mice reach an adult size that is 50–60% of normal body weight (Eicher and Beamer, 1976). Somatotroph cells from *little* mice do not release GH following GHRH treatment in culture but they do secrete GH in response to cAMP or agents that elevate intracellular cAMP levels, suggesting that the defect in the *little* mouse is related to an inability of somatotrophs to bind to or respond to GHRH (Clark and Robinson, 1985; Jansson *et al.*, 1986).

Following the cloning of the GHRH receptor, the molecular defect in the *little* mouse was established to be a missense mutation in the GHRH receptor gene (Godfrey *et al.*, 1993; Lin *et al.*, 1993). As shown in Figure 8A, the mutation changes aspartic acid residue 60 within the amino-terminal extracellular domain of the receptor to glycine. This aspartic acid is highly conserved in other family B-III G protein-coupled receptors. The genetics and the phenotype of the *little* mouse indicated that this would be an inactivating mutation; however, the mutation could impact receptor function at multiple levels, including the expression, localization, or activity of the receptor. Panels B and C of Figure 8 show that the *little* mutant receptor is expressed at normal levels and is efficiently localized to the cell surface in a manner comparable to the wild-type receptor. However, the mutant receptor protein fails to bind mouse GHRH (Figure 8D). As a consequence, this *little* mutant receptor is completely deficient in cAMP-mediated signal transduction (Figure 8E). In agreement with these data on activity of the *little* mouse receptor (Gaylenn *et al.*, 1999), mutation of the conserved aspartic acid residue in the related glucagon or VIP receptors also abolishes ligand binding (Carruthers *et al.*, 1994; Couvineau *et al.*, 1995). This conserved aspartic acid residue appears to play a general structural role in the receptor, rather than providing information necessary for the interaction with specific ligands.

In addition to this cellular defect in signal transduction leading to decreased GH secretion, the loss of GHRH responsiveness has a significant impact on somatotroph proliferation. In elegant developmental studies, Lin and co-workers

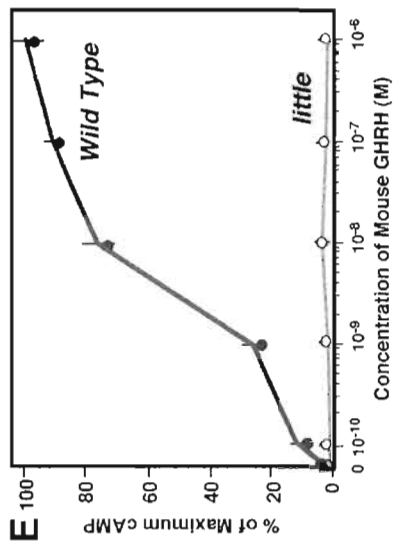
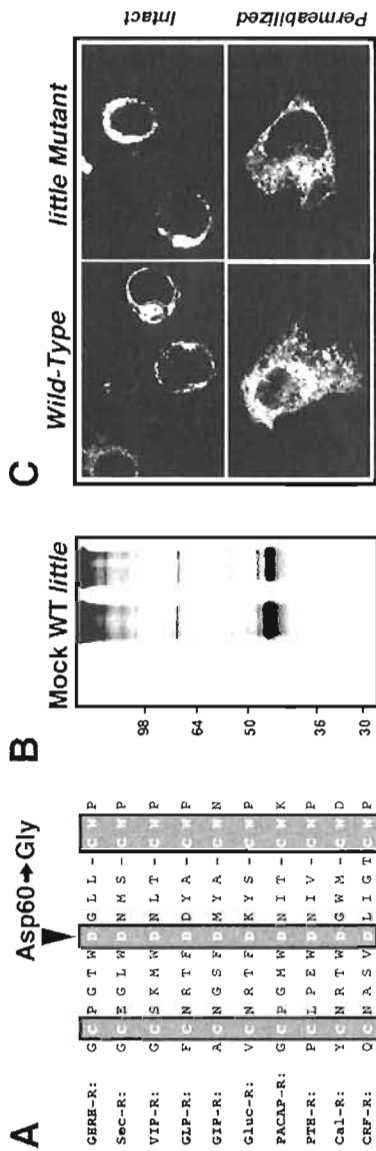


FIG. 8. (See legend on opposite page.)

FIG. 8. Functional analysis of the mutant GHRH receptor from the dwarf *little* mouse. Panel A shows the location of the aspartic acid 60 to glycine mutation in the GHRH receptor from the *little* mouse, indicating the complete conservation of this residue in related family B-III receptors. Panels B and C show appropriate expression and cell surface localization of the wild-type and *little* mutant mouse GHRH receptor proteins in transfected cells. Panel B is an immunoprecipitation of the metabolically labeled and epitope-tagged proteins using an anti-HA (hemagglutinin) antibody. Panel C shows immunofluorescence of the epitope-tagged proteins using an anti-FLAG antibody on intact or permeabilized cells. The locations of the epitope tags are as shown in Figure 2. Panel D is a GHRH-binding competition assay using membranes from transfected 293 cells expressing the wild type of *little* mutant GHRH receptors, while panel E shows GHRH-stimulated cAMP accumulation in these same cells. [Adapted with permission from Gaylinn, B.D., DeAlmeida, V.I., Lyons, C.E. Jr., Wu, K.C., Mayo, K.E., and Thorner, M.O. *Endocrinology* **140**, 5066–5074, 1999. Copyright © 1999 The Endocrine Society.]

(1993) demonstrated that, in the E17.5 fetal pituitary gland, GH or GHRH receptor-positive cells were indistinguishable in wild-type and *little* mice. In contrast, there were far fewer somatotroph cells in the *little* pituitary on postnatal day 60, in agreement with earlier morphometric studies demonstrating pituitary somatotroph hypoplasia in the adult *little* mouse (Wilson *et al.*, 1988). Most of the remaining somatotrophs were found in the anterolateral portion of the pituitary. These investigators proposed that an initial population of GHRH-independent somatotroph stem cells found in the anterolateral aspect of the pituitary proliferate into the caudomedial portions of the gland in a GHRH-dependent fashion and that this proliferation fails in the absence of a functional GHRH receptor (Lin *et al.*, 1993).

## B. PITUITARY HYPERPLASIA IN MICE OVEREXPRESSING GHRH

A second genetic model where the involvement of GHRH in somatotroph proliferation can be observed is the transgenic mouse overexpressing GHRH (Hammer *et al.*, 1985; Mayo *et al.*, 1988; Stefanescu *et al.*, 1989; Asa *et al.*, 1992). Mice ectopically expressing human GHRH from two different transgenes show increased growth rates and gigantism as a result of GH overexpression. In addition, these mice exhibit a profound pituitary hyperplasia that often progresses to adenoma in older transgenic animals (Mayo *et al.*, 1988; Asa *et al.*, 1992; Osamura *et al.*, 1993). This implies that chronic activation of the GHRH-signaling pathway increases proliferation of pituitary somatotroph cells. An example of this phenotype is shown in Figure 9A, which compares the pituitary glands from a control and GHRH-transgenic mouse. There is a five-fold increase in the wet weight of the transgenic gland. These findings in mice correspond to a clinical condition where GHRH is ectopically expressed from pancreatic or other tumors, resulting in somatotroph hyperplasia, increased GH secretion, and acromegaly (Frohman and Szabo, 1981; Thorner *et al.*, 1982).

