Local overexpression of GH and GH/IGF1 effects in the adult mouse hippocampus

Marion Walser¹, Maria Teresa Samà^{1,2}, Ruth Wickelgren³, Maria Åberg^{4,5}, Mohammad Bohlooly-Y⁶, Bob Olsson⁷, Jan Törnell⁸, Jörgen Isgaard¹ and N David Åberg^{1,4}

¹Laboratory of Experimental Endocrinology, Department of Internal Medicine, Sahlgrenska University Hospital, University of Gothenburg, Gröna Stråket 8, SE-41345 Göteborg, Sweden

²Endocrinology, Department of Clinical and Experimental Medicine, University of Piemonte Orientale A. Avogadro, Novara, Italy

³Department of Clinical Chemistry and Transfusion Medicine, Sahlgrenska Academy, University of Gothenburg, SE 41345 Gothenburg, Sweden

⁴Institute of Neuroscience and Physiology, Center of Brain Repair and Rehabilitation and ⁵Department of Primary Health Care, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, SE 41345 Göteborg, Sweden

⁶AstraZeneca Research and Development, SE 43183 Mölndal, Sweden

⁷Clinical Neurochemistry Laboratory, Department of Neurochemistry and Psychiatry, Institute of Neuroscience and Physiology, SE 43180 Mölndal, Sweden

⁸InnoExt AB, Haraldsons väg 10B, SE 42167 Västra Frölunda, Sweden

(Correspondence should be addressed to N D Åberg at Laboratory of Experimental Endocrinology, Department of Internal Medicine, Sahlgrenska University Hospital, University of Gothenburg; Email: david.aberg@medic.gu.se)

Abstract

GH therapy improves hippocampal functions mainly via circulating IGF1. However, the roles of local GH and IGF1 expression are not well understood. We investigated whether transgenic (*TG*) overexpression in the adult brain of bovine GH (*bGH*) under the control of the glial fibrillary acidic protein (GFAP) promoter affected cellular proliferation and the expression of transcripts known to be induced by systemic GH in the hippocampus. Cellular proliferation was examined by 5-bromo-2'-deoxyuridine immunohistochemistry. Quantitative PCR and western blots were performed. Although robustly expressed, *bGH-Tg* did not increase either cell proliferation or survival. However, *bGH-Tg* modestly increased *Igf1* and *Gfap* mRNAs, whereas other GH-associated transcripts were unaffected, i.e. the GH receptor (*Ghr*), IGF1 receptor (*Igf1r*), 2',3'-cyclic nucleotide 3'-phosphodiesterase (*Cnp*), ionotropic glutamate receptor 2a (*Nr2a* (*Grin2a*)), opioid

receptor delta (*Dor*), synapse-associated protein 90/postsynaptic density-95-associated protein (*Sapap2* (*Dlgap2*)), haemoglobin beta (*Hbb*) and glutamine synthetase (*Gs* (*Glul*)). However, IGF1R was correlated with the expression of *Dor*, *Nr2a*, *Sapap2*, *Gs* and *Gfap*. In summary, although local *bGH* expression was robust, it activated local IGF1 very modestly, which is probably the reason for the low response of previous GH-associated response parameters. This would, in turn, indicate that hippocampal GH is less important than endocrine GH. However, as most transcripts were correlated with the expression of IGF1R, there is still a possibility for endogenous circulating or local GH to act via IGF1R signalling. Possible reasons for the relative bio-inactivity of *bGH* include the bell-shaped dose-response curve and cell-specific expression of *bGH*.

Journal of Endocrinology (2012) **215**, 257–268

Introduction

In humans, GH deficiency (GHD) is associated with an impaired quality of life (McGauley 1989, Bengtsson *et al.* 1993, McGauley *et al.* 1996, Burman & Deijen 1998), as well as poor memory and executive functions (Falleti *et al.* 2006). GH treatment alleviates these conditions in humans and in experimental animals (Deijen *et al.* 2005, Aberg *et al.* 2006, Le Greves *et al.* 2006, Devesa *et al.* 2011, Molitch *et al.* 2011, Reimunde *et al.* 2011). In particular, GH therapy may affect hippocampus-related functions, such as spatial memory, both

in humans (Falleti *et al.* 2006) and in animals (Le Greves *et al.* 2006). In the adult rodent brain, systemic GH affects the brain through various mechanisms, including cellular proliferation, neuroprotection and regeneration (Aberg *et al.* 2006). Circulating insulin-like growth factor 1 (IGF1) has been proposed to mediate the effects of endocrine GH. Indeed, peripheral treatment with either GH or IGF1 increases hippocampal neurogenesis almost twofold (Aberg *et al.* 2000, 2010), and antiserum against IGF1 blocks the increase in hippocampal neurogenesis that occurs after physical exercise (Carro *et al.* 2001). These reports support the hypothesis that

circulating IGF1, presumably acting via the IGF1 receptor (IGF1R), is the mediator of endocrine GH in relation to hippocampal cell proliferation.

In recent years, local expression of IGF1 and extra-pituitary GH in the brain has come under increased scrutiny. Indeed, the expression of *GH* mRNA in different parts of the brain has been reported (Donahue *et al.* 2002, 2006, Sun *et al.* 2005, Tang *et al.* 2011). In addition, extrapituitary GH protein has been detected in the brains of hypophysectomised rats, which lack circulating GH, implying local synthesis of GH in the brain (Hojvat *et al.* 1982). Local extrapituitary GH in the brain appears to be the subject of regulation. For example, it is upregulated when circulating GH is depleted (Hojvat *et al.* 1982), in animals with genetic deficiency for GH–IGF1 (Sun *et al.* 2005) and living in a stressful environment (Donahue *et al.* 2006). In analogy, IGF1 (D’Ercole *et al.* 1996, Folli *et al.* 1996) and IGF1R (Yan *et al.* 2011) are expressed locally in diverse areas throughout the brain. Therefore, there is strong evidence of local expression and regulation of both GH and IGF1 in the brain.

While GH treatment of patients and animals with GHD appears to be beneficial (see above), the overexpression of GH may have negative effects. For example, in humans, untreated naïve acromegaly is associated with cognitive impairment (Leon-Carrion *et al.* 2010). In mice, both the systemic transgenic (TG) overexpression of bovine GH (*bGH*) (Soderpalm *et al.* 1999) and (albeit to a lesser degree) local brain overexpression of *GH* affect the brain and the body (Bohlooly-Y *et al.* 2005b). Specifically, brain-specific *bGH* overexpression increases body weight and alters (mostly negatively) several cardiac measures of function (Bohlooly-Y *et al.* 2005a), as well as increases eating behaviour (Bohlooly-Y *et al.* 2005b). Two previous studies using TG models affecting GH signalling in the developing and adult brain have demonstrated an influence on the number of astrocytes in the cerebral cortex (Turnley *et al.* 2002, Ransome *et al.* 2004). Specifically, disruption of GH

signalling, via GH receptor (GHR^{−/−}), decreased astrocyte numbers and increased neuron numbers (Ransome *et al.* 2004), while increasing GH signalling via disrupting the suppressor of cytokine signalling 2 (SOCS2) decreased neuron numbers and presumably increased astrocyte numbers (Turnley *et al.* 2002). Neither from these studies nor from our previous studies using glial fibrillary acidic protein (GFAP)-induced *bGH*-Tg, is it known whether hippocampal function is affected, as hippocampus-related parameters were not investigated.

