MINIREVIEW

Pituitary Hormones as Neurotrophic Signals: Anomalous Hypophysiotrophic Neuron Differentiation in Hypopituitary Dwarf Mice (43719)

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Abstract. **Anterior pituitary hormones are known to exert dynamic negative feedback effects on their respective regulatory ("hypophysiotropic") neurons in the hypothalamus. The purpose of this review is to present the evidence for a theory that the effect of pituitary hormones on these hypophysiotropic neurons is neurotrophic, extending beyond dynamic feedback to influence upon cell survival, phenotypic differentiation, and axonal connectivity. To that end, the adult condition and the development of hypophysiotropic neurons in mutant mice which lack pituitary growth hormone (GH) and prolactin (PRL) are presented as models of the effect of absent specific neurotrophic signals. The expression of the neurohormones which inhibit PRL and GH secretion, dopamine (DA) and somatostatin, respectively, is markedly reduced in the hypothalamus of the hypopituitary dwarf mouse, and this adult condition is the result of postnatal failure to develop or actual regression, which may include neuronal cell death. The deficit in DA may be reversed by PRL replacement, but only if initiated at an identified critical postnatal period. Conversely, expression of the stimulatory GH-releasing hormone (GHRH) is markedly increased in the dwarf mouse hypothalamus. The loss of DA and the increase in GHRH occur in the same hypothalamic area, suggesting neuronal phenotypic plasticity in response to absence of pituitary feedback signals. The axonal terminations of extant GH- and PRL-regulating neurons in external median eminence appear to be reduced, suggesting that pituitary signals are required for appropriate axonal guidance during development, even though an endocrine vascular route intervenes between these regulatory neurons and their target secretory cells. The collective observations indicate that GH and PRL may be regarded as neurotrophic factors for their respective regulatory neurons in the hypothalamus.** [P.S.E.B.M. 1994, Vol 206]

The phenomena of cell selectivity during development, and the requirement for target signals for appropriate connectivity and function, have been studied most thoroughly for neurons (for review see [1]). The classical example is that of developing sympathetic ganglia, which are composed initially of excess numbers of neurons. Subsequently, a subpopulation is selected for survival, based on the neurotrophic signal of nerve growth factor (NGF) from target cells with which the neurons connect by neuromuscular junctions (2, 3). In the central nervous system (CNS) as well, proper neuronal connection and survival appears to depend on target signals (4, 5). Neurotrophic factors such as NGF (6, 7), fibroblast growth

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factor (8) and gangliosides (9, 10) have been shown to promote axonal regeneration or prevent degeneration in several experimental paradigms in adult rodents. In the neuroendocrine hypothalamus, a pertinent observation was reported for the noradrenergic innervation of vasopressin (VP)–secreting neurons. In postnatal development of the genetically VP-deficient Brattleboro rat, the initial robust innervation gradually regresses (11). Whether the signal which normally maintains the innervation is the VP molecule itself (the structure of which is incorrect in the Brattleboro [12]), or is another neurotrophic factor produced by target VP cells, is not known. Importantly, CNS neuronal cell loss and morphological remodeling are not limited to embryonic development, but extend to postnatal events such as sexual maturation, trauma, and pathology in the adult, and neuronal cell loss in aging. Thus, neuronal plasticity in response to target signals may occur after phenotypic differentiation, including specific neurotransmitter production and formation of synaptic contacts.

The principle of target signals promoting cell survival and proper connectivity is not unlike the concept of dynamic endocrine negative feedback, which functions not only between epithelial endocrine cells, such as peripheral target glands and anterior pituitary, but between the target pituitary and CNS hypophysiotropic (pituitary-regulating) neurons. By this feedback mechanism, production and release into hypophysial portal vasculature of hypothalamic stimulatory or inhibitory factors are adjusted according to circulating levels of pituitary hormones, in order to maintain homeostasis, signal reproductive cycling, and respond to environmental cues. It is the purpose of this review to examine a theory that the effect of pituitary hormones on hypophysiotropic neurons extends beyond dynamic feedback, influencing developmental differentiation, survival, and connectivity of these cells.

Evidence for this theory is based on studies of hypophysiotropic neurons in animals with genetic, thus lifelong, absence of specific pituitary hormones. Animals with such deficiencies, whether occurring as spontaneous mutations or transgenically engineered, provide physiological models of the absence of specific target signals which normally "feed back" upon hypophysiotropic neurons and affect their function. Several spontaneous hypopituitary mutants exist in mice; two such models have been described in rats. Each spontaneous mutation has been shown to be inherited as an autosomal recessive allele; pituitary deficiencies are manifested in animals homozygous for the trait (e.g., dw/dw for the Snell dwarf). Siblings that are either heterozygous or homozygous dominant (e.g., DW/dw or DW/DW, for the Snell dwarf allele) are phenotypically normal, and typically are designated +/? (e.g., DW/?). Transgenic hypopituitarism has been produced only in mice. Common to these models are growth hormones (GH) deficiency and dwarfism, as illustrated in the photograph in Figure 1 of an adult Snell dwarf with a DW/? sibling. In addition to reduced size (1/4 to 1/3 of normal, as described by Snell [13]), infantile proportions, especially in the facial cranium (14), are obvious. Like Sir James Barrie's Peter Pan, the dwarfs "don't grow up." They are sexually, as well as somatically, immature, resulting from the typical deficiency of the closely-related prolactin (PRL) molecule also, as described in Table I.

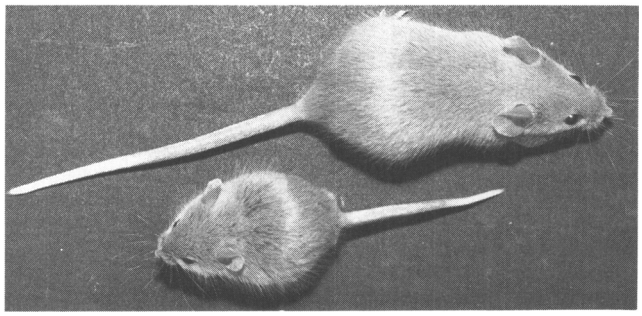

Figure 1. Photograph of a Snell dwarf (dw^j/dw^j) mouse and a phenotypically normal (DW/?) littermate; in mice are approximately 4 months of age.

The information and references in the table are expanded compared with the version given by Phillips and colleagues (15), but remain simplified. The designation "hormone status," for example, combines the results of many different types of measurement of hormones in the pituitary, such as dye histochemical evaluation (24), immunoprecipitation after electrophoresis (16), radioimmunoassay (17, 18, 21), electron microscopy (17), and immunocytochemistry (18, 20, 31, 34); GH and PRL mRNA have been assayed by both Northern blot (17) and *in situ* hybridization (28), and RIA of serum hormone levels (17–19, 21, 34) is an important corollary. Emphasis is placed on reports of direct measurement, rather than inference gathered from studies of hormonal replacement, which have been numerous. In some cases, examination of original reports revealed that assumptions have been made, or that contradictions have not been reconciled. For example, in the case of PRL in *little* mice, disc electrophoresis of pituitary extracts (21) revealed no PRL bands, but PRL ICC (28) showed normal density of PRL cells, albeit in a pituitary of reduced size. PRL ICC on lit/lit pituitaries in this laboratory revealed considerable variation in the density of lactotrophs (unpublished data); quantification of PRL in pituitary or serum of lit/lit mice has not been published. Likewise, quantified levels of TSH, LH, FSH, and ACTH are lacking for most mutants; histochemical or ICC data account for most notations in the table. It should be

Table I. Dwarf Mouse Models of GH/PRL Deficiency (Modified from Phillips *et al.*, 1982 [15])

Mutation	Pituitary hormone status					Chromosome	Defect	Reference
	GH	PRL	TSH	LH/FSH	ACTH			
Snell dwarf mouse[13] (dw)	A[16,17]	A[16–18]	A[19,20]	*N*[20]	*N*[20]	mouse 16[21]	Pit-1[22]; Trp261 → Cys	[13]Snell, 1929 [16]Slabaugh *et al.*, 1981 [17]Cheng *et al.*, 1983 [18]Barkley *et al.*, 1982 [19]Roti *et al.*, 1978 [20]Roux *et al.*, 1982 [22]Li *et al.*, 1990
Snell dwarf mouse[21] -Jackson (dw^j)	A[21]	A*	A*	*N**	*N**	mouse 16[21]	Pit-1 rearrangement[22]	[21]Eicher & Beamer, 1980
Ames dwarf mouse[23] (df)	A[16,17]	A[16–18]	R[24]	*N*[24]	unk	mouse 11[25,26]	unk	[23]Schaible & Gowen, 1961 [24]Bartke, 1964 [25]Bartke, 1965; [26]Camper *et al.*, 1990
little mouse[27] (lit)	R[17,27]	A[27]; *N*[28]	*N***	*N***	unk	mouse 6[27]	GHRH-R[28,29]; Asp60 → Gly	[27]Eicher & Beamer, 1976 [28]Lin *et al.*, 1993 [29]Godfrey *et al.*, 1993
dwarf rat (SD)[30] (dr)	A[31]	*N*[31]	*N*[31]	*N*[31]	*N*[31]	[rat GH]	GH single base pair deletion[32]	[30]Ookuma and Kawashima, 1980 [31]Nogami *et al.*, 1989 [32]Takeuchi *et al.*, 1990
dwarf rat (Lewis)[33]	R[33]	*N*[33]	*N*[33]	*N*[33]	*N*[33]	unk	unk	[33]Charlton *et al.*, 1988
transgenic dwarf[34] (tg Bri 78)	A[34]	R[34]	unk	unk	unk	NA	GH cell ablation[34]	[34]Behringer *et al.*, 1988

N = normal; R = reduced (4%–10% of normal); A = absent (<1% of normal); unk = unknown; NA = not applicable.
TSH = thyroid stimulating hormone; LH = luteinizing hormone; FSH = follicle stimulating hormone; ACTH = adrenocorticotropic hormone; GHRH-R = GH releasing hormone receptor; SD = Sprague Dawley strain; Pit-1 = pituitary specific transcription factor 1 = GHF1.
* presumed to be the same as in dw/dw (Eicher and Beamer, 1980 [21]; Li *et al.*, 1990 [22]); **W. Beamer, personal communication in Phillips *et al.*, 1982 (15).

noted that additional types of transgenic dwarf mice have been produced, such as those with inducible somatotroph ablation (35), and with mutant pituitary cyclic AMP response element binding protein expression (36). However, hypothalamic hormones and neurons have been studied only in the model produced by Behringer and coworkers (34) in which somatroph ablation was achieved by linkage of the diphtheria toxin structural gene to GH promoter in the transgenic construction.

