# MINIREVIEW

# Pituitary Hormones as Neurotrophic Signals: Update on Hypothalamic Differentiation in Genetic Models of Altered Feedback[2] (44427)

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*Abstract.* **Studies of mutant mice that are growth hormone (GH)- and prolactin (PRL)-deficient have provided evidence that these pituitary hormones have trophic, as well as dynamic, feedback effects on the hypothalamic neurons that regulate GH and PRL secretion (1). This review examines further evidence, from those animals and from recent transgenic models, for GH and PRL effects on neuronal differentiation. Characterization of the Ames dwarf (*Prop-1⟨df⟩*) mutation and discovery of other genes important to pituitary differentiation reveal an expression sequence of transcription factors, *Hesx1* (*Rpx*) to *P-Lim* to *Prop-1* to *Pit-1*, that heralds influence on hypothalamic differentiation. Occasional expression of GH and PRL in the Ames dwarf pituitary may result from the "partial loss of function" nature of the Ames *Prop-1* mutation. In transgenic mice with moderately or extremely elevated GH levels, neurons that regulate GH exhibit respective maximum and minimum expression and cell number in inhibitory somatostatin (SRIH) and in stimulatory GH-releasing hormone (GHRH). The phenomenon is inverted in GH-lacking dwarfs, and patterns of SRIH underexpression and GHRH overexpression are established early in postnatal development. The differentiation of PRL-inhibiting dopaminergic (DA) neurons is supported not only by PRL, but by human GH, which is lactogenic in rodents. Transgenic mice with peripherally expressed hGH have increased numbers of DA neurons, as opposed to the decreased DA population in PRL-deficient dwarf mice. Rats engineered to express hGH in GHRH neurons do not show this increase, whereas spontaneously GH-deficient dwarf rats show increased DA neuron number. These findings may be explained by feedback on neurons that co-express GHRH and DA. Current studies suggest that Snell (*Pit-1⟨dw⟩*) dwarf mice show a more severe and earlier DA neuron deficiency than Ames dwarfs, and that PRL feedback must occur prior to 20 days of postnatal age to maintain the DA neuronal phenotype. Insights into the mechanisms of GH and PRL effects on hypophysiotropic neurons include receptor localization on identified neuronal phenotypes, including intermediate neurons that mediate dynamic**

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**feedback, and elucidation of signal transduction pathways for GH and PRL in peripheral cell types. New transgenic models of altered GH, PRL, or receptor expression offer further study of neurotrophic effects.** [P.S.E.B.M. 1999, Vol 222]

This article provides an update of a minireview published in 1994 (1), the purpose of which was to examine a theory that "the effect of pituitary hormones on hypophysiotropic [pituitary-regulating] neurons extends beyond dynamic feedback, influencing developmental differentiation, survival, and connectivity of these cells." The studies described in that review were restricted largely to those that used as models mice bearing spontaneous mutations that result in dwarfism, through failure of the pituitary to produce growth hormone (GH), prolactin (PRL), and thyroid stimulating hormone (TSH). Subsequent work in this and other laboratories has extended examination of the theory using the same dwarf mice, spontaneous dwarf rats, and additional transgenic models of GH or PRL under- or overexpression. The new findings give further support to the classification of anterior pituitary hormones as neurotrophic factors for their respective regulatory neurons. Evidence from another hypothalamic-pituitary system, gonadotropin-releasing hormone neurons and target luteinizing hormone–producing cells, also supports this theory in terms of axonal guidance. Insights into the mechanisms of this feedback/neurotrophic effect have been reported, in localization of GH and PRL receptors and neuronal activation by GH in the hypothalamus. New transgenic, including "knockout," rodent models of altered GH or PRL expression or function have been developed very recently; examination of hypophysiotrophic neuron differentiation in these animals will shed more light on whether pituitary hormones are neurotrophic.

## Developmental Differentiation

The trophic influence of a factor upon differentiation of specific cell types may be deduced initially by assessing the adult condition in an animal that genetically lacks or overproduces that factor, because the condition is lifelong. Assessment of the adult condition of GH- and PRL- regulating hypothalamic neurons in rodent models that either fail to produce or overexpress GH or PRL has been accomplished for an increasing number of spontaneous or transgenically engineered animals. An overview of these assessments is given in Table I, for GH-inhibiting somatostatin (somatotropin release–inhibiting hormone, SRIH), GH-releasing hormone (GHRH), and PRL-inhibiting tuberoinfundibular dopamine (TIDA).

Although SRIH is produced by neurons in many CNS areas and in the gut, the SRIH that inhibits GH release is produced only by median eminence (ME)–projecting cells (27) in the periventricular nucleus (PeN) of the hypothalamus. In contrast, GHRH production in the brain is restricted largely to neurons in the arcuate nucleus (ARC) that project to the ME (28). These antagonistic peptides regulate GH secretory patterns (29, 30), and the feedback effect of GH on their expression is as would be expected, stimulating SRIH (31) and inhibiting GHRH (32, 33). Dopamine produced in the tuberoinfundibular ARC (also known as catecholaminergic area A12), and delivered to the pituitary through the ME, tonically inhibits PRL release (34), and PRL feeds back on TIDA neurons to activate DA synthesis and tyrosine hydroxylase expression (35, 36). The developmental effects of GH and PRL feedback have been studied in the models listed in Table I.

Two types of dwarf mouse, Snell [*dw* (37); $dw^J$ (38)] and Ames [*df* (39)], with absent GH and PRL (40), were used to elucidate physiological roles of GH and PRL long before the nature of the spontaneous mutations was known. With recent characterization as mutations in pituitary transcription factors, as discussed below, the nomenclature for the genetic designations is being changed from the traditional *dw* for Snell and *df* for Ames; both mutations are simple recessives, and dwarfism is manifested in the homozygous condition. "Little" (*lit/lit*) mice (41) have GH levels that are 5%–10% of those in normal (*LIT/?*) mice (41, 42); *lit* was characterized in 1993 as a point mutation in the GHRH receptor (43, 44). In rats, two spontaneous genetic dwarfs have been identified. The *dw* dwarf rat (45) has severely reduced (to 5%) GH levels; the mutation has not been characterized. The *dr* mutation in the Sprague-Dawley strain (46) produces no GH (47) and is a single base deletion in the GH structural gene (48). Production of PRL is not reduced in either dwarf rat type (19, 47).

As opposed to spontaneous mutations, transgenic alteration of GH includes giants as well as dwarfs. Transgenic dwarf mice were produced by ablating somatotrophs through expression of the diphtheria toxin gene driven by the GH promoter (49). Other types of transgenic dwarfs have been produced (50, 51), but hypophysiotropic neurons have been studied only in the "GH-DT" [Tg (GH, DT-A + Mt, GHRH) Bri78] dwarf. Transgenic mice that express human GHRH (52, 53) produce very high levels of endogenous (mouse) GH. Mice engineered to produce heterologous (bovine or human) GH (54) show high circulating levels, depending on gene variant type, copy number, and promoter construct (55); as discussed in a later section, hGH also has PRL-like effects in these animals. In contrast to the giantism resulting from widespread peripheral production of heterologous GH when expression is driven by a ubiquitous promoter such as metallothionein, hGH expression restricted to GHRH neurons suppresses endogenous GHRH

**Table I.** SRIH, GHRH, and TIDA Neurons in Models of Lifelong Altered GH and/or PRL Feedback

| Model | SRIH | Reference | GHRH | Reference | TIDA | Reference |
|---|---|---|---|---|---|---|
| n.d. mGH + n.d. mPRL | ↓ mRNA | [2]Hurley *et al.*, 1997 | ↑ mRNA | [3]Phelps *et al.*, 1993b; [4]Hurley *et al.*, 1998 | ↓ DA | [5]Morgan *et al.*, 1981; [6]Phelps *et al.*, 1985; [7]Phelps *et al.*, 1993a |
| Ames dwarf mouse (*df/df*) | ↓ cell number | [8]Phelps *et al.*, 1996 | ↑ cell number | [3]Phelps *et al.*, 1993b | ↓ cell number | [9]Morgan and Besch, 1990; [10]Phelps *et al.*, 1994a |
| n.d. mGH + n.d. mPRL | ↓ mRNA<br>↓ peptide | [11]O'Hara *et al.*, 1988; [12]Fuhrmann *et al.*, 1985; [13]Webb *et al.*, 1985 | mRNA not determined | | ↓ DA | [5]Morgan *et al.*, 1981; [6]Phelps *et al.*, 1985 |
| Snell dwarf mouse (*dw/dw*) | ↓ cell number | [14]Phelps and Hoffman, 1987 | ↑ cell number | Phelps, unpublished | ↓ cell number | [15]Phelps, 1987; [16]Phelps, *et al.*, 1997 |
| ↓ mGH little mouse (*lit/lit*) | No effect on cell number | [14]Phelps and Hoffman, 1987 | ↑ mRNA<br>↑ cell number | [17]Frohman *et al.*, 1989<br>Phelps, unpublished | no effect on cell number | Phelps, unpublished |
| ↓ rGH dwarf rat (*dw/dw*) | No effect on mRNA | [18]Pellegrini *et al.*, 1997 | ↑ mRNA | [18]Pellegrini *et al.*, 1997 | DA: no effect, ↑ cell number | [19]Thomas *et al.*, 1999 |
| n.d. rGH dwarf rat (*dr/dr*) | ↓ cell number | [20]Sakuma *et al.*, 1990 | ↑ cell number | [20]Sakuma *et al.*, 1990 | not determined | |
| n.d. mGH + ↓ mPRL GH-DT tg dwarf mouse | ↓ mRNA | [21]Hurley and Phelps, 1992 | ↑ mRNA | [22]Hurley and Phelps, 1993 | ↓ DA<br>↓ cell number<br>↓ TH mRNA | [23]Phelps *et al.*, 1991 |
| +bGH tg giant mouse | ↑ mRNA<br>↑ cell number | [24]Hurley *et al.*, 1994 | ↓ mRNA | Hurley, unpublished | No effect on DA, cell number | [25]Phelps and Bartke, 1997 |
| +hGH ("mPRL") tg giant mouse | ↑ mRNA<br>↑ cell number | [24]Hurley *et al.*, 1994 | ↓ mRNA | Hurley, unpublished | ↑ DA<br>↑ cell number | [25]Phelps and Bartke, 1997 |
| ↑ mGH: + hGHRH tg giant mouse | ↑ mRNA | [21]Hurley and Phelps, 1992 | ↓ mRNA | [22]Hurley and Phelps, 1993 | not determined | |
| ↓ rGH: + hGH ("rPRL") Tgr rat | ↑ mRNA | [26]Flavell *et al.*, 1996 | ↓ mRNA | [26]Flavell *et al.*, 1996 | ↑ DA, no effect on cell number | [19]Thomas *et al.*, 1999 |

*Note.* n.d. = not detectable; ↓ = decreased; ↑ = increased; m = mouse; h = human; r = rat; tg = transgenic; Tgr = transgenic growth retarded.

production, resulting in reduced GH levels and dwarfism, in the recently described "transgenic growth-retarded" (Tgr) rat (26). All of these genetic models of altered GH and PRL production represent lifelong conditions, whereby the hormone(s) could affect differentiation during critical periods in the development of hypothalamic neurons. Direct assessment of hypophysiotropic neuron development has been accomplished in the spontaneous dwarf mice. These dwarf models also have been used to elucidate anterior pituitary differentiation.