While there is some evidence that brain *bGH* overexpression affects the brain and that endogenous expression of local GH can be regulated, there are no reports on whether brain-specific GH can actually induce effects that are normally associated with circulating GH. We hypothesised that overexpression of *bGH* in the adult hippocampus would elicit a similar response in selected response elements as circulating GH. Secondly, we hypothesised that these response elements could be associated with the local GH–IGF1 system in the hippocampus. We used a unique model involving *Gfap*–*bGh* TG mice to study whether the local overexpression of *bGh* in the brain influences the adult brain (Bohlooly-Y *et al.* 2001). As it is well known that hippocampus-related behaviours and neurobiology respond to circulating GH, we investigated hippocampal cell turnover by 5-bromo-2′-deoxyuridine (BrdU) immunohistochemistry and we measured the expression of hippocampal mRNA in response to local overexpression of GH. In particular, using quantitative PCR (qPCR), we investigated the expression levels of *Ghr*, *Igf1*, *Igf1r*, 2′,3′-cyclic nucleotide 3′-phosphodiesterase (*Cnp*), glutamine synthetase (*Gs* (*Glul*)), *Gfap*, opioid receptor delta (*Dor*), ionotropic glutamate receptor 2a (*Nr2a* (*Grin2a*)), synapse-associated protein 90/postsynaptic density-95-associated protein (*Sapap2* (*Dlgap2*)) and haemoglobin beta (*Hbb*) (Tables 1 and 2). Western blots of the IGF1, IGF1R, mouse GH (mGH), *bGH* and GFAP

Table 1 Commercially available assay-on-demand mixes of primers and TaqMan MGB probes (FAM dye-labelled)

Symbol	Full name	Acronym in text	Assay number
Transcript data			
<i>Gh</i>	Growth hormone	<i>Gh</i>	Mm00433590_g1
<i>Ghr</i>	Growth hormone receptor	<i>Ghr</i>	Mm00439093_m1
<i>Igf1</i>	Insulin-like growth factor 1	<i>Igf1</i>	Mm00439560_m1
<i>Igf1r</i>	Insulin-like growth factor 1 receptor	<i>Igf1r</i>	Mm00802831_m1
<i>Oprd1</i>	Opioid receptor, delta 1	<i>Dor</i>	Mm00443063_m1
<i>Grin2a</i>	Glutamate receptor, ionotropic, 2a	<i>Nmda2a/Nr2a</i>	Mm00433802_m1
<i>Dlgap2</i>	Discs, large (<i>Drosophila</i>) homologue-associated protein 2 synapse-associated protein 90/postsynaptic density-95-associated protein	<i>Sapap2</i>	Mm00556670_m1
<i>Hbb-b1</i>	Haemoglobin, beta adult major chain	<i>Hbb</i>	Mm01611268_g1
<i>Cnp</i>	2′,3′-Cyclic nucleotide 3′-phosphodiesterase	<i>Cnp</i>	Mm01306640_m1
<i>Glul</i>	Glutamate-ammonia ligase (glutamine synthetase)	<i>Gs</i>	Mm00725701_s1
<i>Gfap</i>	Glial fibrillary acidic protein	<i>Gfap</i>	Mm01253033_m1
<i>Gh</i>	Bovine growth hormone	<i>Bgh</i>	Bt03224658_g1
<i>Ppia</i>	Peptidylprolyl isomerase A=cyclophilin A	<i>Ppia</i>	Mm02342429_g1

Table 2 Key references for the transcripts that are predominantly expressed in glial or neuronal cells

Group	Acronym/abbreviation	Reference(s)
Key references on transcripts		
Neuron-related	<i>Dor</i> <i>Nmda2a/Nr2a</i> <i>Sapap2</i> <i>Hbb</i>	Persson <i>et al.</i> (2003) and Iwata <i>et al.</i> (2007) Le Greves <i>et al.</i> (2002, 2006) Cho <i>et al.</i> (1992) and Takeuchi <i>et al.</i> (1997) Ohyagi <i>et al.</i> (1994), He <i>et al.</i> (2009) and Walser <i>et al.</i> (2011)
Glia-related	<i>Cnp</i> <i>Gs</i> <i>Gfap</i>	Aberg <i>et al.</i> (2007) Albrecht <i>et al.</i> (2007) Pekny <i>et al.</i> (1995)

proteins were performed to further evaluate how bGH-Tg affected the brain. To gain a better understanding of the components of the GH and IGF1 systems to which these transcripts are linked, correlation matrices were generated for the transcripts.

Materials and Methods

Animals

The experimental procedure for the generation of the *bGh* TG mice has been reported previously (Bohlooly-Y *et al.* 2001). Briefly, the *bGh* gene was ligated into a plasmid that contained the GFAP promoter. The *Gfap-bGh* DNA fragment was excised by restriction enzyme SfiI cleavage and microinjected into fertilised C57BL/6J×CBA zygotes to generate the TG mice.

The environment of the animal rooms was maintained in a 12 h light:12 h darkness cycle, relative humidity of 45–55% and temperature of 20 °C. The mice had free access to tap water and standard pellet chow (R-34; Lactamin, Vadstena, Sweden).

Eleven male, adult, Wt mice and 12 male, adult, TG mice that overexpressed *bGH* (bGH-Tg) in the CNS were selected. These animals were from the third back-crossed generation (N3) towards C57/BL. This founder line exhibited the lowest level of *bGH* expression in the brain with the least systemic effect, compared with previous publications that have described a bGH-Tg strain from another founder line. The latter animals exhibited significant increases in body weight, serum bGH and serum IGF1 (Bohlooly-Y *et al.* 2001, 2005a,b), probably due to leakage of bGH from the brain into the circulation. As we wanted to study the local expression of bGH in the brain, we used a founder line without significant effects on body weight or serum IGF1. The animals in both groups were 6 months old at the start of the experiment. For the labelling of mitotic cells, all mice received an i.p. injection of BrdU (50 mg/kg freshly dissolved in sterile water; Boehringer Mannheim, Mannheim, Germany), once per day for 5 days. After the injection of BrdU, mice were killed on day 1 (i.e. at 6 months of age) and day 30 (i.e. at 7 months of age) to evaluate cellular proliferation and survival over time

respectively. For the mRNA expression analysis by qPCR, data from the two time points were pooled. The mice were killed by decapitation. Body weights, total brain weights and hippocampal weights were recorded. This study was performed with the approval of the Ethics Committee for Animal Experimentation of the University of Gothenburg, Sweden.

Serum IGF1 analysis

Serum was collected after decapitation of the mice. The IGF1 concentration in the serum was determined by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) after acid–ethanol extraction, according to the manufacturer's protocol in an assay with double samples of each serum sample (Bohlooly-Y *et al.* 2001). The same assay tested in the central laboratory at the Sahlgrenska University Hospital (University of Gothenburg) showed an inter-assay variability of 4.7% and the intra-assay variability was 3.8%, and the stated detection limit was 0.09 ng/ml when human serum IGF1 was tested.

Immunohistochemistry and cell quantification

Coronal sections (20 µm in thickness) obtained using a freezing microtome were stored in a cryoprotectant (25% ethylene glycol plus 25% glycerine in 0.05 M phosphate buffer) at 22 °C before immunohistochemistry.

