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ORIGINAL ARTICLE

Effects of peripheral administration of GH and IGF-I on gene expression in the hippocampus of hypophysectomised rats

Marion Walser[1], Jan Oscarsson[2], Maria A I Åberg[3], Johan Svensson[1], Jörgen Isgaard[1], N. David Åberg[1]

1 Department of Internal Medicine, Institute of Medicine, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
2 AstraZeneca Gothenburg, Mölndal, Sweden
3 Department of Primary Health Care, Institute of Medicine, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

Correspondence to: Marion Walser
Sahlgrenska University Hospital, Laboratory of Experimental Endocrinology
Bruna Stråket 16, 413 45 Göteborg, Sweden
e-mail: marion.walser@medic.gu.se

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Abstract

OBJECTIVE: Growth hormone (GH) increases insulin-like growth factor I (IGF-I) production and both hormones affect hippocampal plasticity. We have previously shown that *Hbb* and *Alas2* in the rat hippocampus were robustly regulated by GH-infusions for six days, whereas other transcripts were weakly affected. Here, we explored the effects of prolonged GH administration on transcripts linked to neuroprotection and investigated whether serum IGF-I administration may exert similar effects.

DESIGN: Hypophysectomised female rats were infused with GH or IGF-I for 19 days. *Hbb, Alas2* and seven additional GH- and IGF-I-related transcripts were quantified by Q-RT-PCR in rat hippocampus.

RESULTS: Three transcripts, *Hbb, Alas2*, and *Alox15* were increased by both GH and IGF-I administration. The other transcripts were marginally affected.

CONCLUSION: The 19-day GH-infusion induced similar effects as those reported after 6-day GH treatment, with the addition of the regulation of transcript *Alox15*. IGF-I induced altered gene expression in relation to its effect on weight gain. This study underlines that there is an entity of transcripts involved in neuroprotection and vascular tone that is regulated by both systemic GH and IGF-I. For other transcripts, the longer duration of this study did not significantly enhance the marginal effects of GH administration seen previously.

INTRODUCTION

Both growth hormone (GH) and insulin-like growth factor I (IGF-I) improve cognitive function and exert multiple effects in the brain. For instance, systemic GH administration increases cell genesis and number of new-born neurons in the adult brain (Åberg *et al.* 2009; Åberg *et al.* 2010), induces expression of plasticity-related transcripts (for review, see (Åberg *et al.* 2006), exerts neuroprotective effects against brain injury (Zhang *et al.* 2014), and positively affects cognition and memory in rats (Le Greves *et al.* 2006; Schneider-Rivas *et al.* 1995; Ong *et al.* 2018). In general, systemic IGF-I administration exerts effects of a similar type (Guan *et al.* 2003; Guan *et al.* 2001; Kooijman *et al.* 2009).

GH and IGF-I belong to the somatotropic hormone axis. After being released from the anterior pituitary gland, GH stimulates the liver (Chia 2014; Ohlsson *et al.* 2009), and to a lesser extent several other tissues (Mathews *et al.* 1986; Quik 2012; Roelfsema & Clark 2001), to produce and secrete IGF-I. Furthermore, circulating GH may act on the brain as GH receptors are expressed in the brain (Brooks & Waters 2010), and GH has the ability to cross the blood–brain barrier (BBB) (Lopez-Fernandez *et al.* 1996; Pan *et al.* 2005; Ye *et al.* 1997).

IGF-I (D'Ercole *et al.* 1996; Folli *et al.* 1996) and IGF-I receptors (IGF-IR) (Wittwer & Hersberger 2007) are expressed in the brain by neurons, glial and endothelial cells. IGF-I can cross the BBB via carrier-mediated uptake (Armstrong *et al.* 2000; Nishijima *et al.* 2010), and endocrine IGF-I is believed to mediate some of the effects of GH on the brain such as proliferation, cellular differentiation, various plasticity-related processes in several brain regions (Åberg *et al.* 2006), trophic effects on central glutamatergic synapses (Trejo *et al.* 2007), effects on vascular structure and function and synaptic plasticity (Yan *et al.* 2011). However, it has also been suggested that there are some distinct differences between the effects of GH and IGF-I with respect to for example neuroprotection (Åberg *et al.* 2006; Arellanes-Licea *et al.* 2018; Sonntag *et al.* 2005).

