Mode of GH administration and gene expression in the female rat brain

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Abstract

The endogenous secretion of growth hormone (GH) is sexually dimorphic in rats with females having a more even and males a more pulsatile secretion and low trough levels. The mode of GH administration, mimicking the sexually dimorphic secretion, has different systemic effects. In the brains of male rats, we have previously found that the mode of GH administration differently affects neuron–haemoglobin beta (*Hbb*) expression whereas effects on other transcripts were moderate. The different modes of GH administration could have different effects on brain transcripts in female rats. Hypophysectomised female rats were given GH either as injections twice daily or as continuous infusion and GH-responsive transcripts were assessed by quantitative reverse transcription polymerase chain reaction in the hippocampus and parietal cortex (cortex). The different modes of GH-administration markedly increased *Hbb* and 5'-aminolevulinate synthase 2 (*Alas2*) in both brain regions. As other effects were relatively moderate, a mixed model analysis (MMA) was used to investigate general effects of the treatments. In the hippocampus, MMA showed that GH-infusion suppressed glia- and neuron-related transcript expression levels, whereas GH-injections increased expression levels. In the cortex, GH-infusion instead increased neuron-related transcripts, whereas GH-injections had no significant effect. Interestingly, this contrasts to previous results obtained from male rat cortex where GH-infusion generally decreased expression levels. In conclusion, the results indicate that there is a small but significant difference in response to mode of GH administration in the hippocampus as compared to the cortex. For both modes of GH administration, there was a robust effect on *Hbb* and *Alas2*.

Key Words

- ▶ growth hormone
- ▶ injections
- ▶ infusion pumps
- ▶ female
- ▶ gene transcription

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Introduction

Growth hormone (GH) is a pleiotropic hormone, which apart from stimulating growth, improves mental well-being in several ways, such as wakefulness, energy level,

concentration and memory (McGauley 1989, Nyberg & Hallberg 2013, McEwen *et al.* 2015). By analogy, GH administration enhances memory parameters in

rats (Schneider-Rivas *et al.* 1995, Le Greves *et al.* 2006) as well as expression of plasticity-related transcripts (Aberg *et al.* 2010).

When GH is secreted from the pituitary or administered peripherally, it stimulates the liver to release insulin-like growth factor-I (IGF-I) (Mathews *et al.* 1986), which mediates some of the effects of GH. In the brain, both GH and IGF1 have been shown to cross the blood–brain barrier (Armstrong *et al.* 2000, Pan *et al.* 2005, Nishijima *et al.* 2010).

GH receptors are expressed in both glial and neuronal cells, and it is suggested that GH can stimulate both cell types directly (Lobie *et al.* 1993, Hallberg & Nyberg 2012). Many of the effects of GH are related to different aspects of brain plasticity. For instance, GH treatment shows increase in cell genesis and in number of newborn neurons in the adult brain (Aberg *et al.* 2009, 2010). Likewise, IGF-I (D'Ercole *et al.* 1996, Folli *et al.* 1996) and IGF-I receptors are expressed in the brain by neurons, glial and endothelial cells (Yan *et al.* 2011). Furthermore, GH and IGF-I protect the brain against hypoxic-ischaemic injuries (HI) (Gustafson *et al.* 1999, Pathipati *et al.* 2009).

In humans and in rats, GH is secreted from the pituitary in a circadian rhythm that differs between the sexes. In rats, the mechanism of this sex discrepancy has been more studied than in humans. In male rats, maintaining low basal GH levels has been shown to be dependent on neonatal priming by androgens of the hypothalamus as well as by continuous adult presence of testosterone (Jansson & Frohman 1987). In female rats,