These hypopituitary mutants have been used to advantage in determining the roles of GH and PRL in target tissue physiology, such as that of GH in long bone growth (37) and of PRL in pregnancy (38) and in testicular function (39). More recently, dwarf mice have been viewed as models for examining mechanisms underlying the developmental restriction of gene expression that results in pituitary differentiation. This differentiation leads to cell types which produce only a single pituitary hormone, or hormones with structural and evolutionary similarities such as GH and PRL (40, 41) which, with TSH, are absent in Snell and Ames dwarf pituitaries. The structural genes for GH and PRL, located on separate chromosomes, are intact in these mutants (15, 42), except in the Sprague-Dawley dwarf rat (32) where a GH gene mutation is without effect on PRL. A primary mutation that would explain the deficit in several related hormone and cell types was indicated for the dwarf mouse phenotypes. The expression of GH, PRL and TSH has been related to a single transcription factor, Pit-1 or GHF-1, that is common to all three cell types but is not expressed elsewhere in the organism (43). Li and associates reported (22) that both forms of Snell dwarfism stem

from mutations in the Pit-1 gene. The marked hypoplasia of the Snell anterior pituitary and known absence of these specific cell types (24) implicates Pit-1 in proliferation as well as differentiation (44, 45). The primary mutation of lit/lit mice has been described recently (28, 29) as a point mutation in the receptor for GH-releasing hormone (GHRH), a possibility which had been suggested by nonresponsiveness of the *little* pituitary to GHRH (46, 47). Morphological studies suggest that somatotroph proliferation, as well as GH expression, is adversely affected by absence of GHRH receptor in the *little* mouse pituitary (28). Further elucidation of mechanisms of pituitary differentiation using these mutants is surely imminent.

This review is limited to the effect of reduced or absent GH or PRL on the hypothalamic neurons which normally regulate GH or PRL secretion. Although TSH production is also deficient in several GH and PRL-deficient models, study of the CNS TSH-releasing hormone (TRH) has been limited to a single report of assay in Snell dwarf brain (19); TRH also releases PRL, and that study is discussed in a section on PRL-stimulatory neurons. General histological characteristics of brain development in dwarf mice have been reviewed by Noguchi (48).

Prolactin-Regulating Neurons

Prolactin is not produced at any time in development by Snell (dw/dw or dw^j/dw^j) or Ames dwarf mice (df/df) (16, 49); pituitary PRL is reduced severely in transgenic dwarfs (34, 50). The predominant regulation of PRL secretion is inhibitory, by dopamine (DA; for review see Ben Jonathan [51]) produced by neurons in the medial basal hypothalamus (MBH) and delivered to the pituitary via axons terminating in the external median eminence (ME) on hypophysial portal capillaries (52). Although DA is used as a transmitter in extrahypothalamic regions, such as the nigro-striatal pathway, neurons that regulate pituitary function are limited to the tuberoinfundibular arcuate nucleus (thus "TIDA" neurons) and, possibly, to anterior periventricular DA neurons (53); these areas are designated A12 and A14, respectively, according to the catecholaminergic (CA) nomenclature of Dahlström and Fuxe (54). Thus, DA produced in these neurons is the PRL-inhibiting hormone. The existence of an unequivocal PRL-releasing hormone has not been established. Several CNS peptides have been shown to stimulate PRL release, including oxytocin, TRH, and vasoactive intestinal peptide (VIP); DA itself may stimulate PRL release at very low concentrations (55).

Dopaminergic Neuron Deficits in Dwarf Mice. Morgan and colleagues (56) reported the first investigation of hypophysiotropic DA in PRL-deficient dwarf mice, in which DA levels were measured separately in ME (A12 terminals) and hypothalamus (A12 perikarya) in both Snell and Ames dwarfs; ME DA was severely reduced in both dwarf types, but hypothalamic DA was not affected. That study isolated the hypothalamic deficit to DA, because ME norepinephrine (NE) levels were comparable to those in normal mice of each strain. A morphologic assessment of the DA deficit (57) showed that CA histofluorescence in Snell and Ames dwarfs was decreased in cells and terminals of A12 only, while DA in cell bodies in *substantia nigra*, as well as NE terminals in paraventricular nucleus (PVN) and supraoptic nucleus (SON) were comparable to those of normal mice. Morgan and King reported subsequently (58) that DA synthetic rate was decreased markedly in the ME of Ames dwarfs.

Those studies prompted the question of whether the number of A12 DA neurons is reduced in the dwarf hypothalamus. In order to quantify neuronal cell number, A12 perikarya may be marked by immunoreactivity of tyrosine hydroxylase (TH). This enzyme is rate-limiting in DOPA synthesis; i.e., for both DA and NE, but previous studies of DA versus NE systems by microspectrophotometry combined with CNS lesions (53) allow differentiation of DA and NE neuronal groups by location. Figure 2 illustrates TH immunoreactivity in three dopaminergic areas in the hypothalamus of normal (DF/?, A; DW/?, C) and dwarf (Ames, B; Snell, D) mice: A13, in medial *zona incerta*, is most dorsal; intermediate A14 includes periventricular neurons from the anterior commissure rostrally to the level of anterior ME caudally; A12 is the ventral ME-afferent and PRL-inhibitory group. A distinct qualitative decrease in TH immunostaining intensity is shown for both dwarf types, in both ME terminals and in cell bodies of A12, but not of A13 or A14. Quantification of the reduction in A12 cell numbers has been accomplished for Snell, Ames, and transgenic dwarfs. In Snell (dw^j) dwarfs, the number of A12 TH-immunoreactive (TH+) neurons was only 2% of the number in normal siblings (59). In Ames dwarfs, Morgan and Besch (60) reported a decrease in arcuate nucleus TH+ median cell number per coronal section in dwarf females but not males. A morphometric analysis of the A12 deficit in Ames dwarfs (61) showed that total numbers of TH+ A12 neurons were reduced to 48% of normal, and that the deficit was distributed throughout rostral-to-caudal levels; i.e., numbers of TH+ neurons were reduced significantly at each 180 μm rostral-to-caudal interval in df/df mice compared with DF/? siblings. In transgenic dwarf mice (GH, DT-A; Mt, GHRH, [Bri 78]) with reduced but not absent PRL cells (34), the reduction in A12 neuron number was less severe—to 60% of that in normal mice

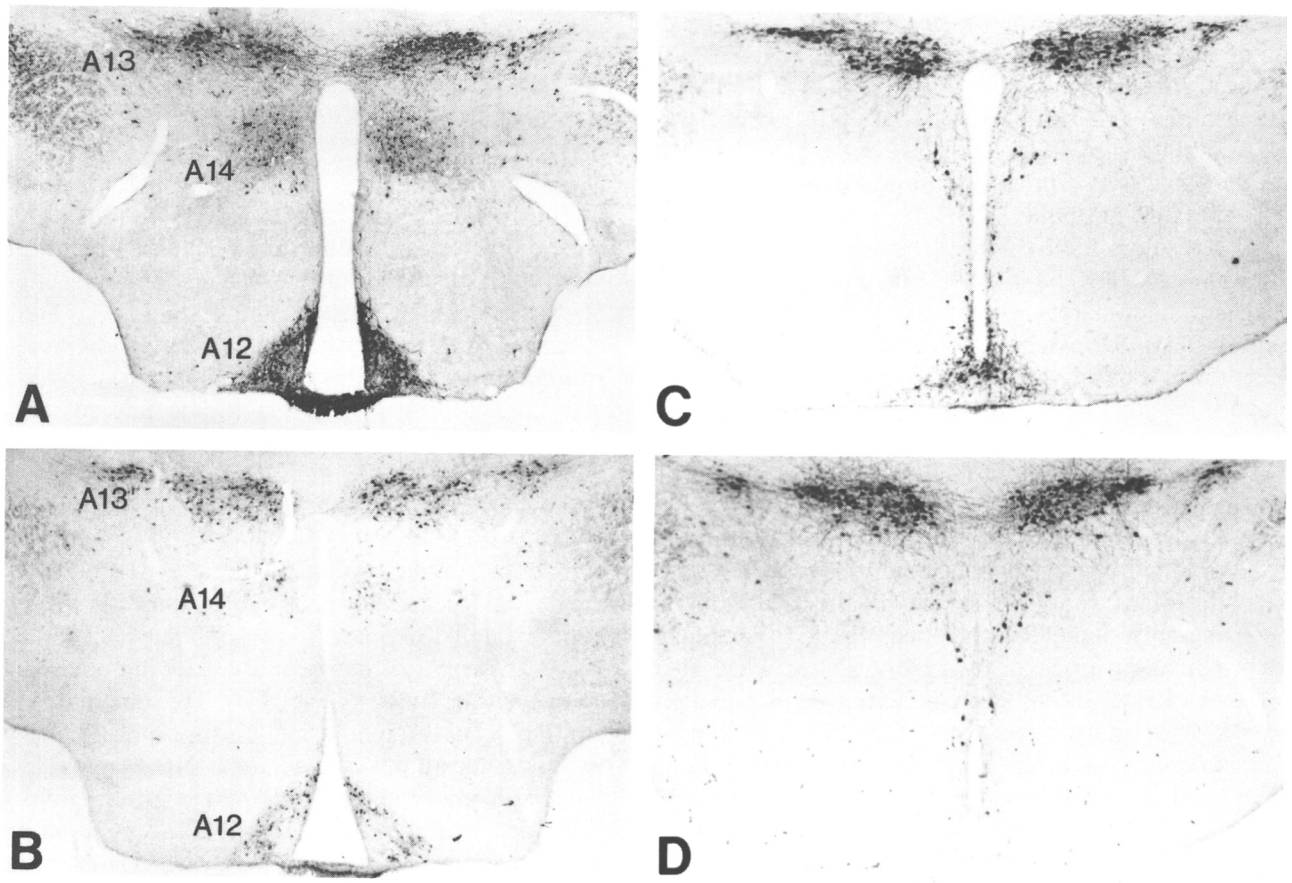

Figure 2. Photomicrographs of coronal sections through the hypothalamus, showing three dopaminergic neuronal groups: dorsally, A13 (medial *zona incerta*), intermediate A14 (anterior periventricular) and ventrally, A12 (arcuate nucleus, ME-projecting). Brains of phenotypically normal mice are shown in A (DF/?) and C (DW/?), for comparison with the same areas in Ames dwarf (df/df, B) and Snell dwarf (dw^j/dw^j, D) hypothalamus. The planes of section are such that a more rostral level of area A12 is shown in C and D, compared with the middle level in A and B. Original objective magnification = ×4 for all.