Immunohistochemistry was performed on free-floating 20 µm-thick sections that were pre-treated with 0.6% H₂O₂ in Tris-buffered saline (TBS; 0.15 M NaCl; 0.1 M Tris–HCl (pH 7.5)) for 30 min to block endogenous peroxidase activity. The DNA was denatured (Aberg *et al.* 2000) before incubation with mouse anti-BrdU antibody (1:400; Boehringer Mannheim). DNA denaturation was performed by incubation in 50% formamide and 2×saline-sodium citrate buffer (SSC; 1×SSC contains 0.3 M NaCl, 0.03 M sodium citrate) for 2 h at 65 °C, rinsing for 15 min in 2×SSC, incubation for 30 min in 2 M HCl at 37 °C and rinsing again for 10 min in 0.1 M boric acid (pH 8.5). Subsequently, the tissue was rinsed several times in TBS, followed by incubation for 30 min in TBS, 0.25% Triton X-100 and 3% normal horse serum (TBS-TS) and incubation with the primary antibody in TBS-TS overnight at 4 °C. The tissue sections were then incubated for 2 h with biotinylated horse anti-mouse

IgG (1:160) secondary antibodies (Vector Laboratories, Burlingame, CA, USA) and rinsed in TBS. Finally, an avidin–biotin–peroxidase complex was applied for 1 h, followed by 5 min of peroxidase detection with 0.25 mg/ml diaminobenzidine, 0.01% H_2O_2 and 0.04% NiCl.

The BrdU-positive cells were counted manually, while the volume of the granule cell layer was determined by digitalised area analysis (Aberg *et al.* 2000). Briefly, area estimations were obtained by planimetry using a Lucivid device (MicroBright-Field, Colchester, VT, USA) attached to a Leica microscope (Leica Microsystems GmbH, Wetzlar, Germany). In the hippocampus, the number of cells in the subgranular and granular zones was counted, and the values obtained were divided by the granule cell layer volume, thereby yielding the cell density (cells/mm³).

A section thickness of 20 µm was used in the dissector estimation of volume. The uppermost focal plane was omitted, and the thickness of the section was focused to avoid oversampling positive cells. For each animal, four to six sections were counted bilaterally, which was equivalent to every sixth section in the particular area. The cells were counted by one and the same person in a blinded fashion.

Western blot analysis

Total protein was extracted from heart tissue using the Tri Reagent solution (Ambion, Austin, TX, USA). For western blot, 15 µg protein was separated on 4–20% Novex Tris–Glycine gels (Invitrogen) and electrophoretically transferred to polyvinylidene difluoride membranes in a tank buffer system with Novex Tris–Glycine Transfer Buffer (Invitrogen). All membranes were blocked in 5% BSA and 0.1% Tween 20 in 0.5 mol/l TBS, (pH 7.5), and incubated for 120 and 30 min with primary and HRP-labelled secondary antibodies respectively. Antigen–antibody complexes were visualised by a chemiluminescence kit, Immuno-Star WesternC kit (Bio-Rad Laboratories), which were subsequently detected with ChemiDoc XRS+ (Bio-Rad Laboratories). The detected immunoreactivities were analysed with Image Lab Software (Bio-Rad Laboratories). The densitometry of a protein sample was always compared with that of samples within the same gel. Six different primary antibodies were used: a polyclonal rabbit antibody against IGF1 (1:200; Santa Cruz Biotechnology), a polyclonal rabbit antibody against IGF1Rβ (1:200; Santa Cruz Biotechnology), a polyclonal goat antibody against mGH (1:200; Santa Cruz Biotechnology), a polyclonal rabbit antibody against bGH (Pierce Biotechnology, Rockford, IL, USA), a polyclonal rabbit antibody against GFAP (1:3000; DAKO Denmark A/S, Glostrup, Denmark) and a monoclonal mouse antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Chemicon–Millipore, Billerica, MA, USA). The secondary antibodies were donkey anti-rabbit IgG–HRP (1:80 000; Amersham Biosciences), donkey anti-goat IgG–HRP (1:5000; Santa Cruz Biotechnology) and sheep anti-mouse IgG–HRP (1:6000; Amersham

Biosciences). All antibodies were used according to the manufacturer's instructions. For each membrane, antibodies against GAPDH were run to ensure that this housekeeping protein was not different in the bGH-Tg vs Wt samples. Several of the membranes were reused after having been stripped in stripping solution (10 mM 2-mercaptoethanol, 2% SDS (w/v) and 62.5 mM Tris–HCl (pH 6.7)) for 30 min, rotating in 50 °C.

RT-PCR

Total RNA was extracted from the hippocampus using the Tri Reagent solution (Ambion). cDNA was prepared from 250 ng total RNA, using conditions recommended by the supplier (High-Capacity cDNA Reverse Transcription Kit; Applied Biosystems). The quantitative real-time PCR analysis was performed using the ABI Prism 7900 Sequence Detection System (Applied Biosystems). Predesigned, TaqMan Gene Expression Assays (Applied Biosystems) were used to detect each transcript (Tables 1 and 2; for details, see <http://www.appliedbiosystems.com>). To analyse the real-time qPCR data, we used the comparative C_T method, where C_T stands for threshold cycle (Bustin *et al.* 2009). All samples were analysed in duplicate. For stability comparisons of candidate reference genes, we used the NormFinder Software (<http://www.mdl.dk>), which for the two evaluated transcripts (*Gapdh* and *Ppia*; see Tables 1 and 2) showed that *Ppia* was the most stable transcript in our experimental series (data not shown; Andersen *et al.* 2004, Bonefeld *et al.* 2008). *Ppia*, which is also known as cyclophilin A, is a major cytosolic protein that possesses peptidylprolyl *cis*–*trans* isomerase activity. The presented data therefore are the C_T values for each respective transcript, normalised to the C_T value for *Ppia*, thereby representing an arbitrary linear amount of each transcript. Thus, a highly expressed transcript approximately represents high-level true expression, e.g. for the comparison of endogenous *mGh* and bGH.

Statistical analysis

All values were expressed as mean ± S.E.M. Comparisons between groups were made using two-tailed Student's *t*-tests. Analysis on proteins with an already expected change of direction (as inferred by changes in mRNA) was performed by one-tailed analysis. Statistical association was tested with a correlation matrix according to Pearson, where the correlation coefficient was expressed as *r*. A *P* value < 0.05 was considered statistically significant.

Results

General effects of local overexpression of GH in the brain

Previously, systemic overexpression of bGH, including overexpression in the pituitary, together with increased levels of circulating GH was shown to enhance body growth and

Table 3 Body weights, brain weights, hippocampal weights and serum IGF1 levels in the Wt and bGH-Tg mice at the indicated time points (ages). The values are given in their respective units $\pm$ S.E.M. Statistical comparisons between Wt and bGH-Tg mice are with Students' *t*-test (*P* values indicated)

Group	Age (months)	Entity	Weights or s-IGF1 unit (values)	<i>P</i>	Normalised to body weight value (%)	<i>P</i>	<i>n</i>
Wt	6	Body	39.5 $\pm$ 7.2 g		NA		5
bGH-Tg	7	Body	41.3 $\pm$ 6.9 g	NS	NA	NA	6
Wt	6	Body	42.2 $\pm$ 4.0 g		NA		6
bGH-Tg	7	Body	46.2 $\pm$ 6.8 g	NS	NA	NA	6
Wt	6	Brain	0.481 $\pm$ 0.014 g		1.25 $\pm$ 0.09		5
bGH-Tg	7	Brain	0.474 $\pm$ 0.0061 g	NS	1.17 $\pm$ 0.07	NS	6
Wt	6	Brain	0.490 $\pm$ 0.0397 g		1.17 $\pm$ 0.09		6
bGH-Tg	7	Brain	0.639 $\pm$ 0.012 g	<0.005	1.40 $\pm$ 0.06	0.06	6
Wt	6	Hipp	13.4 $\pm$ 3.5 mg		0.356 $\pm$ 0.05		5
bGH-Tg	7	Hipp	13.3 $\pm$ 3.03 mg	NS	0.327 $\pm$ 0.035	NS	6
Wt	6	Hipp	13.5 $\pm$ 2.7 mg		0.317 $\pm$ 0.02		6
bGH-Tg	7	Hipp	15.0 $\pm$ 1.5 mg	NS	0.328 $\pm$ 0.02	NS	6
Wt	6+7	s-IGF1	299 $\pm$ 32 μ g/l		NA		6
bGH-Tg	6+7	s-IGF1	241 $\pm$ 14 μ g/l	NS	NA	NA	6