**Anterior Pituitary.** The spontaneous genetic mutations that result in 1) failure of the pituitary to produce GH, PRL, and TSH, and 2) phenotypic dwarfism have provided models for assessing not only similar human deficits (56–62), but differentiation *per se* of this complex organ of diverse and largely independent cell types. The adenohypophysis serves as a model of progressive restriction in gene expression through development and has attracted the attention of numerous research groups recently; the defects in dwarf pituitaries have provided the means and impetus for identifying genes responsible for the differentiation sequence.

The Snell dwarf mutations, *dw* and *dw$^J$*, were characterized in 1990 (63) as, respectively, a base substitution and a sequence rearrangement in the pituitary-specific transcription factor Pit-1 (64, 65), which is necessary for the expression of GH, PRL, and TSH. Because the Ames dwarf (*df/df*) pituitary was phenotypically identical to that of the Snell dwarf, but *df* localized to chromosome 11 (66, 67), rather than 16, it was likely that *df* preceded *dw* in the pituitary differentiation sequence (68, 69).

*Characterization of the Ames dwarf mutation.* Using positional cloning and additional amplification methods, Sornson *et al.* (70) determined that a novel homeobox-containing protein is encoded at the *df* map position on chromosome 11. The protein is a member of the "paired" class of homeobox proteins and is expressed only in the pituitary. The protein binds to DNA *via* the homeodomain in the promoter region of the *Pit-1* gene, suggesting involvement in the activation of *Pit-1* gene transcription. Because the protein thus foretells the production of Pit-1, it was named Prophet of *Pit-1*, abbreviated *Prop-1* (70).

A mutation of *Prop-1* that prevents the proper function of the protein was shown to be present at the *df* allele. Due to a T-to-C transition mutation, the serine residue at codon 83 is changed to proline (S83P), resulting in reduction in the DNA binding affinity of Prop-1 protein by at least 8-fold (70), and leading to failure to properly activate *Pit-1* transcription. The loss of Pit-1 expression due to mutant Prop-1 would lead to the GH-, PRL-, and TSH-deficient phenotype. It is important to note, however, that there may be some residual Prop-1 function in Ames dwarf pituitary because the S83P Prop-1 protein retains some DNA binding capacity

(70, 71). Recently, analysis of patients with multiple pituitary hormone deficiency has revealed *Prop-1* mutations. Although the equivalent of the S83P mutation has not been found, the mutations that were characterized resulted in truncation of the Prop-1 protein, due to a 2-base pair deletion (A301G302), or a C-to-T transition leading to a cysteine substitution for arginine at codon 120 (72).

The genetic nomenclature for the mouse dwarf mutations has been changed officially to reflect characterization of the genes in which they occur, from *dw* to Pit1⟨dw⟩, $dw^J$ to Pit1⟨dwJ⟩, *lit* to Ghrhr⟨lit⟩, and *df* to Prop1⟨df⟩ (73).

*Expression sequence of pituitary differentiation genes.* Beyond the characterization of *Prop-1* as the factor responsible for initiating *Pit-1* expression, several genes that function earlier during anterior pituitary development have been characterized (reviewed in Ref. 71). In temporal order of activation, the first of these is named *Rpx,* for Rathke's pouch homeobox (74), and is also known as *Hesx*1 (75). As the name implies, *Rpx/Hesx*1 expression is eventually restricted to Rathke's pouch, the earliest identifiable precursor of the pituitary. However, *Rpx/Hesx*1 expression initiates during gastrulation at embryonic day (e)7 in the endodermal prechordal plate, then appears in the ectoderm that will become the cephalic neural plate. By e9, restriction of *Rpx/Hesx*1 expression occurs such that transcripts are found exclusively in the ectodermal cells fated to give rise to Rathke's pouch, and expression increases at e11.5, the time of definitive pouch formation. Eventually, by e15.5, after the appearance of some differentiated cell types in the anterior pituitary, *Rpx/Hesx*1 expression is no longer detectable (74). Like *Prop-1, Rpx/Hesx*1 is a member of the paired class of homeobox genes, and the loss of *Rpx* expression by el5.5 depends upon the proper function of *Prop-1*; in adult Ames dwarf mice, *Rpx* expression continues (76). Mutation of the *Hesx*1 gene has been shown to be responsible for familial septo-optic dysplasia (77).

Two other important genes for pituitary development are members of the LIM family of homeobox genes, named *Lhx3* and *Lhx4* (78, 79). *Lhx3,* also named P-*Lim* (80) and *mLIM3* (81), has been shown to be expressed in the pituitary throughout development and in the adult. Transgenic site-directed mutagenesis, or "knock-out," mice lacking *Lhx3* have been shown to initiate but not complete the formation of Rathke's pouch, and to fail to maintain *Rpx/Hesx1* expression beyond el2.5 (78). Pituitaries from these *Lhx3*-deficient mice contain only one differentiated cell type, the corticotroph (78). *Lhx4* is expressed by e9.5 in the developing pituitary, becomes restricted to the future anterior lobe by el2.5, and then decreases to low levels that are maintained through adulthood in the anterior and intermediate lobes (79). Mice in which *Lhx4* has been deleted transgenically show Rathke's pouch formation and elaboration of a hypoplastic anterior pituitary containing a greatly reduced number of differentiated, hormone-producing cells. Thus, *Lhx4* is likely to be a step in the genetic program of pituitary development that functions after *Lhx3* activity, leading to the proliferation of distinct cell types after differentiation from precursors (79).

*GH, PRL, TSH, Pit-1 cells in df/df anterior pituitary.* Gage *et al.* (69) reported that, in Ames dwarf pituitary, GH was detected by immunocytochemistry (ICC) in approximately 100 cells/gland, estimated to be 0.05% the normal number of GH-producing cells (82). Subsequently, the same group reported an analysis of three to four *df/df* pituitaries that showed cells containing immunoreactive GH, PRL, and TSH; immunoreactive Pit-1 was detected in all these cells (83). No GH-, PRL-, or TSH-positive cells were detected in Snell dwarf pituitaries (83). Although cells with ultrastructural features common to somatotrophs and lactotrophs have been observed in the adult Snell dwarf pituitary (84), these "ambiguous" cells do not contain immunoreactive GH, PRL, or TSH (85, 86), supporting the prediction that the Snell Pit-1 mutation would completely block expression of these hormones (63). These ultrastructural studies show maintenance of a possible precursor cell type that remains undifferentiated in the Snell dwarf pituitary (86).

In the studies of Gage *et al.* (83), hormone-positive cells were in "clusters," suggesting clonal origin (69, 83). In another study, Andersen *et al.* (68) reported that no GH-positive cells could be detected by ICC in Ames dwarf pituitary sections, but that GH was detected in 10 to 20 cells per pituitary in dispersed glands (68). Routine pituitary section ICC in this laboratory has also detected occasional GH- and PRL-positive cells in Ames dwarf pituitaries (Phelps, unpublished). The frequency of pituitaries showing "clones" (approximately 20% of over 200 *df/df* glands) has been less than that reported by Gage and colleagues (69, 83), perhaps due to colony differences (69) or to the larger sample. Figure 1 shows examples of GH immunostaining in two adult *df/df* pituitaries that showed "clones" compared with the abundant GH in a *DF/df* gland, and typical absence of GH in a Snell dwarf pituitary. Cell clusters containing PRL also have been found, usually in the same pituitaries in which GH was detected. It is assumed that these cells express *Pit-1.* Although these findings appear to be at odds with the previous reports of undetectable GH or PRL in the dwarf pituitary (40, 42, 87), it is important to note, as does the study by Slabaugh *et al.* (40), that biochemical assays of GH or PRL have a sensitivity of 0.05%, a level of GH cell occurrence that was reached only in the Gage *et al.* (69) study. There are reports that mRNAs for GH, PRL, TSH or Pit-1 are not detectable in Ames dwarf mice, assayed by either RT-PCR (88) or *in situ* hybridization (68). The presence of these clonal cells remains unexplained, but may relate to the fact that the Prop-1 mutation does not completely prevent DNA binding by the protein. Thus, partial function may initiate occasional cellular differentiation in the Ames dwarf pituitary (68, 83). Also, it is likely that other "paired" homeodomain proteins form heterodimers with Prop-1, so it may be important to identify such products and their expression patterns to explain the presence of hormone-positive cells in the Ames dwarf pituitary.