(C57B16 × SJL F_1), and was concentrated in anterior A12. Although A12 DA histofluorescence was virtually absent, TH mRNA in A12, quantified after *in situ* hybridization, was not significantly affected in transgenic dwarfs (50). A comparison of A12/TIDA and A13 neuron number is illustrated graphically in Figure 3, for transgenic (GH, DT-A), Ames, and Snell dwarfs, and for respective normal mice of the same strains. The significant reduction in A13 in Snell dwarfs remains without explanation, but may indicate overall reduction in neuron number in Snell dwarf brain (48), a possibility emphasized by the severity of the A12 reduction in this type.

Development of TIDA Neurons in Dwarf Mice. That the deficit in A12 DA levels and TH immunoreactivity might be due to a developmental failure was proposed by Morgan and colleagues (56, 58, 60). In order to address this hypothesis, studies on both DA (49) and TH immunoreactivity (62) during postnatal development were conducted in Ames dwarfs. Medial basal hypothalamic (MBH) and ventral tegmental midbrain (*substantia nigra*, SN) CA levels were evaluated morphologically, by histofluorescence, and biochemically, by HPLC assay of dissected areas, in df/df and DF/? mice at 7, 14, 21, 30, and 90 days of age (49). In this and other developmental studies, phenotype of mice younger than 21 days was verified by GH and PRL ICC of pituitaries, because dwarfs are not reliably distinguishable from normals at those ages. Concentrations of DA (pg/μg protein) for MBH and SN in DF/? and df/df mice are illustrated in Figure 4. By both morphologic and biochemical methods, A12 DA was comparable for df/df and DF/? through 14 days of age; after 14 days postnatally, DA levels in MBH in df/df mice failed to increase significantly, while levels in DF/? mice continued to increase through 30 days of age. Dwarf DA levels were significantly different from those in DF/? MBH at 30 ($P < 0.01$) and 90 ($P < 0.001$) days of age. In contrast, SN DA was comparable for DF/? and df/df mice at each age examined. Likewise, NE levels were comparable for DF/? and df/df at each age examined, increasing from birth to plateaus at 21 days in SN and 30 days in MBH (49). The results indicated that A12 DA neurons in dwarfs are comparable to those of normal mice neonatally, and that the adult deficit is a result of failure to develop further. Quan-

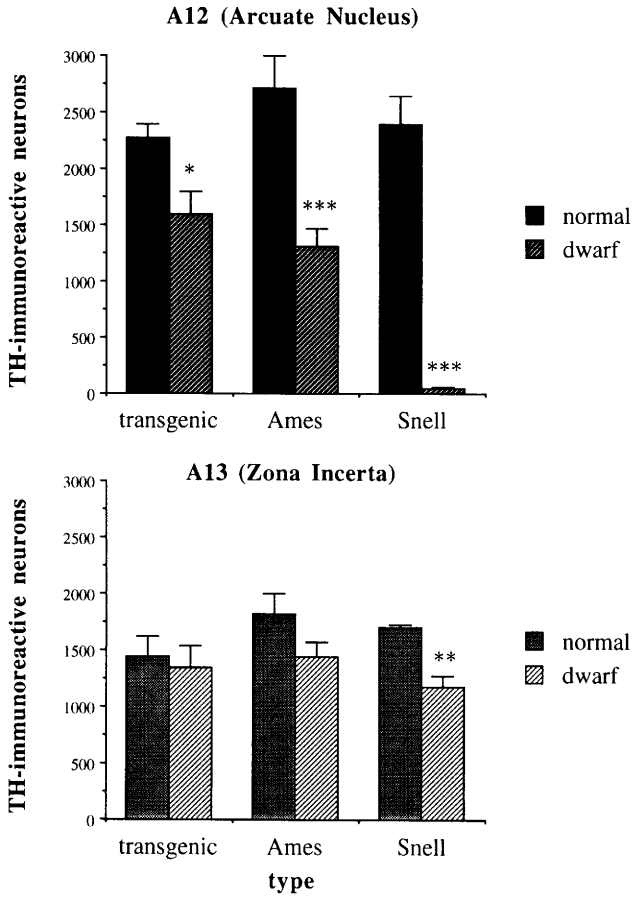

Figure 3. Histograms of TH-immunoreactive neuron number in A12 (upper) and A13 (lower) in three types of dwarf mice: transgenic (GH, DT-A; Mt, GHRF; Bri 78), Ames (df/df) and Snell (dw^j/dw^j), compared with respective normal phenotypes, transgenic control (C57B16 × SJL F_1), DF/? and DW/?. Significant differences between dwarf and control within each type are indicated: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (Student's *t* test). Columns represent means of 5–15 mice; vertical lines on each column are SEM.

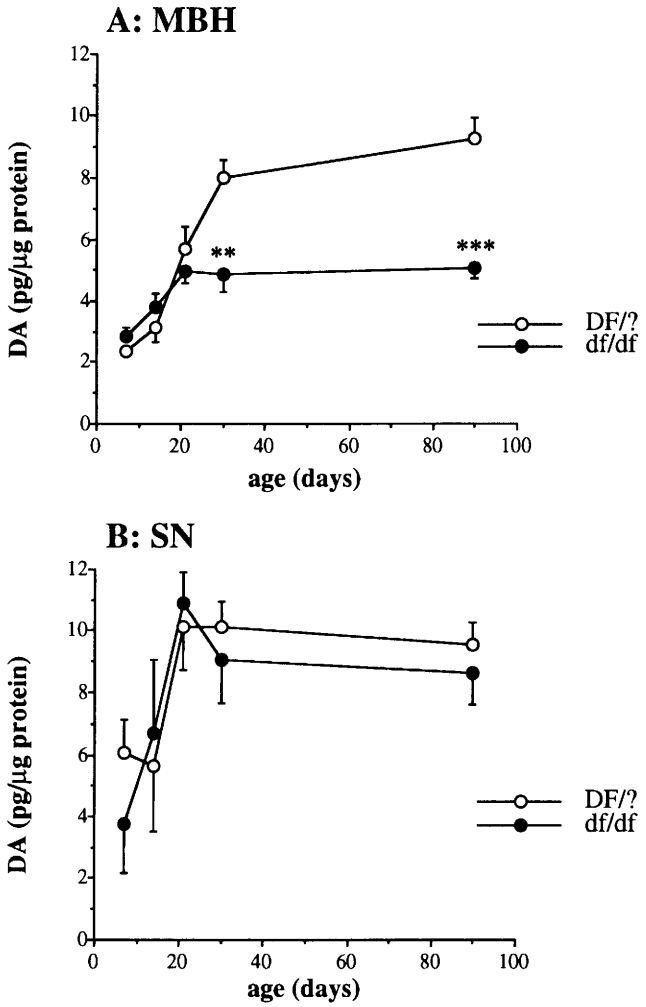

Figure 4. DA concentrations in DF/? and df/df medial basal hypothalamus (MBH, A) and ventral tegmentum/substantia nigra (SN, B) at 7, 14, 21, 30, and 90 days of age. Each point represents the mean of 8–10 animals; vertical lines represent SEM. Asterisks over df/df means show significant difference from DF/? at the same age; $^{**}P < 0.01$, $^{***}P < 0.001$. (From Phelps *et al.*, 1993a [49]; copyright The Endocrine Society.)

tification of TH+ neuron number in Ames dwarfs was accomplished by counting cells at 180 μm rostral-to-caudal intervals through areas A12, A13, and A14 of the brains of df/df and DF/? mice 7, 14, and 21 days and 2 months (42–75 days) of age (62). As shown in Figure 5, TH ICC intensity in dwarf TIDA A12 neurons decreased dramatically between postnatal days 21 and 42. Figure 6 shows graphically the total neuron number in A12 (Fig. 6A) and A13 (Fig. 6B) in the four age groups of DF/? and df/df mice. Cell number in A13 did not vary significantly with age or type. The size of the A12 TH+ cell population increased, and was comparable for df/df and DF/?, through 21 days of age. Thereafter, A12 TH+ neuron number decreased for both normal and dwarf mice; the decrease with age was significant only for df/df ($P < 0.01$ for 21 compared with 60 days; Student-Newman-Keuls test after ANOVA) to 46% of the number in DF/? A12 at 2 months ($P < 0.001$ for means of DF/? and df/df, Student's *t* test). Thus, in the case of TIDA TH+ neuron number, the adult condition in dwarfs represents a regression, the result of an exaggeration of TH+ cell loss that appears to occur postnatally in normal siblings as well. It is important to note that this reduction in cell number does not necessarily represent cell death, and may, instead, show a reduction in perikaryal TH levels below the threshold of ICC detectability.