oestrogen elevates basal plasma GH levels and suppresses GH pulses (Jansson *et al.* 1985). Also in humans, it is believed that the sexual dimorphic GH secretion results from a response to the inhibitory effects of 17 β -oestradiol and the stimulatory effect of testosterone acting on hypothalamic somatostatin release (Devesa *et al.* 1991). This gives rise to low trough levels and high GH peaks with 3- to 4-h intervals in male rodents, whereas more frequent peaks and almost no troughs are observed in female rodents, thereby a more even GH secretory pattern (Eden 1979, MacLeod *et al.* 1991). In rats, the pulsatile mode of GH treatment, which imitates the male endogenous GH secretion (Jansson *et al.* 1982), enhanced *Igf1* mRNA levels more than infusions both in rib growth plate and in skeletal muscle (Isgaard *et al.* 1988). So far, the effects of different modes of GH administration have only been investigated in the hippocampus and in the parietal cortex of male hypophysectomised rats (Walser *et al.* 2014). Specifically, we found that the two brain regions are diversely regulated by different modes of GH administration to a moderate extent with the exception for the highly regulated neuronal–haemoglobin beta (*Hbb*).

However, it is not known whether the mode of GH treatment affects the female rat brain. By analogy with the different effects seen in the periphery, our hypothesis was that different modes of GH administrations, mimicking the specific secretion patterns in males and females, might give rise to responses different from those of male rat brains. Therefore, we administered GH as two daily

Table 1 Abbreviations of probes and gene names, and the names of the transcripts that are used in the present study.

Gene symbol	Fullname	Alias or abbreviation in Ms	Assay number
Transcript data			
Ghr	Growth hormone receptor	<i>Ghr</i>	Rn 00567298_m1
Igf1	Insulin-like growth factor 1	<i>Igf1</i>	Rn 99999087_m1
Igf1r	Insulin-like growth factor 1 receptor	<i>Igf1r</i>	Rn 00583837_m1
Esr1	Estrogen receptor 1	<i>Esr1</i>	Rn 01640372_m1
Hbb-b1	Hemoglobin, beta adult major chain	<i>Hbb</i>	Rn 00583657_g1
Alas2	5'-Aminolevulinate synthase 2	<i>Alas2</i>	Rn 01637175_m1
Grin2a	Glutamate receptor, ionotropic, 2a (N-methyl D-aspartate receptor 2a)	<i>Nr2a (Nmda2a)</i>	Rn 00561341_m1
Dlg4	Discs, large (<i>Drosophila</i>) homolog-associated protein 4/postsynaptic density-95	<i>Psd95</i>	Rn 00571479_m1
Gabbr1	Gamma-aminobutyric acid β receptor, 1	<i>Gabab1</i>	Rn 02586477_m1
Gria1	Glutamate receptor, ionotropic, AMPA 1	<i>Gria1</i>	Rn 00709588_m1
Oprd1	Opioid receptor, delta 1	<i>Dor</i>	Rn 00561699_m1
Cnp	2',3'-Cyclic nucleotide 3' phosphodiesterase	<i>Cnp</i>	Rn 01399463_m1
Gja1	Gap junction alpha-1 protein (connexin 43)	<i>Cx43</i>	Rn 01433597_m1
Gfap	Glial fibrillary acidic protein	<i>Gfap</i>	Rn 00566603_m1
Alas1	5'-Aminolevulinate synthase 1	<i>Alas1</i>	Rn 00577936_m1
Hif1a	Hypoxia-inducible factor 1, alpha subunit	<i>Hif1α</i>	Rn 01472831_m1
Gapdh	Glyceraldehyde 3-phosphate dehydrogenase	<i>Gapdh</i>	Rn 01462662_g1

injections or as infusions by osmotic minipumps, and investigated the effects on previously known transcripts related to plasticity and transcripts related to oxygenation in the hippocampus and parietal cortex (henceforth cortex). The transcripts were divided into four categories (Walser *et al.* 2014): GH-, neuron-, glia-related and neuron-Hbb (Table 1 and Supplementary Table 1 for further details, see section on supplementary data given at the end of this article). Three transcripts, which have not been studied before, were also measured as they are functionally associated with Hbb and oxygenation (Walser *et al.* 2014). These were the rate-limiting enzymes of the haeme synthesis delta-aminolevulinate synthase 1 and 2 (*Alas1*, *Alas2*) and the hypoxia-inducible factor 1-alpha (*HIF1a*), which functions as a master transcriptional regulator of the adaptive response to hypoxia (Table 1, for further details see Supplementary Table 1).