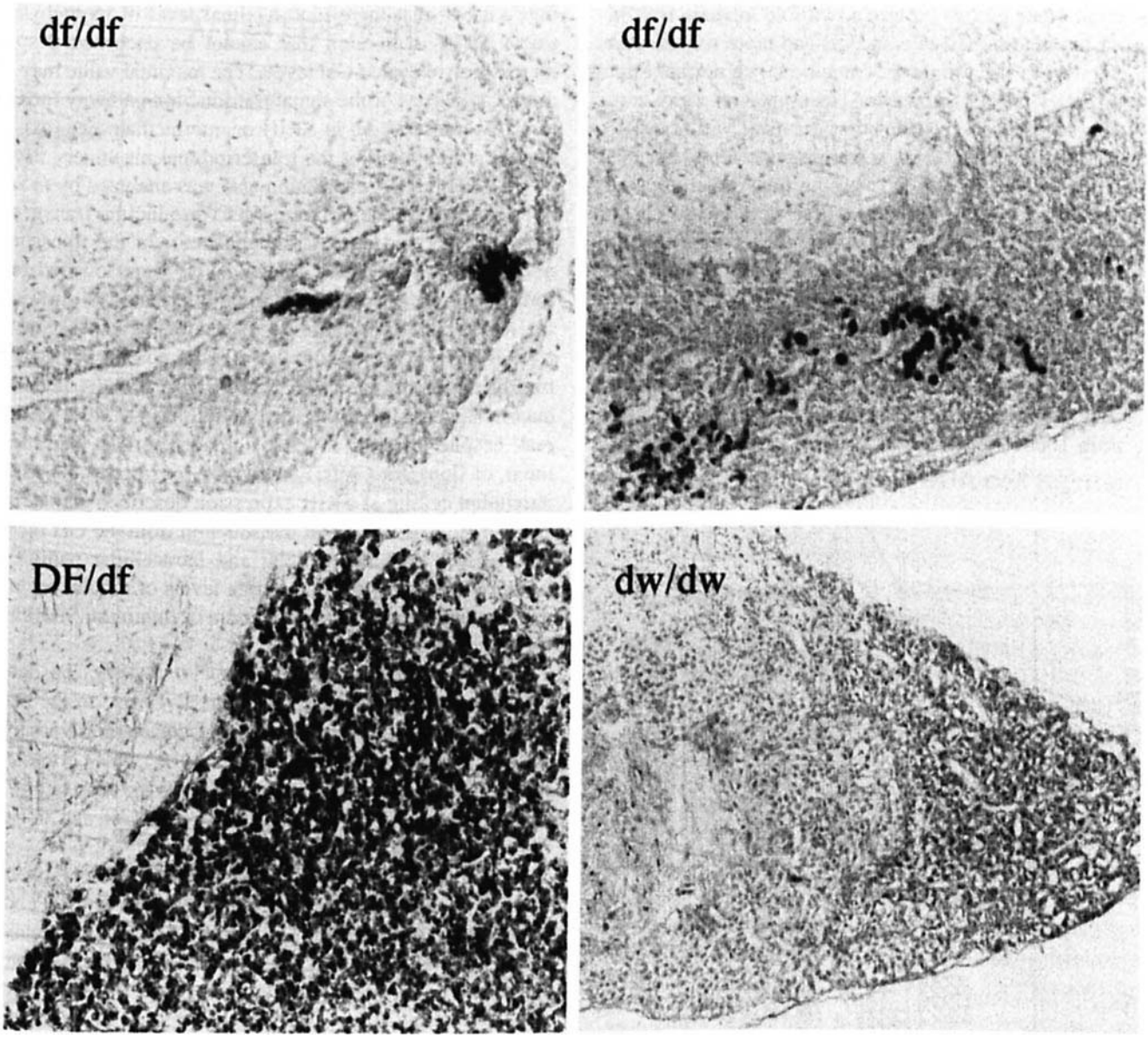


**Figure 1.** Photomicrographs of GH immunoreactivity in Ames dwarf (*df/df*) anterior pituitary (upper panels), compared with GH in normal mouse (*DF/df*) and Snell dwarf (*dw/dw*) pituitaries (lower left and right panels, respectively). All were horizontal 5-μm sections, counterstained with hematoxylin, and photographed at 20× original objective magnification. The neurointermediate lobe appears in the upper left area of each panel.

**GH-Regulating Neurons.** Cell numbers, as well as mRNA and peptide levels, are altered in GH-regulating hypophysiotropic neurons in dwarf and giant mouse hypothalamus. SRIH is reduced in Snell and transgenic dwarfs and is elevated in mice that overproduce endogenous GH because of an hGHRH transgene. Conversely, GHRH is overexpressed in Ames and transgenic dwarfs, and decreased in hGHRH transgenic giant mice (reviewed in Ref. 1). Subsequent studies have addressed the effects of lifelong heterologous GH and the developmental patterns of SRIH and GHRH in the spontaneous dwarf.

*SRIH and GHRH expression in GH-excess transgenic mice.* Several GH-transgenic mouse types were used to evaluate the feedback effect of differing levels of heterologous GH on SRIH expression (24). The models were mice with the metallothionein (*MT*) promoter directing expression of the bovine (b) GH gene (*MT-bGH*), mice with *MT* promoter controlling the human (h) GH gene (*MT-hGH*), and mice with the *bGH* gene fused to the phosphoenolpyruvate carboxykinase (PEPCK) promoter (*PEPCK-bGH*). Because these constructs direct GH expression in multiple peripheral sites, and lack upstream SRIH and GHRH regulatory regions, GH production is unregulated and excessive. Circulating levels of heterologous GH ranged from a mean of 19.9 ng/ml in MT-hGH, to 30 ng/ml in MT-bGH, to 4381 ng/ml in PEPCK-bGH mice. Body weights were increased in proportion to GH levels. SRIH expression was examined by both peptide ICC and mRNA *in situ* hybridization, in hypophysiotropic PeN and nonhypophysiotropic medial basal hypothalamus (MBH). All

three transgenic groups showed a two-fold increase in numbers of immunostained cells in PeN and more robust staining intensity in ME terminals, compared with normal littermates. SRIH mRNA expression, measured on x-ray autoradiographs for total hybridization intensity, did not differ in the MBH among normal and transgenic mice, but PeN SRIH mRNA was increased in all the transgenic groups to 200%–240% that of normals. These results are shown graphically in the upper panel of Figure 2, compared with normal, dwarf and hGHRH transgenic mice. Hypophysiotropic SRIH expression increased in the presence of elevated GH, but not in proportion to circulating GH levels, as was the case for body weight. Previously, PeN SRIH mRNA was found to increase to ~200% of normal in hGHRH transgenic mice with circulating mGH levels ranging from 166–1095 ng/ml (21). Taken together, these findings suggest that there is a maximal level of hypophysiotropic SRIH expression that cannot be exceeded despite increasingly elevated GH levels. The maximal value may be due to properties of the signal transduction pathway for GH to affect transcription in SRIH or intermediate neurons, or may be a limitation of the transcriptional machinery itself.

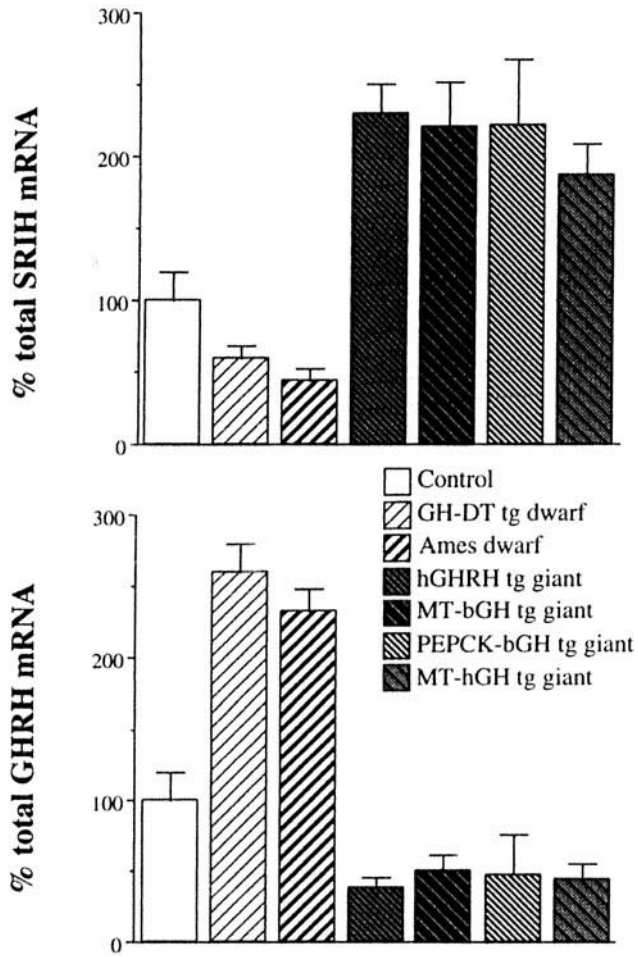


**Figure 2.** Mean total levels, as assessed by *in situ* hybridization, of hypophysiotropic SRIH (top panel) or GHRH (bottom panel) mRNA in control, GH-deficient and GH-excess spontaneous or transgenic mice. Expression for each experimental model has been normalized after setting control (normal sibling) values equal to 100% for each analysis using data presented separately (2–4, 21, 22, 24, and Hurley, unpublished data). Vertical bars on each column represent the standard error of the mean (SEM) for each group, also normalized to percentage. tg = transgenic, GH-DT = GH-diphtheria toxin [Tg (GH,DT-A + Mt, GHRH) Bri 78], hGHRH = human GH-releasing hormone [Tg (Mt, GHRH) Bri 11], MT-bGH = metallothionein promoter – bovine GH, PEPCK-bGH = phosphoenolpyruvate carboxykinase promoter – bovine GH, MT-hGH = MT – human GH.

GHRH mRNA expression also was analyzed by *in situ* hybridization in heterologous GH-producing transgenic mice (Hurley and Phelps, unpublished). In the transgenic mice, total ARC GHRH mRNA levels were lowered uniformly, to ~40% of those in normal littermates, as shown in the lower panel of Figure 2. As also shown in Figure 2, GHRH mRNA expression was 40% of normal in hGHRH transgenic giant mice (22). Thus, GHRH mRNA level reduction appeared to reach a minimum level of 40% of normal, despite differing levels of circulating GH. The minimum, or floor, for GHRH expression may be similar to the maximum ceiling of SRIH expression described above. Because hypothalamic signal transduction from the GH receptor involves both intercellular and intracellular pathways, further elucidation of the multiple levels of regulation may explain the phenomena of maximum or minimum SRIH and GHRH expression.

*SRIH neuronal development in dwarfs.* To determine the developmental pattern that gives rise to the adult state of severely reduced number of detectable hypophysiotropic SRIH neurons (14), SRIH mRNA levels (2) and cell numbers (8) were determined from postnatal day 1 (day of birth) through 90 days of age in Ames dwarf and normal mice.