Response of Dwarf TIDA Neurons to PRL Treatment. The foregoing developmental studies indicated that the onset of the A12 DA and TH deficit occurred after 14–21 days of age in dwarf mice. In order to test directly whether the absence of PRL was important in the hypophysiotropic DA decrease, PRL (oPRL, 50 μg/day for 30 days) or vehicle treatment of half-dwarf litters was initiated at 12 days of age (63). The dwarf phenotype was recognizable at later ages, even among PRL-treated df/df mice. Brains of df/df and DF/? sib-

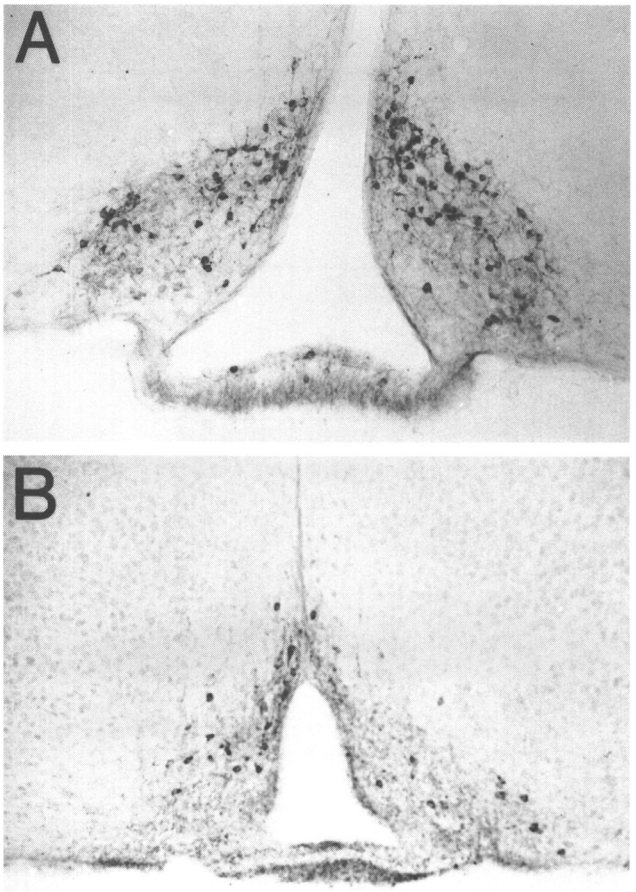

Figure 5. Photomicrographs of TH immunoreactivity in Ames dwarf (df/df) TIDA/A12 neurons at 21 (A) and 42 (B) days of age. Coronal 30 μm sections; original objective magnification = ×10.

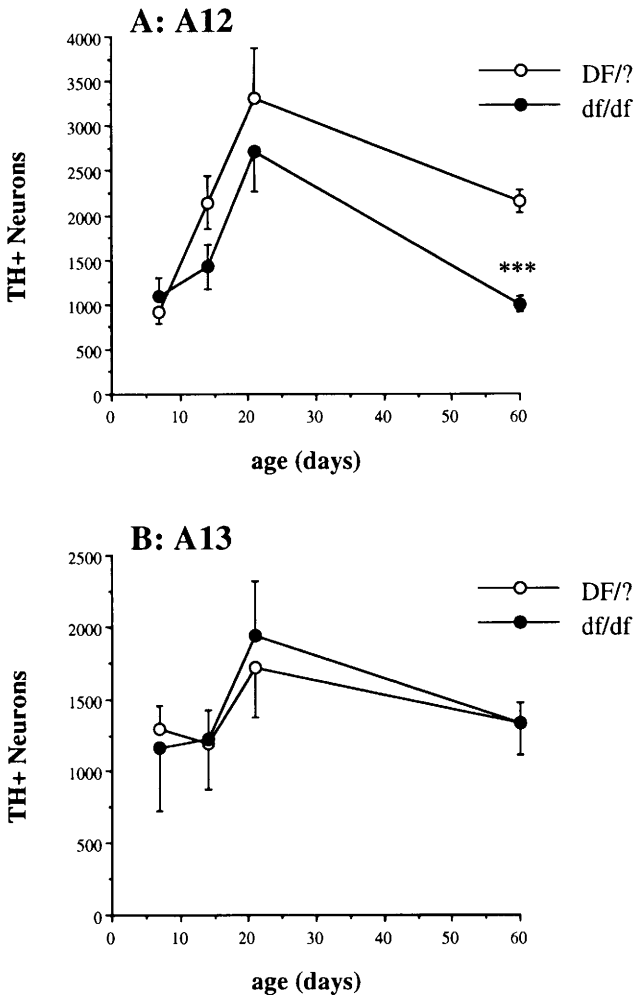

Figure 6. Numbers of TH-immunoreactive neurons in hypothalamic dopaminergic areas A12 (A) and A13 (B) of Ames dwarf (df/df) and normal (DF/?) mice at 7, 14, 21 days and 2 months (42–75 days) of age. Each point represents the mean of 6–10 animals; vertical lines represent SEM. ***df/df mean significantly different ($P < 0.001$) from DF/? mean at the same age.

lings were assessed by histofluorescence and TH ICC at the end of treatment. Perikaryal and ME DA histofluorescence was markedly enhanced in A12 neurons of PRL-treated compared with vehicle-treated dwarfs. A similar enhancement in TH immunoreactivity occurred, as shown in Figure 7, which depicts middle A12 in both DF/? (Fig. 7, A and B) and df/df (Fig. 7, C and D) treated with vehicle (Fig. 7, A and C) or PRL (Fig. 7, B and D). Figure 8 illustrates total numbers of TH+ cells in A12 and nonhypophysiotropic area A13 in the same four treatment groups. Quantification of TH+ populations showed that A12 neuronal numbers in PRL-treated dwarfs were maintained at a level comparable to that of phenotypically normal siblings. Differential cell counts within A12 showed that the deficit in untreated dwarfs was greater in ventrolateral (A12v in Fig. 7A) than in dorsomedial (A12d in Fig. 7A) regions, but that cell numbers in both areas were "rescued" by early PRL treatment. This may suggest differential involvement of these subpopulations in PRL regulation, and in dependence on PRL feedback for normal development.

Morgan and coworkers (56) reported that PRL treatment of adult dwarf mice resulted in significantly increased ME DA, but to levels still lower than those in normal mice. A similar result also was reported for studies of DA synthesis in ME after PRL treatment of dwarfs (58). Morgan and Besch (60) reported an increase in TH+ A12 cells for df/df females, but not males, after PRL injection or renal pituitary grafts in adult dwarfs. A very recent study in this laboratory (64) of PRL treatment of adult df/df mice extended the reports from Morgan's laboratory (56, 58, 60). Dwarf and normal mice 60 days of age were subjected to the same protocol of PRL treatment that was effective in "rescuing" A12 TH/DA cells when treatment was begun at 12 days (63). Histofluorescence was evaluated for qualitative DA levels, and hypothalamic TH+ cells were quantified in groups of dwarfs treated with PRL (30d), PRL (30d) + saline (15d), PRL (30d) + saline (15d) + PRL (30d). The results were qualitatively striking: PRL treatment repeatedly enhanced in-

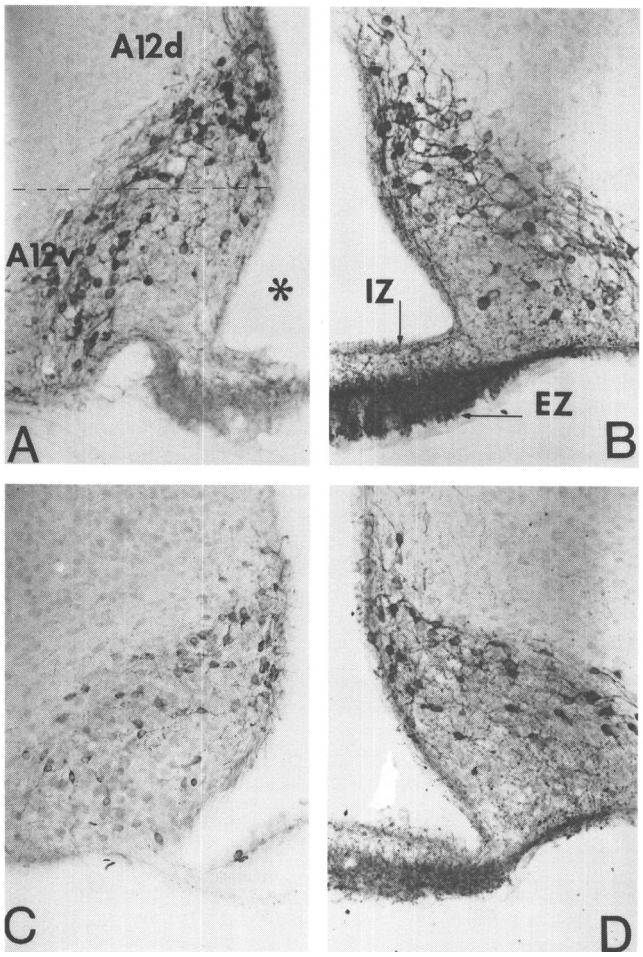

Figure 7. TH immunoreactivity in TIDA/A12 neurons in phenotypically normal (DF/?; A, B) and Ames dwarf (df/df; C, D) mice treated for 30 days, beginning at 12 days of age, with either ovine PRL (B, D) or saline vehicle (A, C). A12d = dorsomedial group; A12v = ventrolateral group; horizontal broken line indicates division between the two cell groups; *third ventricle; IZ = ME internal zone; EZ = ME external zone. All 30 μm coronal sections, ×20 original objective magnification. (From Romero and Phelps, 1993a [63]; copyright, The Endocrine Society.)

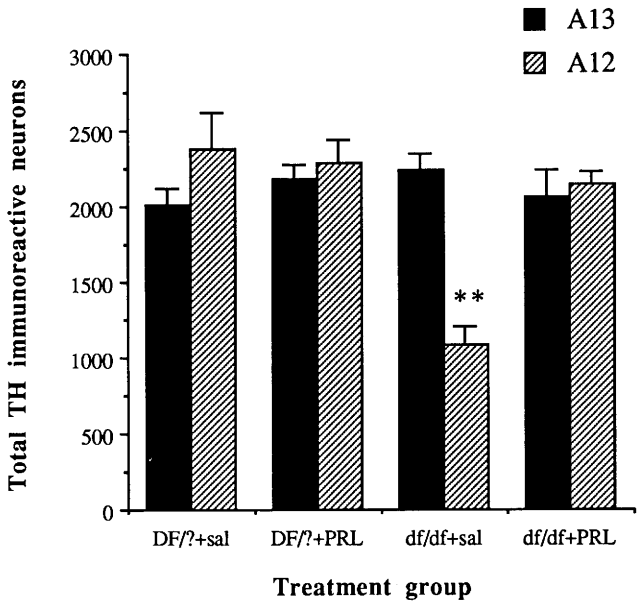

Figure 8. TH-immunoreactive cell number in hypothalamic dopaminergic areas A13 and A12 in normal (DF/?) and Ames dwarf (df/df) 42 day-old mice treated with either vehicle (+sal) or ovine PRL (+PRL) for 30 days beginning at 12 days of age. Each column represents the mean of 5–7 mice; vertical lines represent SEM. The df/df + sal A12 group differed from A12 means in all other A12 groups, $P < 0.01$ (**, SNK). (From Romero and Phelps, 1993a [63]; copyright, The Endocrine Society).

tensity of CA histofluorescence and TH immunoreactivity in A12 (as shown in Figure 9), but not in A13, neurons. However, A12 TH+ neuron number was not increased after PRL in df/df mice, in any treatment group, as shown graphically in Figure 10. In addition, neither CA fluorescence nor TH immunoreactivity in ME (Fig. 9) was enhanced by PRL treatment of adult dwarfs.

