

GH in the Central Nervous System: Lessons from the Growth Hormone Receptor Knockout Mouse

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Abstract: Various central nervous system (CNS) tissues express both growth hormone (GH) and its receptor (GHR), including those involved in memory and cognition. Studies show the presence of GHR in the pituitary, choroid plexus, hypothalamus, hippocampus, pituitary and the spinal cord during development and, to a lesser extent, in adults. This expression implies a role of GH signaling in growth, development and functionality of the CNS. While data on the function of GH in the CNS is sparse, several studies have been conducted using the GHR knockout (-/-) mouse in order to better understand this role. Abnormal growth hormone signaling in humans is the cause of various diseases that include Laron syndrome, GH deficiency and acromegaly. This article will review the research conducted using the GHR-/- mouse on the role of GH signaling in the CNS. Where possible, we will attempt to contextualize the animal data with respect to human disease.

Keywords: Growth hormone, growth hormone receptor, central nervous system, acromegaly, Laron syndrome, brain.

1. INTRODUCTION

Studies in multiple species have shown expression of growth hormone (GH) and GH receptors (R) in multiple tissues of the central nervous system (CNS), implicating a role for GH signaling in the development and maintenance of neural tissues as well as memory and cognition [1-4]. Despite several lines of evidence from both *in vivo* and *in vitro* studies suggesting a role for GH in the CNS [5-8], the data are often contradictory. For example, studies of animals with elevated GH have shown improvements in memory and learning [9,10], but similar results have been reported using animal models of reduced GH action [11-13]. Human subjects that are GH-deficient or resistant have not provided clarity, as some show normal or high intelligence and others have impaired cognitive function [14]. In addition, various mouse lines with altered GH signaling do not show obvious developmental or functional abnormalities in the CNS.

In humans, disease states exist for GH excess (acromegaly), GH deficiency (GHD) and GH insensitivity (Laron syndrome). In our laboratory and those of others, genetically engineered mouse lines (see Fig. 1) have been established that approximate these human diseases and have been useful in the study of the role of GH in the brain. Mice with excess GH production and secretion, such as the bovine GH transgenic animal (bGH), serve as models for acromegaly [15]. To approximate GHD, a transgenic dwarf mouse line has been generated that expresses a GHR antagonist (GHA) discovered in our laboratory [16]. Lastly, we have disrupted the GHR gene (GHR-/-) to generate a

mouse model of Laron syndrome [17]. It is the only mouse that has complete inhibition of GH-GHR signaling [17]. Together, these mice provide unique tools to study brain development and function in the presence or absence of GHR signaling from conception through old age. A review of the studies conducted with the GHR-/- dwarf mouse strain, along with data from several other animal models, will be the primary subject of this review, with reference to human disease where appropriate.

2. EXPRESSION OF GHR IN THE BRAIN

Early experimental evidence established the presence of GH binding sites in the hypothalamus of rabbits [18] and chickens [19]. Another study revealed the GH binding capacity of human brain tissue [20]. In this initial study, adult male and female brain specimens were obtained 15-48 hours post mortem at autopsy and assayed for radio-labeled human GH (hGH) binding capacity [20]. The choroid plexus possessed the highest amount of GH binding, followed by the pituitary, with no significant difference between males and females. In a follow-up study, GH binding in multiple areas of the human brain was examined as a function of age [21]. GH binding was observed in the choroid plexus, hippocampus, hypothalamus, pituitary, putamen and thalamus. Co-treatment with an excess of prolactin (PRL) was performed to exclude lactogenic binding sites, which also bind hGH. GH binding was again observed to be highest in the choroid plexus and the pituitary. A decline in GH binding as a function of age was observed in all areas except for the thalamus [21]. GH-binding sites have also been found in multiple regions of the rat brain by Zhai *et al.* [22], who showed binding sites in the choroid plexus, hypothalamus, hippocampus, pituitary and spinal cord with a decrease in binding in an age dependent manner.

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Fig. (1). Mice with alterations of GH action. Left to right are WT, bGH transgenic, GHA transgenic and GHR^{-/-} mice.