In previous studies, we have investigated how GH affects male (Walser *et al.* 2014) and female (Walser *et al.* 2017) hypophysectomised (Hx) rats after six days of GH-treatment. In both reports, the two transcripts neuron–haemoglobin beta *(Hbb)* and 5′-aminolevulinate synthase 2 *(Alas2)*, the rate-limiting enzymes of the haeme synthesis, were considerably decreased in the hippocampus by Hx and robustly restored by GH administration.

In this study, to further examine whether the responsiveness differs between GH and IGF-I administered for a longer period of time, we investigated female Hx rats treated for 19 days with GH- and IGF-I infusions (Åberg *et al.* 2000). Since we previously observed that the *Igf1, Igf1r, Hif1a, Alas1, Gfap* and *Grin2a* transcripts in the female hippocampus were moderately affected by six days of GH-infusion (Walser *et al.* 2017), we included these transcripts in this study, with the hypothesis that a longer treatment could possibly exert larger effects. We also studied arachidonate 15-lipoxygenase *(Alox15)*, also called 15-LOX-1 (or 15-LOX), since it modulates vascular inflammation (Shalini *et al.* 2018; Wittwer & Hersberger 2007) and neuroprotection (Palacios-Pelaez *et al.* 2010), and has been found to increase in the hippocampus in response to GH treatment (Yan *et al.* 2011). Information and references on the transcripts are found in Table 1.

MATERIALS AND METHODS

Animals and hormonal treatment

Female Sprague-Dawley rats (Møllegaard Breeding Center Ltd., Ejby, Denmark), hypophysectomised (Hx) at 50 days of age, with commencement of experiments

Tab. 1. Gene names and abbreviations of commercially available probes. Key references for the transcripts.

Gene symbol	Fullname	Assay number	Reference(s)
Igf1	insulin-like growth factor 1	Rn 99999087_m1	Lopez-Fernandez, 1996
Igf1r	insulin-like growth factor 1 receptor	Rn 00583837_m1	Le Greves, 2006; De Geyter, 2016
Hbb-b1	hemoglobin, beta adult major chain	Rn 00583657_g1	He, 2009; Walser, 2017
Alas2	5′-aminolevulinate synthase 2	Rn01637175_m1	Sadlon, 1999, Walser, 2017
Alox15	arachidonate 15-lipoxygenase	Rn01646191_m1	Yan, 2011; Jung, 2015
Grin2a	glutamate receptor, ionotropic, 2a (N-methyl D-aspartate receptor 2a)	Rn 00561341_m1	Le Greves, 2002; Le Greves, 2006
Gfap	glial fibrillary acidic protein	Rn 00566603_m1	Pekny, 1995
Alas1	5′-aminolevulinate synthase 1	Rn00577936_m1	Thunell, 2006
Hif1a	hypoxia-inducible factor 1, alpha subunit	Rn01472831_m1	Viacava, 2003
Gapdh	glyceraldehyde 3-phosphate dehydrogenase	Rn 01462662_g1	

The probes are assay-on-demand mixes of primers and TaqMan MGB probes (FAM dye-labeled). Further details can be obtained at http://www.appliedbiosystems.com

Tab. 2. Weight gain and serum IGF-I levels of experimental animals

	Serum IGF-I	*p*	n	Weight gain	*p*	n
	(μg/l)			(g)		
Intact	1183 ± 161	< 0.001	3	58.7 ± 9.6	< 0.001	3
Hx	67 ± 7		6	0 ± 3.4		6
Hx + GH	712 ± 94	< 0.001	8	51.9 ± 6.7	< 0.001	8
Hx + IGF-I	145 ± 30	< 0.001	5	9.3 ± 3.4	0.014	7

T-tests are performed relative to Hx. Variation is given in 95% CIs. The number of rats in each group = n.