Analysis of SRIH mRNA expression was accomplished using both total hybridization intensity in the entire PeN or ARC, and grain counts indicating SRIH mRNA per neuron. Although total SRIH mRNA increased from day 3 through adulthood in the PeN of normal mice, there was no increase until 60 days in Ames dwarf mice, resulting in a reduction to 40% in adult dwarfs compared with normal littermates; data are graphed in Figure 3A. Thus, the reduced total hypophysiotropic SRIH mRNA in GH-deficient Ames dwarf mice appears developmentally shortly after initial detectability of SRIH in the PeN, because SRIH mRNA levels fail to increase during postnatal development. However, these studies also showed that there was no difference between dwarf and normal SRIH expression levels per neuron, indicating that extant SRIH neurons in Ames dwarf mice produce normal levels of SRIH mRNA (2).

Detection of SRIH peptide, especially in neonatal mice, required immunostaining with a more sensitive ratio of avidin-biotin reagents (89) (Vectastain "Elite" kit, Vector Laboratories, Burlingame, CA) than had been used initially (14). In addition, some adult animals were treated with intracerebroventricular colchicine to block axonal peptide transport and thus increase perikaryal immunostaining. Neurons in PeN, and terminals in ME, were detectable in *DF/?* mice at 3 days of age, and in *df/df* mice by day 7. Cell numbers increased thereafter, reaching adult numbers by

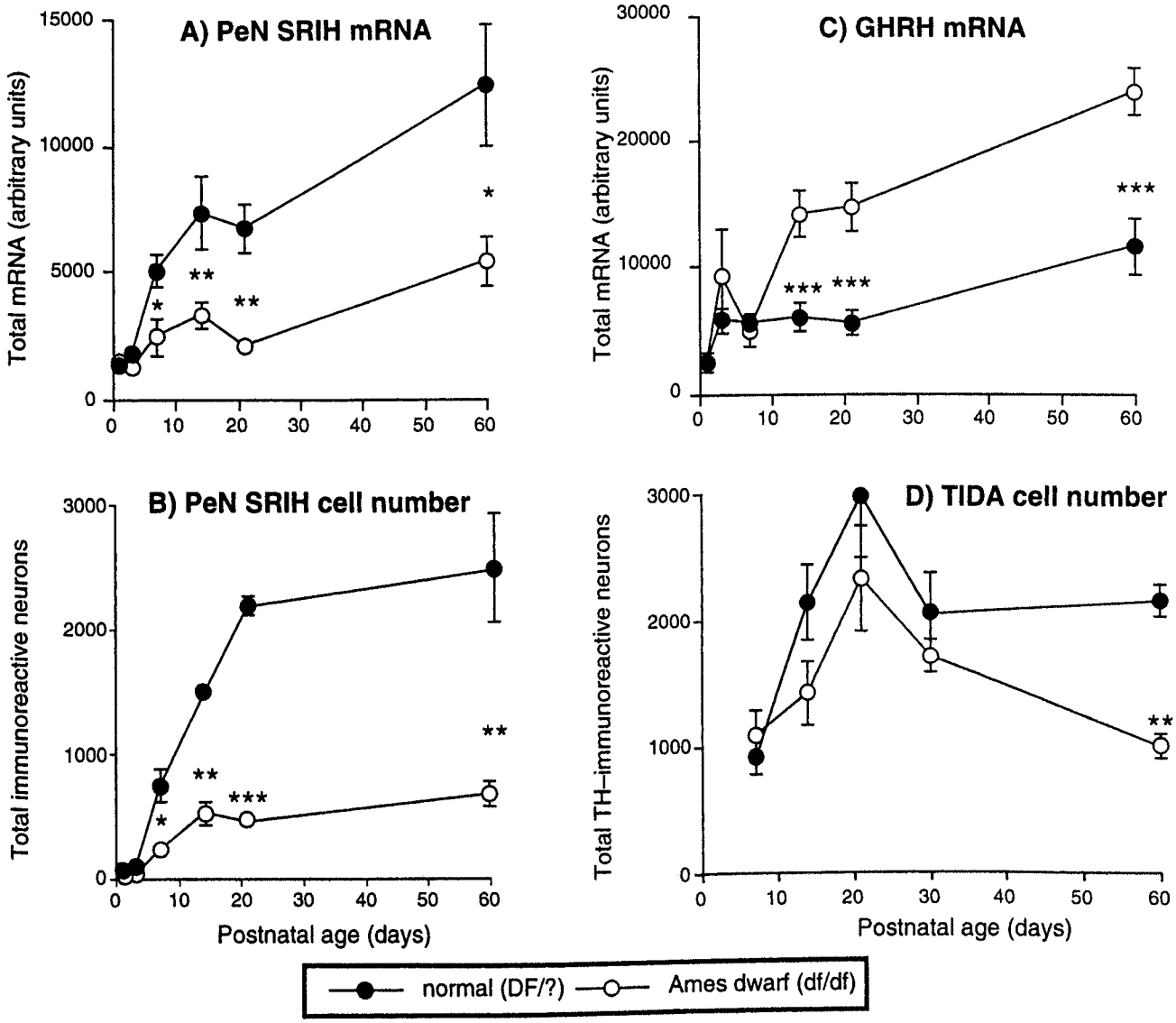


**Figure 3.** Developmental patterns of PeN SRIH (left panels) mRNA (A) and cell number (B), GHRH mRNA (C) and TIDA neuron number (D) in Ames dwarf versus normal mice. Each point represents the mean, and each vertical bar the SEM. In this and subsequent figures, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, compared with normals of the same age.

day 21 in *DF/?*, and day 14 in *df/df*, as shown in Figure 3B. Numbers of SRIH-immunoreactive neurons in dwarf PeN were markedly lower than normal from day 7 through 60–90 days, at which age *df/df* cells numbered 28% of those in *DF/?*. In colchicine-treated adult dwarfs, PeN SRIH neurons numbered 47% of those in normals. Thus, the reduction in SRIH expression to approximately 40% in Ames dwarf mice appears to be the result of a smaller population of SRIH-positive neurons (8), rather than a change in the level of expression per neuron (2). The developmental pattern that results in a reduced number of detectable hypophysiotropic SRIH cells in Ames dwarf mice thus differs markedly from that of PRL-inhibiting TIDA neurons, which show an increase in number that is comparable to that of normals through 3 weeks of age, then a precipitous decline by adulthood (90), as shown in Figure 3D and as discussed further below. The difference in pattern may reflect a difference in timing of the GH and PRL trophic effects because GH production in the pituitary begins before birth, whereas PRL is not detectable in the mouse pituitary until 7–8 days postnatally (10, 90).

*GHRH development in dwarfs.* Studies of adult Ames dwarf mice showed that GHRH mRNA and peptide are overexpressed in the ARC (3). To examine the developmental time course of GHRH overexpression, GHRH mRNA was quantified in dwarf and normal mice at 1, 3, 7, 14, 21, and 60 postnatal days by *in situ* hybridization (4). This was the first study of the developmental pattern of GHRH expression in mice. Both total mRNA and mRNA expression per neuron were quantified. As shown in Figure 3C, total GHRH mRNA was the same in dwarf and normal mice at 1, 3, and 7 days, then GHRH mRNA in dwarf increased at day 14 to 240% of that in normal littermates, and remained ≥200% through 60 days, although total GHRH mRNA increased in both dwarfs and normals during this period. GHRH mRNA per neuron is shown in Figure 4,

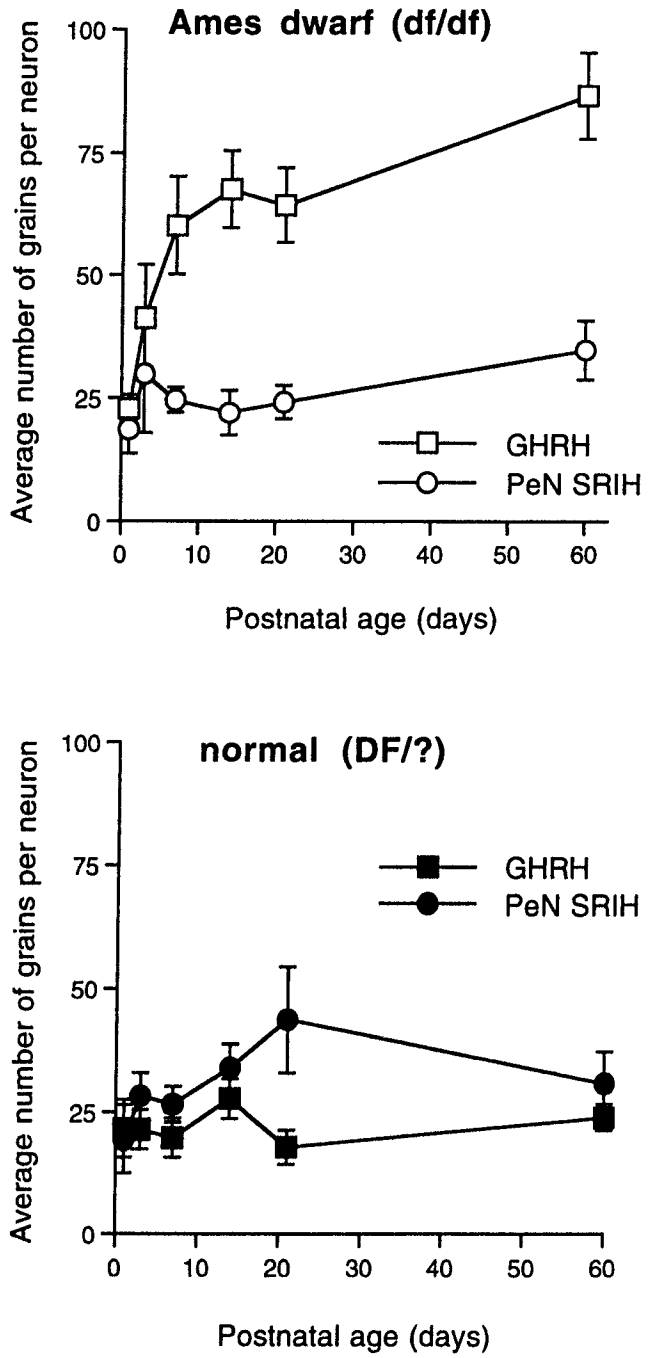


**Figure 4.** Quantification during development of GHRH and PeN SRIH mRNA per neuron in Ames dwarf (upper panel) and normal mice (lower panel). Each point represents the mean, and vertical bars represent the SEM.

plotted with PeN SRIH mRNA per cell in Ames dwarf (top panel) and normal mice (bottom panel). GHRH mRNA per neuron was the same in normal and dwarf mice at 1 day, increased in dwarfs to 190% of that in normals at 3 days, and rose to 300% of normal levels by 7 days and beyond. In normal mice (lower panel, Fig. 4), neither GHRH nor SRIH per neuron changed during the postnatal period examined. Thus the increase in GHRH mRNA per neuron in Ames dwarf mice that is first detectable at 3 days occurs 7 days after the failure to initiate GH production, which occurs normally at el7.5. This onset of GHRH overexpression (i.e., expression levels per cell) occurs earlier than the failure of SRIH to increase in total (Fig. 3A) or per cell (Fig. 4), which may be pertinent to the influence of SRIH on GHRH. Subsequent to the early per-cell increase in GHRH mRNA in response to absent inhibition by GH, long-term GH absence may lead to recruitment of neurons to a GHRH-expressing phenotype to increase the total production of GHRH.