The expression of GHR mRNA in the adult rat brain was shown by *in situ* hybridization, indicating that the GH binding sites that had been observed were likely due to specific binding to the GHR [23]. As anti-GHR antibodies became available, immunohistochemical techniques were used to analyze rat CNS tissue for GHR protein expression [1]. Rats at 2, 10, 25 and 90 days of age were examined. Ten day old rats showed near ubiquitous presence of GHR in varying degrees in the CNS, with immunoreactivity in all regions and cell types. There was a decline in GHR expression with increasing postnatal age of the animals. The presence of GHR mRNA was measured in all age groups. As with GHR immunoreactivity, there was a marked decline in GHR mRNA expression in the older groups of animals. As discussed by the authors [1], sites of GHR expression correlated with previously reported sites of insulin-like growth factor 1 (IGF-1) expression, implying that one of GH's actions in the brain may be the stimulation of local IGF-1 production. The presence of GHR mRNA in various regions of rat CNS was reported in the brain stem and spinal cord [24]. *In situ* hybridization experiments identified GHR in the arcuate nucleus of the hypothalamus, where GHR is known to be involved in the negative feedback loops that regulate GH production and secretion from somatotrophs of the pituitary. GHR was also found to be expressed in various regions of the pons, spinal cord and medulla oblongata. In these areas, GHR expression was detected in regions known to regulate food intake and sleep patterns, suggesting that GH may affect these biological processes by direct GH signaling in the brain [24]. The widespread presence of GH binding sites in various brain areas from multiple species indicates a biological role for GH in the CNS. The presence

of GHR protein in multiple brain areas further indicates the capacity for GH signaling in the brain.

3. GH IN BRAIN GROWTH AND DEVELOPMENT

Growth hormone has long been hypothesized to be important for growth and development of the brain [3]. If GH has a critical role in brain growth, there should be obvious abnormalities in GHR^{-/-} brain tissues. Most tissues in the GHR^{-/-} animals show allometric scaling. The brain, however, is one of few organs that are disproportionate in size relative to the size of this dwarf animal. While smaller in absolute terms, brains of GHR^{-/-} mice are approximately 50% larger than WT when normalized to body weight [25, 26]. Another model of reduced GH action, the dwarf GHA transgenic mouse, also displays an increase in brain weight relative to total body weight [27]. Concordant with this data, giant bGH transgenic mice display a decrease in brain size relative to body weight [28]. To further define the effect of GH disruption on brain growth, cross sectional areas were measured in GHR^{-/-} mice [26]. In striatal sections, the cortical, subcortical and striatal cross sectional areas were all significantly smaller than WT in absolute terms, ranging from a ~13% to ~22% reduction in size. In hippocampal sections, the cortical and subcortical areas showed a ~26% and ~23% reduction in size, respectively, compared to WT controls. Cortical somatosensory thickness, however, was not different in GHR^{-/-} animals. Even though GHR is widely expressed in the developing brain, these data indicate that GH is not a predominant regulator of early brain growth, which occurs largely prenatally, during a period of GH-independent somatic growth. Therefore, the function of GHR in the developing brain is not clear.

