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New Insights Into the Regulation of Somatotrope Function Using Genetic and Transgenic Models

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Growth hormone (GH) secretion is under the control of the hypothalamic hormones GH-releasing hormone (GHRH) and somatostatin (SRIF), and is regulated by feedback effects of GH and insulin-like growth factor (IGF-1). GHRH and SRIF act on somatotropes by binding to G-protein–coupled receptors. GHRH activates the stimulatory G protein (G_s), leading primarily to activation of adenylyl cyclase and protein kinase A. SRIF activates the inhibitory G protein (G_i). Several animal models enable the study of various disorders of GH secretion in vivo. Genetic models of impaired GH secretion include the little (*lit*) mouse, the dwarf (*dw*) rat, the fatty (*fa*) rat, and the high-growth (*hg*) mouse. Transgenic models of impaired and excessive GH secretion, respectively, include the tyrosine hydroxylase-human GH (TH-hGH) transgenic mouse and the metallothionein-human GHRH transgenic mouse. These models encompass a wide spectrum of disorders of GH secretion, involving defects of hypothalamic regulation, feedback control at the pituitary level, or the mechanism of GHRH action in the somatotrope. They may provide insights into our understanding of human GH secretory disorders.

THE NEUROENDOCRINE regulation of growth hormone (GH) secretion is under the control of a complex mechanism that is mediated by two hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin (SRIF). Neural, hormonal, and metabolic factors participate in the regulatory mechanisms, which include both central and peripheral components. Among the latter, feedback effects of GH and insulin-like growth factor (IGF-1) occur at both pituitary and hypothalamic levels. While extensive studies have been performed in animal models to elucidate the various components of this regulatory system, studies in humans have been restricted because of the inaccessibility of the critical tissue components (hypothalamus and pituitary) and their vascular connection (hypothalamic-hypophyseal portal vascular system).

The regulation of GH secretion at the cellular level has also been extensively studied in animal models, primarily in normal tissue, or in pituitary tumor cell lines. The mechanism of action of both GHRH and SRIF has been, to a great extent, clarified during the past decade. The effects of both hormones are initiated by their binding to G-protein–coupled receptors on the somatotrope cell membrane. Although several signal transduction pathways are utilized, the major effect of GHRH occurs by activation of adenylyl cyclase and protein kinase A, as a consequence of increased activity of the stimulatory G protein (G_s). Other important effects include the phosphatidylinositol protein kinase C pathway and Ca^{2+} channel activity. The inhibitory effects of SRIF are mediated primarily by an inhibitory G protein (G_i). The eventual cascade of these effects alters GH release under both basal and stimulated conditions. In addition, GHRH also enhances GH synthesis and serves as a mitogenic factor for the somatotrope.

Despite these advances, however, our understanding of the pathogenesis of commonly observed disorders of GH secretion has been limited. These disorders include impaired GH secretion unassociated with known structural abnormalities (idiopathic GH deficiency of childhood) and the consequences of excessive GHRH secretion (ectopic GHRH secretion and possibly other forms of acromegaly). Several animal models (both genetic and transgenic) have recently become available and have provided insight into these and other clinical disorders of GH secretion.

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IMPAIRED GH SECRETION MODELS

Little (lit) Mouse

The *lit* mouse exhibits moderately severe growth retardation and a decrease in pituitary GH and GH mRNA.[1] Plasma GH levels are barely detectable and do not increase after stimulation with GHRH. In vitro, dispersed anterior pituitary cells also fail to increase GH release or synthesis or cyclic adenosine monophosphate (cAMP) accumulation in response to GHRH.[2] However, responses to other probes of signal transduction, including cholera toxin (a direct stimulator of the α-subunit of G_s), forskolin (a direct activator of adenylyl cyclase), and dibutyryl cAMP, are intact. The recent reports of a point mutation in the extracellular domain of the GHRH receptor that inhibits the binding of GHRH[3,4] explain the defects observed, which limit GH secretion to basal release in this model.