NA, not applicable; NS, not significant. *P* > 0.1 not supplied.

alter behaviour (Soderpalm *et al.* 1999). By contrast, bGH overexpression that was restricted to the CNS showed less prominent effects on the body, and it was thought that these effects were manifested mainly via mechanisms other than circulating GH (Bohlooly-Y *et al.* 2005b). Still, theoretically, low levels of extrapituitary CNS-produced bGH could enter the circulation, which would then be expected to increase body growth and/or increase serum levels of IGF1. However, we did not find any differences in body weights or serum levels of IGF1 between the TG and Wt mice (Table 3). The total weight of the brain was not different between the 6-month-old bGh-Tg mice and the Wt mice. There was a minor increase in brain weight in the 7-month-old bGH-Tg mice, which turned out to not be statistically significant when brain weight was normalised to body weight. We observed no significant differences in total hippocampal weight between the groups. Taken together, these findings indicate that systemic growth and serum IGF1 are not affected by the GFAP-controlled bGH-Tg, neither were hippocampal nor were total brain weights.

Local overexpression of GH in the brain and hippocampal cell proliferation

Previously, systemic treatment with GH was shown to increase adult hippocampal cell proliferation and cell survival (Aberg *et al.* 2010). In the 6-month-old bGH-Tg mice, the number of BrdU-positive cells in the dentate gyrus of the hippocampus was not significantly different between the bGH-Tg mice and Wt mice, indicating that local bGH does not affect cellular proliferation (Fig. 1). Similarly, there was no difference between the bGH-Tg and Wt groups regarding the number of remaining BrdU-positive cells in the 7-month-old mice.

Local overexpression of GH in the brain and its effects on known GH-induced transcripts

As local GFAP-induced overexpression of bGH in the brain had only a modest effect on total brain weight and cell proliferation, we used qPCR analysis of transcripts to determine the presence of other effects that have been previously attributed to systemic GH. In addition, we analysed components of the local GH-IGF1 system. The transcripts were categorised as follows: 1) neuron- or synapse-associated transcripts; 2) glia-associated transcripts and 3) transcripts that are components of the local GH-IGF1 system (see also Tables 1 and 2 for references). Furthermore, as there were no significant differences in transcript levels between day 1 (i.e. 6 months of age) and day 30 (i.e. 7 months of age) after BrdU injection (data not shown), in either the TG or the Wt group, we pooled the transcript data from the two time-points to increase statistical power (bGH-Tg and Wt respectively).

Figure 1 Quantification of BrdU-positive cells in the adult mouse hippocampus. The densities of the BrdU-positive cells were determined stereologically in 6-month-old Wt (*n* = 5) and bGH-Tg (*n* = 6) and in 7-month-old Wt (*n* = 6) and bGH-Tg (*n* = 6) animals. Data are presented as mean $\pm$ S.E.M.

Figure 2 Levels of transcripts involved in the GH/IGF1 axis, analysed by qPCR, in the hippocampal samples from mice pooled from 6 and 7 months (Wt, $n=11$ and bGH-Tg, $n=12$). All transcript levels are normalised to the level of *PPIA* transcripts, the levels are therefore quantitative arbitrary. Data are presented as mean $\pm$ S.E.M. * $P<0.05$.

Regarding the local GH–IGF1 system, there were no significant differences in the levels of *Ghr* and *Igf1r* expression between the bGH-Tg and the Wt mice (Fig. 2). However, the level of *Igf1* mRNA was 30% higher in the bGH-Tg mice than in the Wt mice (Fig. 2). We also analysed the mRNA levels for hippocampal endogenous *mGh* and bGH. As expected, the expression of mGH was 91% lower in the bGH-Tg mice compared with the Wt mice. The bGH-Tg mice showed high expression of *bGh* mRNA, which confirms that the transgene was robustly transcribed. The amount of *bGh* mRNA in the bGH-Tg mice exceeded by 38-fold the amount of endogenous mGH in the Wt mice. To further analyse whether the transcripts in the GH–IGF1 system actually gave rise to changed net protein levels, we performed densitometry of western blots. These experiments revealed that hippocampal IGF1 protein was not changed other than marginally (+9%, NS) comparing bGH-Tg and Wt (Fig. 3). Neither was IGF1R changed (+33%, NS) comparing bGH-Tg and Wt. Unexpectedly, mGH protein was not decreased (+5%, NS), while bGH protein was expectedly increased by 66% comparing bGH-Tg and Wt. As can be observed from the western blot panel, bGH protein immunoreactivity was unexpectedly present in the Wt mice (Fig. 3, lower panel), probably representing that the antibody also recognised mGH to a certain degree (see Discussion).

Next, we analysed four transcripts that are primarily related to neuronal functions previously known to be regulated by systemic GH: *Dor*, *Nr2a*, *Sapap2* and *Hbb* (Tables 1 and 2, Fig. 4). Comparing the Wt and bGH-Tg animals, we found that local overexpression of *bGH* did not affect the expression levels of these previously known systemically GH-induced transcripts.

Finally, as *bGH* was expressed under the control of the GFAP promoter, which is expressed in glial cells, we hypothesised that bGH might affect the transcripts found in astrocytes (or oligodendrocytes). Therefore, we analysed three glia-related transcripts: *Cnp*, *Gs* and *Gfap* (Tables 1 and 2, Fig. 5). However, only *Gfap* showed a significantly higher expression level (26% higher) in the TG group, while there were no significant differences in the levels of *Cnp* or *Gs* mRNA. As *Gfap* mRNA levels were increased in bGH-Tg animals, we also quantified GFAP protein in western blots (Fig. 3). This analysis showed that GFAP was not statistically increased (+4.2% in both the 6- and 7-month animals, NS).

Relationship between specific transcript expression and activities of the GH–IGF1 system

The local expression of bGH under the control of the GFAP promoter weakly affected local IGF1 expression and GFAP abundance and had no effects on cellular proliferation, cell survival or the expression of five other known GH-regulated genes. We hypothesised that other local components of the GH–IGF1 system might be more important in terms of modulating these transcripts. To address this question, we performed correlation analysis with respect to possible interactions between the specific transcripts and the local GH–IGF1 system (Table 4). With the exceptions of endogenous *mGh*, which was suppressed in the bGH-Tg group, and bGH (found only in the bGH group), the correlation patterns were similar in the Wt and bGH-Tg groups at both time points (data not shown). Therefore, the datasets were pooled (as earlier).

Figure 3 Densitometry of western blots and representative examples of the bands analysed. Western blots of IGF1, IGF1R, mGH, bGH, GFAP and GAPDH are shown as indicated (Wt, $n=11$ and bGH-Tg, $n=12$). All protein levels are normalised to the level of short-term Wt (6 months old). Representative panels of the western blots are shown. The estimated molecular weights were 7 kDa for IGF1, 98 kDa for IGF1R, 18 and 22 kDa for mGH, 18 and 22 kDa for bGH, 55 kDa for GFAP and 44 kDa for GAPDH. Both bands of the western blots of mGH were used for densitometry while only the 22 kDa band was used for densitometry in western blots of bGH (see Discussion). Data are presented as mean $\pm$ S.E.M. ** $P<0.01$.