**PRL-Regulating Neurons.** Studies of PRL-deficient dwarf mice indicate that TIDA neuron number is reduced (10) and that this deficit has a postnatal developmental onset (90). Because the dwarf TIDA neuron deficiency could be averted by PRL replacement initiated neonatally (91) but not in adults (92), a specific developmental role for PRL was indicated. Subsequent studies have addressed whether excess PRL (in the guise of transgenic human GH) production in early development affects TIDA neuron differentiation.

*Effect of peripheral hGH on TIDA neurons.* Transgenic mice developed to produce heterologous (human or bovine) GH driven by either *MT* or *PEPCK* promoters (54, 93, 94) have been used to study the effects of excessive GH on reproduction as well as growth and metabolism in both sexes (55). The effects of hGH and bGH on reproduction in these mice differ, probably because hGH has lactogenic, as well as somatogenic, properties in rodents (95, 96). An investigation of the possible differential effects of PRL-like hGH versus only-somatogenic bGH on TIDA neurons (25) showed that both catecholamine fluorescence and numbers of neurons detectable by ICC for the catecholamine synthetic enzyme tyrosine hydroxylase (TH) were increased significantly in mice bearing either *MT-hGH* or *PEPCK-hGH,* but not *bGH,* transgenes, as shown in Figure 5. In the particular lines of mice assessed, hGH levels were higher in PEPCK-hGH than in MT-hGH transgenics; numbers of

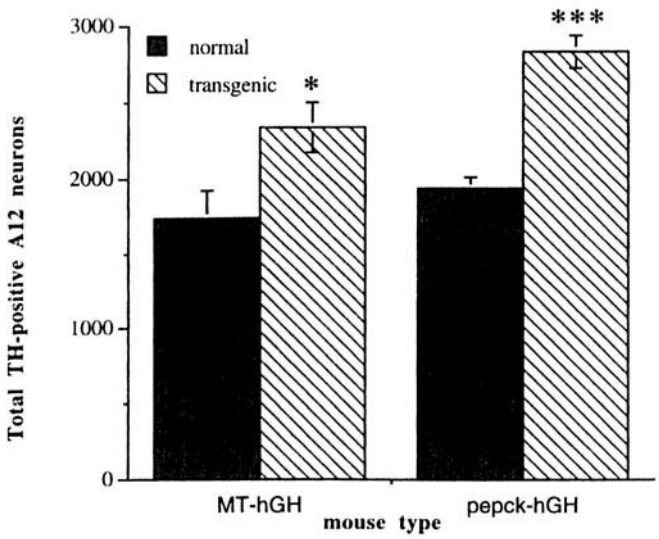


**Figure 5.** TIDA neuron numbers, as assessed by TH immunocytochemistry, in transgenic hGH-expressing mice, compared with cell numbers in normal littermates. Columns represent the mean, and vertical bars the SEM.

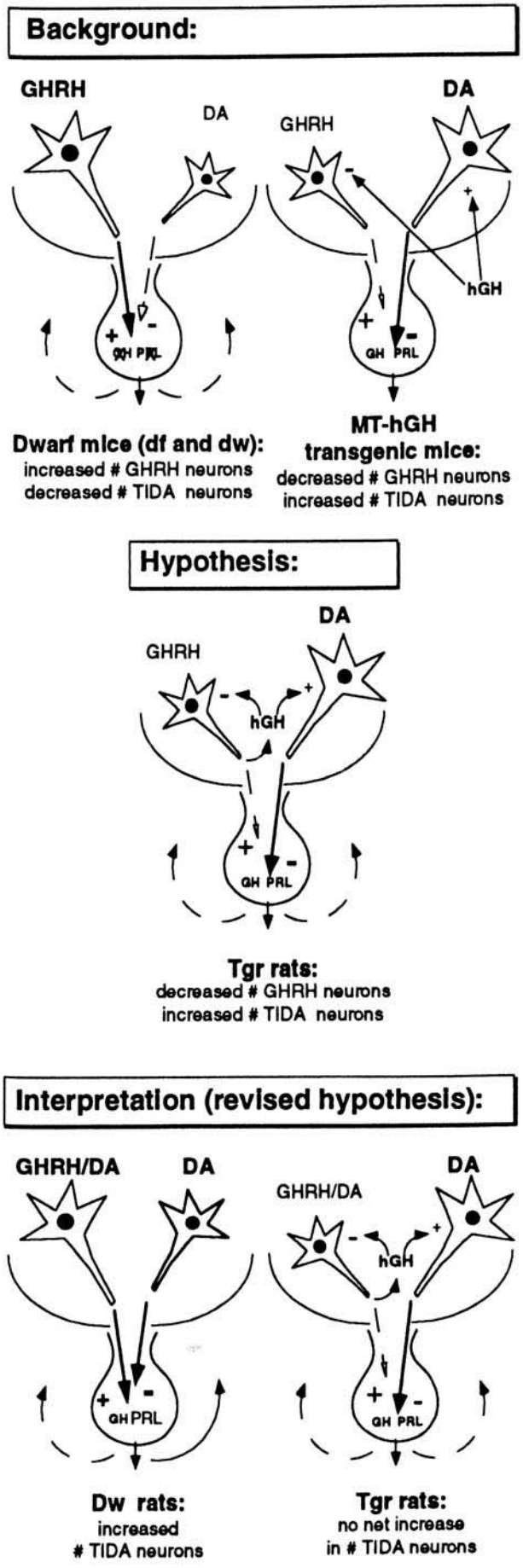


**Figure 6.** The precedent, hypothesis, and outcome of studies of TIDA neurons in *Tgr* and *dw* dwarf rats.

TIDA neurons were 134% of normal in MT-hGH transgenics, and 147% of normal in the PEPCK-hGH mice. These findings indicate that not only is the stimulatory feedback signal necessary for a normal complement of PRL-regulating TIDA neurons, but that a larger population results if an excess PRL stimulus is present during TIDA development. Because the high circulating levels of hGH ("PRL") are the result of peripheral (liver, gut, kidney) production, the studies support the role of circulating, rather than retrogradely transported or hypothalamically produced, PRL in TIDA development.

*hGH in GHRH/(TIDA) neurons: Tgr rats.* Prompted by the interesting effect of transgenic hGH overexpression on TIDA neurons described above, TIDA neurons were evaluated in rats bearing a transgenic construct consisting of structural hGH downstream of a sequence containing the rat GHRH promoter, resulting in hGH expression in GHRH neurons (26). These animals were designed to produce GH deficiency by intrahypothalamic feedback suppression of GHRH, resulting in an animal that would respond to exogenous GHRH and synthetic GH secretagogues, as opposed to pituitary GH-deficient *dw/dw* rats (45) or mice. These transgenic growth-retarded (Tgr) rats exhibit decreased hypothalamic GHRH (and increased SRIH) expression (26) as opposed to the increased GHRH expression of *dw/dw* rats (18) and *df/df* mice (3).

It was hypothesized that the intrahypothalamic production of hGH in Tgr rats would have a PRL-like stimulatory effect on TIDA neurons, as had circulating hGH in MT- or PEPCK-hGH transgenic mice, especially within neurons that express both GHRH and DA, a subpopulation of the ARC that has been demonstrated in rats (97). This background and hypothesis are illustrated by the cartoons of Figure 6. Brains of Tgr, normal (AS parent strain), and *dw/dw* rats prepared by paraformaldehyde/glutaraldehyde (98) perfusion in the laboratory of Dr. I. C. A. F. Robinson at the NIMR, London, were shipped to New Orleans (in a significant adventure with transatlantic couriers and the USDA) for assessing TIDA catecholamine fluorescence and neuron number by TH ICC. Although increased DA fluorescence in Tgr individual TIDA neurons (as shown in Fig. 7) indicated stimulation, TH-immunoreactive neuron numbers did not differ between Tgr and AS controls. However, the TIDA neuronal population was increased significantly in *dw/dw* rats. To test whether the 37% increase in TIDA neurons resulted from increased GHRH expression, a group of *dw/dw* rats was treated with GH to reduce GHRH (99). The treatment did not decrease TIDA neuron numbers (i.e., TH production) significantly, suggesting that a permanent developmental effect had occurred. The results have been interpreted to indicate that, in the *dw/dw* rat, with isolated pituitary GH deficiency, ongoing PRL secretion stimulates differentiation of a normal population of neurons that produce DA only, whereas the greatly decreased GH negative feedback results in an increased population of GHRH-producing neurons, a portion of which also produce DA,