approximately one week later. The rats weighed 150 g at arrival, were maintained under standard conditions of temperature (24–26 °C) and humidity (50% to 60%) and a 14 h light: 10 h darkness cycle. The rats had free access to standard laboratory chow and water (Rat and mouse standard diet, B&K Universal Ltd., Sollentuna, Sweden). Body weight was measured every second day (0800 h) and Hx rats gaining in weight more than 0.5 g/day were excluded prior to experiments. All Hx rats received substitution therapy with cortisol phosphate (400 μg/kg/24h; Solucortef, Upjohn, Puurs, Belgium) and L-thyroxine (10 μg/kg/24h; Nycomed, Oslo, Norway) as a daily subcutaneous injection at 0800 h. The rats were divided into a control group (designated Hx) (n=6) and two treatment groups, which either received bovine GH (bovine GH recombinant) (n=8), a gift from American Cyanamide Co. (Princeton, NJ), or rhIGF-I (n=7) provided by Genentech, Inc. (South San Francisco, CA). Hormone administration, was maintained for 19 days, with continuous infusion of bGH (1 mg/kg/24h), or rhIGFI (0.85 mg/kg/24h) via mini-osmotic pumps implanted sc in the neck (0.25 μL/h, total volume 232 μL, model Alzet 2004 model, Alza Corp.). The samples from hippocampus were frozen, and stored until preparation. In a previous report the cerebral cortex (n=8) from this experiment have been used for assessment of expression (Åberg *et al.* 2000). Due to the use of hippocampal samples in the present study and cerebrocortical samples in the previous study, the samples were derived from partially different rats. Therefore, in the present report, this resulted in slightly different weight gains (n=7) and s-IGF-I (n=5) in the IGF-I group compared to previously reported results. All treatment procedures were approved by the Board of Animal Ethics at the University of Gothenburg.

Quantitative reverse transcription polymerase chain reaction (Q-RT-PCR)

Q-RT-PCR was used to quantify the transcripts. Total RNA was extracted from the hippocampus essentially according to Chomczynski and Sacchi, with minor modifications as described previously (Blomstrand *et al.* 1999; Chomczynski & Sacchi 1987). For assessment of RNA prior to downstream experiments, the optical density (OD) of 260/280 measurement via NanoDrop 1000 (Thermo Scientific) was used for quantification and Experion™ RNA analysis kit was used for quality assessment (Fleige & Pfaffl 2006). These assessments indicated a high quality of RNA with RQI >7.8. cDNA was prepared from 250 ng total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Q-RT-PCR analysis was performed using an ABI Prism 7900 Sequence Detection System (Applied Biosystems). Predesigned, TaqMan Gene Expression Assays were used (Applied Biosystems) (Table 1, for further details see http://www.appliedbiosystems.com). For information on the calculations of the Q-RT-PCR, see previously published Supplementary information (Walser *et al.* 2014). All transcript levels were normalized to the expression of GAPDH, and are therefore arbitrary but linear for each transcript, and comparisons between treatment groups represent actual linear changes.

Serum IGF-I analysis

Serum IGF-I concentrations were determined by a hydrochloride acid–ethanol extraction radioimmunoassay, using human IGF-I for labelling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) (Åberg *et al.* 2000; Rivero *et al.* 1994), with an inter-assay variability of 4.7% and an intra-assay variability of 3.8%.

Statistical analysis

Values are presented as the mean ± 95% confidence interval (CI). The values were log-transformed before evaluating effects of GH and IGF-I administrations as compared to Hx controls using two-tailed t-tests (in our case identical to ANOVA throughout the study). The Q-RT-PCR values of gene expression were logarithmically transformed to obtain better normal distribution, where appropriate. Post-hoc comparisons were not corrected for multiple comparisons, as the primary aim was to assess whether a change in the GH-treatment group was also present in the IGF-I-treatment group.

RESULTS

Systemic effects of GH and IGF-I

The GH-infusion group resulted in 88% of the weight gain in intact rats, and the IGF-I infusion group reached

16% of the weight gain in intact rats (Table 2). The s-IGF-I levels in the GH-treated group increased 10.5-fold relative to Hx after 19 days of treatment, while in the IGF-I group, there was a 2.2-fold increase of s-IGF-I relative to Hx. The comparatively small increase in s-IGF-I corresponded to modest weight gain in the IGF-1 treated rats. Among the IGF-I treated rats, there were clearly two populations; those who increased their weight gain and those who did not change their weight during the study. Therefore, in a posthoc analysis, data were separated for the group of the rats that exceeded the weight gains of the hx-group (i.e. weight gain > 6 g, n=4). These rats are shown below as "IGF-I- higher weight gain" (IGF-I-hwg) and had an average weight gain of 12.5 ± 3.0 g ($p < 0.001$). The low number of s-IGF-I observations (hwg, n=2 and non-hwg, n=3) precluded significance testing of s-IGF-I in the hwg and non-hwg-groups.