Figure 4 Levels of four glia-related transcripts, analysed by qPCR, in the hippocampal samples from mice pooled from 6 and 7 months (Wt, $n=11$ and bGH-Tg, $n=12$). All transcript levels are normalised to the level of *PPIA* transcripts, the levels are therefore quantitative arbitrary. Data are presented as mean $\pm$ S.E.M.

We uncovered three types of associations. First, we observed a series of statistically significant correlations between the *Igf1r* and *Nr2a*, *Sapap2*, *Gs* and *Gfap* and a near-significant association to *Dor* (Table 4). Secondly, it is noteworthy that there were no significant correlations between *Ghr* and any of the specific transcripts. However, there could still be some possibility that local endogenous GH could have a role as we observed that *Ghr* expression correlated highly with the local expression of *Igf1* (see Discussion). In addition, *Ghr* expression in the 6-month-old bGH-Tg mice was positively correlated with circulating levels of serum IGF1 ($r=0.844$, $P=0.035$) while data on the serum levels of IGF1 were not available for the 7-month-old bGH-Tg mice. Thirdly, *bGH* expression was negatively correlated with *Ghr* expression in the bGH-Tg mice. It is noteworthy that this association was not found for the Wt mice regarding mGH and *Ghr*, which could indicate a local negative feedback in response to high local concentrations of bGH.

In summary, it appears that neither local bGH nor mGH is directly associated with the expression of previously known, systemically administered GH-induced transcripts, whereas IGF1R is robustly correlated with most of these transcripts.

Discussion

The primary objective of this study was to investigate whether local CNS-driven overexpression of bGH induces effects previously found to be exerted by circulating GH in the hippocampus. Surprisingly, we found that local *bGH* overexpression did not influence cell proliferation. While BrdU-positive cells are usually used as a measure of cellular proliferation, they are also used to assess cell survival,

provided two known time points after the BrdU injections (Kempermann *et al.* 2004). Therefore, as the proportion of surviving cells did not increase in bGH-Tg vs Wt animals 30 days compared with 1 day after the last BrdU injection, we conclude that cell survival was not affected. Although bGH-Tg modestly increased the expression of hippocampal IGF1, it did not affect the levels of other transcripts known to be induced by circulating GH with the exception of GFAP. In summary, the GFAP-driven overexpression of bGH affected the adult hippocampus to a limited degree, contrary to the primary hypothesis of the project (Fig. 6). In the secondary analysis, however, the robust association between *Igf1r* and several of the analysed transcripts, such as *Nr2a*, *Sapap2*, *Gs*, *Gfap* and *Dor*, indicates an important role of the local GH-IGF1 system.

Why is the effect of bGH overexpression modest in the hippocampus?

The fact that *Igf1* mRNA was only increased by 30% indicates that bGH is a weak activator of local IGF1 signalling. The modest effects of bGH-Tg in our study is surprising considering that local overexpression of bGH in the CNS has previously been shown to modify cardiac function (Bohlooly-Y *et al.* 2005a) and eating behaviour (Bohlooly-Y *et al.* 2005b). Although changes in hypothalamic gene expression were observed, the authors also speculated that there was a change in sympathetic outflow, possibly in conjunction with an increase in serum corticosterone (Bohlooly-Y *et al.* 2001) and serum leptin (Bohlooly-Y *et al.* 2005b). In these studies, there was an endocrine leakage of

Figure 5 Levels of three GH-affected transcripts, analysed by qPCR, in the hippocampal samples from mice pooled from 6 and 7 months (Wt, $n=11$ and bGH-Tg, $n=12$). All transcript levels are normalised to the level of *Ppia* transcripts, the levels are therefore quantitative arbitrary. Data are presented as mean $\pm$ S.E.M. * $P<0.05$.

Table 4 Two-tailed correlation matrix using the Pearson analysis for the correlation of amounts of RNA transcripts (see Materials and Methods). The correlations shown represent analysis of all animals pooled together, for the Wt and bGH-Tg mice (*n*=23), except for mGH and bGH, whose correlations are performed as indicated. Significant correlations are shown in bold text. Values of *r* and *P* as indicated

Transcript	GHR	IGF1	IGF1R
Correlations			
<i>Ghr</i>			
<i>r</i>	1.00	0.54	0.37
<i>P</i>		0.01	0.08
<i>Igf1</i>			
<i>r</i>	0.54	1.00	0.37
<i>P</i>		0.01	0.08
<i>Igf1r</i>			
<i>r</i>	0.37	0.37	1.00
<i>P</i>	0.08	0.08	
<i>Hbb</i>			
<i>r</i>	0.07	0.03	0.04
<i>P</i>	0.74	0.89	0.85
<i>Cnp</i>			
<i>r</i>	0.21	0.05	0.30
<i>P</i>	0.33	0.84	0.17
<i>Dor</i>			
<i>r</i>	0.11	0.30	0.40
<i>P</i>	0.62	0.16	0.06
<i>Nr2a</i>			
<i>r</i>	0.11	0.07	0.77
<i>P</i>	0.62	0.74	<0.001
<i>Sapap2</i>			
<i>r</i>	0.35	0.34	0.84
<i>P</i>	0.10	0.11	<0.001
<i>Gs</i>			
<i>r</i>	0.23	−0.05	0.43
<i>P</i>	0.28	0.82	0.04
<i>Gfap</i>			
<i>r</i>	0.30	0.25	0.439*
<i>P</i>	0.16	0.26	0.04
<i>mGh</i> (correlation only in Wt)			
<i>r</i>	−0.15	−0.15	−0.05
<i>P</i>	0.67	0.66	0.89
<i>mGh</i> (correlation only in bGH-Tg)			
<i>r</i>	0.24	0.07	−0.08
<i>P</i>	0.46	0.82	0.81
<i>bGH</i> (correlation only in bGH-Tg)			
<i>r</i>	−0.59	−0.06	−0.37
<i>P</i>	0.04	0.85	0.24

bGH with a slight increase in circulating bGH levels (Bohlooly-Y *et al.* 2001), which led to a progressive increase in body weight to a total of 21–27% comparing postnatal days 30 and 130 (Bohlooly-Y *et al.* 2005b). To minimise the endocrine effects of *bGH* overexpression, we used an alternative founder line with less robust overexpression of bGH that did not affect the serum IGF1 levels or body weights (see Materials and Methods and Table 3). Accordingly, our founder line of bGH-Tg should have addressed whether brain GH affects the brain locally (with minimal secondary endocrine bGH).

A mechanism that could account for the modest effects of bGH-Tg is the so-called bell-shaped dose response. The net effect of GH then becomes negative at high concentrations of

GH (Lyuh *et al.* 2007, Aberg *et al.* 2009). This phenomenon occurs when excessive concentrations of GH homo-dimerise, thereby preventing binding to the GHR. However, the fact that western blot experiments showed that bGH was not increased excessively (only +66%, Fig. 3) in bGH-Tg mice contradicts the presence of the negative part of bell-shaped dose response. On the other hand, the local micro-environment could allow cell-specific high GH concentrations causing such a phenomenon.

Most previous studies have shown that exogenous GH expression or treatment, regardless of species differences, causes profound growth effects (Groesbeck & Parlow 1987, Soderpalm *et al.* 1999, Bohlooly-Y *et al.* 2001). Therefore, we believe that the central effects of bGH-Tg in terms of altered

Figure 6 Graphical summarising of the hypothesis, major results and the possible options compatible with the results. Top: the investigation tested the hypothesis that local overexpression of bGH would elicit a response in a number of previously known effectors of circulating GH. Middle: the major result was that bGH only elicited a very limited response, and there may be several options that may explain this (see Discussion). Below: the relatively high correlation between IGF1R and many of the previously known GH-induced transcripts are still compatible with an endogenous activation stemming from either local or circulating GH (see Discussion for how this could be acting).

cardiac function and metabolism (Bohlooly-Y *et al.* 2005a) are confined to brain regions other than the hippocampus and that the previously reported hippocampus-specific effects of systemic GH are weakly affected by local bGH-Tg.