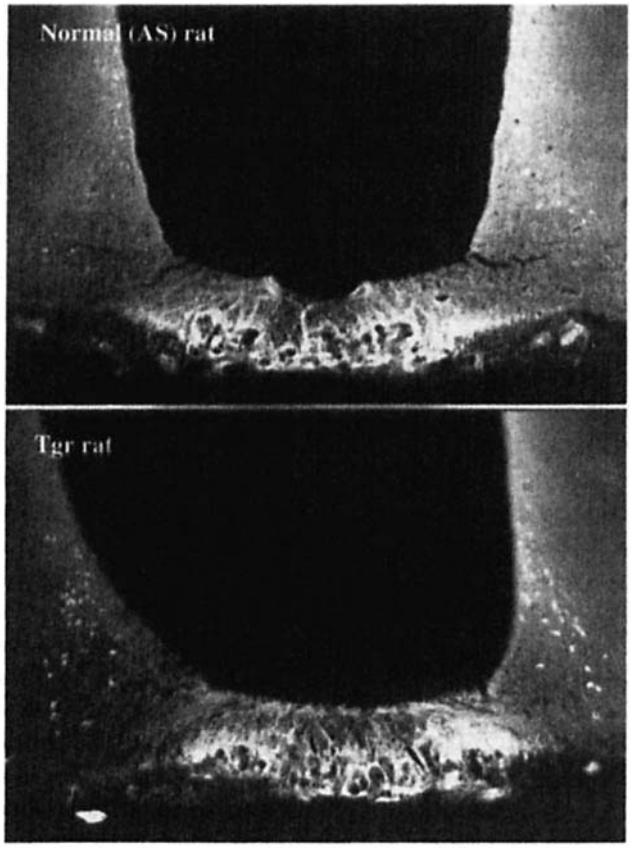


**Figure 7.** Paraformaldehyde-induced catecholamine histofluorescence in normal (parent strain AS) rat (top panel) and Tgr rat (bottom panel) basal hypothalamus. Sections were coronal, 30 μm thick, and photographed at original objective magnification of 10×.

resulting in an increase in total numbers of TIDA neurons. In the Tgr rat hypothalamus, hGH appears to exert a developmental suppression of the GHRH population, a portion of which are DA-producing. It is likely that the hGH also exerted a stimulatory effect on TIDA (DA-only) neuronal number, or the resulting TH-immunoreactive population in Tgr rats would have been reduced compared with normal AS rats, rather than comparable. The latter interpretation is supported both by the increased DA fluorescence in extant TIDA neurons in Tgr, and by reduced pituitary levels of PRL that would result from increased DA secretion. However, GHRH and DA/TH expression have been analyzed only independently in Tgr rats, and confirmation of this interpretation awaits quantification of the neuronal population that expresses both GHRH and DA/TH. The interpretation/revised hypothesis (19) is illustrated in Figure 6.

*TIDA neuron development in Snell dwarf mice.* The developmental studies that were summarized previously (1) of hypophysiotropic neurons in dwarf mice were conducted using Ames dwarf mice reared in a colony established from breeding pairs contributed by Dr. Andrzej Bartke, Southern Illinois University College of Medicine. A devastating fire at the Jackson Laboratory in 1989 rendered Snell dwarf mice or mutation-bearing breeding pairs essentially unavailable for a number of years, but recent establishment of new breeding colonies now allows developmental studies of *dw/dw* hypophysiotropic neurons. Because an earlier quantification of TIDA neurons in adult Snell dwarf (15) indicated that the population deficit was more severe than that in Ames dwarf (10), it was of interest to compare the developmental patterns of the two dwarf types. As shown in Figure 3D, numbers of TIDA neurons in *df/df* as well as normal (*DF/?*) mice increase comparably between 7 and 21 postnatal days of age; subsequently, the population in *df/df* declines to <50% of that in *DF/?*. A comparable study was conducted recently for Snell dwarfs and normal siblings, by assessing catecholamine fluorescence (98) and TIDA neuron number by TH ICC between 7 and 60 days postnatally (16). Qualitatively, TIDA fluorescence and TH immunostaining appeared reduced in *dw/dw* compared with *DW/?* at early ages, which differed from the pattern in *df/df* (7, 90). TH ICC is compared for *DW/?* and *dw/dw* at 14 days and as adults in Figure 8. Neuron numbers were identical for *dw/dw* and *DW/?* at 7 days. Subsequently, the TIDA population increased in *DW/?* hypothalamus, reaching the adult complement by 14 days. The population size did not increase in dwarfs beyond 7 days, was significantly lower than that in *DW/?* at 14 days, and, in fact, decreased at 30 and 60 days from numbers present at 7–21 days. The number of TH-immunoreactive TIDA neurons in adult *dw/dw* was 23% the number in *DW/?* mice, as opposed to 48% of *DF/?* in *df/df*. These data are graphed in Figure 9, superimposed over the pattern in Ames dwarfs (see Fig. 3). An explanation for the difference in developmental pattern and severity of deficit between the two mutations is elusive because the PRL-deficient phenotype is identical for the two mutations, except for the findings of Gage *et al.* (83) and Phelps (unpublished) described previously. A similarity in the developmental pattern of TIDA neurons in Ames and Snell dwarfs is the precipitous decrease that occurs after 21 days in both mutants, albeit from a lower population size in Snell dwarf; it is as if a previous maintenance ceases at that age, when the failure to produce endogenous PRL in dwarfs becomes significant, because PRL is only first detectable at 7–8 days in normal mice (7, 87), and circulating levels are low prior to 21 days (Romero and Phelps, unpublished results).

It had been shown that the decline in numbers of TIDA neurons that occurs in Ames dwarfs between 21 and 60 days of age (90) could be prevented by ovine (o) PRL treatment initiated at 12 (91), but not at 60 (92), days of age. Further studies (Romero and Phelps, unpublished) tested whether PRL replacement initiated at 21 or 30 days would prevent the decline in TIDA cell number; it did not. Dwarf mice treated with PRL beginning at 21 or 30 days had TIDA populations comparable to those of untreated dwarfs, approximately 50% of the normal population. These findings are summarized in Table II. That PRL treatment beginning at 21 days was not effective in maintaining the TIDA neurons suggests that the phenomena responsible for the loss of cells or phenotype (TH and DA production) are already operating at 21 days of age.

The developmental patterns of hypophysiotropic neurons that are affected by GH or PRL feedback signals are summarized in the graphs of Figure 3. For SRIH and GHRH expression, total mRNA levels are shown (Figs. 3A and 3C); the patterns of divergence of dwarf from normal development suggest an effect of the absence of GH influence at early postnatal ages. The stimulus for overexpression of GHRH in the absence of GH feedback must include signaling through the GH receptor and is likely to include an inhibitory mediator that is stimulated by GH. For detectable cell numbers in postnatal development, the patterns by which PeN SRIH neurons (Fig. 3B) and TIDA perikarya (Fig. 3D) result in the adult deficit differ markedly. Although both neuronal types are inhibitory, and require the positive influence of GH and PRL, respectively, SRIH neurons fail to differentiate, whereas TIDA neuron number regresses from the neonatal complement. All the patterns suggest dependence on neurotrophic signals at critical developmental intervals.

## Mechanisms of Neurotrophic Feedback

**GH and PRL Receptors in Hypothalamus.** A direct trophic effect of GH and PRL on respective hypophysiotropic neurons would require the presence of hormone receptors in those specific neuron populations. It is remarkable that hypothalamic GH and PRL receptor localization has been only recent, despite considerable information on the nature of these receptors (100–102).

In the case of CNS GH receptor (GHR), localization to both PeN and ARC predominates (103). However, subsequent studies showed that, whereas GHR is expressed by SRIH neurons in PeN, the majority of GHR-expressing neurons in ARC are not GHRH-producing (104), but have a neuropeptide Y (NPY) phenotype (105, 106), consistent with a role established earlier for NPY in GH regulation (107). The *dw* dwarf rat has been shown to express GHR mRNA in ARC at markedly reduced levels, which could be reversed by peripheral GH treatment (108). Studies of GHR in the CNS of mouse models are lacking completely.

In the case of PRL receptor (PRLR), expression in the adult hypothalamus is very low (109, 110), and regional mapping (111–114) has not indicated particularly greater expression in ARC, compared with other hypothalamic or extrahypothalamic areas. Indeed, a uniform finding in all the PRLR localization studies is the remarkable abundance of PRLR mRNA or protein in the ventricular choroid plexus, which may both indicate an access route of PRL to the brain and emphasize that the feedback route may, at least in part, be indirect. Until very recently, morphologic *in situ* localization studies omitted neuronal phenotype identification. In 1997, Arbogast and Voogt (115) reported colocalization of PRLR and TH in fetal rat hypothalamic neurons *in vitro*. In August, 1998, Lerant and Freeman (116) published a thorough report of PRLR ICC in subdivisions of TH-immunoreactive hypothalamic neurons, as influenced by the ovarian steroid *milieu*. Expression of the PRLR in hypophysiotropic DA neurons appeared to correlate with both estrogen and PRL levels in the circulation, at least in certain regions. Frequency of PRLR localization was quantified within counts of 50 TH-positive cells in each region; percentage co-localization was rather low (<20%) in ovariectomized rats, but rose to 80% in dorsal TIDA neurons after estrogen. It was not addressed whether a significant population of nondopaminergic PRLR neurons exists. Studies of PRLR localization in mouse have not been reported.

**Table II.** Timing and Specificity of PRL Replacement Effect in Ames Dwarf Mice

| Treatment | TIDA neurons |
|---|---|
| oPRL beg. 12d | ↑ perikaryal DA/TH, ↑ cell number (to normal), ↑ DA/TH in ME[a] |
| oPRL beg. 21d | No effect on cell number[b] |
| oPRL beg. 30d | No effect on cell number[b] |
| oPRL beg. 60d | ↑ perikaryal DA/TH, no effect on cell number, ↑ DA/TH in ME[c] |
| mPRL beg. 60d (pituitary graft) | ↑ perikaryal DA/TH, no effect on cell number, ↑ DA/TH in ME[c] |

[a] Romero and Phelps, 1993 (92); [b]Romero and Phelps, unpublished; [c]Romero and Phelps, 1995 (93).

Thus, it appears that the substrates exist for direct influence of PRL on TIDA, and GH on SRIH, but not GHRH, neurons, suggesting that signaling among these hypophysiotropic populations, as well as from other, indirect, pathways and molecules, may be important in the feedback effects of GH and PRL.