Materials and methods

Animals and hormonal treatment

The experiments were performed in female ($n=21$) Sprague–Dawley rats (Møllegaard Breeding Center Ltd, Ejby, Denmark), as previously described ((Walser *et al.* 2011) and Supplementary information). Normal pituitary-intact rats (henceforth intact) and hypophysectomised rats (henceforth Hx) were kept to monitor effects of Hx *per se*, and to evaluate whether bovine-GH (bGH) restored specific transcript expression to relevant physiological levels.

The effects of hormonal administration were assessed in rats, which were Hx at 60 days of age ($n=5-6$ in each group). Hormone administration was maintained for 7 days and initiated 10 days after Hx, to sort out rats with incomplete Hx as determined by weight gain. All Hx rats received substitution therapy with cortisol phosphate (C; 400 µg/kg/day; Solucortef, Upjohn, Puurs, Belgium) and L-thyroxine (T_4 ; 10 µg/kg/day; Nycomed, Oslo, Norway), which were diluted in saline and administered subcutaneously once daily at 08:00 h (Jansson *et al.* 1982). These rats were randomised into a control group (Hx) and two bovine GH (bGH)-groups. Bovine GH (recombinant), donated by American Cyanamide Co (Princeton, NJ, USA), was prepared as described before and given as a subcutaneous continuous infusion (0.7 mg/kg per day) for 7 days using mini-osmotic pumps (Alzet 2004 model) implanted subcutaneously in the neck (henceforth GH)

or as subcutaneous injections (henceforth GHx2; i.e. 0.35 mg/kg, twice daily, equalling a total of 0.7 mg/kg per 24 h) (Oscarsson *et al.* 1999). Tissues were immediately dissected and frozen in liquid nitrogen and stored at -80°C . All treatment procedures were approved by the Board of Animal Ethics of the University of Gothenburg. Of note is that samples from the intact, Hx and GH groups have been used in previous experiments (Walser *et al.* 2011) but the transcripts assessed then (*Hbb*, *Gabbr1*) have here been re-analysed with quantitative reverse transcription polymerase chain reaction (Q-RT-PCR) using new primers.

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

To quantify the different transcripts, we used the Q-RT-PCR. Total RNA was extracted from hippocampus and cortex using the Tri Reagent solution (Ambion) and quantified by spectrophotometric analysis of absorption at 260 vs 280 nm using a Nanodrop 1000 (Thermo Scientific). cDNA was prepared from 250 ng total RNA, (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>).

Detailed information and references on the transcripts are found in Table 2. For information on the calculations of the Q-RT-PCR, Supplementary information (Walser *et al.* 2014).

Statistical analysis

Values are presented as the mean $\pm$ 95% confidence interval (CI). Comparisons between any two groups were made using unpaired *t*-tests (Table 4). As the primary objective was to compare GH and GHx2, these analyses were not corrected for multiple comparisons. Furthermore, as the transcripts were selected for previous GH-responsiveness, other secondary comparisons are shown for convenience but are not corrected for multiple comparisons. Correlations were calculated according to the Pearson correlation coefficient expressed as *r*.

A mixed model analysis (MMA) was used for all other statistical comparisons, such as to evaluate effects of GH and GHx2 related to each of the categories of transcripts. For further information on the MMA, Supplementary