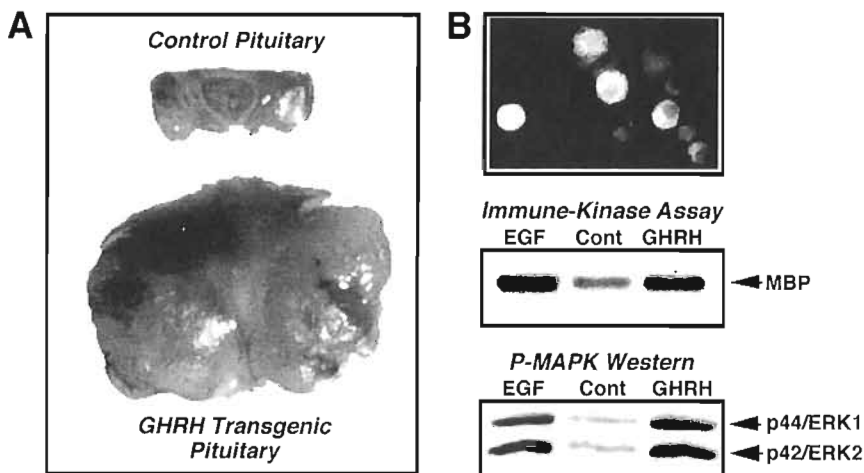


FIG. 9. Pituitary hyperplasia and stimulation of the MAP kinase pathway in pituitary cells by GHRH. (A) Pituitary glands from a female transgenic mouse expressing an MT-GHRH transgene or from a control female littermate, at 8 months of age. (B) Activation of MAP kinase by GHRH in primary rat pituitary cell cultures. The upper panel shows GH staining of somatotroph cells in this population, the center panel is an immune-kinase assay using myelin basic protein (MBP) as the substrate, and the lower panel is a Western protein blot probed with an antibody selective for the phosphorylated and activated form of MAP kinase. Epidermal growth factor (EGF) is used as a positive control.

### C. ACTIVATION OF THE MAP KINASE PATHWAY BY GHRH

In addition to previously discussed genetic models, direct evidence exists for a proliferative action of GHRH. GHRH stimulates DNA synthesis in a subset of pituitary somatotroph cells maintained in primary culture (Billestrup *et al.*, 1986). GHRH also can directly activate the MAP kinase pathway in pituitary cells. MAP kinase activation by growth factors is associated with cell proliferation in many cases, although this pathway has more recently also been linked to cell differentiation (Cobb, 1999). Figure 9B shows an example of the activation of MAP kinase by GHRH in cultured pituitary cells. Both GHRH and epidermal growth factor (EGF) stimulate a rapid increase in MAP kinase phosphorylation, assessed using antibodies specific to the phosphorylated forms of the MAP kinases ERK1 and ERK2 for Western protein blotting, and in MAP kinase activity, assessed by examining the phosphorylation of a MAP kinase substrate, myelin basic protein, in an immune-kinase assay. The signaling pathways leading to MAP kinase activation and proliferation in somatotroph cells remain unknown but they are likely to be mediated by cAMP, in that the cholera toxin A1 chain (an activator of  $G_s$ ), targeted to the somatotroph in transgenic mice, results in pituitary hyperplasia

and gigantism (Burton *et al.*, 1991), while a dominant-negative form of the cAMP-responsive transcription factor CREB, targeted to the somatotroph in transgenic mice, leads to pituitary hypoplasia and dwarfism (Struthers *et al.*, 1991).

## V. Signaling Through the GHRH Receptor

### A. GHRH-RECEPTOR INTERACTION

Given the strong structural homologies between receptors in family B-III of the G protein-coupled receptor superfamily—and the physiological importance of ligands such as GHRH, VIP, PACAP, glucagon, GLP-1, secretin, and GIP—substantial effort has gone into understanding mechanisms of receptor-ligand interaction with the goal of facilitating the design of new ligands of therapeutic value. Much of this focus has been on the amino-terminal extracellular domains of these receptors. There are six conserved cysteine residues in this domain that are common to all family B-III receptors and are believed to form a disulfide-linked structure important for hormone interaction (Gaudin *et al.*, 1995; Vilardaga *et al.*, 1997). This is also the domain in which a naturally occurring mutation of the GHRH receptor in the *little* mouse leads to a loss of hormone binding. Additionally, the amino-terminal extracellular domain of the PACAP receptor, the receptor most closely related to the GHRH receptor, is reported to bind ligand when tethered to the cell via the first membrane-spanning domain (Cao *et al.*, 1995). Although substantial evidence suggests an important role for the amino-terminal domain in hormone binding, in general, this domain alone is not sufficient to confer high-affinity binding. Additional residues in the transmembrane domains or extracellular loops appear to be important (Horn *et al.*, 1998).

Consistent with these general findings for family B-III receptors, studies of the GHRH receptor indicate important roles for both the amino-terminal domain and additional residues in specific hormone binding (DeAlmeida and Mayo, 1998). To investigate the role of the amino-terminal domain, chimeric receptors between the GHRH receptor and the related VIP receptor were generated and their ligand-binding and signaling properties studied. An example of this analysis is presented in Figure 10. Figure 10A shows the structures of several mutant and chimeric GHRH receptors that were made and expressed in HeLaT4 cells. Figure 10B shows that these receptor proteins were expressed at levels comparable to the wild-type receptor and are localized to the cell surface. A chimeric receptor with the amino-terminal domain of the GHRH receptor and the remainder of the VIP receptor ( $G_NV_C$ ) binds GHRH and signals only very weakly, at levels comparable to the related VIP receptor (Figure 10C). In contrast, a chimeric receptor with the membrane-spanning regions and associated extracellular loops of the GHRH receptor and the amino-terminal domain of the VIP receptor ( $V_NG_C$ ) binds GHRH at levels comparable to the wild-type GHRH receptor and stimulates a robust cAMP response similar to that of the wild-type receptor (Figure 10C).

These data suggest a critical role for the carboxyl-terminal half of the receptor, including the transmembrane domains and associated loops, in selective ligand interaction.

Although the amino-terminal domain of the GHRH receptor can be replaced with the homologous domain from the VIP receptor, it is not dispensable for ligand binding. As shown in Figure 10D, a mutant receptor in which most of the amino-terminal domain has been deleted (GHRHR $\Delta$ N) fails to bind GHRH or to signal. Similarly, insertion of a small epitope tag into the amino-terminal extracellular domain of the GHRH receptor disrupts ligand binding (DeAlmeida and Mayo, 1998). An  $\alpha$ -helical region within this amino-terminal domain of family B-III receptors is hypothesized to be involved in a coiled-coil interaction with an  $\alpha$ -helical region in the respective hormone (Momany and Bowers, 1996), perhaps explaining the general requirement for this domain but its apparent lack of complete specificity. These and other studies suggest that, although the amino-terminal extracellular domain is essential for ligand binding, the transmembrane domains and associated extracellular loop regions of the GHRH receptor provide critical information necessary for specific interaction with GHRH.