**Mediators of GH and PRL Feedback.** Related to studies of GHR localization were concurrent reports of c-*fos* expression in hypothalamic neurons after acute GH treatment; activation occurred in both PeN and ARC (104, 117). Kamegai *et al.* (118) reported that c-*fos* expression induced by exogenous GH in PeN was in SRIH neurons, but that NPY-producing cells in ARC were activated by GH. Subsequently, Chan *et al.* (119) showed that hypothalamic NPY expression reduced by hypophysectomy was restored by treatment with GH. Total ARC levels of NPY mRNA are reduced significantly in the *dw* dwarf rat, and are elevated after peripheral treatment with bGH (120). Suzuki *et al.* (121) reported that intracerebroventricular administration of agonists specific for NPY receptors Y1 and Y2 were as effective as NPY in suppressing pulsatile GH secretion in conscious male rats. In the hypothalamus, both receptor subtypes are expressed in ARC, and the Y2 receptor is also found in PeN (122, 123), where synaptic connections of NPY axons on SRIH neurons have been demonstrated (124). In the study by Suzuki *et al.* (121), knifecut anterolateral deafferentation of ARC abolished the inhibitory effect of NPY on GH release, indicating that the effect of NPY on GH is mediated by SRIH, as had been postulated before (125).

NPY may be involved in PRL feedback mediation as well. In most studies of GH stimulation of c-*fos* expression

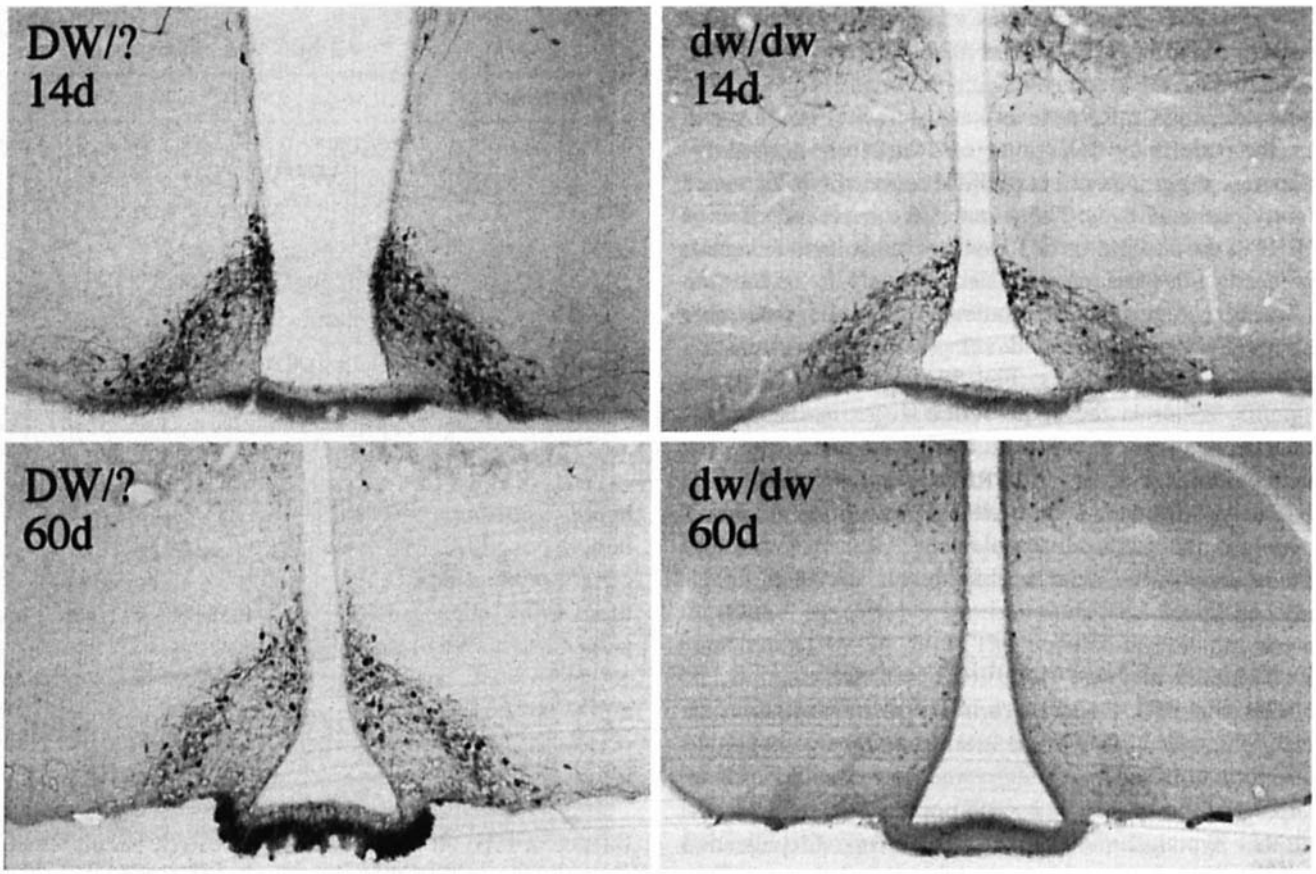


**Figure 8.** Photomicrographs of TH immunostaining in Snell dwarf (*dw/dw*) and normal (*DW/?*) hypothalamus, at 14 days postnatally (top panels) and as adults (bottom panels). The sections were coronal, 30 μm, and photographed at 10× original objective magnification.

in hypothalamus (104, 106, 117, 119), recombinant human GH was used. Because hGH is lactogenic in rodents, including binding to PRLR (126) and trophic effects on TIDA neurons (19, 25), NPY neuron activation may result from PRL stimulation. Increased hypothalamic NPY expression has been measured during lactation (127–130), and acutely after suckling (131). Circulating PRL levels are elevated after administration of antiserum to NPY (132), suggesting a stimulation of DA by NPY. Numbers of NPY-immunoreactive neurons are reduced in the hypothalamus of Ames and Snell dwarf mice (Phelps, unpublished data).

Galanin-producing hypothalamic neurons may also be involved in GH feedback to GHRH neurons; hypothalamic galanin expression is reduced in Ames dwarf mice (133) and *dw* rats (134), and galanin expression reduced by hypophysectomy, like NPY, can be restored by peripheral GH treatment (134). Also similar to NPY, galanin receptor is expressed on PeN SRIH, but not in GHRH, neurons (134). There is evidence that galanin is involved directly in regulating PRL secretion because mice engineered to lack functional galanin have reduced PRL production, including response to estrogen, and fail to lactate (135).

Other endogenous GH secretagogues (GHS) may be involved in GH effects in hypothalamus. The recent characterization (136, 137) of a specific receptor (GHSR) for synthetic GH-releasing peptides such as GHRP-6 (138), and demonstration that GHS acts in the hypothalamus (139) fostered study of GHSR expression in the CNS as influenced by GH (120). GHSR is expressed in arcuate and ventromedial nuclei, but not PeN; GHSR mRNA levels are increased markedly in *dw/dw* rats and reduced after bGH treatment to normal or lower levels (120).

It would be logical to suggest that feedback effects of GH in the hypothalamus are mediated by peripheral or local production of IGF-I stimulated by GH, because IGF-I has been shown to mediate the biological effects of GH *via* both endocrine (production of IGF-I in liver) and paracrine (local IGF-I production) routes. IGF-I receptor has been localized to the hypothalamus in several species using radiolabeled ligand-binding techniques (140–142), and in rat CNS using nucleotide probes and antisera (143). Circulating levels of IGF-I are extremely low in dwarf mice, but rise in response to GH treatment (144). Although one report exists of IGF-I ICC in mouse brain, including reduction in Snell dwarf (145), this laboratory has been unable to detect IGF-I in brains of adult normal or dwarf mice, using several polyclonal and monoclonal antisera (unpublished results). It might be valuable to assess IGF-I receptor expression in brains of GH (and PRL)-deficient models. However, Minami *et al.* (146) found that injection of rat GH, but not human IGF-I, into PeN or ARC inhibited duration and amplitude of GH secretory pulses.

In all these studies, there is evidence that the feedback effects of GH are upon SRIH directly, including SRIH to

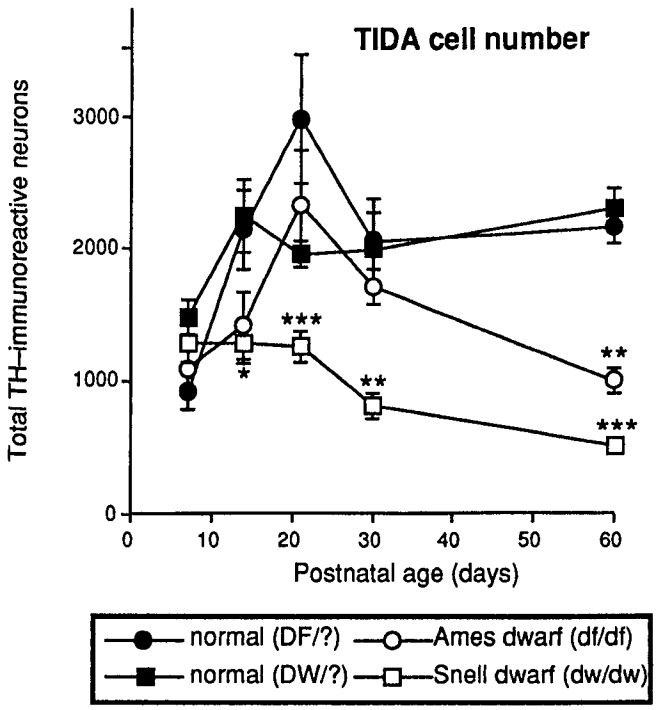


**Figure 9.** The pattern of TIDA neuron development and loss, compared for Snell and Ames dwarf mice and with normal counterparts.

GHRH, even when NPY or galanin is involved. There is abundant anatomical evidence for SRIH terminals on GHRH neurons (147–150), and the pattern of GH release after intracerebroventricular NPY, compared to that after ARC deafferentation, suggests effects on GHRH that are mediated by anterior neuronal elements (121). Although the effect could result from a number of possible transmitters, specific inhibition of SRIH has been shown to result in increased GHRH expression (151). Thus, while GH feedback is effected by many peptide transmitters, SRIH may be the final common mediator of this feedback (119, 152) for GH secretory patterns (153, 154) and GH trophic effects.