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

Abbreviation	Primarily in cell type	Designated category in Ms	Main function	Reference(s)
Ghr	Neuron/glia	GH-related (GH)	Brain plasticity	Le Greves <i>et al.</i> (2002)
Igf1	Neuron/glia	GH-related (GH)	Brain plasticity	Lopez-Fernandez <i>et al.</i> (1996)
Igf1r	Neuron/glia	GH-related (GH)	Brain plasticity	De Geyter <i>et al.</i> (2016)
Esr1	Neuron/glia	GH-related (GH)	Brain plasticity	Pons & Torres-Aleman (1993)
Hbb	Neuron	Neuron/Hbb-related (N-Hbb)	O2-regulatory protein	He <i>et al.</i> (2009), Walser <i>et al.</i> (2014)
Alas2	Neuron	Neuron/Hbb-related (N-Hbb)	Rate-controlling enzyme of heme biosynthesis	Sadlon <i>et al.</i> (1999)
Nr2a	Neuron	Neuron – related (N)	Hippocampal synaptic plasticity	Le Greves <i>et al.</i> (2002, 2006)
Psd95	Neuron	Neuron – related (N)	Hippocampal synaptic plasticity	Le Greves <i>et al.</i> (2006)
Gabab1	Neuron	Neuron – related (N)	Inhibitory neurotransmitter/ neuroprotection	Xu <i>et al.</i> (2008)
Gria1	Neuron	Neuron – related (N)	Excitatory neurotransmitter receptors	Martin <i>et al.</i> (1993)
Dor	Neuron (glia)	Neuron – related (N)	Neuroprotective effects	Persson <i>et al.</i> (2003)
Cnp	Glia	Glia-related (G)	Formation of the myelin sheath	Aberg <i>et al.</i> (2007)
Cx43	Glia	Glia-related (G)	Cell communication	Aberg <i>et al.</i> (2000)
Gfap	Glia	Glia-related (G)	Excitatory neurotransmitter/ morphogenesis	Pekny <i>et al.</i> (1995)
Alas1	Glia	Glia-related (G)	Rate-controlling enzyme of heme biosynthesis	Thunell (2006)
Hif1a	Glia	Glia-related (G)	Hypoxia-induced signaling protein	Ziello <i>et al.</i> (2007)

For more details on exact effects and previous effects by GH/IGF-I administration, see [Supplementary Table 1](#).

information (Walser *et al.* 2014). $P < 0.05$ were considered statistically significant.

Results

GH exerts systemic effects

Hx rats received bGH either as subcutaneous infusion (GHi) or injections twice daily (GHx2) for 7 days. bGH normalised weight gains in Hx rats to slightly above the weight gain observed in intact rats ($P < 0.001$) (Table 3), indicating that the administered GH had the expected systemic effect on body growth. Of note is that GHx2 increased weight gain significantly more (+20%) than GHi.

Table 3 Weight gains of experimental animals treated with bGH for 7 days.

	Weight gain (g)	T-test (P-values)	
		Each vs hx	Ghi vs GHx2
Intact	3.60 ± 0.38	<0.001	0.022
Hx	−0.01 ± 0.11		
Hx + GHi	3.76 ± 1.78	<0.001	
Hx + GHx2	4.53 ± 0.10	<0.001	

Two-tailed T-tests are performed relative Hx and between GHi and GHx2. Values are given as means ± 95% confidence intervals (CIs).

GH administration produces a robust response in *Hbb* and *Alas2* expression but has a moderate influence on other transcripts in both the hippocampus and cortex

The selection of transcripts investigated in this study is based on results from previous studies showing that they are regulated by GH treatment in the adult male rat brain, and divided into GH-, neuron- or glia-related transcripts (Tables 1, 2 and [Supplementary Table 1](#)).

Overall, both GHi and GHx2 induced small and in most cases non-significant changes in transcript abundance, except for the robust increases in *Hbb* and *Alas2* (Table 4). In most cases, there was some agreement with previous reports but not necessarily in both brain regions or for both modes of administration. Hx *per se* only significantly affected a few transcripts, namely *Alas2* ($P = 0.001$) in the hippocampus and *Hbb* ($P = 0.005$), *Esr1* ($P = 0.023$), *Gria1* ($P = 0.029$) and *Alas2* ($P = 0.042$) in the cortex.

When testing our primary hypothesis, whether there was different magnitude in response to GHi or GHx2, only a few specific transcripts exhibited statistically significant differences *Gfap*, *Igf1r*, *Psd95* and *Alas1* in the hippocampus, and *Hbb*, *Psd95* and *Hif1a* in the cortex (Table 4). It is of note that in all these cases, GHx2 showed a higher response than GHi in the hippocampus, whereas the opposite, except for *Hbb*, was the case in the cortex.

Table 4 The relative mean values for the transcripts $\pm$ 95% confidence intervals (CIs).

