Early-onset GH deficiency results in spatial memory impairment in mid-life and is prevented by GH supplementation

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Abstract

GH levels increase to high concentrations immediately before puberty then progressively decline with age. GH deficiency (GHD) originating in childhood is treated with GH supplementation to foster somatic development during adolescence. It is not clear if or how early GH replacement affects memory in adulthood, or whether it can prevent the cognitive deficits commonly observed in adults with childhood-onset GHD (EOGHD). Rats homozygous for the Dw-4 mutation (dwarf) do not exhibit the normal increase in GH at 4 weeks of age when GH levels normally rise and are used to model childhood or early-onset GHD (EOGHD). One group of these rats was injected with GH from 4 to 14 weeks of age to model GH supplementation during adolescence with GHD beginning in adulthood (adult-onset GHD; AOGHD). Another group received GH from 4 weeks throughout the lifespan to model normal lifespan GH (GH-replete). Age-matched, Dw-4 heterozygous rats (HZ) do not express the dwarf phenotype and were used as controls. At 8 and 18 months of age, spatial learning in the water maze was assessed. At 8 months of age all experimental groups were equally proficient. However, at 18 months of age, the EOGHD group had poor spatial learning compared to the AOGHD, GH-replete, and HZ groups. Our data indicate that GHD during adolescence has negative effects on learning and memory that emerge by middle-age unless prevented by GH supplementation.


Introduction

The levels of GH and its effector insulin-like growth factor 1 (IGF1) vary substantially throughout the lifespan (Nyberg 2000). Postnatal GH and IGF1 levels are low, but increase to higher concentrations immediately before puberty, and then progressively decline with increasing age. Deficits of GH in childhood can be due to a congenital deficiency or acquired in either childhood or adulthood due to hypothalamic–pituitary tumors, GH insensitivity, or disorders involving GH secretion. The clinical emphasis on treating childhood-onset GH deficiency (COGHD) currently focuses on the somatic role of GH in increasing body size (mainly height) during puberty (Clayton et al. 2007, Nilsson et al. 2007). Continued GH treatment during this important transition period between adolescence and adulthood may be important for the normal development of bone and muscle in adulthood (20–30 years old) (reviewed in Clayton et al. (2007) and Nilsson et al. (2007)). As the body continues to develop into young adulthood in humans, the brain also continues to mature (Rice & Barone 2000). The effects of COGHD on cognition in adulthood indicate that it causes impairments on measures of hippocampal/medial temporal lobe function. For example, a study of 24-year-old subjects with COGHD and age-matched controls indicated that COGHD results in poor performance on delayed verbal memory when compare to age-matched controls (van Dam et al. 2005). However, it is not clear if the adult-onset of this memory impairment can be remediated by GH supplementation earlier in the lifespan.

Dwarf (Dw-4/Dw-4) rats have chronically low levels of GH and IGF1 and make an excellent animal model of COGHD (Charlton et al. 1988). These rats have a spontaneous mutation that results in decreased GH secretion from the pituitary (Charlton et al. 1988, Carter et al. 2002a,b). A relatively circumscribed exposure of the dwarf rats to GH during postnatal weeks 4–14 (peripubertal to young adulthood life stages) influences the timing of intra-cerebral hemorrhage or cardiac thrombus later in life and increases the lifespan by 15% (Sonntag et al. 2005). This has led to the conclusion that manipulation of GH levels for a brief transitional period starting just around puberty and ending in young adulthood.
is sufficient to alter age-related pathology later in life. It is not known if this circumscribed exposure also influences cognition in adulthood and midlife.

The present study was designed to further explore the consequences of alterations in the peripubertal rise in GH on memory in adult animals. In the Lewis dwarf rats, puberty begins around postnatal day (PD) 35–36, just after the increase in GH pulsatility and the subsequent IGF1 increase, which begins around PD28. Brain development continues through adolescence to young adulthood (PD63–70) (McCutcheon & Marinelli 2009). Because of the high density of GH and IGF1 receptors in the hippocampus (Adem et al. 1989, Lai et al. 1993, Sonntag et al. 1999) and the finding that IGF1 infusion can restore the age-related decline in hippocampal-dependent memory (Markowska et al. 1998), hippocampal-dependent memory was assessed using a standardized assessment of spatial learning in a water maze that has been demonstrated to be sensitive to the effects of age (Gallagher et al. 2003, LaSarge & Nicolle 2009). For this study, we examined spatial learning at 8 and 18 months of age in dwarf animals treated with vehicle alone to model COGHD (early-onset GHD; EOGHD). The spatial learning ability of the EOGHD rats was then compared with dwarf rats treated from 4 to 14 weeks of age to model a GH intervention in the periadolescent stage (adult-onset GHD; AOGHD). Long-term GH replacement (GH-replete) throughout the lifespan of dwarf rats was used to model lifetime supplementation with GH. In addition to the manipulations of GH in the dwarf rats, littermates that were heterozygous (HZ) for the autosomal recessive Dw-4 mutation (non-dwarf phenotype) were used as controls to model normal lifetime supplementation with GH. Our results support the conclusion that GHD phenotype) was used as controls to model normal life-time supplementation with GH. In addition to the manipulations of GH in the dwarf rats, littermates that were heterozygous (HZ) for the autosomal recessive Dw-4 mutation (non-dwarf phenotype) were used as controls to model normal life-time levels of GH. Our results support the conclusion that GHD during the periadolescent transition period results in deficits in learning and memory that are manifest at midlife. Furthermore, GH supplementation during this period, but not beyond, is sufficient to prevent and/or reduce this early decline in learning and memory.