4. PRENATAL AND PERINATAL MOTONEURON DEVELOPMENT

Both GH and GHR have been shown to be widely expressed in the CNS of developing embryos, a finding highly suggestive of a role for GH signaling in neural development [1, 2, 29]. Additionally, GH has been shown to enhance proliferation of certain cells of the CNS. For example, incubation of fetal rat cerebral cortical cells with recombinant hGH results in increased proliferation in a time and dose-dependent manner [8]. GH is also reported to increase the proliferation of cortical cell precursors and astrocytes [8]. In mice, expression of GH and GHR peaks during embryonic day 14-18 (E14-E18), coincident with a period of CNS remodeling by way of a process known as programmed cell death (PCD), in which selective cell death and survival occur [30]. It is likely that this process involves both pro-apoptotic and protective anti-apoptotic signals. Given the well known anti-apoptotic actions of GH, and building upon previous work indicating neuroprotective properties of GH, Parsons *et al.* [30] hypothesized that GH may provide survival signals to selective cells during the process of PCD. To test this, they undertook studies using GHR^{-/-} mice to investigate the potential neurotrophic role of GH in motoneurons during prenatal and perinatal CNS development [30]. They reasoned that if GH possesses an anti-apoptotic role in the developing CNS, then mice lacking GHR signaling would show obvious differences in brain cell apoptosis compared to WT animals. Three time points were examined for gross morphology, E13.5, E18.5 and postnatal day 2 (P2). Body weight, spinal cord volume, and muscle fiber diameter were found to be no different from controls. Additionally, no differences were reported in muscle fiber organization, innervations or motor axonal branching. Also, no obvious differences in motoneuron nuclei appearance and position, spinal cord appearance or motoneuron location within the lateral motor columns were found. Finally, to evaluate whether GH has a neurotrophic effect during PCD, the total number of motoneurons was quantified at the beginning and end of PCD. No difference in motoneuron number in the brachial and lumbar lateral motor columns between WT and GHR^{-/-} embryos was reported [30]. While the number of cells was the same, the nuclear area (an indirect measure of cell size) was marginally but significantly smaller in GHR^{-/-} mice at E13.5 and E18.5, indicative of a role of GH in motoneuron maturation. These data show only minor differences in motoneuron development in mice that possess complete disruption of GH-induced GHR signaling. This is somewhat unexpected given the fact that GH is expressed in the CNS during development and both *in vitro* and *in vivo* evidence suggest a role for GH in neuronal survival. One possibility is that an intact PRL signaling pathway, which shares many downstream components with GH signaling, is potentially able to compensate for the lack of GHR activity in GHR^{-/-} mice.

5. NEURON POPULATIONS OF THE CNS

The near ubiquitous expression of GH and GHRs within the brain during development [1] suggests that alterations of

GH signaling may affect various neuron populations in the adult animals. Turnley and colleagues demonstrated that suppressor of cytokine signaling-2 (SOCS2), a negative regulator of Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling, is expressed in neuroepithelial cells and neurons during development of the CNS [31]. SOCS2^{-/-} mice, which have a loss of negative regulation of GHR-induced JAK/STAT signaling and thus increased GHR signaling, display a decrease of ~30% in neuron density in the somatosensory cortex when compared to WT littermates [31]. Conversely, GHR^{-/-} mice display a 23-26% increase over WT controls in overall neuron cell density in the same region, when quantified by immunohistochemical staining for neuronal nuclei protein, while total cell number was not different from WT [26].

Staining for specific types of neurons was performed in order to determine if the increased neural density was due to changes in specific subpopulations of cells [26]. Cortical interneuron subpopulations expressing calretinin and calbindin were both increased in density, along with cholinergic neurons in the striatum, while the density of parvalbumin positive neurons was not significantly altered in GHR^{-/-} animals. Not only was there an increase in the density of calretinin and calbindin positive interneurons, but cell counts revealed that there was also an increase in the number of these cells in the GHR^{-/-} cortex [26]. Astrocytes, on the other hand, were decreased in cell number in GHR^{-/-} animals. In addition, measures of cell soma size revealed a general decrease in cell size for both neurons and astrocytes.

These data indicate differential effects of GH signaling on subpopulations of CNS cells. While the GHR^{-/-} brain is smaller than WT brain in absolute terms, it appears that in general, the same number of neurons are packed in at a greater density. On the other hand, glial cells were decreased in both size and number, resulting in a decreased glial to neuronal ratio in GHR^{-/-} mice. Further studies are necessary to determine the neurological impact of this imbalance and to further define the exact roles that GH plays in astrocyte development and neurogenesis, as well as its role in various other sub-populations of neural cells.