## B. GHRH RECEPTOR SIGNAL TRANSDUCTION

The GHRH receptor appears to couple predominantly to a stimulatory G protein, leading to the activation of adenylyl cyclase and the second messenger cAMP. Little is known about the GHRH receptor sequences that might mediate G protein interaction but, in general, the third intracellular loop and cytoplasmic tail are important determinants of this coupling for many G protein-coupled receptors (Spiegel, 1995). Evidence for the importance of the third intracellular loop of the GHRH receptor in signaling comes from the analysis of a naturally occurring splice variant of the rat receptor. During the cloning of the GHRH receptor, two cDNA species were identified that differ by the insertion of 123 nucleotides encoding 41 amino acids in the third cytoplasmic loop of the receptor (Lin *et al.*, 1992; Mayo, 1992). As shown in Figure 4, the mRNA represented by the larger cDNA results from alternative RNA processing to include exon 11 of the gene in the mature mRNA. In the adult pituitary, the alternatively spliced "long" form of the receptor is a minor product, compared to the 423-amino-acid "short" form. However, there are indications that this alternative processing may be regulated. The long variant was reported to be more abundant in the pituitary of an older, estrogen-treated animal (Lin *et al.*, 1992). The pituitary somatotroph cell line MtT/S expresses the long-form mRNA at higher levels than the short-form mRNA under some growth conditions (Miller *et al.*, 1999).

The functional properties of the two isoforms of the rat GHRH receptor were compared in studies summarized in Figure 11. The short and long receptor isoforms are expressed, glycosylated, and localized to the cell surface equivalently

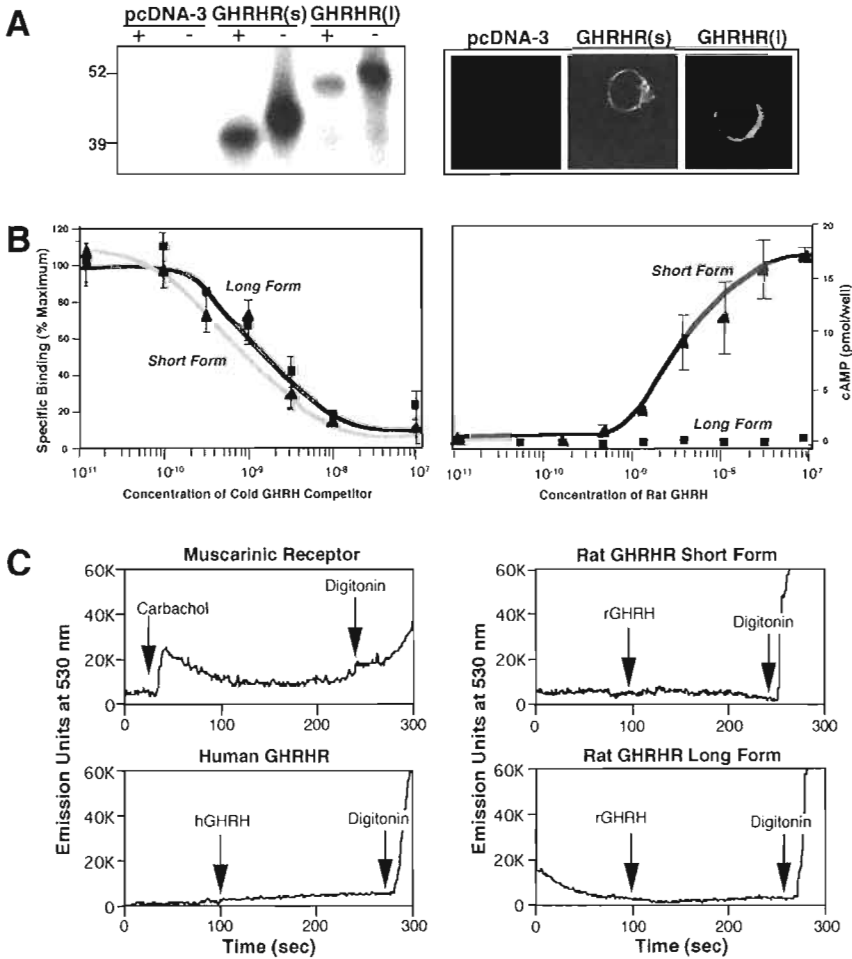


FIG. 11. GHRH binding and signaling by the “short” and “long” isoforms of the rat GHRH receptor. Panel A (left) is an immunoprecipitation gel of labeled proteins from cells expressing the two receptor isoforms. Samples were treated (+) or not (-) with PNGase, to show glycosylation of both receptor isoforms. The right portion of the panel is immunofluorescence of intact cells expressing the two receptor isoforms, showing cell surface localization. Panel B shows GHRH-binding and cAMP-signaling assays with HeLaT4 cells expressing the two receptor isoforms. Panel C shows calcium mobilization studies using the indicator Fluo-3AM, with cells transfected with the indicated GHRH receptor isoforms or with the muscarinic M3 receptor as a positive control. [Adapted with permission from Miller, T.L., Godfrey, P.A., DeAlmeida, V.I., and Mayo, K.E. *Endocrinology* **140**, 4152–4165, 1999. Copyright © 1999 The Endocrine Society.]

(Figure 11A). Both bind GHRH with nearly equivalent affinity, as shown in Figure 11B. However, the long isoform of the receptor fails to stimulate intracellular cAMP production and appears to be completely inactive with respect to signal transduction (Figure 11B).

Similar alternative RNA processing of the PACAP receptor is observed at the analogous exon-intron boundary in the third cytoplasmic loop (Spengler *et al.*, 1993). In this case, five splice variants are generated. These differ in their activation of the PKA or PKC signaling pathways when expressed in *Xenopus laevis* oocytes. Indeed, many of the receptors in family B-III of the G protein-coupled receptor superfamily—including the VIP and PACAP receptors—are able to activate phospholipase C through a  $G_{q/11}$ -class G protein (Trimble *et al.*, 1987; Delporte *et al.*, 1995). Signaling through this pathway by the two isoforms of the rat GHRH receptor was investigated by measuring cellular calcium flux with the indicator Fluo-3AM (Merritt *et al.*, 1990). As shown in Figure 11C, neither isoform of the GHRH receptor activated calcium flux, although a control M3 muscarinic receptor was active in this assay.

Although the long form of the GHRH receptor has been reported only in the rat, additional RNA processing variants have been identified in other species. Alternative processing of the human GHRH receptor transcript results in the inclusion of an intron in the mRNA and generation of a premature stop codon that leads to a truncation of the protein after the fifth transmembrane domain (Hashimoto *et al.*, 1995; Tang *et al.*, 1995). This variant has been reported in both normal pituitary and in pituitary adenoma; the truncated receptor appears to exert a dominant inhibitory effect on signaling by the normal receptor (Motomura *et al.*, 1998).