Materials and Methods

Subjects and experimental design

Subjects are male Lewis rats that are heterozygous or homozygous for the spontaneous autosomal recessive Dw-4 mutation which causes a decrease in GH secretion from the pituitary gland (Charlton et al. 1988). Female heterozygous (Dw-4/−) Lewis rats were bred with male homozygous Lewis dwarf rats (Dw-4/Dw-4) to generate heterozygous (Dw-4/−) offspring with a normal phenotype (HZ) or homozygous rats (Dw-4/Dw-4) with a dwarf phenotype (dwarf). Classification as HZ or dwarf was based on their body weight at 28 days of age. Beginning on day 30, dwarf rats were divided into three experimental groups: 1) EOGHD given saline, 2) AOGHD with GH administered between 4 and 14 weeks of age, or 3) GH-replete with GH administered beginning at 4 weeks of age and continued throughout the lifespan. Saline or GH (200 μg recombinant porcine GH, Alpharma, Victoria, Australia) was s.c. injected twice daily. The HZ rats were used as controls and given saline injections twice daily from 4 weeks of age to the end of life. Terminology and treatment groups are described in Table 1. Two experimental cohorts were studied, one with water maze testing at 8 months of age and another with testing at 18 months of age. The first cohort had the following number of subjects per groups: HZ, 9; EOGHD, 5; AOGHD, 10; and GH-replete, 10. The second cohort had the following number of subjects per group: HZ, 14; EOGHD, 5; AOGHD, 13; and GH-replete, 13. Rats had access to food and water ad libitum and were housed in pairs in the vivarium of the Department of Physiology and Pharmacology at Wake Forest University Medical School. Body weights were measured prior to spatial learning assessment. All studies were approved by the Wake Forest University Institutional Animal Care and Use Committee.

Spatial learning assessment

Rats were tested in a water maze at either 8 or 18 months of age based on a standard assessment of spatial learning ability (Gallagher et al. 1993). The maze consisted of a circular pool (diameter, 1.83 m and height, 0.58 m) with an escape platform centered in one of the four maze quadrants. Rats were trained over 12 sessions (three trials/day) to locate an escape platform hidden just below the water’s surface. There were two types of trials, training trials and probe trials. During a training trial, the animal was allowed to swim for 90 s to locate the platform. Every sixth trial consisted of a 30 s probe trial to assess the development of a spatially localized search for the escape platform. During the probe trial, the platform was made unavailable for escape. The accuracy of the search

<table>
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<tr>
<th>Group/model</th>
<th>Genetic background</th>
<th>Treatment: 4–14 weeks of age</th>
<th>Treatment: 14 weeks of age until sacrifice</th>
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<tbody>
<tr>
<td>Heterozygous (HZ)</td>
<td>Dw-4/−</td>
<td>Saline vehicle, twice daily</td>
<td>Saline vehicle, twice daily</td>
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<td>Early-onset GH deficiency (EOGHD)</td>
<td>Dw-4/Dw-4</td>
<td>Saline vehicle, twice daily</td>
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<td>Dw-4/Dw-4</td>
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<td>GH replete</td>
<td>Dw-4/Dw-4</td>
<td>GH, twice daily</td>
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for the platform location was measured by the proximity to the location for 30 s with the subject’s position sampled ten times/s and summed. There were four interpolated probe trials, the sum of which was used to generate a composite score. Lower proximity scores represent a more accurate search. Cue training to a visible platform occurred on the last day of training to test for sensorimotor and motivational factors independent of spatial learning. Cue training consisted of six sequential trials with randomized start and visible platform locations.