6. THE PITUITARY AND HYPOTHALAMUS

The hypothalamic-pituitary axis functions in the coordinated release of endogenous hormones for the regulation of metabolism, gonadal function, and homeostasis. The anterior pituitary is composed of two populations of cells: basophiles and acidophiles. Basophile-derived hormones include follicle-stimulating hormone (FSH), leutinizing hormone (LH), adrenocorticotrophic hormone (ACTH), and thyroid stimulating hormone (TSH). Acidophile-derived hormones include GH and PRL. GH synthesis and secretion is tightly regulated by a number of feedback systems; several reviews [32-34] expound upon specific control mechanisms of endocrine GH synthesis and regulation. In short, neurons from the hypothalamus project to the pituitary where GH releasing hormone (GHRH) secretion stimulates GH gene expression and GH secretion while somatostatin (SRIF) secretion from another subset of hypothalamic neurons inhibits GH release. While GHRH and

SRIF hypothalamic neurons represent primary sites of GH feedback control, there also exists a “long-loop” feedback by which GH synthesis and secretion is inhibited by serum IGF-1. In addition, GH can attenuate its own production by participating in a “short-loop” feedback system in which GH acts *via* the hypothalamus to stimulate SRIF and inhibit GHRH secretion; GH also participates in an “ultra-short” feedback system in which GH acts directly upon somatotrophs. Ghrelin, a regulator of satiety, has also been shown to regulate GH production in the pituitary *via* the GH secretagogue receptor (GHS-R). In addition to these control mechanisms reviewed by Wong *et al.* [34], the organized three-dimensional network of somatotrophs within the pituitary may regulate GH secretion *via* direct cell to cell communication [35].

Feedback inhibition of GH production is predicated upon proper function of the GHR signaling cascade. Asa *et al.* [36] provided the first evidence that GH could directly inhibit GH production in somatotrophs. The study compared somatotroph morphology of giant transgenic mice expressing a GH agonist (E117L), dwarf transgenic mice expressing a GHA (G119K), and GHR^{-/-} mice. The dwarf GHR^{-/-} and GHA transgenic mice both demonstrated pituitary somatotroph hypersecretion. This was denoted by well-developed endoplasmic reticulae and large juxtannuclear Golgi complexes, in addition to increased populations of sparsely granulated focal-globular staining somatotrophs. The GHR^{-/-} mice additionally exhibited mild pituitary somatotroph hyperplasia signified by an increase in the number of GH-immunoreactive cells and a disrupted reticulin network with obvious acini expansion. The giant transgenic mice, while grossly larger than their littermates, showed no morphological changes in the somatotroph population. In subsequent studies, an overall increase in the number of GH-immunoreactive cells in GHR^{-/-} mice was confirmed [37, 38], with the total number of GH cells being disproportionately increased when normalized to body weight.

While populations of TSH and ACTH pituitary cells were not significantly different in GHR^{-/-} mice when compared to wild type littermates [37], the number of PRL cells was greatly reduced. This is surprising because plasma PRL levels have been shown to be normal or elevated in GHR^{-/-} mice [39]. Elevated serum PRL levels have also been demonstrated in Laron syndrome patients [40] and bGH mice [41]. These results imply that intact GHR signaling may be required to preserve normal PRL levels.

The loss of GH negative feedback in GHR^{-/-} mice results in hypothalamic and pituitary stimulation of GH production. Neuropeptide Y (NPY) is a hypothalamic inhibitor of GHRH release. Peng *et al.* [38] established that GHR^{-/-} mice express decreased levels of NPY mRNA and increased levels of GHRH mRNA. In addition, pituitary mRNA levels for GHRH receptor (GHRH-R) and GHS-R were significantly increased. Together these results further support the autoregulatory abilities of GH. Interestingly, NPY plays a role in stress management and may be central to the pathophysiology of psychiatric disorders such as anxiety and

PTSD. Decreased NPY levels have been associated with impaired memory, learning, and cognition [42].

In summary, the regulation of endocrine GH synthesis and secretion is complex — involving both central and peripherally derived modulators. GH feedback mechanisms are paramount to proper functioning of the pituitary. Loss of GH feedback causes changes in gross morphology of the pituitary, dysregulation of other pituitary hormones, and may influence psychiatric state and brain function.