GH and IGF-I administration robustly increase Hbb, Alas2 and Alox15

The expression of *Hbb, Alas2* and *Alox15* transcripts in the Hx group was well below the level of the intact group (Hx compared to intact group: 0.6-fold, 0.6-fold, and 0.7-fold, respectively). The three transcripts were affected in the same direction by both GH and IGF-I administration as compared to Hx. GH administration significantly increased *Hbb, Alas2, Alox15* and *Gfap* (Hx compared to GH group: 3.1-fold, 3.2-fold, 3.8-fold, and 1.3-fold, respectively) (Figure 1), whereas only the Alox15 transcript was significantly increased by IGF-I (1.4-fold). However, in the IGF-I-hwg group, *Hbb, Alas2,* and *Alox15* were significantly raised as compared to hx (1.7-fold, 1.7-fold, and 1.7-fold, respectively), whereas GFAP was not significantly increased (p=0.11).

The transcripts *Igf1, Igf1r, Grin2a, Hif1a* and *Alas1* were not significantly affected by either GH or IGF-I administration.

DISCUSSION

In this report, we show that 19-day GH-infusion increased the expression of three transcripts, *Hbb, Alas2* and *Alox15*. For *Hbb* and *Alas2*, the effect size was of a similar magnitude as compared to that after 6 days of GH treatment (Walser *et al.* 2017), indicating that the GH effect is not markedly decreased over time. In addition, we show for the first time that IGF-I-treatment could increase expression of *Hbb, Alas2* and *Alox15*, but only in IGF-I treated rats gaining weight. For the other transcripts, the longer duration of this study did not significantly influence transcript levels.

Robustly increased transcripts and their functional consequences

The three transcripts with increased expression following GH and IGF-I treatment (*Hbb, Alas2* and Alox15), are all related to oxidative stress; *Hbb* and *Alas2* by increased oxygenation (Sadlon *et al.* 1999; Schelshorn *et al.* 2009) of the tissue and *Alox15* by regulating vascular tone, local blood flow, and blood pressure (Chawengsub *et al.* 2009). Based on these findings, we speculate that the basal metabolic rate may be lowered in Hx rats and that the administration of GH triggers the restoration of the basal metabolism leading to increased oxygen consumption, thereby affecting neuronal