**Measurement of plasma IGF1**

Rats were anesthetized with isoflurane and plasma was obtained via tail bleed within 1 week of behavioral assessment. Total IGF1 levels in plasma tissue homogenates were determined using R&D Systems Quantikine mouse IGF1 Immunoassay (MG100; Minneapolis, MN, USA) as previously described (Adams et al. 2009). Briefly, blood was collected into tubes with 38 units porcine heparin per milliliter, incubated on ice, and centrifuged. The plasma was collected and stored in aliquots at −80 °C. For IGF1 measurement, samples were thawed to room temperature, gently vortexed, diluted serially in kit calibrator diluent at 1:2091 final, and assayed according to the manufacturer’s instructions. Results are reported as nanogram IGF1 per milliliter plasma.

**Statistical analysis**

Training trial (hidden platform) data from the water maze were analyzed using repeated-measures ANOVA with group as a factor. Significant interactions were followed with Fisher’s protected least significant difference (PLSD). Other measures were analyzed using a one-way (group) ANOVA unless otherwise specified. Data were analyzed within a specific age group (8 or 18 months of age) and not compared across ages due to the design of the experiment.

**Results**

**Spatial learning**

Spatial learning ability was assessed using a standardized protocol in the water maze (Gallagher et al. 2003, LaSarge & Nicolle 2009). At 8 months of age, all groups became more proficient with increased training during the acquisition phase of the water maze indicated by a main effect of training trial block (F(3,4) = 137.81, P < 0.0001; Fig. 1A). Overall, there were no differences between the groups in acquisition of the task during training trials (Fig. 1A) or in the accuracy of their searches during probe trials (Fig. 1B). At 18 months of age (Fig. 1C), a main effect of block indicated that all groups performed more accurately by the end of training and acquired the task (F(3,3) = 174.63, P < 0.0001; Fig. 1C). A main effect of group (F(3,41) = 8.88, P ≤ 0.0001), an interaction between group and block (F(3,9) = 3.85, P < 0.0005) and a subsequent Fisher’s PLSD post hoc, however, indicated that the HZ group performed significantly better than all other groups (P < 0.05, each) and that the EOGHD group performed significantly poorer than all other groups (P < 0.05, each; Fig. 1C). There were no significant differences in spatial learning between AOGHD or GH-replete groups indicating no further effect of continuing GH supplementation after the 10-week treatment period. Overall, the data indicate that by 18 months of age, AOGHD rats receiving GH supplementation from 4 to 14 weeks of age demonstrate cognitive performance that is better than that of EOGHD rats, but not fully improved to the level of the HZ group.

The probe trial data in Fig. 1D shows a similar pattern to the acquisition data described above: the HZ group had a significantly more accurate search for the escape platform compared to all other groups (main effect: F(3,41) = 10.37, P < 0.0001, post hoc, P < 0.05) and the EOGHD group had the least accurate search in finding the location of the platform.
compared to all other groups ( Fisher’s PLSD post hoc, P<0.05). No significant differences in search accuracy were observed between AOGHD and GH-replete rats, indicating that additional GH supplementation after 14 weeks of age were not of added value in the prevention of spatial learning decline at 18 months of age.

Swim speed and visual ability were measured during the visible platform test (cue training). HZ and the dwarf groups were not different, nor was there an effect of GH treatment in the dwarfs at either 8 or 18 months of age (data not shown). These data indicate that the differences in spatial learning and memory ability are due to deficits in learning, and not due to group differences in sensory or motor ability.

Plasma IGF1 levels and body weight

IGF1 levels were measured in plasma obtained from tail blood 1 week after water maze testing. Figure 2 shows that at both 8 and 18 months of age, HZ and dwarf GH-replete rats had significantly higher plasma IGF1 levels compared to the EOGHD and AOGHD rats (P<0.05, each), indicating that twice daily administration of GH to the dwarf rats increases plasma IGF1 and that discontinuation of GH administration at 14 weeks of age results in a return to GH-deficiency (main effect of group, 8 months: F(3,27)=56.66, P<0.0001; 18 months: F(3,39)=12.73, P<0.0001). At 8 months of age, the GH-replete group had slightly higher levels of IGF1 compared to the HZ group (P<0.005), whereas at 18 months the GH-replete group had slightly lower levels than the HZ group (P<0.05). These data could indicate an upregulation of some signaling mechanisms at 8 months of age that become less responsive by 18 months of age. There were no significant differences in IGF1 plasma levels between the AOGHD and EOGHD groups at either age. The HZ and GH-replete rats expressed the typical phenotype of adequate GH levels, indicated by increased body weight (Fig. 3). At both ages, the HZ and GH-replete rats weighed significantly more than the AOGHD and EOGHD groups (8 months; main effect of AOGHD) (van Dam et al. 2005). GH supplementation in dwarf rats during the 10-week period to model AOGHD improved memory at 18 months of age, as indicated by the comparable spatial learning and memory ability between the HZ and AOGHD groups. Interestingly, the additional supplementation after 14 weeks of age (GH-replete) did not provide any additional therapeutic benefit. These data provide supportive evidence for the existence of an important period of the lifespan during which elevated levels of GH are necessary to delay or prevent cognitive impairment in mid-life.