The latter results of PRL treatment of adult dwarfs suggest that normal development of A12 DA neurons is dependent on PRL feedback during a critical postnatal period (between 12 and 60 days), and that postnatal PRL absence in dwarfs results in a permanent deficiency of A12 TH-producing neurons. This differs from the report by Morgan and Besch (60) in which increased numbers of TH+ A12 neurons were counted in female df/df mice after PRL. Those authors concluded that A12 neurons in dwarfs were present in nearly normal numbers, but were dormant with regard to TH production, which could be reversed by PRL treatment. Results of the recent study in this laboratory suggest instead that A12 neurons with very weak TH immunoreactivity in untreated dwarfs were not counted in Morgan and Besch's study, because PRL treatment enhanced TH immunostaining in both experiments. Alternatively, the conflicting results may be due to different PRL treatment protocols, in that Morgan and Besch (60) used a higher dose of oPRL (175 μg/day) or pituitary grafts that produce mouse PRL, either of which might be more effective in enhancing A12 TH levels. The results from this laboratory indicate that A12 neurons that are not detectable with TH ICC in Ames dwarf mice are refractory to "rescue" to the TH+ phenotype by PRL treatment at 60 days of age, although TH immunoreactivity and steady-state DA histofluorescence levels may be enhanced by PRL in extant neurons.

A distinct qualitative difference in response to PRL treatment in neonates versus adults was observed in the perivascular zone of external ME. As shown in Figure 7D, the intensity of ME TH immunostaining in dwarfs treated with PRL beginning at 12 days was at least comparable to that of untreated DF/? animals (Figure 7A). In dwarfs treated with PRL as adults (Figure 9, A and C), ME TH immunoreactivity remained extremely low. Similar observations were made with regard to DA histofluorescence in the external ME of dwarfs treated with PRL neonatally or as adults. The

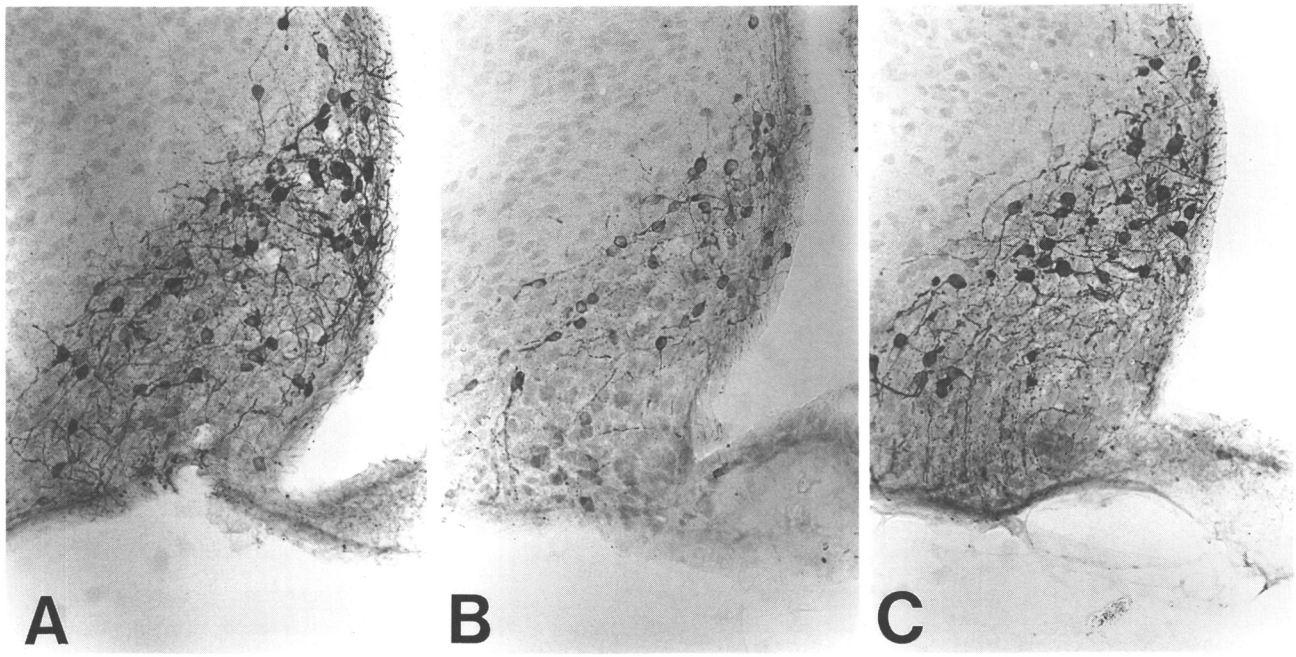

Figure 9. Photomicrographs of TH immunoreactivity in TIDA/A12 area neurons in adult Ames dwarf (df/df) mice treated beginning at 60 days of age with ovine (o) PRL for 30 days (A), oPRL for 30 days followed by saline vehicle for 15 days (B), or oPRL (30 d), sal (15 d) and oPRL (30 d; Panel C). All 30 μm coronal sections, ×20 original objective magnification.

photomicrographs of TH immunoreactivity in PRL-treated adult dwarfs also showed very low ME intensity in the report of Morgan and Besch (60). The observation of increased perikaryal DA or TH after PRL treatment of adult dwarfs, without comparable enhancement in the terminal ME region, suggests an abnormality in A12 axon connectivity in dwarfs. Tract-tracing studies are underway currently to determine whether extant A12 terminals in dwarfs reach the external ME; preliminary data suggest that ME projections are reduced.

It is important to consider why the initial development of TIDA/A12 neurons in dwarfs is comparable to that of normal mice. Does this observation indicate that early postnatal TIDA neuron development is independent of PRL? One possible answer to this question may be inferred from related studies in rats. Production of PRL by the mouse pituitary is not detectable until 7–8 days postnatally (49, 65). In rat pups, significant levels of PRL are present in the circulation from Day 1 through 21 of age (66). Although PRL production may be initiated earlier in rats than in mice (67), a majority of this PRL is likely to be derived from PRL in maternal milk which is absorbed by pups (68) because maternally-derived PRL has been shown to be important for normal development of TIDA neuron function. Experimental induction of milk PRL deficiency, by treatment of dams with bromocriptine at 2–5 days postpartum, has been shown to result in deficient ME DA levels and turnover at 35 days of age (69) and hyperprolactinemia which persisted until 100 days of age (70) in offspring. In rats, the intestinal

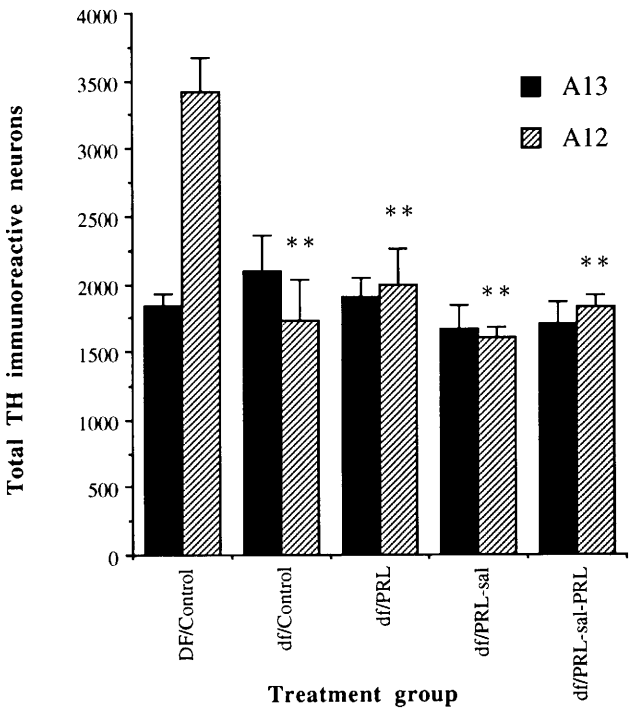

Figure 10. Histogram of numbers of TH-immunoreactive neurons in hypothalamic dopaminergic areas A13 and A12 in Ames dwarf mice treated as adults (60 days of age) with PRL and/or vehicle. Treatment groups (5–7 mice/group) were: DF/Control = normal mice, no treatment, 90 days of age; df/Control = dwarf mice, no treatment, 90 days of age; df/PRL = dwarfs treated with oPRL for 30 d; df/PRL-sal = 30 d oPRL followed by 15 d vehicle; df/PRL-sal-PRL = 30 oPRL followed by 15 d vehicle followed by 30 d oPRL (135 days of age). Columns represent means; vertical lines = SEM. A12 cell numbers in all df/df groups were lower than A12 cell numbers in normal mice, $P < 0.01$ (**, SNK).

mucosa undergoes closure via formation of intercellular tight junctions at 18–21 days of age, preventing absorption of molecules as large as PRL thereafter (71); at this age, endogenous PRL production is significant and circulating PRL levels rise (66), such that endogenous PRL could supplant the influence of maternal hormone on TIDA neuron development. This substitution of PRL source could not occur in dwarf mice, in which endogenous PRL production is absent, and further influence on TIDA neuron development would cease after removal of maternally-derived PRL.

The collective data in PRL-deficient df/df mice indicate that TIDA PRL-inhibiting neurons are not only deficient in adults, but exhibit a critical developmental period for maintenance of normal population size and transmitter content by PRL feedback. The results suggest actual cell loss and aberrant terminal contacts in A12 when dwarfs fail to produce the PRL signal.

Prolactin-Stimulating Neurons. Although an unequivocal PRL-releasing hormone has not been confirmed, several hypothalamic peptides have been shown to stimulate PRL secretion in certain species or circumstances. These include TRH (72, 73), oxytocin (74), galanin (75), and VIP (76).