## VI. Summary

### A. GHRH RECEPTOR ACTIONS AND REGULATION

The identification of the GHRH receptor has provided a framework for beginning to understand the molecular and cellular mechanisms by which GHRH regulates somatotroph cell function. Characterization of the receptor confirmed the hypothesis that GHRH activates a stimulatory G protein, leading to activation of adenylyl cyclase and stimulation of the cAMP/protein kinase A pathway. Somatostatin is thought to oppose the actions of GHRH through inhibition of adenylyl cyclase; the identification of a family of G protein-coupled somatostatin receptors, several of which are coupled to an inhibitory G protein, supports this concept (Yamada *et al.*, 1992; Bell *et al.*, 1995).

GHRH acts to stimulate both the synthesis and secretion of GH from the somatotroph cell (Guillemin *et al.*, 1982; Rivier *et al.*, 1982; Barinaga *et al.*, 1983). GHRH-stimulated GH secretion is calcium dependent (Spence *et al.*, 1980) and may involve receptor-induced G protein activation of cellular ion channels

as well as PKA-mediated phosphorylation of ion channels (Kato and Sakuma, 1997). Current views on how PKA activation causes increased GH synthesis are that the stimulation of PKA leads to the phosphorylation and activation of the transcription factor CREB (Gonzalez and Montminy, 1989) and that one of the target genes for transcriptional stimulation by CREB is that encoding the pituitary-specific transcription factor Pit-1 or GHF-1, which acts to increase GH gene transcription (McCormick *et al.*, 1990; Shepard *et al.*, 1994). Consistent with this, GHRH increases the expression of Pit-1 in pituitary cells (Soto *et al.*, 1995; Gonzalez-Parra *et al.*, 1996). As discussed in this chapter, GHRH also activates the MAP kinase pathway and stimulates somatotroph cell proliferation, although the signaling pathways mediating this response are largely uncharacterized.

GHRH receptor expression in the somatotroph cell appears to be very dynamically modulated. Expression is developmentally regulated, sexually dimorphic, and strongly controlled by glucocorticoid hormones. In addition to these forms of regulation (discussed earlier in this chapter), thyroid hormone potently stimulates GHRH receptor gene expression (Miki *et al.*, 1995; Korytko and Cutler, 1997). Recent studies suggest that GHRH itself can regulate GHRH receptor gene expression both *in vivo* and in cell culture. Decreased expression of receptor mRNA is detected after chronic passive immunization of neonatal rats with GHRH antibodies (Horikawa *et al.*, 1996), while treatment of primary pituitary cell cultures with GHRH decreases GHRH receptor gene expression (Aleppo *et al.*, 1997). These effects are likely to be mediated by cAMP-dependent pathways. In this regard, it is interesting to note the presence of several potential cAMP response elements in the rat GHRH receptor promoter (Figure 4).

## B. RELEVANCE TO DISEASES OF GH SECRETION

Historically, diseases or disorders of the GH neuroendocrine axis in both mouse and human have revealed much about normal physiological mechanisms of GH expression, secretion, and actions on target cells. Some of the genes that are known or speculated targets for mutation in disorders of the GH axis are illustrated in Figure 12. Several genetic lesions lead to pleiotropic effects within the pituitary gland that involve alterations in GH secretion and growth. Included among these are activating mutations in the stimulatory G protein  $G_{\alpha s}$  in pituitary adenoma (Landis *et al.*, 1989) and in McCune-Albright syndrome (Weinstein, 1991), inactivating mutations in the transcription factor Pit-1/GHF-1 in the Snell and Jackson dwarf mice (Li *et al.*, 1990) and in human panhypopituitary dwarfism (Radovick *et al.*, 1992), and inactivating mutations of the transcription factor Prop-1 in the Ames dwarf mouse (Sornson *et al.*, 1996) and in human familial combined pituitary hormone deficiency (Wu *et al.*, 1998). Other mutations are very specific to the GH system, most notably, mutations in the GH structural gene

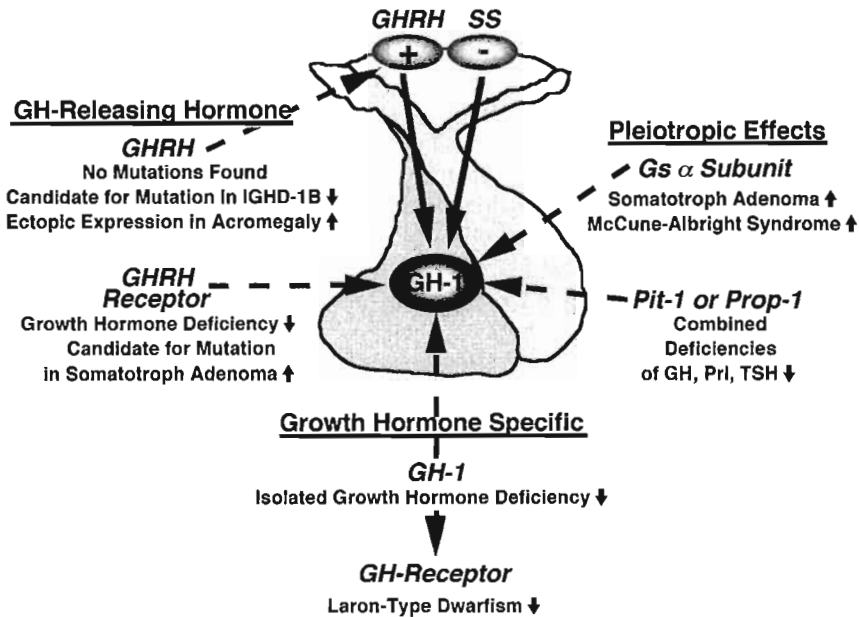


FIG. 12. Genetic diseases of GH secretion. The schematic shows the regulation of pituitary GH synthesis and secretion by the hypothalamic peptides GHRH and somatostatin. Genes that are known or speculated targets for mutation in diseases affecting GH production or action are indicated, along with brief comments. Mutations are classified as pleiotropic in nature, specific to GH and its receptor, or as involving the GHRH system. The arrows indicate either activating ( $\uparrow$ ) or inactivating ( $\downarrow$ ) mutations, effects, or predicted changes in function. Mutations in all genes shown, with the exception of GHRH, have been identified in human disease.

(Phillips and Vnencak-Jones, 1989) or in the GH receptor/binding protein, in the case of Laron-type dwarfism (Godowski *et al.*, 1989).