An established CNS neurotrophin that might mediate the effects of PRL on TIDA neurons is glial cell line–derived neurotrophic factor, GDNF. Originally shown to promote survival and differentiation of cultured midbrain DA neurons (155), GDNF has been shown to protect and stimulate the nigrostriatal (155) and other motor pathways (156) in several experimental paradigms. Intranigral injection of GDNF in adult rats has been shown to protect against neuron loss after axotomy (157), 6-hydroxydopamine (158, 159), or methamphetamine (160), and to restore functional deficits and DA levels after MPTP in mice (161) and monkeys (162). GDNF promotes fiber outgrowth of fetal midbrain DA grafts *in oculo* (163) and *in situ* (164). Mice engineered to bear a null mutation in the GDNF locus (165) showed no deficits in brainstem catecholamine neuron groups, but GDNF-null mice die within 12–24 hr of birth, so examination of the late-developing A12 DA neurons was not possible. Choi-Lundberg and Bohn (166) found low GDNF levels in diencephalon at P0 and P10, but the potential role of GDNF in development of hypothalamic DA neurons is yet to be explored.

## Current and Indicated Directions

**Connectivity.** It had been noted by this and other laboratories (5, 8, 9, 13, 91, 92) that the levels or appearance of GH- and PRL-regulating factors in the dwarf mouse hypothalamic ME were uniformly low, even for GHRH that is overexpressed (3, 4). This observation prompted a hypothesis that hypophysiotropic neurons in the dwarfs may not terminate appropriately near the hypophysial portal vasculature. Such a hypothesis may be tested using retrograde transport of peripherally injected fluorogold (FG) combined with immunocytochemical identification of neuron phenotypes (27, 28). FG is taken up by axon terminals not protected by the blood-brain barrier in areas such as the ME, and is subsequently sequestered in neuronal perikarya, where it is visible using either ultraviolet illumination or immunostaining (167).

As shown in Table III, the reduction of SRIH in the dwarf ME appears to be proportional to the reduction in detectable PeN perikarya, because 83% of the PeN SRIH neurons in dwarfs were labeled with FG, compared with 87% of the larger population in normal mice. Also, 73% of the large (>2× normal) dwarf GHRH-producing population showed FG labeling, compared with 76% of the lower normal population. The low GHRH in the dwarf ME suggests increased GHRH release. It must be cautioned that SRIH or GHRH perikarya that sequestered peripherally administered FG may terminate in internal layers of ME or in ventral ARC because these areas also lack blood-brain barrier (168). Among TIDA neurons, the percentage of cells showing FG label appears to be lower in dwarf (~50%) than in normal mice (70%–90%), such that a smaller proportion of neurons, in a population that is already reduced in size, terminate appropriately in the ME.

**Table III.** ME-Projecting Neurons (Percentage of Total Populations in Ames Dwarf Mice)

| Neuron type | *DF/df* %FG+ | *df/df* %FG+ | *df/df* % of *Df/df* ME-projecting cells | Total *df/df* neuronal population (as % of *DF/df*) | Total % *df/df* ME-projecting cells |
|---|---|---|---|---|---|
| GHRH[a] | 76 ± 3 | 73 ± 4 | 96 | 225 | 203 |
| SRIH[a] | 87 ± 2 | 83 ± 2 | 95 | 46 | 44 |
| TIDA[b] | 86 ± 4 | 52 ± 2 | 62 | 51 | 32 |

[a] Ref. 169
[b] Romero and Phelps, unpublished

The mechanisms and cues involved in hypophysiotropic innervation of the ME during normal development have not been elucidated, but the phenomenon has been demonstrated in transplantation studies. Following grafts of normal fetal hypothalamus into the third ventricle of gonadotropin-releasing hormone (GnRH)–deficient hypogonadal mice (169, 170) or of rats deficient in GHRH because of neonatal monosodium glutamate treatment (171), the adult host ME exhibited respective GnRH- or GHRH-immunoreactive axon terminals. Whether pituitary hormones acted as attractive cues or induced the expression of cues in the ME is not known. When adenohypophysis was co-grafted with fetal preoptic area to the third ventricle of hypogonadal mice, GnRH-positive axons invaded host pituitary as well as ME (172). *In vitro,* GnRH-positive axons have been shown to be attracted to anterior pituitary, in culture of separate explants (173) and in slices of early embryonic brain, in which growth cones were oriented consistently toward hypothalamus and pituitary, although ME was not yet developed (174).

Tanycytic morphology has been shown to affect hypophysiotropic axon contacts with ME and change with hormonal *milieu* (e.g., GnRH terminals in ME are surrounded by tanycytic end feet, and do not reach the pericapillary basal lamina, except during the preovulatory LH surge) (175, 176). Lerant and Freeman (116) found that tanycytes show immunoreactivity for the ligand-binding portion of the PRL receptor, and suggested that PRL may act on tanycytes to regulate DA release from ME terminals.

**Signal Transduction by GH and PRL in Other Systems.** Intracellular signaling by GH and PRL has been studied in detail in the liver and lymphocytes (reviewed in Refs. 100–102). GH and PRL each bind to specific receptor proteins that are members of the cytokine receptor superfamily (177). Ligand binding leads to receptor dimerization and resulting alteration of the intracellular portions of the receptor molecule, enabling signaling from the receptors *via* receptor-associated protein kinases (178). The Janus Associated Kinase (JAK)-1 and -2 proteins have been shown to be receptor-associated enzymes that become activated by GH or PRL binding to receptors (102, 179, 180). The JAK kinases, while bound to the activated receptor, act as protein tyrosine kinases on cytosolic regions of the receptor and the *src* homology (SH)-2 domains of selected Signal Transducers and Activators of Transcription (STAT) protein subtypes. Phosphorylation of the SH-2 domains of the STAT proteins promotes interactions forming homo- or heterodimers of the STATs, which then translocate to the nucleus, bind to cognate DNA response elements, and regulate transcription (178). The specificity of signaling by this mechanism is inherently subject to multiple levels of control, including selectivity of cell types expressing receptor, receptor-JAK complex preference for certain STAT members as preferred substrates, the possibility of homo- or heterodimeric STAT combinations, and sequence-selective DNA binding for a subset of gene targets (178). In the liver, the STAT subtypes that are activated in response to GH are 3 and 5b, and analysis of the DNA elements bound by these specific STATs is being used to identify potential genomic targets that are activated (179–182).

It is likely that JAK/STAT signaling pathways also are activated by GH and PRL in hypothalamic neurons. Signaling through another cytokine receptor ObR in hypothalamus has been shown by STAT3 activation after leptin treatment in mice and rats (183, 184). Activation was restricted to STAT3 as shown by DNA binding assays (183) and by immunodetection of phosphorylated STAT3 (184). Darnell (178) mentioned an unpublished finding that GH treatment resulted instead in hypothalamic STAT5 activation, emphasizing the selectivity and specificity of cytokine signaling pathways.

**New Transgenic Models.** Although animal models will always be required for studying certain aspects of neuronal differentiation such as cytoarchitecture and connectivity, transgenic technology is providing the means to manipulate the neuroendocrine *milieu in vivo* tantamount to that *in vitro.* Like spontaneous mutations in specific transcription factors or structural genes, factors that are transgenically overexpressed or subjected to disruption (knockout) have lifelong, thus developmental, effects. Several new models pertinent to the neurotrophic feedback of GH or PRL have been reported recently, in terms of general phenotypic effects.

*GH receptor knock-out.* Unlike the availability of rodent models for panhypopituitary dwarfism such as Snell and Ames, and for isolated GH deficiency as in the little mouse or dwarf rats, human Laron dwarfism, the result of GHR dysfunction, knows no spontaneous animal counterpart. A mouse model for the syndrome has been produced by targeted disruption of the GHR binding protein gene (185). The mice exhibit severe growth retardation with proportional dwarfism, very low circulating IGF-I levels, and elevated GH. These mice are hyperprolactinemic, show diminished responses to GnRH and to LH, and reduced fertility in males (186). Whether these additional deficits are the result of low IGF-I is not known.

*Disruption of PRL and PRLR.* Mice with transgenic null mutations in PRLR (187) and ligand (188) do not show identical phenotypes. Female PRLR knockout mice are sterile, showing multiple reproductive abnormalities such as irregular cyclicity, lack of pseudopregnancy, and defective implantation. Lactation and maternal behavior were compromised in females heterozygous for the mutation, and half of the PRLR-null male mice showed reduced or absent fertility (187). In PRL-null mice, reproductive deficits were less severe; whereas females were infertile, with absent post-pubertal mammary development, they displayed maternal behavior, and males were fertile (188). Further study of neuroendocrine function in the PRL knock-out mice showed that reduction in gonadotropin secretion did not compromise male fertility, and that testosterone secretion was unaffected (189). Preliminary morphological studies in

this laboratory (190) indicate that TIDA cell numbers are unaffected in PRL-null mice of either sex. These findings are surprising, considering that PRL can repair infertility (191, 192) and maintain TIDA neurons (91) in spontaneous dwarf mice. The animals may provide a model for redundancy in feedback, and further studies are warranted.

*Other models.* Transgenic mice that overexpress rat PRL have been reported and show markedly enlarged prostate (193). Interestingly, circulating IGF-I levels in those animals were elevated to the same extent as in bGH-transgenic mice. Disruption of the NPY gene in leptin-deficient mice has been shown to attenuate obesity (194), whereas NPY receptor Y5- or Y1-null mice become obese, suggesting that these receptors are not essential to NPY effects (195, 196).

In summary, recent studies of additional spontaneous and transgenic models of lifelong altered production of GH or PRL have shown that the differentiation of pertinent hypophysiotropic neurons in the hypothalamus is affected by the alterations, but not as consistently as would be predicted. The solutions to certain questions, such as the nature of the *df* mutation, were juxtaposed with new perplexities, such as GH expression in the *df* pituitary. Interesting contradictions surfaced, such as increased TIDA cells in the dwarf rat, opposed to profound decrease in dwarf mice; the endocrine *milieu* in the two dwarfs is not the same, and the feedback/neurotrophic results refuse neat categorization. Likewise, GH and PRL effects are not distinct, especially between species and when expression is altered experimentally. There seem to be limits in magnitude for the developmental neuronal response to altered GH and PRL. The findings have stimulated alternative interpretation and further study, the intricacy and redundancy in physiological signaling continuously revealing more complexity than predicted. New data and model systems in intracellular signaling and genetic engineering portend exciting novel approaches for addressing general feedback and specific trophic mechanisms in hypothalamic-pituitary interactions.

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