Discussion

The data presented in the current study demonstrate that the absence of the rise of GH in dwarf rats between 4 and 14 weeks of age (EOGHD) results in spatial learning and memory impairment at 18 months of age compared to HZ rats. These results are in accordance with human studies that show a greater disturbance in cognitive function in EOGHD patients than in those that become GH deficient later in life (AOGHD) (van Dam et al. 2005). GH supplementation in dwarf rats during the 10-week period to model AOGHD improved memory at 18 months of age, as indicated by the comparable spatial learning and memory ability between the HZ and AOGHD groups. Interestingly, the additional supplementation after 14 weeks of age (GH-replete) did not provide any additional therapeutic benefit. These data provide supportive evidence for the existence of an important period of the lifespan during which elevated levels of GH are necessary to delay or prevent cognitive impairment in mid-life.

The peripubertal rise in GH, independent of GH levels in adulthood, may exert an unique effect on the brain and permanently alter brain neurochemistry and/or the neuronal circuitry necessary for the development and maintenance of neural circuitry in adulthood. This hypothesis is congruent with the clinical data indicating that continued GH administration throughout the transitional period between puberty and adulthood in COGHD patients may be necessary for normal somatic development manifest later in adulthood (Clayton et al. 2007, Nilsson et al. 2007, McCutcheon & Marinelli 2009). In the human brain, diffusion tensor magnetic resonance imaging has shown continued maturation.
of certain brain connections, particularly fronto-temporal connections, into the second and third decades of life (Lebel et al. 2008). There is also evidence in humans and rodents that myelination and changes in dopaminergic neuron firing rate occur throughout the transitional adolescent period (Wiggins 1982, Hunter et al. 1997, Giedd et al. 1999). In the rat, there is evidence of continued brain maturation, measured by dopamine receptor levels, until postnatal day 60 (PND 60, 8 weeks of age) (Tarazi & Baldessarini 2000), and some forms of hippocampal-dependent learning, such as spatial delayed alternation, are not evident until PND 40 (Stanton 2000). Thus, endocrine modulators such as GH and IGF1 reach peak levels just at precisely the time of formation and maintenance of critical connections. Dysfunctional regulation of brain development may be subtle and not fully apparent until revealed by the additional insult of increased age.

In humans, the effect of COGHD or AOGHD on cognition in adulthood generally has been associated with attentional and memory deficits along with mood disorders (Frankel & Laron 1968, Laron & Galatzer 1981, Galatzer et al. 1993, Falleti et al. 2006, van Nieuwpoort & Drent 2008). For example, individuals with COGHD exhibit delayed verbal memory recall and deficits in the Trail Making Test A (a test of planning, processing speed and attention) and these deficits are associated with reductions in the brain N-acetylaspartate/choline ratio, a marker of neuronal integrity (van Dam et al. 2005). The optimal GH supplementation strategy to improve deficits in cognition in GHD patients is still highly debatable but there is some evidence that discontinuation of supplementation during the transitional adolescent period may impact somatic development (e.g. see Clayton et al. (2007)). GH substitution therapy does improve long-term and working memory in 27-year-old COGHD patients treated for 6 months (Arwert et al. 2006). Whether or not GH supplementation during the adolescent stage has greater impact than supplementation in adulthood is not known.

Previously published findings and the results reported in the present study provide compelling evidence that the administration of GH to deficient individuals in adolescence results in pleiotropic actions that are manifest late during the lifespan. For example, replacement of GH to dwarf rats only from postnatal week 4 through week 14 produces AOGHD and increases lifespan by 15% (Sonntag et al. 2005). This relatively circumscribed exposure to GH during postnatal weeks 4–14 also appears to influence the timing of intracerebral hemorrhage or cardiac thrombus in the older brain (Sonntag et al. 2008). These results have led to the conclusion that manipulation of GH for a relatively brief period during the transitional period between puberty and young adulthood is sufficient to alter age-related pathology later in life, although future studies are needed to identify the explicit boundaries of the optimal treatment time. The present data add to our understanding of this important period of brain growth by indicating that the absence of GH during this time period is also a risk factor for the early emergence of cognitive deficits at middle age.

In conclusion, our results indicate that GHD in adolescence deleteriously impacts brain function later in life and may result in the early emergence of age-related deficiencies in learning and memory. Supplementation of dwarf rats with GH during this period results in performance comparable to that of HZ rats and additional GH supplementation after 14 weeks of age has no additional benefits. The specific mechanisms for the effects of GH during the transitional period remain to be determined, but may include the formation or stabilization of the microcircuitry of essential synaptic transmission (Ramsey et al. 2005, Mahmoud & Grover 2006).

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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