7. GH IN MEMORY & COGNITION

More than 30 years ago, animal studies began to examine the role of GH in memory [9]. In a mouse study, GH was shown to decrease memory retention when administered prior to a learning event [9]. The presence of GHR in brain regions that participate in memory formation and recall, the hippocampus and amygdala [1], is suggestive of a role for GH in memory and cognition. More recently, GH mRNA expression has been shown to be induced in the hippocampus by a memory formation exercise termed trace conditioning [43]. Data on cognitive function in human patients with abnormalities in GH signaling have provided contradictory data. In one cohort of 48 GHD patients, the intelligence quotient (IQ) was found to be lower than the control group and hence, an interrelation between GH deficiency and cognitive dysfunction was proposed [44]. A deficit in memory in these GHD patients was also observed. Other research, however, shows high or normal cognitive ability in GHD patients, as reviewed by Forshee [14].

The GHR^{-/-} mice are long lived, surviving an average of 38-55% longer than WT controls [45]. Ames dwarf mice, another long-lived animal with impaired GH signaling, are similarly long-lived, surviving up to a year and a half longer than controls [46]. It is of interest to examine whether this extended longevity is a delay in the aging processes, which includes a decline in cognitive function, or simply an extension of “old age.” This has been evaluated by examination of memory retention as a function of age in animals with altered GH action [11, 12]. In a foot shock inhibitory avoidance test, 22-29 month old Ames dwarf mice performed as well as young WT or young Ames dwarf mice, while old WT mice had reduced performance compared to either young group [12]. In accordance with this data, young (6 months old) bGH transgenic mice resemble the aged 25 month old WT controls in exhibiting poor memory retention compared to young WT controls [47]. Memory retention has also been examined in a study of GHR^{-/-} mice [11]. In this experiment, both young (2-4 months) and old (17-20 months) GHR^{-/-} mice were examined, along with WT controls. The test performed consisted of applying an equivalent foot shock to the mice (adjusted for the reduced size of GHR^{-/-} animals) as they entered a compartment. Memory retention and resulting avoidance was measured at 24 hours, 7 days and 28 days after the initial shock. After 24 hours, there was no significant difference in avoidance behavior between the four groups, but by 7 days, the old WT mice showed a decline in memory retention compared to the young WT mice. This decline in avoidance was even more pronounced at the 28 day time point. Old GHR^{-/-} mice, on

the other hand, did not show any such decline in memory retention compared to young GHR^{-/-} or WT mice [11]. Therefore, it is concluded that memory retention is improved in old mice with absent or impaired GH signaling, suggesting not only that GH is not necessary for memory formation and retention, but also that the absence of GH signaling has beneficial effects on memory retention.

Further analysis was conducted by performing a water maze test with young and old WT and GHR^{-/-} mice [13]. In this experiment, spatial learning and memory were measured by recording the time taken to repeatedly swim to a hidden under water platform from different starting locations at different intervals following a training regimen. Old WT animals had lower spatial learning and memory performance than young WT controls, while the old GHR^{-/-} mice performed as well as young animals of both genotypes. While the mechanism of GH's effects on cognition are unknown, GH has been postulated to correlate with the activity and concentration of certain neurotransmitters in the cerebrospinal fluid (CSF) that may be involved. For example, a study of 24 patients with adult onset GH deficiency showed that rhGH administration caused a decrease in the CSF concentration of homovanillic acid (a metabolite of dopamine) and an increase in aspartate (an excitatory neurotransmitter involved in memory processing from the hypothalamus)[48].

The experimental observations described above indicate a putative role of GH in memory performance and cognition. Further investigations using animal models of altered GH action and human subjects will be helpful in defining the role of GH in the brain and the molecular mechanism behind this effect.