Cat.	Transcript	Group	Hippocampus	N	Cortex	N
GH	<i>Ghr</i>	Intact	100.0 (77.6–122.4)	6	100.0 (65.2–134.8)	5
		Hx	98.2 (75.7–120.7)	6	95.4 (64.1–126.6)	6
		GHi	95.6 (71.0–120.2)	5	91.7 (57.5–126.0)	5
		GHx2	128.8 (101.3–156.4)	4	107.1 (72.9–141.4)	5
GH	<i>Igf1</i>	Intact	100.0 (74.9–125.1)	6	100.0 (59.5–140.5)	5
		Hx	76.3 (54.3–98.3)	6	119.7 (82.8–156.6)	6
		GHi	97.2 (73.1–121.4)	5	109.1 (68.7–149.6)	5
		GHx2	103.2 (76.2–130.2)	4	78.3 (37.8–118.7)	5
GH	<i>Igf1r</i>	Intact	100.0 (79.9–120.1)	6	100.0 (72.4–127.6)	5
		Hx	97.1 (76.9–117.2)	6	73.7 (47.0–100.4)	6
		GHi	80.1 (58.0–102.2) [†]	5	99.9 (70.6–129.1)	5
		GHx2	114.7 (90.1–139.4)	4	71.0 (41.7–100.3)	5
GH	<i>Esr1</i>	Intact	100.0 (76.2–123.8)	6	100.0 (73.6–126.4)	5
		Hx	109.7 (83.7–135.7)	6	57.7 (32.1–83.3) [‡]	6
		GHi	101.2 (72.7–129.7)	5	56.5 (25.9–87.1)	4
		GHx2	115.9 (84.0–147.7)	4	69.3 (34.7–103.8)	3
N-Hbb	<i>Hbb</i>	Intact	100.0 (47.9–152.1)	6	100.0 (64.3–135.7)	5
		Hx	34.3 (–31.8 to 100.4)	6	22.5 (–10.5 to 55.6) ^{‡‡}	6
		GHi	147.1 (74.7–219.5) [*]	5	55.3 (19.1–91.5) [†]	5
		GHx2	131.0 (50.0–211.9)	4	111.4 (75.2–147.6) ^{**}	5
N-Hbb	<i>Alas2</i>	Intact	100.0 (79.5–120.5)	5	100.0 (42.9–157.1)	4
		Hx	47.7 (–15.4 to 110.8) ^{‡‡}	6	22.9 (–6.5 to 52.4) [‡]	6
		GHi	127.1 (57.9–196.2)	5	75.1 (42.8–107.3) [*]	5
		GHx2	190.7 (113.4–268.0) ^{**}	4	98.2 (65.9–130.5) ^{**}	5
N	<i>Grin2a</i>	Intact	100.0 (78.4–121.6)	6	100.0 (68.7–131.3)	5
		Hx	91.8 (67.5–116.2)	6	71.1 (44.5–97.7)	6
		GHi	73.9 (47.3–100.6)	5	100.5 (71.4–127.9)	5
		GHx2	113.6 (83.8–143.4)	4	65.4 (36.3–94.6)	5
N	<i>Dlg4</i>	Intact	100.0 (79.5–120.5)	6	100.0 (55.8–144.2)	5
		Hx	97.9 (79.9–115.8)	6	113.1 (75.4–150.8)	6
		GHi	78.0 (58.4–97.7) [†]	5	131.6 (90.3–172.9) [†]	5
		GHx2	111.9 (89.9–133.9)	4	67.3 (26.0–108.6)	5
N	<i>Gabbr1</i>	Intact	100.0 (68.7–131.3)	6	100.0 (62.7–137.3)	5
		Hx	137.9 (111.7–164.1)	6	93.2 (61.8–124.7)	6
		GHi	101.8 (73.1–130.4)	5	129.7 (95.2–164.1)	5
		GHx2	120.9 (88.8–152.9)	4	81.7 (47.2–116.2)	5
N	<i>Gria1</i>	Intact	100.0 (78.0–122.0)	6	100.0 (78.9–121.1)	5
		Hx	96.4 (76.4–116.5)	6	67.4 (42.0–92.7) [‡]	6
		GHi	78.9 (56.9–100.8)	5	99.1 (71.4–126.9)	5
		GHx2	109.4 (84.9–133.9)	4	77.6 (49.8–105.4)	5
N	<i>Oprd1</i>	Intact	100.0 (70.3–129.7)	6	100.0 (71.3–128.7)	5
		Hx	112.3 (86.0–138.5)	6	84.4 (35.8–133.0)	6
		GHi	77.6 (48.9–106.3)	5	126.9 (73.7–180.1)	5
		GHx2	116.5 (84.4–148.6)	4	67.2 (14.0–120.4)	5
G	<i>Cnp</i>	Intact	100.0 (72.1–127.9)	6	100.0 (74.4–125.6)	5
		Hx	96.0 (76.2–115.8)	6	82.0 (57.6–106.5)	6
		GHi	92.4 (70.7–114.1)	5	95.4 (68.6–122.1)	5
		GHx2	116.7 (92.4–141.0)	4	69.1 (42.3–95.8)	5
G	<i>Gja1</i>	Intact	100.0 (75.5–124.5)	6	100.0 (77.2–122.8)	5
		Hx	116.0 (95.8–136.2)	6	71.1 (51.9–90.4)	6
		GHi	96.4 (74.3–118.5)	5	81.3 (60.2–102.4)	5
		GHx2	109.6 (84.9–134.4)	4	92.9 (71.8–114.0)	5
G	<i>Gfap</i>	Intact	100.0 (73.6–126.4)	6	100.0 (65.3–134.7)	5
		Hx	111.1 (88.1–134.2)	6	110.4 (74.3–146.6)	6
		GHi	91.8 (66.5–117.0) ^{‡‡}	5	158.9 (119.3–198.5)	5
		GHx2	147.5 (119.2–175.7)	4	106.6 (67.0–146.2)	5
G	<i>Alas1</i>	Intact	100.0 (77.0–123.0)	6	100.0 (62.0–138.0)	4
		Hx	95.2 (77.7–112.7)	6	107.8 (71.1–144.4)	6
		GHi	73.0 (53.9–92.2) [*]	5	133.5 (93.4–173.7)	5
		GHx2	103.3 (81.9–124.7)	4	87.1 (46.9–127.2)	5
G	<i>Hif1a</i>	Intact	100.0 (76.4–123.6)	5	100.0 (55.0–145.0)	4
		Hx	97.2 (77.4–117.0)	6	134.7 (98.4–171.0)	6
		GHi	75.5 (53.8–97.2)	5	163.1 (123.3–202.8) [†]	5
		GHx2	105.1 (80.8–129.3)	4	93.2 (53.5–133.0)	5