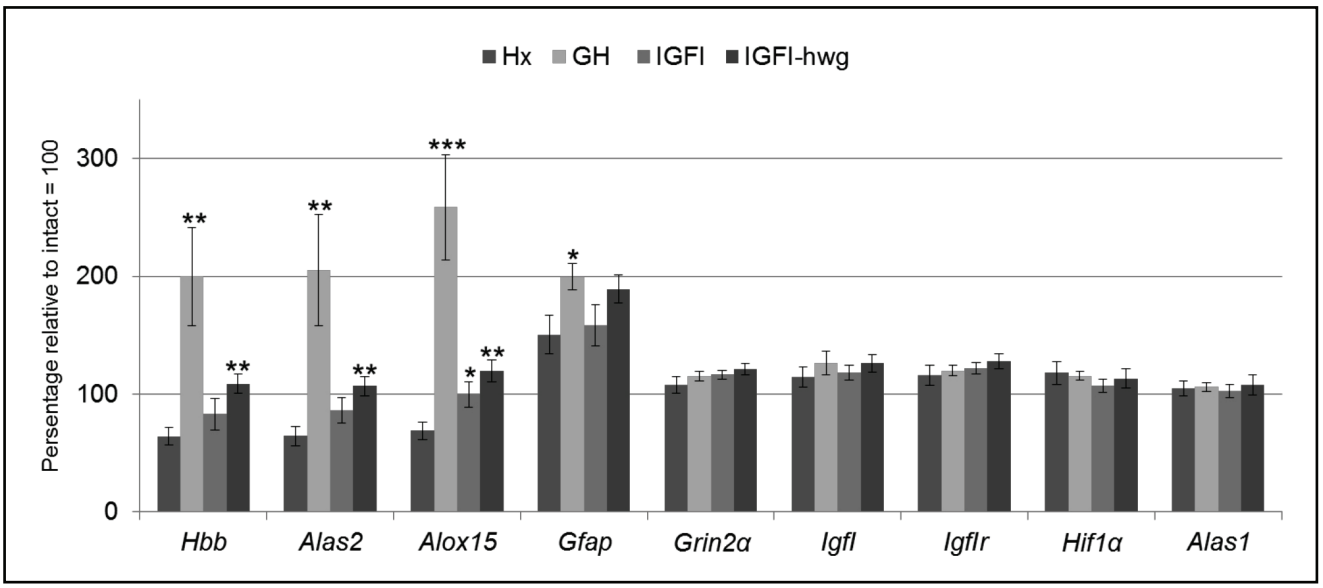

Fig. 1. Level of transcripts in hippocampus

All transcript levels are normalised to the level of the *Gapdh* transcript, the levels are therefore arbitrary but quantitative. For easier comparison, the average levels for intact rats have been set to 100%. Data are presented as means ± 95% confidence intervals (CIs). Statistically significant differences calculated by t-tests are thus indicated by non-overlapping CI. Number of rats in the respective groups; Hx (n=6), GH (n=8), IGF-I (n=7) and IGF-I-hwg = IGF-I-higher weight gain (n=4). * represents Hx vs GH, IGF-I or IGF-I-hwg; $p < 0.05$, ** represents Hx vs GH, IGF-I or IGF-I-hwg; $p < 0.01$, *** represents Hx vs GH, IGF-I or IGF-I-hwg; $p < 0.001$.

oxygen-binding capacity as well as vascular-associated transcripts (Goodman & Grichting 1983; Walser *et al.* 2017). Bovine GH transgenic mice show an increase in energy expenditure as compared to littermate controls indicating that GH treatment would increase basal metabolic rate (Olsson *et al.* 2005). In a rat model of GH / IGF-I deficiency [Lewis dwarf (dw / dw)], GH administration increased hippocampal expression of *Alox15, Hba-a2* and α and β globins transcripts (Yan *et al.* 2011). Furthermore, neuronal *Hbb* is upregulated following ischemia-induced hypoxia (He *et al.* 2009), which suggests that the regulation of this transcript may have consequences for previously reported GH- and IGF-I-induced neuroprotection (Åberg *et al.* 2006; Zhang *et al.* 2014).

GH and IGF-I and the interactions with insulin and estrogen

Apart from affecting IGF-I, GH also interacts with insulin and indirectly via IGF-I, the estrogen levels may be affected. In previous investigations we have shown the effect of rhIGF-I treatment in hypophysectomised rats given T4 and cortisol as background therapy on serum insulin and C-peptide levels (Frick *et al.* 2000). In that study, a marked and significant reduction in both serum insulin and C-peptide was observed following rhIGF-I treatment. Since GH is known to increase serum insulin levels (Gosteli-Peter *et al.* 1994), and rhIGF-I and GH had similar effects on several mRNA species in the present study, it is concluded that these effects are not likely explained by changed insulin levels. The level of IGF-I binding protein (IGFBP) may influence the half-life and action of IGF-I. Also, nutritional status as well as insulin regulates IGFBPs and therefore IGF-1 half-life and action, (for review see, (Thissen *et al.* 1999). In this study IGFBPs were not measured. However, it could be speculated that low IGFBP levels in this study could have affected IGF-I half-life and therefore IGF-I action on brain transcripts.

In the present study, the animals are hypophysectomised and therefore the ovaries produce very low levels estrogens. Since there is an interaction between estrogen and IGF-I, it would be of interest to investigate the combined effect of estrogens and IGF-I on brain transcripts in future studies. Ligand bound estrogen receptor α is required for rapid activation of the IGF-I-receptor signalling cascade (Kahlert *et al.* 2000). Both IGF-I and estradiol activate the MAPK/ERK and the PI3K/Akt pathways involved in IGF-I signalling (Cardona-Gomez *et al.* 2001). Therefore, it is fully possible that additional supplementation with 17beta-estradiol in the IGF-I administration group, would have increased the effect of IGF-I on the studied hippocampal transcripts.