Roti and colleagues (19) measured TRH by RIA in brains of male Snell (dw/dw) dwarfs, and found a significant reduction compared with DW/? littermates in hypothalamus, but not in extrahypothalamic areas; treatment of dwarfs with thyroxine was without effect on TRH levels. Emphasizing the pituitary site of the primary mutation, TRH treatment of Snell dwarfs does not elicit TSH synthesis (77). Immunocytochemical studies of TRH in dwarfs are lacking. Oxytocin in dwarf hypothalamus has not been investigated.

Galanin has been localized in both hypothalamic arcuate nucleus (78) and in PRL-, GH-,and TSH-producing cells of the anterior pituitary (79), and has been shown to increase GH as well as PRL release (80). Considering the foregoing evidence, one might well predict significant alteration in galanin levels in PRL-, GH-, and TSH-deficient mice. Hyde and associates (81) measured galanin peptide and mRNA in hypothalamus, anterior pituitary, and neurointermediate pituitary in adult female Ames dwarf and normal sibling mice; the study included estrogen treatment for 2 weeks. Neither galanin peptide nor mRNA were detectable in dwarf anterior pituitary; galanin in neurointermediate lobe was similar for df/df and DF/? mice. In the hypothalamus, galanin mRNA was comparable for df/df and DF/?, but peptide levels in dwarfs were 30% lower than in normals. Estrogen treatment did not affect galanin expression in dwarf pituitary or hypothalamus. The absence of anterior pituitary galanin in dwarfs is understandable, because dwarfs lack all the cell types in which galanin is produced. In hypothalamus, the galanin peptide reduction is surprising for a proposed stimulatory factor; one would predict elevation of a releasing factor in PRL- and GH-deficient mice. (It should be noted that this was the first published report of separate assay of anterior and posterior pituitary in dwarfs; the dissection is microscopic.)

Like galanin, VIP has been localized to both hypophysiotropic areas such as parvocellular paraventricular nucleus (PVN) in hypothalamus (82, 83) and to anterior pituitary (84). Whether hypothalamic or pituitary VIP is more important in PRL release remains unresolved (76). Immunocytochemical studies of the Snell dwarf hypothalamus (85) and pituitary (unpublished data) in this laboratory have revealed no significant differences from normal siblings; Noguchi and colleagues (86) reported significantly decreased radioimmunoassayed VIP in the pituitaries of both Snell dwarf and *little* mice, corrected for pituitary weight in pooled samples. In Ames dwarfs, RIA of dissected dorsal (including PVN) and ventral (including ME) hypothalamus showed df/df and DF/? VIP levels to be comparable (87). In the Ames dwarf pituitary, ICC for VIP revealed immunoreactivity that was qualitatively comparable to that in DF/?, and anterior pituitary VIP measured by RIA showed df/df levels at less than 2% (6.9 ± 1.0 pg/gland) of DF/? levels (391.3 ± 86.2 pg); total VIP in neurointermediate lobe was comparable for df/df (7.6 pg) and DF/? (6.9 pg) (88).

Because the changes in confirmed hypophysiotropic hormones (DA, above; somatostatin and GHRH, discussed below) are so profound in PRL- and GH-deficient mice, the animals may be considered a model for testing whether a proposed hypophysiotropic factor is physiologically relevant, or, at least, subject to feedback effects by pituitary hormones. Among proposed PRL-releasing factors, TRH, galanin, and VIP are virtually unaffected by PRL absence in dwarf mice.

Growth Hormone–Regulating Neurons

Like PRL, GH is not detectable at any stage of development in Snell or Ames dwarf mice (16). In addition to absence of protein, GH mRNA transcripts are undetectable in adults (17) or neonates (89). GH secretion is regulated by two confirmed and antagonistic hypothalamic peptides, inhibitory somatostatin (somatotropin release–inhibiting hormone; SRIH) and GHRH. These two neurohormones interact to maintain homeostatic GH levels and, as has been shown in male rats, to generate the ultradian pulsatile pattern of GH secretion (90, 91). The expression of both peptides has been investigated in both spontaneous and transgenically engineered GH-deficient dwarf mouse hypothalamus.

Somatostatin. Like DA, SRIH exists in many extrahypothalamic areas, such as neocortex and amygdala, although the only confirmed physiological

function of SRIH is inhibition of pituitary GH release. Neurons that serve this function, and project to ME, are confined to anterior periventricular areas in hypothalamus (92), often collectively designated as a periventricular nucleus (PeN). In fact, SRIH-producing neurons are found in the arcuate nucleus, an area containing several pituitary-regulating factors, but these SRIH neurons do not project to ME (92), although they may communicate with adjacent arcuate nucleus GHRH neurons (93–95).

Tissue SRIH levels assessed by RIA in Snell dwarfs (dw/dw) are significantly reduced compared with levels in DW/? mice in ME, but not in pineal or in gut (96), and in hypothalamic, but not extrahypothalamic, brain (97). An immunocytochemical study of SRIH in Snell dwarf (dw^j/dw^j) hypothalamus showed severe qualitative reduction in PeN, as shown in Figure 11, and in external ME (98). In that same study, ICC assessment of SRIH in *little* mice indicated normal expression of the peptide in both neuronal perikarya (Fig. 12) and ME terminals, suggesting that

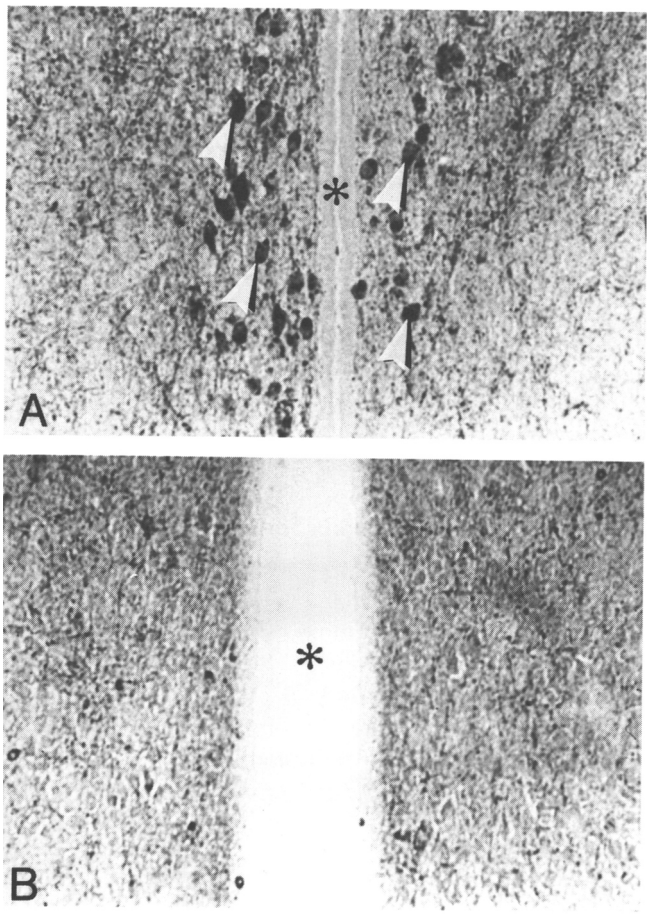

Figure 11. Photomicrographs of SRIH immunoreactivity in the anterior hypothalamic periventricular nucleus (PeN) in phenotypically normal (DW/?, A) and Snell dwarf (dw^j/dw^j, B) mice. Arrowheads indicate SRIH-positive neuronal cell bodies, *third ventricle. Coronal 20 μm sections, ×20 original objective magnification. (From Phelps and Hoffman, 1987 [98]; copyright, Pergamon Press.)

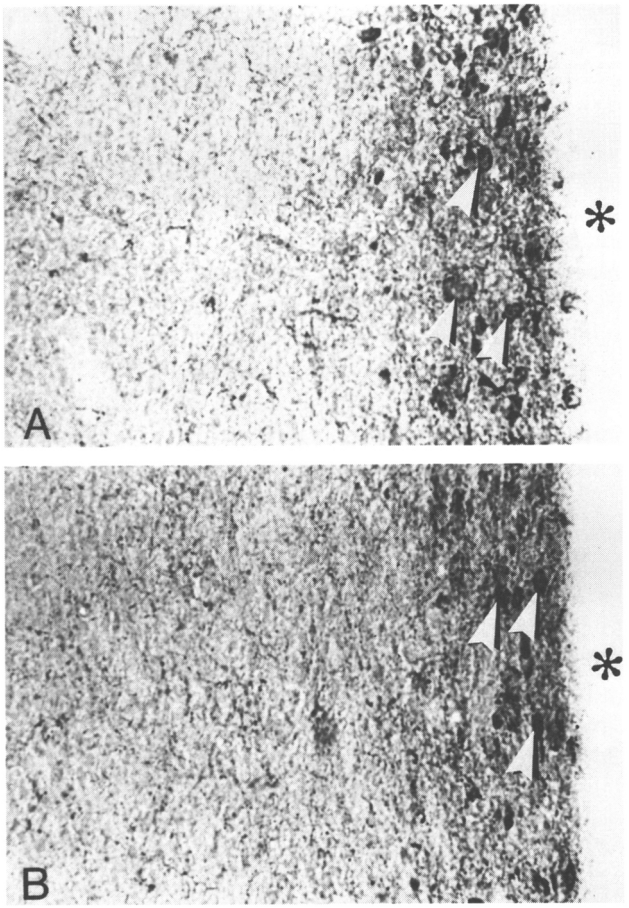

Figure 12. SRIH immunoreactivity in PeN of normal (LIT/?,A) and *little* (lit/lit, B) mice. Arrowheads indicate SRIH-positive neuronal cell bodies; *third ventricle. Coronal 20 μm sections, ×20 original objective magnification. (From Phelps and Hoffman, 1987 [98]; copyright, Pergamon Press.)

the low levels of GH found in *little* mice (10%; [17]) were adequate for maintaining SRIH expression. In transgenic dwarfs (34), total PeN SRIH mRNA assessed by quantification of *in situ* hybridization signal was reduced to 40% of that in sibling mice (99). In the same study, transgenic "giant" mice, with excess endogenous mouse GH production stimulated by transgenic incorporation of human GHRH, showed markedly increased (250% of normals) SRIH mRNA in PeN neurons. The GH absence or excess in transgenic mice is lifelong, as in spontaneous dwarf mutants, and is likely to affect developmental hypothalamic differentiation. The reductions in SRIH in both Snell and transgenic dwarfs, however, are quantitatively similar to reductions in SRIH expression after acute GH removal by hypophysectomy in rats (100–102).