With respect to the GHRH system, it is surprising that mutations in GHRH itself associated with human disease have not been identified. There is evidence that a significant proportion of GH-deficient children respond to treatment with exogenous GHRH (Grossman *et al.*, 1984), suggesting that they might produce insufficient amounts of GHRH and indicating the potential for mutations in the GHRH gene. The GHRH receptor is a target for mutation in the dwarf *little* mouse (Godfrey *et al.*, 1993; Lin *et al.*, 1993). In addition, there are numerous reports of GHRH receptor mutations in human GH deficiency syndromes. Three different groups have reported an identical mutation in different kindreds in which a premature stop codon is introduced at position 72 of the receptor coding sequence, resulting in a severely truncated inactive receptor (Wajnrajch *et al.*, 1996; Baumann and Maheshwari, 1997; Netchine *et al.*, 1998). There is a more-recent report of an additional mutation that appears to affect the splicing of the first intron,

again leading to a premature truncation of the translational reading frame and generation of an inactive receptor protein (Salvatori *et al.*, 1999).

Many of these mutations that affect the GH axis were first identified in mouse models but then rapidly found in association with human disease. Thus, there has been a rapid translation of basic research information into the clinic that promises to enhance the diagnosis and treatment of syndromes of GH deficiency and excess. In turn, naturally occurring mutations in transcriptional regulatory proteins and signal transduction proteins of the GH axis in human disease promise to provide a wealth of new information that will enhance our basic understanding of the structure and function of these key regulatory proteins.

#### ACKNOWLEDGMENT

The studies described in this report were supported by National Institutes of Health grant DK-48071 to K.E.M.

#### REFERENCES

- Abribat, T., Deslauriers, N., Brazeau, P., and Gaudreau, P. (1991). *Endocrinology* **128**, 633–635.
- Aleppo, G., Moskal, S.F., De Grandis, P.A., Kineman, R.D., and Frohman, L.A. (1997). *Endocrinology* **138**, 1058–1065.
- Andersen, B., and Rosenfeld, M. (1994). *J. Biol. Chem.* **269**, 29335–29338.
- Asa, S.L., Kovacs, K., Stefaneanu, L., Horvath, E., Billestrup, N., Gonzalez-Manchon, C., and Vale, W. (1992). *Endocrinology* **131**, 2083–2089.
- Bagnato, A., Moretti, C., Ohnishi, J., Frajese, G., and Catt, K.J. (1992). *Endocrinology* **130**, 1097–1102.
- Barinaga, M., Yamonoto, G., Rivier, C., Vale, W., Evans, R., and Rosenfeld, M.G. (1983). *Nature* **306**, 84–85.
- Baumann, G., and Maheshwari, H. (1997). *Acta Paediatrica. (suppl.)* **423**, 33–38.
- Bell, G.I., Yasuda, K., Kong, H., Law, S.F., Raynor, K., and Reisine, T. (1995). *Ciba Found. Symp.* **190**, 65–79.
- Berry, S.A., and Pescovitz, O.H. (1988). *Endocrinology* **123**, 661–663.
- Bilezikjian, L.M., and Vale, W.W. (1983). *Endocrinology* **113**, 1726–1731.
- Billestrup, N., Swanson, L.W., and Vale, W. (1986). *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6854–6857.
- Bourdouresque, F., Guillaume, V., Grino, M., Strbak, V., Chautard, T., Conte-Devolx, B., and Oliver, C. (1988). *Neuroendocrinology* **48**, 417–422.
- Bowers, C.Y., Momany, F.A., Reynolds, G.A., and Hong, A. (1984). *Endocrinology* **114**, 1537–1545.
- Burton, F.H., Hasel, K.W., Bloom, F.E., and Sutcliffe, J.G. (1991). *Nature* **350**, 74–77.
- Campbell, R.M., and Scanes, C.G. (1992). *Growth Regulat.* **2**, 175–191.
- Cao, Y.J., Gimpl, G., and Fahrenholz, F. (1995). *Biochem. Biophys. Res. Comm.* **212**, 673–680.
- Carruthers, C.J., Unson, C.G., Kim, H.N., and Sakmar, T.P. (1994). *J. Biol. Chem.* **269**, 29321–29328.
- Chatterjee, T.K., Liu, X., Davisson, R.L., and Fisher, R.A. (1997). *J. Biol. Chem.* **272**, 12122–12131.
- Cheng, T.C., Beamer, W.G., Phillips, J.A., Bartke, A., Mallonee, R.L., and Dowling, C. (1983). *Endocrinology* **133**, 1669–1678.
- Ciampani, T., Fabbri, A., Isidori, A., and Dufau, M.L. (1992). *Endocrinology* **131**, 2785–2792.
- Clark, R.G., and Robinson, I.C. (1985). *J. Endocrinol.* **106**, 1–5.