8. GH DYSREGULATION AND THE HUMAN BRAIN: ACROMEGALY, GHD AND LARON SYNDROME

The study of GH function in the human brain presents many unique obstacles. Deijen and Arwert review these difficulties [49]. In order to assess an individual's cognitive function, many branches of cognition must be addressed. Inherent to this assessment is the first problem: comparison among studies is difficult, as different batteries of exams are used to assess cognitive function. Secondly, differences among sampled patient populations might represent a confounding factor. For example, within GHD patients, isolated GH deficiency (IGHD) is often a disease that begins in childhood and manifests chronically while adult onset GHD (AO-GHD) is more acute, often due to a pituitary tumor, pituitary surgery, radiotherapy, or physical trauma to the head. This means that in AO-GHD other pituitary hormone levels are affected concurrently. Finally, there is the problem of ensuring an adequate control group. Most studies reference normal healthy individuals as a control group, effectively leading to open, non-placebo controlled, non-blinded studies.

In addition to procedural limitations, other difficulties lie in the complex biochemical effects of GH. For example, GH is known to increase the peripheral conversion of thyroxine (T4) to the more biologically active triiodothyronine (T3),

inhibit 11-beta hydroxysteroid dehydrogenase 1 (11-BHSD) which is responsible for the conversion of cortisone to cortisol, and enhance overall androgen production [49]. Elevated T3 levels have been linked to obesity [50], while changes in T3 levels have been associated with depression [51]. On the other hand, high levels of 11-BHSD1 are expressed in areas of the brain known to affect cognition such as the hippocampus and prefrontal cortex, and increased levels may play a role in depression [52, 53]. Also, local aromatization of testosterone to estrogens in the brain has been implicated in sex specific effects on cognition and higher level functions [54].

Hyper GH secretion after cessation of vertical growth and loss of a diurnal GH secretion pattern characterize acromegaly. Clinically, the most commonly presented symptoms are coarsened facial features, enlarged hands and feet, hypertension, and macroglossia [55]. Acromegaly and its effects on brain function are reviewed by Werner [56]. In short, there are no documented improvements in cognition due to chronically increased GH secretion. Additionally, there have been no reports of increased psychiatric morbidity or depression in patients with newly diagnosed, untreated acromegaly. What has been documented is a decrease in the quality of life in patients with acromegaly, probably due to the changes in habitus, decreased motility, and decreased energy levels. Interestingly, approximately 30% of patients with acromegaly also exhibit hyperprolactinemia which has been shown to decrease libido and cause difficulty in concentration and memorization. Finally, acromegaly patients have increased risk of neuropathies due to hypertrophy of perineural and endoneural tissue. One striking example is a case study report of a patient presenting with symptoms of both acromegaly and the rare Chiari-I malformation, likely due to neural covering and bony tissue changes from increased GH secretion [57].

While hypersecretion of GH, as evidenced by acromegaly patients, has yet to show any detrimental effects on cognition, studies on GHD patients demonstrate mixed results, with some studies indicating subnormal cognition and others reporting normal or enhanced cognition. One randomized, double-blind, placebo-controlled study in 40 male AO-GHD patients showed that after 18 months of GH replacement there were no significant improvements in cognition [58]. Deijen and Arwert [49] provide a thorough review on the cognitive status of AO-GHD patients. In general, AO-GHD patients show a decrease in attention and concentration with increased forgetfulness. Long term studies indicate a subnormal IQ. A meta-analysis of literature between 1985 and 2004 reported patient outcomes in GHD adults after GH replacement therapy [59]. Out of 15 studies in the meta-analysis only four reported data on cognitive function. Of these four, only two studies were placebo controlled. Results demonstrated improvement in patient-reported outcomes after 3, 6 and 12 months of GH replacement when compared to baseline. When compared to placebo, however, no significant improvement was recognized. Furthermore, cognitive function, after a median GH treatment duration of 6 months, also showed no significant improvement. Due to lack of controlled studies,

however, the effect of GH replacement on cognitive function was not compared to placebo. The authors conclude that further analysis is necessary to not only differentiate between CO-GHD and AO-GHD, but also to distinguish the effects of IGF-1 from those of GH in a standardized and controlled study [59].