CONCLUSION

The three transcripts *Hbb, Alas2* and *Alox15* were decreased by Hx and effectively restored by GH administration. The effect of GH treatment on these transcripts does not seem to be transient since the longer 19-day treatment period of GH-infusion resulted in similar effects as the 6-day treatment. IGF-I infusions had a smaller effect than GH, but when related to weight gain the effect was rather similar. Neuronal *Hbb, Alas2* and *Alox15* all have links to the oxygen-binding capacity or regulation of vascular tone and may therefore be vital to neuroprotection and recovery after brain injuries. For further exploration, it would be of interest to analyse the role of *Hbb, Alas2*, and *Alox15* in the functional recovery after brain injuries treated with GH/IGF-I infusions.

CONFLICT OF INTEREST STATEMENT

Jan Oscarsson is employed by AstraZeneca. The other authors declare no conflicts of interest.

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REFERENCES

1 Arellanes-Licea EC, Avila-Mendoza J, Ramirez-Martinez EC, Ramos E, Uribe-Gonzalez N, Aramburo C, Morales T, Luna M (2018). Upregulation of GH, but not IGF1, in the hippocampus of the lactating dam after kainic acid injury. Endocrine connections **7**: 258-267.
2 Armstrong CS, Wuarin L, Ishii DN (2000). Uptake of circulating insulin-like growth factor-I into the cerebrospinal fluid of normal and diabetic rats and normalization of IGF-II mRNA content in diabetic rat brain. J Neurosci Res **59**: 649-660.
3 Blomstrand F, Åberg ND, Eriksson PS, Hansson E, Ronnback L (1999). Extent of intercellular calcium wave propagation is related to gap junction permeability and level of connexin-43 expression in astrocytes in primary cultures from four brain regions. Neuroscience **92**: 255-265.
4 Brooks AJ, Waters MJ (2010). The growth hormone receptor: mechanism of activation and clinical implications. Nature reviews Endocrinology **6**: 515-525.
5 Cardona-Gomez GP, Mendez P, Doncarlos LL, Azcoitia I, Garcia-Segura LM (2001). Interactions of estrogens and insulin-like growth factor-I in the brain: implications for neuroprotection. Brain research Brain research reviews **37**: 320-334.
6 Chawengsub Y, Gauthier KM, Campbell WB (2009). Role of arachidonic acid lipoxygenase metabolites in the regulation of vascular tone. American journal of physiology Heart and circulatory physiology **297**: H495-507.
7 Chia DJ (2014). Minireview: mechanisms of growth hormone-mediated gene regulation. Mol Endocrinol **28**: 1012-1025.
8 Chomczynski P, Sacchi N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem **162**: 156-159.