Fuhrmann and colleagues (97) reported significantly elevated SRIH in Snell dwarf neocortex, striatum, hippocampus, and medulla, compared with levels in DW/? mice, and suggested that increased overall expression of SRIH in early development might account for the failure of GH cell differentiation in dwarf pituitary. This theory was tested by O'Hara and co-

workers (103) by treating newborn normal mice with a potent SRIH analog; pituitary differentiation was unaffected by this treatment. Two additional important findings in that report were that SRIH mRNA in Snell dwarf hypothalamus, quantified by signal intensity after *in situ* hybridization, was reduced to 60% of levels in normal siblings, and that the SRIH gene, although located on Chromosome 16, was nonallelic with the dw mutant gene.

Studies of the development of SRIH mRNA (104) and peptide (105) in normal (Balb/cByJ) mice have shown that SRIH in PeN first appears at postnatal Day 3. Only preliminary studies of SRIH development in dwarf mice have been published (106, 107). Current studies in this laboratory indicate that the developmental patterns of SRIH mRNA and peptide expression differ in dwarf hypothalamus. Somatostatin mRNA is detectable in both Ames dwarf and normal sibling PeN as early as the day of birth. In DW/? mice, SRIH peptide ($SRIH_{1-14}$) is first detected immunocytochemically at 3 days of age, and immunostaining increases in intensity thereafter, in both PeN and ME. The peptide is not detectable in dwarf PeN perikarya at any age (unpublished data) although ME SRIH can be detected in some dwarfs as early as 3 days, and is detectable in most dwarfs as adults ($\geq$60 days). Although terminal (ME) SRIH is detectable in adult dwarfs, perikaryal SRIH mRNA in PeN appears to decline during postnatal development, as shown in Figure 13, which compares *in situ* hybridization signal at 4 and 21 days postnatally (107).

The collective data on SRIH in dwarfs indicate a significant reduction, compared with normal mice, in ME-afferent neurons and terminals; the developmental sequence of this reduction indicates an earlier onset, in terms of SRIH mRNA, than for PRL-inhibitory DA, which may coincide with the earlier onset of normal GH-production at E16–17 in normal mice and its absence in dwarfs (16). It is important to note that the role of the GH signal in development has not been addressed directly, as has the role of PRL in TIDA development, or with regard to the delivery of the hormone to pups in maternal milk.

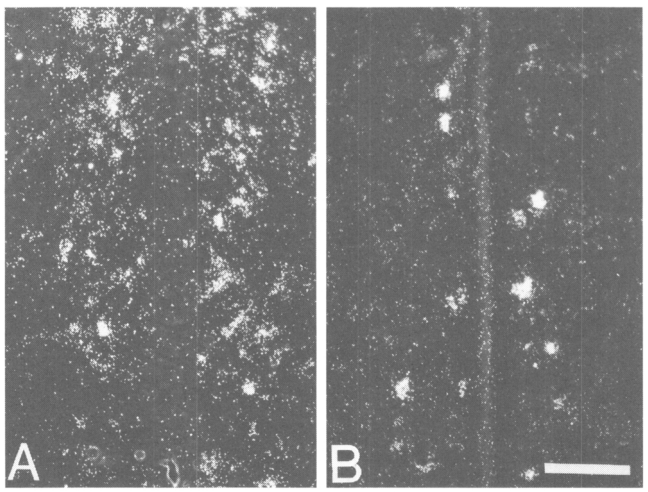

Figure 13. Preprosomatostatin mRNA detected by *in situ* hybridization in the PeN of Ames dwarf (df/df) mice at 4 (A) and 21 (B) days of age. Reduced silver grains (bright dots) indicate neuronal cell bodies expressing SRIH mRNA. Darkfield photomicrographs of 30 μm coronal brain sections; bar = 100 μm. (From Phelps and Hurley, 1993 [107]; copyright, Academic Press.)

GHRH. The hypothalamic peptide that enhances pituitary GH transcription (108, 109) as well as release (see review by Frohman and Jansson [110]) was first characterized in human pancreatic tumors (111, 112), but has been localized as a hypophysiotropic hormone to the hypothalamic arcuate nucleus (113, 114) in several species (see also review by Frohman and Jansson [110]). The feedback effect of GH on SRIH has been studied primarily by acute removal of GH by hypophysectomy in adult rats, which has resulted uniformly in elevated hypothalamic GHRH mRNA (115, 116), and reduced GHRH peptide, whether assessed by ICC (113, 117) or by RIA (102, 115, 118, 119). The discrepant effects of GH removal on GHRH peptide and mRNA have been hypothesized to be the result of increased release of peptide, which would lower hypothalamic content, but direct measurement of GHRH release from hypothalami of hypophysectomized rats has failed to show increased release (115, 119).

Mouse GHRH cDNA has been cloned and sequenced (120, 121) and mGHRH mRNA has been localized in normal mouse hypothalamus and placenta (120), and shown to be increased in the hypothalamus of *little* mice (121). Studies in this laboratory have addressed the effect of lifelong GH absence in spontaneous and in transgenically engineered models. In transgenic dwarf mice, GHRH mRNA is overexpressed, to 282% ($P < 0.001$) compared with normal controls (34); in contrast, transgenic giant mice (Mt, GHRH; Bri 11) with excess endogenous GH, underexpress GHRH mRNA to 42% ($P < 0.001$) of normals (122). In Ames dwarf mice, GHRH mRNA is elevated compared to levels in DF/? siblings, whether quantified as total expression in the arcuate nucleus or as mRNA per neuron, as shown in Figure 14 (123). Studies of GHRH mRNA expression in states of GH deficiency (hypophysectomy or dwarfism) are consistent, showing increased steady-state levels of GHRH message in response to absent GH signal and negative feedback.

The study of GHRH peptide in mice has been hampered by the absence of a mouse-specific antiserum. The predicted amino acid sequence of mGHRH shows 38% nonhomology with human and, importantly, 30% nonhomology with rat GHRH (121); most nonhomology in the GHRH peptide is found in carboxy-terminal residues (110). The unique mGHRH structure may account for the inability to detect mGHRH by RIA using anti-rat GHRH (unpublished

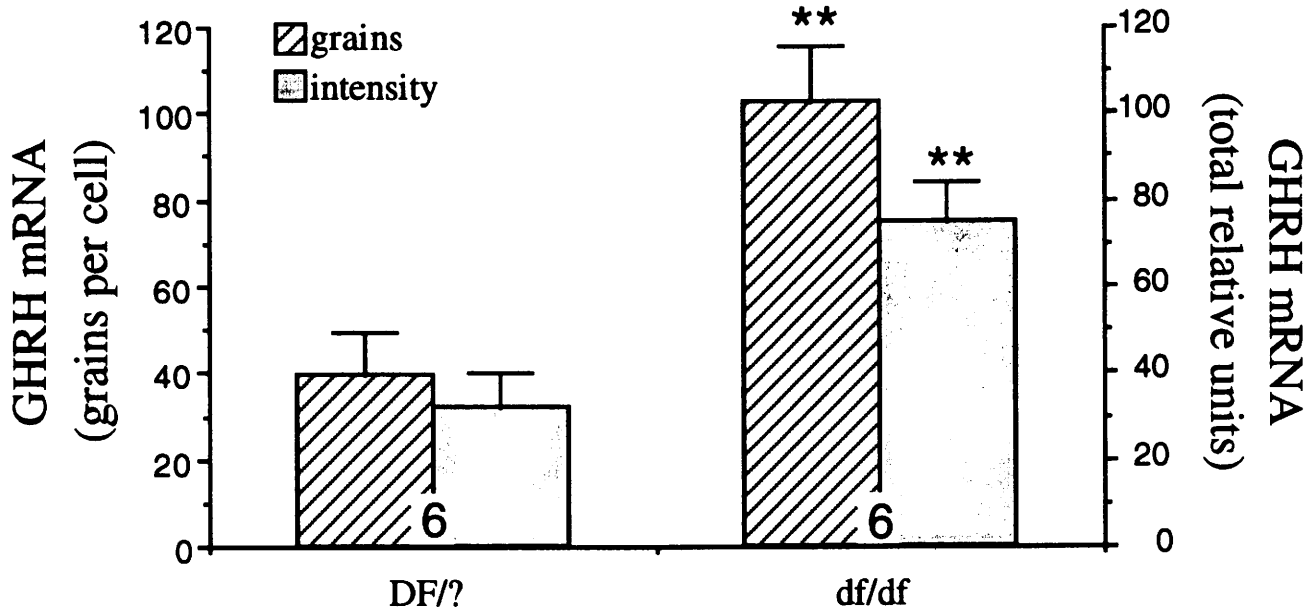

Figure 14. Quantification of mGHRH mRNA from *in situ* hybridization. Values of intensity of hybridization for *DF/?* and *df/df* are shown; numbers at the base of the columns indicate animals per group, and vertical bars represent SEM. Striped bars indicate grain counts per cell (left axis); shaded bars represent total image intensity from X-ray film (right axis). **different from DF/? phenotype ($P < 0.01$) in the same parameter. (From Phelps *et al.*, 1993b [123]; copyright, The Endocrine Society.)

data), or aberrant ICC localization using antisera directed against human (124) or rat GHRH (125, 126). On the assumption that GHRH was similar among species, preliminary reports on GHRH ICC which employed heterologous antisera identified unusual sites for GHRH immunoreactivity in mouse brain. Using antiserum directed against human pancreatic (hp) $GHRH_{1-44}$, ICC conducted in garden dormice by Fellman and associates (124) localized strong immunoreactivity in ME, but perikaryal GHRH in only PVN. In 1986 (126), this laboratory reported a decrease in Snell dwarf (dw^j/dw^j) hypothalamic GHRH compared with that in normal animals, in an ICC study that used antiserum directed against rat GHRH (127). In subsequent attempts to verify that study, additional antisera directed against rat GHRH from several laboratories were found to be unable to detect GHRH in mouse hypothalamus (unpublished data). Very recently, Dr. Hisako Endo, working in the laboratory of Dr. Frank Talamantes, developed a rabbit antiserum specific to a synthetic peptide consisting of the predicted 25 carboxy-terminal amino acids of mGHRH. The specificity of the antiserum was verified by RIA of mouse hypothalamic as well as placental extracts, and the antiserum did not detect several hypothalamic peptides, including $hGHRH_{44}$ or $_{1-32}$ or $rGHRH_{43}$ (128). This antiserum proved to be excellent for ICC, and was used to quantify an increase ($P < 0.005$) in the number of GHRH-expressing neuronal cell bodies in the arcuate nucleus of Ames dwarfs, as shown in Figure 15 (123). GHRH-positive neurons numbered 342.5 ± 54.4 in DF/? and 976.4 ± 123.4 in df/df A12. Despite