- Cobb, M. (1999). *Prog. Biophys. Mol. Biol.* **71**, 479–500.
- Couvineau, A., Gaudin, P., Maoret, J.J., Rouyer, F.C., Nicole, P., and Laburthe, M. (1995). *Biochem. Biophys. Res. Comm.* **206**, 246–252.
- Daughaday, W.H. (1995). In "Endocrinology" (L.J. DeGroot, ed.), vol. 1, pp. 303–329. W.H. Saunders, Philadelphia.
- DeAlmeida, V.I., and Mayo, K.E. (1998). *Mol. Endocrinol.* **12**, 750–765.
- Delporte, C., Poloczek, P., de Neef, P., Vertongen, P., Ciccarelli, E., Svoboda, M., Herchuelz, A., Winand, J., and Robberecht, P. (1995). *Mol. Cell. Endocrinol.* **107**, 71–76.
- Devesa, J., Lima, L., and Tresguerres, J.A.F. (1992). *Trends Endocrinol. Metab.* **3**, 175–183.
- Dupouy, J.P., Coffigny, H., and Marge, S. (1974). *J. Endocrinol.* **65**, 347–352.
- Eicher, E.M., and Beamer, W.G. (1976). *J. Hered.* **67**, 87–91.
- Evans, R., Birnberg, N., and Rosenfeld, M. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7659–7663.
- Frohman, L.A., and Jansson, J.O. (1986). *Endocr. Rev.* **7**, 223–253.
- Frohman, L.A., and Szabo, M. (1981). *Prog. Clin. Biol. Res.* **74**, 259–271.
- Frohman, M., Downs, T., Chomczynski, P., and Frohman, L. (1989). *Mol. Endocrinol.* **3**, 1529–1536.
- Gatford, K.L., Egan, A.R., Clarke, I.J., and Owens, P.C. (1998). *J. Endocrinol.* **157**, 373–389.
- Gaudin, P., Couvineau, A., Maoret, J.J., Rouyer, F.C., and Laburthe, M. (1995). *Biochem. Biophys. Res. Comm.* **211**, 901–908.
- Gaylinn, B.D., Harrison, J.K., Zysk, J.R., Lyons, C.E., Lynch, K.R., and Thorner, M.O. (1993). *Mol. Endocrinol.* **7**, 77–84.
- Gaylinn, B.D., DeAlmeida, V.I., Lyons, C.E. Jr., Wu, K.C., Mayo, K.E., and Thorner, M.O. (1999). *Endocrinology* **140**, 5066–5074.
- Girard, N., Boulanger, L., Denis, S., and Gaudreau, P. (1999). *Endocrinology* **140**, 2836–2842.
- Godfrey, P., Rahal, J.O., Beamer, W.G., Copeland, N.G., Jenkins, N.A., and Mayo, K.E. (1993). *Nature Genet.* **4**, 227–232.
- Godowski, P.J., Leung, D.W., Meachum, L.R., Galgani, J.P., Hellmis, R., Keret, R., Rotwein, P.S., Parks, J.S., Laron, Z., and Wood, W.I. (1989). *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8083–8087.
- Gonzalez, C., and Jolin, T. (1981). *Horm. Res.* **14**, 130–137.
- Gonzalez, G.A., and Montminy, M.R. (1989). *Cell* **59**, 675–689.
- Gonzalez-Parra, S., Chowen, J.A., Garcia-Segura, L.M., and Argente, J. (1996). *Neuroendocrinology* **63**, 3–15.
- Grossman, A., Savage, M.O., Lytras, N., Preece, M.A., Sueiras-Diaz, J., Coy, D.H., Rees, L.H., and Besser, G.M. (1984). *Clin. Endocrinol.* **21**, 321–330.
- Gubler, U., Monahan, J.J., Lomedico, P.T., Bhatt, R.S., Collier, K.J., Hoffman, B.J., Bohlen, P., Esch, F., Ling, N., Zeytin, F., Brazeau, P., Poonian, M.S., and Gage, L.P. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4311–4314.
- Guillemin, R., Brazeau, P., Bohlen, P., Esch, F., Ling, N., and Wehrenberg, W.B. (1982). *Science* **218**, 585–587.
- Hammer, R.E., Brinster, R.L., Rosenfeld, M.G., Evans, R.M., and Mayo, K.E. (1985). *Nature* **315**, 413–416.
- Hashimoto, K., Koga, M., Motomura, T., Kasayama, S., Kouhara, H., Ohnishi, T., Arita, N., Hayakawa, T., Sato, B., and Kishimoto, T. (1995). *J. Clin. Endocrinol. Metab.* **80**, 2933–2939.
- Hemming, A.J., Begeot, M., Dubois, M.P., and Dubois, P.M. (1984). *Endocrinology* **114**, 2107–2113.
- Horikawa, R., Hellmann, P., Cella, S.G., Torsello, A., Day, R.N., Muller, E.E., and Thorner, M.O. (1996). *Endocrinology* **137**, 2642–2645.
- Horn, F., Bywater, R., Krause, G., Kuipers, W., Olivera, L., Paival, A.C.M., Sander, C., and Vriend, G. (1998). *Receptors and Channels* **5**, 305–314.
- Hsiung, H.M., Smith, D.P., Zhang, X.Y., Bennett, T., Rosteck, P.J., and Lai, M.H. (1993). *Neuropeptides* **25**, 1–10.

- Jansson, J.O., Downs, T.R., Beamer, W.G., and Frohman, L.A. (1986). *Science* **232**, 511–512.
- Jansson, J.O., and Frohman, L.A. (1987). *Endocrinology* **120**, 1551–1557.
- Kato, M., and Sakuma, Y. (1997). *Endocrinology* **138**, 5096–5100.
- Kineman, R.D., Faight, W.J., and Frawley, L.S. (1992). *Endocrinology* **130**, 3289–3294.
- Korytko, A., Zeitler, P., and Cuttler, L. (1996). *Endocrinology* **137**, 1326–1331.
- Korytko, A. I., and Cuttler, L. (1997). *J. Endocrinol.* **152**, R13–R17.
- Labrie, F., Gagne, B., and Lefevre, G. (1983). *Life Sci.* **33**, 2229–2233.
- Lam, K.S., Lee, M.F., Tam, S.P., and Srivastava, G. (1996). *Neuroendocrinology* **63**, 475–480.
- Landis, C.A., Masters, S.B., Spada, A., Pace, A.M., Bourne, H.R., and Vallar, L. (1989). *Nature* **340**, 692–696.
- Li, H., Zeitler, P., Valerius, M., Small, K., and Potter, S. (1996). *EMBO J.* **15**, 714–724.
- Li, S., Crenshaw, E.B.D., Rawson, E.J., Simmons, D.M., Swanson, L.W., and Rosenfeld, M.G. (1990). *Nature* **347**, 528–533.
- Lin, C., Lin, S.C., Chang, C.P., and Rosenfeld, M.G. (1992). *Nature* **360**, 765–768.
- Lin, S.C., Lin, C.R., Gukovsky, I., Lusic, A.J., Sawchenko, P.E., and Rosenfeld, M.G. (1993). *Nature* **364**, 208–213.
- Margioris, A.N., Brockmann, G., Bohler, H.J., Grino, M., Vamvakopoulos, N., and Chrousos, G.P. (1990). *Endocrinology* **126**, 151–158.
- Matsubara, S., Sato, M., Mizobuchi, M., Niimi, M., and Takahara, J. (1995). *Endocrinology* **136**, 4147–4150.
- Mayo, K.E., Vale, W., Rivier, J., Rosenfeld, M.G., and Evans, R.M. (1983). *Nature* **306**, 86–88.
- Mayo, K.E., Cerelli, G.M., Rosenfeld, M.G., and Evans, R.M. (1985). *Nature* **314**, 464–467.
- Mayo, K.E., Hammer, R.E., Swanson, L.W., Brinster, R.L., Rosenfeld, M.G., and Evans, R.M. (1988). *Mol. Endocrinol.* **2**, 606–612.
- Mayo, K.E. (1992). *Mol. Endocrinol.* **6**, 1734–1744.
- McCormick, A., Brady, H., Theill, L.E., and Karin, M. (1990). *Nature* **345**, 829–832.
- McKee, K.K., Palyha, O.C., Feighner, S.D., Hreniuk, D.L., Tan, C.P., Phillips, M.S., Smith, R.G., Van der Ploeg, L.H., and Howard, A.D. (1997). *Mol. Endocrinol.* **11**, 415–423.
- Merchenthaler, I., Vigh, S., Schally, A.V., and Petrusz, P. (1984). *Endocrinology* **114**, 1082–1085.
- Merritt, J.E., McCarthy, S.A., Davies, M.P.A., and Moores, K.E. (1990). *Biochem. J.* **269**, 513–519.
- Miki, N., Ono, M., Murata, Y., Ohsaki, E., Tamitsu, K., Ri, T., Demura, H., and Yamada, M. (1995). *Biochem. Biophys. Res. Comm.* **217**, 1087–1093.
- Millard, W.J., Politch, J.A., Martin, J.B., and Fox, T.O. (1986). *Endocrinology* **119**, 2655–2660.
- Miller, T.L., Godfrey, P.A., DeAlmeida, V.I., and Mayo, K.E. (1999). *Endocrinology* **140**, 4152–4165.
- Miller, T.L., and Mayo, K.E. (1997). *Endocrinology* **138**, 2458–2465.
- Momany, F.A., and Bowers, C.Y. (1996). *Ann. N.Y. Acad. Sci.* **805**, 172–181.
- Moore, D., Marks, A., Buckley, D., Kapler, G., Payvar, F., and Goodman, H. (1985). *Proc. Natl. Acad. Sci. U.S.A.* **82**, 699–702.
- Motomura, T., Hashimoto, K., Koga, M., Arita, N., Hayakawa, T., Kishimoto, T., and Kasayama, S. (1998). *Metabolism: Clin. Exper.* **47**, 804–808.
- Netchine, I., Talon, P., Dastot, F., Vitaux, F., Goossens, M., and Amselem, S. (1998). *J. Clin. Endocrinol. Metab.* **83**, 432–436.
- Nogami, H., and Tachibana, T. (1993). *Endocrinology* **132**, 517–523.
- Nogami, H., Yokose, T., and Tachibana, T. (1995). *Am. J. Physiol.* **268**, E262–E267.
- Ono, M., Miki, N., Murata, Y., Osaki, E., Tamitsu, K., Ri, T., Yamada, M., and Demura, H. (1995). *Biochem. Biophys. Res. Comm.* **216**, 1060–1066.
- Osamura, R., Oda, K., Utsunomiya, H., Inada, K., Umemura, S., Shibuya, M., Katakami, H., Voss, J., Mayo, K., and Rosenfeld, M. (1993). *Endocr. J.* **40**, 133–139.
- Pei, L. (1997). *Reg. Peptides* **71**, 153–161.