9 D'ercole AJ, Ye P, Calikoglu AS, Gutierrez-Ospina G (1996). The role of the insulin-like growth factors in the central nervous system. Mol Neurobiol **13**: 227-255.
10 De Geyter D, De Smedt A, Stoop W, De Keyser J, Kooijman R (2016). Central IGF-I Receptors in the Brain are Instrumental to Neuroprotection by Systemically Injected IGF-I in a Rat Model for Ischemic Stroke. CNS Neurosci Ther. **22**(7): 611-616.
11 Fleige S, Pfaffl MW (2006). RNA integrity and the effect on the real-time qRT-PCR performance. Molecular Aspects of Medicine **27**: 126-139.
12 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. Mol Neurobiol **13**: 155-183.
13 Frick F, Oscarsson J, Vikman-Adolfsson K, Ottosson M, Yoshida N, Eden S (2000). Different effects of IGF-I on insulin-stimulated glucose uptake in adipose tissue and skeletal muscle. Am J Physiol Endocrinol Metab **278**: E729-737.
14 Goodman HM, Grichting G (1983). Growth hormone and lipolysis: a reevaluation. Endocrinology **113**: 1697-1702.
15 Gosteli-Peter MA, Winterhalter KH, Schmid C, Froesch ER, Zapf J (1994). Expression and regulation of insulin-like growth factor-I (IGF-I) and IGF-binding protein messenger ribonucleic acid levels in tissues of hypophysectomized rats infused with IGF-I and growth hormone. Endocrinology **135**: 2558-2567.
16 Guan J, Bennet L, Gluckman PD, Gunn AJ (2003). Insulin-like growth factor-1 and post-ischemic brain injury. Prog Neurobiol **70**: 443-462.
17 Guan J, Miller OT, Waugh KM, Mccarthy DC, Gluckman PD (2001). Insulin-like growth factor-1 improves somatosensory function and reduces the extent of cortical infarction and ongoing neuronal loss after hypoxia-ischemia in rats. Neuroscience **105**: 299-306.
18 He Y, Hua Y, Liu W, Hu H, Keep RF, Xi G (2009). Effects of cerebral ischemia on neuronal hemoglobin. J Cereb Blood Flow Metab **29**: 596-605.
19 Jung JE, Karatas H, Liu Y, Yalcin A, Montaner J, Lo EH, Van Leyen K (2015). STAT-dependent upregulation of 12/15-lipoxygenase contributes to neuronal injury after stroke. J Cereb Blood Flow Metab **35**: 2043-2051.
20 Kahlert S, Nuedling S, Van Eickels M, Vetter H, Meyer R, Grohe C (2000). Estrogen receptor alpha rapidly activates the IGF-1 receptor pathway. J Biol Chem **275**: 18447-18453.
21 Kooijman R, Sarre S, Michotte Y, De Keyser J (2009). Insulin-like growth factor I: a potential neuroprotective compound for the treatment of acute ischemic stroke? Stroke **40**: e83-88.
22 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. Proc Natl Acad Sci U S A **99**: 7119-7123.
23 Le Greves M, Zhou Q, Berg M, Le Greves P, Fholenhag 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. Exp Brain Res **173**: 267-273.
24 Lopez-Fernandez J, Sanchez-Franco F, Velasco B, Tolon RM, Pazos F, Cacicedo L (1996). Growth hormone induces somatostatin and insulin-like growth factor I gene expression in the cerebral hemispheres of aging rats. Endocrinology **137**: 4384-4391.
25 Mathews LS, Norstedt G, Palmiter RD (1986). Regulation of insulin-like growth factor I gene expression by growth hormone. Proc Natl Acad Sci U S A **83**: 9343-9347.
26 Nishijima T, Piriz J, Duflot S, Fernandez AM, Gaitan G, Gomez-Pinedo U, Verdugo JMG, Leroy F, et al. (2010). Neuronal Activity Drives Localized Blood-Brain-Barrier Transport of Serum Insulin-like Growth Factor-I into the CNS. Neuron **67**: 834-846.
27 Ohlsson C, Mohan S, Sjogren K, Tivesten A, Isgaard J, Isaksson O, Jansson JO, Svensson J (2009). The role of liver-derived insulin-like growth factor-I. Endocr Rev **30**: 494-535.
28 Olsson B, Bohlooly YM, Fitzgerald SM, Frick F, Ljungberg A, Ahren B, Tornell J, Bergstrom G, et al. (2005). Bovine growth hormone transgenic mice are resistant to diet-induced obesity but develop hyperphagia, dyslipidemia, and diabetes on a high-fat diet. Endocrinology **146**: 920-930.
29 Ong LK, Chow WZ, Tebay C, Kluge M, Pietrogrande G, Zalewska K, Crock P, Åberg ND, et al. (2018). Growth Hormone Improves Cognitive Function After Experimental Stroke. Stroke **49**: 1257-1266.
30 Palacios-Pelaez R, Lukiw WJ, Bazan NG (2010). Omega-3 essential fatty acids modulate initiation and progression of neurodegenerative disease. Mol Neurobiol **41**: 367-374.
31 Pan W, Yu Y, Cain CM, Nyberg F, Couraud PO, Kastin AJ (2005). Permeation of growth hormone across the blood-brain barrier. Endocrinology **146**: 4898-4904.