Dwarf (dw) Rat

The spontaneous *dw* rat is moderately growth-retarded and also has decreased pituitary GH stores. However, plasma GH levels do respond, although subnormally, to GHRH. In vitro, the GH response to GHRH is moderately impaired, while that of cAMP is barely detectable.[5] Responses to dibutyryl cAMP and forskolin are intact, although stimulation by cholera toxin and prostaglandin E_1 is no more effective than is that by GHRH, indicating a postreceptor defect. Pituitary somatotrope number is also decreased, reflecting the lack of a normal mitogenic effect of GHRH. Extensive studies of $G_s\alpha$ structure and function in the *dw* rat revealed no abnormalities, and the molecular defect in these animals is presently unknown.[6] Among the possibilities are an abnormality in the βγ-subunit of G_s, an alteration in an intracellular protein required for $G_s\alpha$ function, or a mutation in adenylyl cyclase, affecting $G_s\alpha$ binding, but not forskolin activation.

Fatty (fa) Rat

The *fa* rat exhibits moderate obesity, hyperinsulinemia, and hyperlipidemia. It is also growth-retarded and has a decreased pituitary GH content. Pituitary cells from *fa* rats exhibit decreased sensitivity to GHRH in vitro, although the molecular defect is not known. The animals also have decreased hypothalamic GHRH and GHRH mRNA levels, indicating a primary hypothalamic defect in GH regulation, presumably related to their obesity.[7]

Tyrosine Hydroxylase-Human GH Transgenic Mouse

Transgenic mice bearing a tyrosine hydroxylase-human GH (TH-hGH) transgene have targeted expression of the reporter gene in the CNS and adrenal medulla and are growth-retarded.[8] The mice have decreased GH and GH mRNA in the pituitary and decreased hepatic IGF-1 mRNA and serum IGF-1 levels. Within the CNS, hGH is expressed in both the periventricular and arcuate nuclei of the hypothalamus. Feedback effects occur at both sites, leading to increases in both SRIF and SRIF mRNA and decreases in both GHRH and GHRH mRNA, respectively.[9] The reduction of GHRH during the fetal period decreases levels of GHRH receptors on somatotropes and also impairs somatotrope development. This transgenic mouse serves as a model of idiopathic GH deficiency in humans and provides a means of studying factors mediating somatotrope proliferation.

High Growth (hg) Mouse

A strain of mice with a high-growth gene (*hg*), characterized by increased growth rates, has been developed by selective inbreeding.[10] The mice exhibit increased plasma IGF-1 levels, and linkage studies have placed the gene on chromosome 10, near, although distinct from, the position of the IGF-1 gene.[11] The mice have decreased pulsatility of plasma GH and reduced levels of pituitary GH and GH mRNA. The absence of demonstrable changes in GHRH or SRIF mRNA levels suggests a direct inhibitory effect of IGF-1 at the level of the pituitary. The mutation is believed to involve the regulatory region of the gene, although it does not lead to constitutive activation, since the expected decrease in IGF-1 levels is seen in response to food deprivation.

EXCESSIVE GH SECRETION MODEL

Metallothionein-Human GHRH Transgenic Mouse

The most dramatic example of increased somatotrope function yet described is the mouse metallothionein-hGHRH transgenic mouse.[12] Mice expressing this transgene have allomorphic increases in tissue size, markedly increased pituitary GH and GH mRNA content and GH secretion, and increased IGF-1 levels. Massive somatotrope hyperplasia and, eventually, foci of adenomatous transformation are among the most striking changes observed.[13] The hGHRH gene is expressed in many tissues, including the pituitary and the arcuate nucleus of the hypothalamus.[14] Mouse hypothalamic GHRH content is decreased, most likely due to the effects of the high circulating levels of GH, though also possibly attributable to a local (paracrine) effect of the transgene. This model of GHRH-induced somatotrope proliferation can be used to study the molecular mechanisms of mitogenesis in this cell type.

CONCLUSIONS

The models described encompass a wide spectrum of disorders of GH secretion, from hypothalamic regulation, and feedback control at the pituitary level, to the mechanism of action of GHRH in the somatotrope. Although more information has been accumulated on GHRH than SRIF, it is clear that both neurohormones must be considered in the regulatory mechanism, at least with respect to GH secretion. The role of SRIF in somatotrope mitogenesis remains to be clarified, and this will require new models. While human counterparts of the genetic models described here remain to be identified, the models may provide new insight into currently unexplained human GH secretory disorders.

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