- Petersenn, S., Rasch, A.C., Heyens, M., and Schulte, H.M. (1998). *Mol. Endocrinol.* **12**, 233–247.
- Phillips, J.A.I., and Vnencak-Jones, C.L. (1989). *Adv. Hum. Genet.* **18**, 305–363.
- Pong, S.S., Chaung, L.Y., Dean, D.C., Nargund, R.P., Patchett, A.A., and Smith, R.G. (1996). *Mol. Endocrinol.* **10**, 57–61.
- Radovick, S., Nations, M., Du, Y., Berg, L.A., Weintraub, B.D., and Wondisford, F.E. (1992). *Science* **257**, 1115–1118.
- Rivier, J., Spiess, J., Thorner, M., and Vale, W. (1982). *Nature* **300**, 276–278.
- Rosenfeld, M.G., Bach, I., Erkman, L., Li, P., Lin, C., Lin, S., McEvilly, R., Ryan, A., Rhodes, S., Schonemann, M., and Scully, K. (1996). In "Recent Progress in Hormone Research," vol. 51, pp. 217–238. The Endocrine Society, Bethesda, Md.
- Salvatori, R., Hayashida, C.Y., Aguiar-Oliveira, M.H., Phillips, J.A. III, Souza, A.H., Gondo, R.G., Toledo, S.P., Conceicao, M.M., Prince, M., Maheshwari, H.G., Baumann, G., and Levine, M.A. (1999). *J. Clin. Endocrinol. Metab.* **84**, 917–923.
- Sawchenko, P.E., Swanson, L.W., Rivier, J., and Vale, W.W. (1985). *J. Comp. Neurol.* **237**, 100–115.
- Segre, G.V., and Goldring, S.R. (1993). *Trends Endocrinol. Metab.* **4**, 309–314.
- Seifert, H., Perrin, M., Rivier, J., and Vale, W. (1985a). *Nature* **313**, 487–489.
- Seifert, H., Perrin, M., Rivier, J., and Vale, W. (1985b). *Endocrinology* **117**, 849–854.
- Shepard, A.R., Zhang, W., and Eberhardt, N.L. (1994). *J. Biol. Chem.* **269**, 1804–1814.
- Smith, R.G., Cheng, K., Schoen, W.R., Pong, S.S., Hickey, G., Jacks, T., Butler, B., Chan, W.W., Chaung, L.Y., Judith, F., et al. (1993). *Science* **260**, 1640–1643.
- Smith, R.G., Van der Ploeg, L.H., Howard, A.D., Feighner, S.D., Cheng, K., Hickey, G.J., Wyvrat, M.J. Jr., Fisher, M.H., Nargund, R.P., and Patchett, A.A. (1997). *Endocr. Rev.* **18**, 621–645.
- Sornson, M.W., Wu, W., Dasen, J.S., Flynn, S.E., Norman, D.J., O'Connell, S.M., Gukovsky, I., Carriere, C., Ryan, A.K., Miller, A.P., Zuo, L., Gleiberman, A.S., Andersen, B., Beamer, W.G., and Rosenfeld, M.G. (1996). *Nature* **384**, 327–333.
- Soto, J.L., Castrillo, J.L., Dominguez, F., and Dieguez, C. (1995). *Endocrinology* **136**, 3863–3870.
- Spence, J.W., Sheppard, M.S., and Kraicer, J. (1980). *Endocrinology* **106**, 764–769.
- Spengler, D., Waeber, C., Pantaloni, C., Holsboer, F., Bockaert, J., Seeburg, P.H., and Journot, L. (1993). *Nature* **365**, 170–175.
- Spiegel, A.M. (1995). *Ann. Rev. Physiol.* **58**, 143–170.
- Spiess, J., Rivier, J., and Vale, W. (1983). *Nature* **303**, 532–535.
- Stefaneanu, L., Kovacs, K., Hovarth, E., Asa, S.L., Losinski, L.E., Billestrup, N., Price, J., and Vale, W.W. (1989). *Endocrinology* **125**, 2710–2118.
- Struthers, R.S., Vale, W.W., Arias, C., Sawchenko, P.E., and Montminy, M.R. (1991). *Nature* **350**, 622–624.
- Suhr, S.T., Rahal, J.O., and Mayo, K.E. (1989). *Mol. Endocrinol.* **3**, 1693–1700.
- Takuma, N., Sheng, H.Z., Furuta, Y., Ward, J.M., Sharma, K., Hogan, B.L., Pfaff, S.L., Westphal, H., Kimura, S., and Mahon, K.A. (1998). *Development* **125**, 4835–4840.
- Tang, J., Lagace, G., Castagne, J., and Collu, R. (1995). *J. Clin. Endocrinol. Metab.* **80**, 2381–2387.
- Tannenbaum, G.S., and Ling, N. (1984). *Endocrinology* **115**, 1952–1957.
- Terry, L.C., Saunders, A., Audet, J., Willoughby, J.O., Brazeau, P., and Martin, J.B. (1977). *Clin. Endocrinol.* **6**, 19S–28S.
- Thorner, M.O., Peryman, R.L., Cronin, M.J., Rogol, A.D., Draznin, M., Johanson, A., Vale, W., Hovarth, E., and Kovacs, K. (1982). *J. Clin. Invest.* **70**, 965–977.
- Treier, M., Gleiberman, A.S., O'Connell, S.M., Szeto, D.P., McMahon, J.A., McMahon, A.P., and Rosenfeld, M.G. (1998). *Genes Dev.* **12**, 1691–1704.
- Trimble, E.R., Bruzzone, R., Biden, T.J., Meehan, C.J., Andreu, D., and Merrifield, R.B. (1987). *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3146–3150.