Cell-type-specific local GHR and IGF1R circuits in the hippocampus?

Cell-specific differences in *Ghr* expression could account for the weak effects of bGH-Tg. Indeed, GHR isoforms exist, both in human brains and in rat brains (Lai *et al.* 1993), which may mediate different functions in the CNS and periphery (Nyberg 2000). Furthermore, it cannot be excluded that although bGH is properly transcribed, the levels of final bioactive bGH peptide could be much lower. However, the latter is unlikely, as bGH overexpression caused down-regulation of endogenous mGH and as the existence of bGH protein was shown. It should, however, be pointed out that, although the existence of bGH protein was shown, precise quantification is difficult due to antibody cross-reactivity (Fig. 3). The antibody against mGH detected an 18 kDa band robustly and a 22 kDa band weakly, and the antibody against bGH detected the 18 kDa weakly and the 22 kDa robustly. As the antibody against bGH detected the 22 kDa relatively best, this band was the one analysed in the densitometries. It is also likely that the antibody against mGH also detected some bGH, as otherwise mGH would probably have been lower in the bGH-Tg group (as inferred from the mRNA levels).

There is also the possibility of cell-specific expression of IGF1R. As bGH was expressed under the control of the GFAP promoter, it may be that primarily the IGF1R in astrocytes was activated. Indeed, the fact that bGH-Tg

increased mRNAs of *Gfap* (+26%) and *Igf1* (+30%) indicates an effect localised to glial cells. Although IGF1 total protein in the hippocampus (stemming from both local and circulating IGF1) was not increased, astroglial IGF1 protein may have been increased, causing the observed increase in GFAP. Although our investigation was targeting adult effects of local GH, it has to be kept in mind that the bGH-Tg came into effect in the early postnatal life when GFAP started to become expressed. As previous TG models that either increase or decrease GH signalling affect astrocyte numbers (Turnley *et al.* 2002, Ransome *et al.* 2004), at least in the cerebral cortex, it is plausible that astrocyte numbers may have slowly accumulated during the development. Such a slow accumulation may not have been detected in our assays of proliferating cells. However, as GFAP protein did not increase (+4.2% in both the 6- and 7-month animals, Fig. 3), it is not plausible that astrocyte numbers would have increased by more than that amount.

The increase in *Igf1* mRNA in the bGH-Tg mice could be due to an autocrine or intracrine function of bGH-Tg acting on the astrocytes to increase the synthesis of local IGF1. However, the effect on glial cells of GH overexpression does not appear to be sufficient to influence the expression of other transcripts. Either the increase of IGF1 was too low and/or the IGF1 did not enter other cell types, such as neurons, in the vicinity of the GFAP-associated (astroglial) cells. This is in line with the findings that NR2A and SAPAP2 are mainly located in neurons and that their transcripts are not affected by bGH-Tg. Therefore, the endogenous two- to four-fold regulation of local GH and IGF1 in the brain caused by age, sex and stress (Donahue *et al.* 2006) may act differently than in our investigation. Also, it may be that bGH-Tg may respond differently to a physiological stimulus than just comparing bGH-Tg and Wt with no stimulus.

Numerous studies have confirmed that astrocytes are a target for circulating IGF1 (for review, see Fernandez *et al.* (2007)). Moreover, astroglial cells appear to express an IGF1R similar to that found in peripheral cells (Baron-Van Evercooren *et al.* 1991). Interestingly, these receptors differ from the IGF1Rs found in neurons, which have distinct glycosylated residues, as evidenced by *in vitro* studies (Burgess *et al.* 1987). While the astroglial IGF1R is concentrated in the glial end-feet covering the brain vessels, the neuronal IGF1R is found both pre- and post-synaptically (Garcia-Segura *et al.* 1997). It has also been suggested that IGF1 elicits in astrocytes a functionally distinct response that is both anti-apoptotic and neuroprotective, while neurons respond to IGF1 by increasing synaptic plasticity and neuritic outgrowth (D'Ercole & Ye 2008). If IGF1R is the final mediator of both local and endocrine GH, this may explain why astroglial transcripts and not neuronal transcripts were affected by bGH-Tg in this study.

The role of IGF1R for endocrine and local GH actions?

The local circuits for GH-IGF1 signalling may depend on endocrine or local GH (see also Fig. 6). In both cases,

IGF1R may mediate the final effect. Indeed, our results demonstrate that previously identified GH-induced transcripts, such as *Nr2a*, *Sapap2*, *Gs* and *Gfap*, have strong correlations with *Igf1r* and that there is also a strong correlation between *Ghr* and *Igf1*. This indicates that local IGF1 and IGF1R are limiting factors for promoting these GH-induced transcripts. Our results could imply that circulating or local GH acts via local or endocrine IGF1/IGF1R and to a lesser extent by direct stimulation of the brain GHR.

However, the lack of association between mGH and GHR does not exclude that local GH has a less prominent effect that is difficult to discriminate from the effect of local IGF1. First, local GH may act without necessarily affecting the levels of GHR. Secondly, high-level overexpression of bGH appears to downregulate GHR (Table 4). Thirdly, the finding that hippocampal GHR and IGF1 are associated ($r=0.54$, Table 4) indicates a dependence of IGF1 on GHR and provides indirect evidence of local GHR–IGF1 signalling. For example, it is known that systemic GH treatment induces an increase in the expression of the hippocampal mRNA for NR2A (Le Greves *et al.* 2002, 2005), an effect likely mediated via either circulating or local IGF1. Finally, the robust association observed between serum IGF1 and local hippocampal GHR ($r=0.844$) underlines that local GH and circulating IGF1 signalling are linked.

Conclusions

The results of this study indicate that local GH overexpression in astrocytes does not influence cellular proliferation or neuronal transcripts and has only a modest effect on astroglial transcripts. However, the transcripts are strongly associated with hippocampal IGF1R. Therefore, although bGH produced in astrocytes exerted a limited effect, there is still a possibility that endocrine or local endogenous GH exerts its effects by circulating IGF1 or by activating local IGF1 in the brain. In either case, the signalling occurs via the hippocampal IGF1R. However, that the results are also compatible with local GH is of less importance than endocrine GH. The exact nature of regulation, especially with regard to cell type-specific regulation, needs further investigation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The authors are grateful for grants from the Swedish Medical Society (Svenska Läkaresällskapet) and the Swedish Government (ALFGBG-74640).

Acknowledgements

The English grammar was corrected by scientific editor Dr Vincent Collins (University of Gothenburg).