32 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 J **14**: 1590-1598.
33 Quik EH (2012). The somatotropic axis: Effects on brain and cognitive functions. Utrecht University Repository. Utrecht University, Utrecht University.
34 Rivero F, Goya L, Pascual-Leone AM (1994). Comparison of extraction methods for insulin-like growth factor-binding proteins prior to measurement of insulin-like growth factor-I in undernourished neonatal and adult rat serum. J Endocrinol **140**: 257-263.
35 Roelfsema V, Clark RG (2001). The growth hormone and insulin-like growth factor axis: its manipulation for the benefit of growth disorders in renal failure. J Am Soc Nephrol **12**: 1297-1306.
36 Sadlon TJ, Dell'oso T, Surinya KH, May BK (1999). Regulation of erythroid 5-aminolevulinate synthase expression during erythropoiesis. The international journal of biochemistry & cell biology **31**: 1153-1167.
37 Schelshorn DW, Schneider A, Kuschinsky W, Weber D, Krüger C, Dittgen T, Bürgers HF, Sabouri F, et al. (2009). Expression of hemoglobin in rodent neurons. Journal of Cerebral Blood Flow and Metabolism **29**: 585-595.
38 Schneider-Rivas S, Rivas-Arancibia S, Vazquez-Pereyra F, Vazquez-Sandoval R, Borgonio-Perez G (1995). Modulation of long-term memory and extinction responses induced by growth hormone (GH) and growth hormone releasing hormone (GHRH) in rats. Life Sci **56**: PL433-441.
39 Shalini SM, Ho CF, Ng YK, Tong JX, Ong ES, Herr DR, Dawe GS, Ong WY (2018). Distribution of Alox15 in the Rat Brain and Its Role in Prefrontal Cortical Resolvin D1 Formation and Spatial Working Memory. Mol Neurobiol **55**: 1537-1550.
40 Sonntag WE, Ramsey M, Carter CS (2005). Growth hormone and insulin-like growth factor-1 (IGF-1) and their influence on cognitive aging. Ageing research reviews **4**: 195-212.
41 Thissen JP, Underwood LE, Ketelslegers JM (1999). Regulation of insulin-like growth factor-I in starvation and injury. Nutrition reviews **57**: 167-176.
42 Thunell S (2006). (Far) Outside the box: genomic approach to acute porphyria. Physiological research / Academia Scientiarum Bohemoslovaca **55 Suppl 2**: S43-66.
43 Trejo JL, Piriz J, Llorens-Martin MV, Fernandez AM, Bolos M, Leroith D, Nunez A, Torres-Aleman I (2007). Central actions of liver-derived insulin-like growth factor I underlying its pro-cognitive effects. Molecular psychiatry **12**: 1118-1128.
44 Walser M, Schioler L, Oscarsson J, Aberg MA, Svensson J, Åberg ND, Isgaard J (2014). Different modes of GH administration influence gene expression in the male rat brain. J Endocrinol **222**: 181-190.
45 Walser M, Schioler L, Oscarsson J, Aberg MA, Wickelgren R, Svensson J, Isgaard J, Åberg ND (2017). Mode of GH administration and gene expression in the female rat brain. J Endocrinol **233**: 187-196.
46 Viacava P, Gasperi M, Acerbi G, Manetti L, Cecconi E, Bonadio AG, Naccarato AG, Acerbi F, et al. (2003). Microvascular density and vascular endothelial growth factor expression in normal pituitary tissue and pituitary adenomas. Journal of endocrinological investigation **26**: 23-28.
47 Wittwer J, Hersberger M (2007). The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins, leukotrienes, and essential fatty acids **77**: 67-77.
48 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. J Endocrinol. **211**: 27-37.

49 Ye P, Umayahara Y, Ritter D, Bunting T, Auman H, Rotwein P, D'ercole AJ (1997). Regulation of insulin-like growth factor I (IGF-I) gene expression in brain of transgenic mice expressing an IGF-I-luciferase fusion gene. Endocrinology **138**: 5466-5475.
50 Zhang H, Han M, Zhang X, Sun X, Ling F (2014). The effect and mechanism of growth hormone replacement on cognitive function in rats with traumatic brain injury. PLoS ONE **9**.
51 Åberg DN, Lind J, Isgaard J, Georg Kuhn H (2010). Peripheral growth hormone induces cell proliferation in the intact adult rat brain. Growth Horm Igf Res **20**: 264-269.
52 Åberg ND, Brywe 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. ScientificWorldJournal **6**: 53-80.
53 Åberg ND, Carlsson B, Rosengren L, Oscarsson J, Isaksson OG, Ronnback L, Eriksson PS (2000). Growth hormone increases connexin-43 expression in the cerebral cortex and hypothalamus. Endocrinology **141**: 3879-3886.
54 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. J Endocrinol **201**: 141-150.