- Vale, W., Vaughan, J., Yamamoto, G., Spiess, J., and Rivier, J. (1983). *Endocrinology* **112**, 1553–1555.
- Vallar, L., Spada, A., and Giannattasio, G. (1987). *Nature* **330**, 566–568.
- Villardaga, J.P., Di Paolo, E., Bialek, C., De Neef, P., Waelbroeck, M., Bollen, A., and Robberecht, P. (1997). *Eur. J. Biochem.* **246**, 173–180.
- Wajnrajch, M.P., Gertner, J.M., Harbison, M.D., Chua, S.C. Jr., and Leibel, R.L. (1996). *Nature Genet.* **12**, 88–90.
- Watkins-Chow, D.E., and Camper, S.A. (1998). *Trends Genet.* **14**, 284–290.
- Wehrenberg, W., Baird, A., and Ling, N. (1983). *Science* **221**, 556–558.
- Weinstein, L.S., Shenker, A., Gejman, P.V., Merino, M.J., Friedman, E., and Spiegel, A.M. (1991). *N. Engl. J. Med.* **24**, 1688–1695.
- Wilson, D.B., Wyatt, D.P., Gadler, R.M., and Baker, C.A. (1988). *Acta Anatomica* **131**, 150–155.
- Wu, W., Cogan, J.D., Pfaffle, R.W., Dasen, J.S., Frisch, H., O'Connell, S.M., Flynn, S. E., Brown, M.R., Mullis, P.E., Parks, J.S., Phillips, J.A. Jr., and Rosenfeld, M.G. (1998). *Nature Genet.* **18**, 147–149.
- Yamada, Y., Hayami, T., Nakamura, K., Kaisaki, P.J., Someya, Y., Wang, C.Z., Seino, S., and Seino, Y. (1995). *Genomics* **29**, 773–776.
- Yamada, Y., Post, S., Wang, K., Tager, H., Bell, G., and Seino, S. (1992). *Proc. Natl. Acad. Sci. U.S.A.* **89**, 251–255.
- Zeitler, P., Argente, J., Chowen, B.J., Clifton, D.K., and Steiner, R.A. (1990). *Endocrinology* **127**, 1362–1368.

#### DISCUSSION

**Gordon Cutler:** Have there been studies to look at combining inactivating heterozygous mutations of the growth hormone-releasing hormone (GHRH) receptor (or GHRH itself) with similar mutations at other levels of the pathway, such as GH, GH receptor, or insulin-like growth factor-1 (IGF-1)? An analogy in diabetes research comes from crossing heterozygous knockout mice for insulin and the insulin receptor, which leads to diabetes for the double heterozygote, even though the single heterozygote has little or no phenotype.

**Kelly Mayo:** Not that I am aware of. I agree that this could be a valuable approach and have followed with interest the exciting studies of this type with the insulin system. One limitation is that we currently do not have mouse knockout or mutation models for several of the principle players, including GHRH and GH. Experiments related to this have been performed by other investigators, by crossing transgenic mouse lines that overexpress some of the hormones of the GH axis or by crossing transgenic and gene knockout lines. For example, a GH transgene was introduced into the GHRH receptor-deficient *little* mouse and an IGF-1 transgene was introduced into the GH-deficient GH-diphtheria toxin transgenic mouse.

**Gordon Cutler:** Do you think that the Alton giant might have had an activating mutation of the GHRH receptor? He grew at an extraordinary rate in the first year of life, weighing 60 pounds at one year, suggesting a genetic cause that was acting from a very early age, as opposed to a sporadic tumor, which would be extraordinarily rare at such a young age. Presumably, this could be tested if one could find stored pathological tissue or obtain a tissue sample from his gravesite in Alton, Illinois.

**Kelly Mayo:** I do not think I'd want to resort to sampling the gravesite but I agree that, if tissue was available, this would be a very interesting case to examine! With respect to potential activating mutations in the GHRH receptor, I am aware of several groups that have looked for such mutations. I do not think that there are any clear indications for activating mutations of the receptor in association with GH excess or pituitary adenoma. I certainly expect that such mutations will be found but suspect that they will be relatively rare, given that they have not been reported to date. An alternative approach that we are now taking to ask whether the receptor can be constitutively activated is to introduce

random mutations into the third cytoplasmic domain and sixth transmembrane of the receptor (areas where activating mutations in other G protein-coupled receptors are commonly found). Then, these mutant proteins are tested for their hormone-binding and signaling properties.

**Christin Carter-Su:** If I recall correctly, you showed that glucocorticoids increase levels of GHRH receptor mRNA. Have you looked at GHRH receptor binding or protein levels to know that changes in GHRH receptor mRNA levels translate into changes in GHRH binding?

**Kelly Mayo:** Part of our rationale for looking for changes in GHRH receptor gene expression induced by glucocorticoids stemmed from studies by Seifert and Vale, performed prior to the cloning of the receptor, showing that the binding of GHRH to pituitary cells and the sensitivity of the pituitary to GHRH were enhanced by glucocorticoids or repressed by adrenalectomy. So, we do know that the changes in gene expression that we observe correlate with this physiological end-point. We have not yet examined changes in protein levels. Specific antibodies for detection of the GHRH receptor have been difficult to make, although several reasonable antibodies are becoming available. I agree that it will be important to use these to confirm changes in protein expression.

**Christin Carter-Su:** Glucocorticoid therapy in children is associated with diminished growth. Consistent with this, we have shown that glucocorticoids decrease binding in a variety of cell lines, although mRNA levels of GH receptors have been reported to be increased by glucocorticoids in some target tissues. Do you then view the stimulatory effect of glucocorticoids on GHRH receptor as a compensatory mechanism?

**Kelly Mayo:** You bring up the important point that glucocorticoid hormones have complex and somewhat paradoxical effects in the GH axis. While they are generally growth suppressive, they act directly on the pituitary to stimulate somatotroph differentiation, increase GH gene expression, and augment basal and GHRH-induced GH secretion. I imagine that the net effects of glucocorticoids on the GH axis are suppressive, at least in part, because critical end-points, such as GH-binding studies that you just mentioned, are repressed. I do not really view the increased expression of the receptor as compensatory to these suppressive effects. Rather, I think that the important physiological effects of glucocorticoids on the somatotroph and on the GHRH receptor are likely to be developmental effects, establishing early expression of the receptor and increasing responsiveness to the trophic effects of GHRH manifested during the perinatal period. I would guess that regulation of the receptor by glucocorticoids in the adult animal is less important, although that is just speculation.

**Lawrence Frohman:** An abstract by Collu *et al.* described a presumed activating mutation in the GHRH receptor from a pituitary GH-secreting adenoma. Have you had an opportunity to evaluate the mutant receptor?

**Kelly Mayo:** We have not evaluated this potentially interesting GHRH receptor mutation, which was reported in abstract form at the 1995 Endocrine Society meeting. The mutation, which was identified in a GH-secreting pituitary tumor, was complex in that the patient was heterozygous and had both wild-type and mutant alleles of the receptor. I am not aware of any subsequent studies on the hormone-binding and signaling properties of this receptor.