References

- Åberg MA, Åberg ND, Hedbacker H, Oscarsson J & Eriksson PS 2000 Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *Journal of Neuroscience* **20** 2896–2903. (doi:10.1210/en.2006-1556)
- Åberg ND, Bryve KG & Isgaard J 2006 Aspects of growth hormone and insulin-like growth factor-I related to neuroprotection, regeneration, and functional plasticity in the adult brain. *Scientific World Journal* **6** 53–80. (doi:10.1100/tsw.2006.22)
- Åberg ND, Johansson UE, Åberg MA, Hellstrom NA, Lind J, Bull C, Isgaard J, Anderson MF, Oscarsson J & Eriksson PS 2007 Peripheral infusion of insulin-like growth factor-I increases the number of newborn oligodendrocytes in the cerebral cortex of adult hypophysectomized rats. *Endocrinology* **148** 3765–3772. (doi:10.1210/en.2006-1556)
- Åberg ND, Johansson I, Åberg MA, Lind J, Johansson UE, Cooper-Kuhn CM, Kuhn HG & Isgaard J 2009 Peripheral administration of GH induces cell proliferation in the brain of adult hypophysectomized rats. *Journal of Endocrinology* **201** 141–150. (doi:10.1677/JOE-08-0495)
- Åberg ND, Lind J, Isgaard J & Kuhn HG 2010 Peripheral growth hormone induces cell proliferation in the intact adult rat brain. *Growth Hormone & IGF Research* **20** 264–269. (doi:10.1016/j.ghir.2009.12.003)
- Albrecht J, Sonnewald U, Waagepetersen HS & Schousboe A 2007 Glutamine in the central nervous system: function and dysfunction. *Frontiers in Bioscience: a Journal and Virtual Library* **12** 332–343. (doi:10.2741/2067)
- Andersen CL, Jensen JL & Orntoft TF 2004 Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Research* **64** 5245–5250. (doi:10.1158/0008-5472.CAN-04-0496)
- Baron-Van Evercooren A, Olichon-Berthe C, Kowalski A, Visciano G & Van Obberghen E 1991 Expression of IGF-I and insulin receptor genes in the rat central nervous system: a developmental, regional, and cellular analysis. *Journal of Neuroscience Research* **28** 244–253. (doi:10.1002/jnr.490280212)
- Bengtsson BA, Eden S, Lonn L, Kvist H, Stokland A, Lindstedt G, Bosaeus I, Tolli J, Sjöström L & Isaksson OG 1993 Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *Journal of Clinical Endocrinology and Metabolism* **76** 309–317. (doi:10.1210/jc.76.2.309)
- Bohlooly-Y M, Olsson B, GritliLinde A, Brusehed O, Isaksson OGP, Ohlsson C, Söderpalm B & Tornell J 2001 Enhanced spontaneous locomotor activity in bovine GH transgenic mice involves peripheral mechanisms (vol 142, pp 4560, 2001). *Endocrinology* **142** 5293.
- Bohlooly-Y M, Bollano E, Mobini R, Soussi B, Tornell J & Omerovic E 2005a Selective cerebral overexpression of growth hormone alters cardiac function, morphology, energy metabolism and catecholamines in transgenic mice. *Growth Hormone & IGF Research* **15** 148–155. (doi:10.1016/j.ghir.2004.12.007)
- Bohlooly-Y M, Olsson B, Bruder CE, Linden D, Sjogren K, Bjursell M, Egicioglu E, Svensson L, Brodin P, Waterton JC *et al.* 2005b Growth hormone overexpression in the central nervous system results in hyperphagia-induced obesity associated with insulin resistance and dyslipidemia. *Diabetes* **54** 51–62. (doi:10.2337/diabetes.54.1.51)
- Bonefeld BE, Elfving B & Wegener G 2008 Reference genes for normalization: a study of rat brain tissue. *Synapse* **62** 302–309. (doi:10.1002/syn.20496)
- Burgess SK, Jacobs S, Cuatrecasas P & Sahyoun N 1987 Characterization of a neuronal subtype of insulin-like growth factor I receptor. *Journal of Biological Chemistry* **262** 1618–1622.
- Burman P & Deijen JB 1998 Quality of life and cognitive function in patients with pituitary insufficiency. *Psychotherapy and Psychosomatics* **67** 154–167. (doi:10.1159/000012276)