Each group value is set in accordance with the intact group's value set to 100 for each transcript.

*Represents Hx vs GHi or GHx2 $P < 0.05$; [†]represents GHi vs GHx2 $P < 0.05$; [‡]represents Hx vs intact $P < 0.05$. Double symbols (**/†) represent P -values < 0.01 .

General effects in the hippocampus

To further study the general effect of mode of administration and category of transcript, a MMA statistical analysis was used. For the specific categories of transcripts, the MMA revealed a significant difference in the neuron- and glia-related group between GH_i and GHx2 groups, with a higher expression for GHx2 (Fig. 1A). In the neuron-Hbb category, there were significant differences between both Hx vs GH_i and Hx vs GHx2 (Fig. 1A). Furthermore, the MMA did not reveal any significant differences in the GH-related transcripts (Fig. 1A).

General effects in the cortex

Specifically, there was a significant difference between GH_i and GHx2 (Fig. 1B) in the neuron-related category, with negative effects of GHx2 as compared to the positive effects of GH_i. This finding was in contrast to the results found in hippocampal expression where the effect of GHx2 was in general larger than the effect of GH_i. In the neuron-Hbb category, there were significant differences between Hx and both of GH_i and GHx2 and between GH_i vs GHx2 (Fig. 1B), with a higher expression for GHx2, consistent with the results in the hippocampus. The GH-related and glia-related transcripts were not significantly affected by the mode of GH-administration (NS, Fig. 1B).

Figure 1

Levels of categories of transcripts in hippocampus (A) and cortex (B), as analysed by Q-RT-PCR. Significance levels and variation are given by mixed model analysis (MMA), see methods. All transcript levels are normalised to the level of the *Gapdh* transcript, the levels are therefore arbitrary but quantitative within each figure. For easier comparison, the average levels for intact rats have been set to 100%. Data are presented as means $\pm$ 95% confidence intervals (CIs). *P*-values are given when below 0.05.