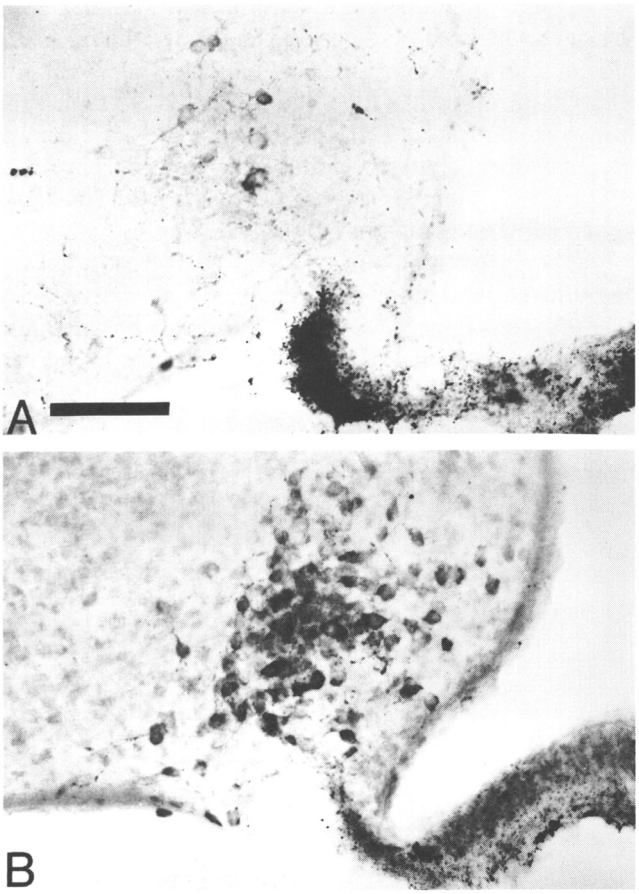

Figure 15. Immunocytochemical detection of mGHRH peptide in colchicine-pretreated normal (*DF/?*; A) and dwarf (*df/df*; B) left hypothalamic arcuate nucleus and median eminence; the dwarf section is at a slightly posterior level. Bar = 100 μm. (From Phelps *et al.*, 1993b [123]; copyright, The Endocrine Society.)

marked increase in GHRH-positive neurons in dwarfs, immunoreactivity of GHRH in ME was consistently reduced in dwarfs compared with normals, as may be noted in Figure 15. As for TIDA neurons, retrograde tract-tracing studies are under way to determine whether GHRH axonal terminations in ME are reduced in dwarfs.

The only other report of GHRH peptide in a spontaneously GH-deficient animal was an ICC study of the Sprague-Dawley dwarf rat, in which the number of GHRH-positive neurons was increased only after correction for the decreased brain weight of the dwarfs (129). This difference in quantitative results suggests that the difference in pituitary status (i.e., signals) may be responsible; the dwarf rat is deficient in GH, but not PRL. It is tempting to speculate that the reduction in arcuate nucleus DA neurons in Ames dwarfs is related to the marked increase in GHRH neurons in the same area (compare TH ICC in Figures 7C and 9B with GHRH ICC in Figure 15B). Because GHRH and TH have been colocalized in ventrolateral arcuate neurons (130), phenotype plasticity in response to target signals may occur.

The studies on Ames dwarfs provide the first evaluation of mGHRH using a homologous antiserum which is in itself important because of the prevalence of models of GH deficiency or excess in this species. In addition, these studies are the first to quantify both GHRH mRNA and peptide in the same spontaneously GH-deficient model, and the results differ from the effects of hypophysectomy for peptide, but not for mRNA. The opposite results may be related to the duration of GH deficiency, which is lifelong in Ames dwarf mice. Thus, increased hypothalamic GHRH in a genetically GH-deficient animal may reflect response to absent pituitary feedback signal at critical developmental periods, as has been shown for PRL-regulating dopamine neurons. Whether GH treatment in spontaneous dwarfs can reverse the GHRH abnormality awaits the identification of a specific developmental period for manifestation of the GHRH overexpression.

Two studies of the ontogeny of GHRH expression in rat hypothalamus have used ICC assessment. Several studies have shown that enhancement of peptide concentration in perikarya is necessary for GHRH ICC detection, and this enhancement is most often accomplished by blocking axonal transport with intracerebroventricular injection of colchicine. Daikoku and coworkers (131) administered intraventricular colchicine to rat fetuses *in utero* 24 hr prior to sacrifice, on Day 17.5–20.5 of gestation, and first detected GHRH perikarya in the arcuate nucleus on Day 19.5 of gestation. Ishikawa and associates (132) first detected GHRH-immunoreactivity perikarya in colchicine-treated 1-day-old rat pups. Both studies detected GHRH in ME at Day 18–19 of gestation. Experience in this laboratory indicates that mouse pups as old as 14 days do not survive intracerebroventricular colchicine, and developmental assessment therefore has been restricted to quantification of hypothalamic GHRH mRNA. It has been documented in a preliminary report (133) that neonatal (1–14 days of age) GHRH mRNA in Ames dwarf and normal hypothalamus is similar, even though GHRH mRNA levels are markedly elevated in adult dwarf mice (123). Further quantification of GHRH mRNA *in situ* hybridization signal indicates that Ames dwarf GHRH expression is higher than that of DF/? mice at 21 days of age, and continues to be elevated through 3 and 6 months of age (unpublished data). The collective data on SRIH and GHRH expression in Ames dwarf mice indicate that the hypothalamic defects in these neurohormones, as is the case for PRL-regulatory neurons, also develop postnatally.

An appropriate footnote to the collective studies cited is that immunoreactivity and distribution of the hypothalamic neurons which stimulate LH and FSH secretion by production of LH- or gonadotropin-releasing hormone (LHRH/GnRH) appear qualitatively comparable to those of phenotypic normals in both Snell and Ames dwarfs (unpublished data). As noted in Table 1, LH and FSH are expressed at essentially normal levels in dwarf pituitary.

The mechanism by which either PRL or GH affect their respective hypophysiotropic neurons is not known. Localization of receptors for PRL and GH, particularly in the hypothalamus of rats, has been problematic for some time, although lactogenic receptors have been localized in rabbits (134). Very recently, rat PRL receptor has been detected in hypothalamus by polymerase chain reaction amplification (135), and Crumeyrolle-Arias and associates (136) have reported localization of lactogenic receptors in rat hypothalamus by both specific ICC and hGH binding. Burton and colleagues (137) have reported GH receptor mRNA localization by *in situ* hybridization in both SRIH and GHRH perikaryal sites. For GH feedback, sites of insulin-like growth factor (IGF-I) production or IGF-I receptor sites are also potential mediators. Although studies of localization of PRL or GH receptors in brain are completely lacking for mouse, Noguchi and coworkers (138) have reported qualitatively decreased ICC for IGF-I protein in Snell dwarf compared with normal mice. In this laboratory, ICC localization of IGF-I, using a number of antisera, has been uniformly negative in brains of normal or Ames dwarf mice (unpublished data). The avenue of access of PRL or GH to hypophysiotropic neurons is also unknown, but includes for consideration the absence of blood-brain barrier in ventral arcuate nucleus (33, 139), the presence of pituitary hormones in CSF (140) of the third ventricle near all these hypophysiotropic

neurons, and possible retrograde flow from the hypophysial portal vasculature (141, 142).

The studies described above indicate that the deficient hypophysiotropic DA and SRIH found in adult dwarf hypothalamus represent divergence from the normal pattern during postnatal development of these pituitary-inhibiting neurons. Hypophysiotropic PRL-inhibiting DA fails to increase in dwarfs after 14 days, while it continues to increase in phenotypically normal siblings (49). Numbers of tyrosine hydroxylase-immunoreactive TIDA neurons actually decrease in number in dwarf mice after 21 days of age (62). Prolactin treatment initiated prior to 14 days stimulates continued DA expression and prevents the postnatal regression of TH immunoreactivity (63); PRL treatment of adult dwarfs is ineffective in restoring the deficient TIDA TH+ neuron number (64). In addition, the continued reduction of ME TH and DA after PRL in adult dwarfs (60) suggests deficient appropriate axonal connectivity. SRIH mRNA expression also recedes during postnatal development of dwarfs (106); GH replacement experiments await confirmation by further developmental studies of a critical age when the deficit appears.

A converse response have been found in the hypophysiotropic neurons which stimulate GH secretion. GH-releasing hormone (GHRH), the structure of which has been characterized only recently for the mouse, is overexpressed in adult dwarfs, in both mRNA and peptide (123). The increase in dwarf GHRH also appears to develop postnatally, with divergence of dwarf and normal GHRH expression occurring by 21 days of age, but not being present in younger animals (133). Thus, for all three types of hypophysiotropic neurons studied, initial development and phenotype differentiation are indistinguishable for GH- and PRL-deficient dwarfs and their normal siblings. Subsequently, with absence of endogenous GH or PRL feedback signals, further development ceases or regression occurs. In addition, it appears that the nature of the hypophysiotropic signal; i.e., whether the neuron is inhibitory or stimulatory to pituitary secretion, dictates the type of response to absent target pituitary signals, a phenomenon which has not been explored from the viewpoint of neurotrophic signals in developmental differentiation. From the viewpoint of neuroendocrine feedback, however, this difference is logical because neurotrophic effects of PRL and GH simply mirror lifelong dynamic effects, activating inhibitory neurons and inhibiting stimulatory neurons.

Collectively, the findings indicate that chronic developmental absence of pituitary target signals affect not only dynamic neuroendocrine feedback, as may be documented in acute experimental studies, but may interfere with differentiation in hypophysiotropic neurons, with regard to connectivity, survival and phenotypic plasticity. Thus, GH and PRL may be regarded as neurotrophic signals for their regulatory neurons.

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