Laron syndrome, or primary GH insensitivity, is caused by mutations in the gene coding for the GHR or in genes encoding proteins involved in intracellular signaling downstream of the receptor. Clinically, patients present just as those with IGHD with the only difference being that Laron patients will have high serum GH and undetectable IGF-1 levels. In a review by Laron [60], brain growth and motor development were delayed in Laron syndrome patients. MRI and CT scans showed various degrees of tissue loss, from normal to diffuse atrophy. Mental development also varied from normal to mental retardation. Untreated patients, when compared to age matched groups, demonstrated a lower than average IQ. In this review, Laron notes that hypoglycemia in infancy may contribute to the low intelligence scores. In a more recent study, specific molecular defects in the GHR gene were correlated with phenotypical findings on brain MRI [61]. In particular, the combined deletion of exons 3, 5, and 6 was associated with cerebellar atrophy, parenchymal loss and low IQ scores, while mutations in exon 6 resulted in no abnormal findings on brain MRI and a normal IQ score. Although these associations are based on only 10 patients, these results bring light to the idea that the different genetic dysfunctions of the GHR gene potentially have distinct biological effects, the nature of which is unknown. Further study of larger cohorts of patients that includes precise genetic and molecular characterization of the GHR gene and expression products could bring a better understanding to the mechanism by which GH signaling affects brain development and function.

9. SUMMARY

For more than 25 years, the brain has been known to possess the capacity to bind GH [62]. Subsequent characterization has shown that both GH and GHR are expressed in the CNS in a temporally and spatially regulated fashion. The precise role of GH in the CNS has been a challenging topic to study. Much of the data gathered has been contradictory and difficult to reconcile. Studies using the GHR^{-/-} “Laron Mouse” have provided some important clues. Brain and pituitary tissues from GHR^{-/-} animals are larger than controls, relative to body weight, which suggests that growth of these tissues may occur in a GH-independent manner. At the same time, morphologic changes in these tissues are evident, including changes in neural cell density. While the GHR^{-/-} brain is significantly smaller than the WT brain in absolute weight (though larger relative to body weight), the total number of neurons was equivalent. Also, changes in the pituitary due to GHR disruption include a decrease in lactotrophs accompanied by an increase in circulating PRL levels, suggesting that intact GHR signaling is necessary to maintain normal PRL levels. Dysregulation of the complex GH mediated feedback loops in the hypothalamus and pituitary causes significant changes in

somatotroph morphology in GHR^{-/-} and dwarf GHA mice. Prenatal and perinatal motoneuron development in the GHR^{-/-} animals is nearly indistinguishable from controls by most measures taken, including spinal cord volume, muscle fiber diameter and organization as well as the number of motoneurons.

In order to use this research to further our understanding of human disease, it is helpful to contextualize the data with respect to acromegaly, GHD, and Laron syndrome, which represent primary dysfunctions of GH secretion and/or signaling. Supraphysiologic GH levels in acromegaly have neither proved to be beneficial nor detrimental to brain function. Some researchers believe that the emerging picture for adult GHD patients is one of impaired cognition, however, there is limited data and further study is needed [63]. In Laron patients, the variety of psychological presentations, particularly the diverse phenotypes associated with different molecular defects of the GHR gene, further confounds the role of GH in the brain. The recent production of a Cre-lox mediated GHR disruption mouse, which allows for tissue specific deletion of GHR, will allow more refined studies to be undertaken [64]. Fundamental questions regarding the role of GH in brain development and function remain largely unanswered, including even the most basic question of whether intact GH signaling is necessary for proper brain development and function. While much progress has been made, perhaps the data to date has generated more questions than answers. There are currently mouse models that approximate acromegaly [15], Laron syndrome [17] and GHD [16]. Further use of these and other animal models, as well as continued work with controlled studies of human patients with acromegaly, GHD, and Laron syndrome, will help elucidate the intricate role that GH and IGF-1 play in the development and function of the brain.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENT

Declared none.

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Received: June 3, 2011

Revised: May 4, 2012

Accepted: May 4, 2012

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