- Bustin SA, Benes V, Garson JA, Hellems J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL *et al.* 2009 The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry* **55** 611–622. (doi:10.1373/clinchem.2008.112797)
- Carro E, Trejo JL, Busiguina S & Torres-Aleman I 2001 Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. *Journal of Neuroscience* **21** 5678–5684.
- Cho KO, Hunt CA & Kennedy MB 1992 The rat brain postsynaptic density fraction contains a homolog of the *Drosophila* discs-large tumor suppressor protein. *Neuron* **9** 929–942. (doi:10.1016/0896-6273(92)90245-9)
- Deijen JB, Arwert LI, Witlox J & Drent ML 2005 Differential effect sizes of growth hormone replacement on quality of life, well-being and health status in growth hormone deficient patients: a meta-analysis. *Health and Quality of Life Outcomes* **3** 63. (doi:10.1186/1477-7525-3-63)
- D'Ercole AJ & Ye P 2008 Expanding the mind: insulin-like growth factor I and brain development. *Endocrinology* **149** 5958–5962. (doi:10.1210/en.2008-0920)
- D'Ercole AJ, Ye P, Calikoglu AS & Gutierrez-Ospina G 1996 The role of the insulin-like growth factors in the central nervous system. *Molecular Neurobiology* **13** 227–255. (doi:10.1007/BF02740625)
- Devesa P, Reimunde P, Gallego R, Devesa J & Arce VM 2011 Growth hormone (GH) treatment may cooperate with locally-produced GH in increasing the proliferative response of hippocampal progenitors to kainate-induced injury. *Brain Injury* **25** 503–510. (doi:10.3109/02699052.2011.559611)
- Donahue CP, Jensen RV, Ochiishi T, Eisenstein I, Zhao M, Shors T & Kosik KS 2002 Transcriptional profiling reveals regulated genes in the hippocampus during memory formation. *Hippocampus* **12** 821–833. (doi:10.1002/hipo.10058)
- Donahue CP, Kosik KS & Shors TJ 2006 Growth hormone is produced within the hippocampus where it responds to age, sex, and stress. *PNAS* **103** 6031–6036. (doi:10.1073/pnas.0507761103)
- Falletti MG, Maruff P, Burman P & Harris A 2006 The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: a meta-analysis of the current literature. *Psychoneuroendocrinology* **31** 681–691. (doi:10.1016/j.psyneuen.2006.01.005)
- Fernandez S, Fernandez AM, Lopez-Lopez C & Torres-Aleman I 2007 Emerging roles of insulin-like growth factor-I in the adult brain. *Growth Hormone & IGF Research* **17** 89–95. (doi:10.1016/j.ghir.2007.01.006)
- Folli F, Ghidella S, Bonfanti L, Kahn CR & Merighi A 1996 The early intracellular signaling pathway for the insulin/insulin-like growth factor receptor family in the mammalian central nervous system. *Molecular Neurobiology* **13** 155–183. (doi:10.1007/BF02740639)
- Garcia-Segura LM, Rodriguez JR & Torres-Aleman I 1997 Localization of the insulin-like growth factor I receptor in the cerebellum and hypothalamus of adult rats: an electron microscopic study. *Journal of Neurocytology* **26** 479–490. (doi:10.1023/A:1018581407804)
- Groesbeck MD & Parlow AF 1987 Highly improved precision of the hypophysectomized female rat body weight gain bioassay for growth hormone by increased frequency of injections, avoidance of antibody formation, and other simple modifications. *Endocrinology* **120** 2582–2590. (doi:10.1210/endo-120-6-2582)
- He Y, Hua Y, Liu W, Hu H, Keep RF & Xi G 2009 Effects of cerebral ischemia on neuronal hemoglobin. *Journal of Cerebral Blood Flow and Metabolism* **29** 596–605. (doi:10.1038/jcbfm.2008.145)
- Hojvat S, Baker G, Kirsteins L & Lawrence AM 1982 Growth hormone (GH) immunoreactivity in the rodent and primate CNS: distribution, characterization and presence posthypophysectomy. *Brain Research* **239** 543–557. (doi:10.1016/0006-8993(82)90529-7)
- Iwata M, Inoue S, Kawaguchi M, Nakamura M, Konishi N & Furuya H 2007 Effects of delta-opioid receptor stimulation and inhibition on hippocampal survival in a rat model of forebrain ischaemia. *British Journal of Anaesthesia* **99** 538–546. (doi:10.1093/bja/aem220)
- Kempermann G, Jessberger S, Steiner B & Kronenberg G 2004 Milestones of neuronal development in the adult hippocampus. *Trends in Neurosciences* **27** 447–452. (doi:10.1016/j.tins.2004.05.013)
- Lai Z, Roos P, Zhai O, Olsson Y, Fohlenhag K, Larsson C & Nyberg F 1993 Age-related reduction of human growth hormone-binding sites in the human brain. *Brain Research* **621** 260–266. (doi:10.1016/0006-8993(93)90114-3)
- Le Greves M, Steensland P, Le Greves P & Nyberg F 2002 Growth hormone induces age-dependent alteration in the expression of hippocampal growth hormone receptor and N-methyl-D-aspartate receptor subunits gene transcripts in male rats. *PNAS* **99** 7119–7123. (doi:10.1073/pnas.092135399)
- Le Greves M, Le Greves P & Nyberg F 2005 Age-related effects of IGF-1 on the NMDA-, GH- and IGF-1-receptor mRNA transcripts in the rat hippocampus. *Brain Research Bulletin* **65** 369–374. (doi:10.1016/j.brainresbull.2005.01.012)
- Le Greves M, Zhou Q, Berg M, Le Greves P, Fohlenhag K, Meyerson B & Nyberg F 2006 Growth hormone replacement in hypophysectomized rats affects spatial performance and hippocampal levels of NMDA receptor subunit and PSD-95 gene transcript levels. *Experimental Brain Research* **173** 267–273. (doi:10.1007/s00221-006-0438-2)
- Leon-Carrion J, Martin-Rodriguez JF, Madrazo-Atutxa A, Soto-Moreno A, Venegas-Moreno E, Torres-Vela E, Benito-Lopez P, Galvez MA, Tinahones FJ & Leal-Cerro A 2010 Evidence of cognitive and neurophysiological impairment in patients with untreated naive acromegaly. *Journal of Clinical Endocrinology and Metabolism* **95** 4367–4379. (doi:10.1210/jc.2010-0394)
- Lyu H, Kim HJ, Kim M, Lee JK, Park KS, Yoo KY, Lee KW & Ahn YO 2007 Dose-specific or dose-dependent effect of growth hormone treatment on the proliferation and differentiation of cultured neuronal cells. *Growth Hormone & IGF Research* **17** 315–322. (doi:10.1016/j.ghir.2007.03.002)
- McGauley GA 1989 Quality of life assessment before and after growth hormone treatment in adults with growth hormone deficiency. *Acta Paediatrica Scandinavica. Supplement* **356** 70–72 (discussion 73–74). (doi:10.1111/j.1651-2227.1989.tb11249.x)
- McGauley G, Cuneo R, Salomon F & Sonksen PH 1996 Growth hormone deficiency and quality of life. *Hormone Research* **45** 34–37. (doi:10.1159/000184756)
- Molitch ME, Clemmons DR, Malozowski S, Merriam GR & Vance ML 2011 Evaluation and treatment of adult growth hormone deficiency: an endocrine society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism* **96** 1587–1609. (doi:10.1210/jc.2011-0179)
- Nyberg F 2000 Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance. *Frontiers in Neuroendocrinology* **21** 330–348. (doi:10.1006/frne.2000.0200)
- Ohya Y, Yamada T & Goto I 1994 Hemoglobin as a novel protein developmentally regulated in neurons. *Brain Research* **635** 323–327. (doi:10.1016/0006-8993(94)91455-9)
- Pekny M, Leveen P, Pekna M, Eliasson C, Berthold CH, Westermark B & Betsholtz C 1995 Mice lacking glial fibrillary acidic protein display astrocytes devoid of intermediate filaments but develop and reproduce normally. *EMBO Journal* **14** 1590–1598.
- Persson AI, Aberg ND, Oscarsson J, Isaksson OG, Ronnback L, Frick F, Sonesson C & Eriksson PS 2003 Expression of delta opioid receptor mRNA and protein in the rat cerebral cortex and cerebellum is decreased by growth hormone. *Journal of Neuroscience Research* **71** 496–503. (doi:10.1002/jnr.10515)
- Ransome MI, Goldshmit Y, Bartlett PF, Waters MJ & Turnley AM 2004 Comparative analysis of CNS populations in knockout mice with altered growth hormone responsiveness. *European Journal of Neuroscience* **19** 2069–2079. (doi:10.1111/j.0953-816X.2004.03308.x)
- Reimunde P, Quintana A, Castanon B, Casteleiro N, Vilarnovo Z, Otero A, Devesa A, Otero-Cepeda XL & Devesa J 2011 Effects of growth hormone (GH) replacement and cognitive rehabilitation in patients with cognitive disorders after traumatic brain injury. *Brain Injury* **25** 65–73. (doi:10.3109/02699052.2010.536196)
- Soderpalm B, Ericson M, Bohlooly M, Engel JA & Tornell J 1999 Bovine growth hormone transgenic mice display alterations in locomotor activity and brain monoamine neurochemistry. *Endocrinology* **140** 5619–5625. (doi:10.1210/en.140.12.5619)

- Sun LY, Al-Regaiey K, Masternak MM, Wang J & Bartke A 2005 Local expression of GH and IGF-1 in the hippocampus of GH-deficient long-lived mice. *Neurobiology of Aging* **26** 929–937. (doi:10.1016/j.neurobiolaging.2004.07.010)
- Takeuchi M, Hata Y, Hirao K, Toyoda A, Irie M & Takai Y 1997 SAPAPs. A family of PSD-95/SAP90-associated proteins localized at postsynaptic density. *Journal of Biological Chemistry* **272** 11943–11951. (doi:10.1074/jbc.272.18.11943)
- Tang H, Zhang Y, Yu X, Song J, Xu C & Wan Y 2011 Changes in growth hormone (GH), GH receptor, and GH signal transduction in hippocampus of congenital hypothyroid rats. *Journal of Neuroscience Research* **89** 248–255. (doi:10.1002/jnr.22540)
- Turnley AM, Faux CH, Rietze RL, Coonan JR & Bartlett PF 2002 Suppressor of cytokine signaling 2 regulates neuronal differentiation by inhibiting growth hormone signaling. *Nature Neuroscience* **5** 1155–1162. (doi:10.1038/nn954)
- Walser M, Hansen A, Svensson PA, Jernas M, Oscarsson J, Isgaard J & Aberg ND 2011 Peripheral administration of bovine GH regulates the expression of cerebrocortical β -globin, GABAB receptor 1, and the Lissencephaly-1 protein (LIS-1) in adult hypophysectomized rats. *Growth Hormone & IGF Research* **21** 16–24. (doi:10.1016/j.ghir.2010.11.002)
- Yan H, Mitschelen M, Bixler GV, Brucklacher RM, Farley JA, Han S, Freeman WM & Sonntag WE 2011 Circulating insulin-like growth factor (IGF)-1 regulates hippocampal IGF-1 levels and brain gene expression during adolescence. *Journal of Endocrinology* **211** 27–37. (doi:10.1530/JOE-11-0200)

Received in final form 10 August 2012

Accepted 22 August 2012

Made available online as an Accepted Preprint

23 August 2012