Possible functional associations indicated by correlation patterns

Statistical correlation can be used to indicate which transcripts have a functional association. Thus, a statistical association between a transcript and weight increase may indicate one type of effect of GH, possibly by peripheral or direct effects of GH. In contrast, the other associations to components of the GH-IGF1 system may indicate a regulation by local components. Accordingly, it is of note that the expression of *Hbb* and *Alas2* was highly associated with weight gain in both brain regions (Table 5), whereas they were neutral or negatively associated with *Igf1r*. For all other transcripts, there was a neutral or negative association with weight gain, except for *Gja1* in the hippocampus (showed a positive association with weight gain). Further, these transcripts all showed a robust positive association with *Igf1r*. This was most evident in the hippocampus.

Discussion

This study aimed to investigate responses to different modes of GH administration to hypophysectomised female rats with respect to previously known GH-induced plasticity-related transcripts in the hippocampus and the cortex. The study shows that GH affects the selected transcripts differently in the hippocampus compared with the cortex albeit to a moderate degree. One exception was the neuron-Hbb category, where GH_i and to larger degree GHx2 increased expression by 2–3 fold in both brain regions (Fig. 1 and Table 4). For the other transcripts, primary statistical analysis for each of the transcripts revealed only a few instances of significant differences

Table 5 Correlation matrix for weight gain, and the GH-related transcripts vs all transcripts.

Cat.	Transcript	Hippocampus		Cortex	
	Correlations	Weight (Δ)	<i>Igf1r</i>	Weight (Δ)	<i>Igf1r</i>
N/A	weight (Δ)				
	<i>r</i>	1	-0.009	1	0.112
	<i>P</i>		0.969		0.630
GH	<i>Ghr</i>				
	<i>r</i>	0.124	0.734**	0.046	0.538*
	<i>P</i>	0.602	<0.001	0.842	0.012
GH	<i>Igf1</i>				
	<i>r</i>	0.205	0.464*	-0.461*	0.306
	<i>P</i>	0.386	0.039	0.035	0.177
GH	<i>Igf1r</i>				
	<i>r</i>	-0.009	1	0.112	1
	<i>P</i>	0.969		0.630	
GH	<i>Esr1</i>				
	<i>r</i>	-0.165	0.726**	0.312	0.741**
	<i>P</i>	0.476	<0.001	0.207	<0.001
N-Hbb	<i>Hbb</i>				
	<i>r</i>	0.540*	-0.468*	0.627**	0.146
	<i>P</i>	0.014	0.037	0.002	0.526
N-Hbb	<i>Alas2</i>				
	<i>r</i>	0.599**	-0.141	0.569**	-0.162
	<i>P</i>	0.005	0.553	0.009	0.495
N	<i>Grin2a</i>				
	<i>r</i>	0.105	0.843**	0.104	0.771**
	<i>P</i>	0.659	<0.001	0.653	<0.001
N	<i>Dlg4</i>				
	<i>r</i>	-0.012	0.881**	-0.318	0.359
	<i>P</i>	0.958	<0.001	0.159	0.110
N	<i>Gabbr1</i>				
	<i>r</i>	-0.449*	0.688**	-0.127	0.758**
	<i>P</i>	0.041	0.001	0.582	<0.001
N	<i>Gria1</i>				
	<i>r</i>	0.052	0.796**	0.293	0.790**
	<i>P</i>	0.823	<0.001	0.197	<0.001
N	<i>Oprd1</i>				
	<i>r</i>	-0.105	0.775**	-0.036	0.789**
	<i>P</i>	0.651	<0.001	0.877	<0.001
G	<i>Cnp</i>				
	<i>r</i>	0.054	0.534*	-0.037	0.427
	<i>P</i>	0.815	0.015	0.873	0.054
G	<i>Gja1</i>				
	<i>r</i>	-0.331	0.676**	0.442*	0.521*
	<i>P</i>	0.143	0.001	0.045	0.015
G	<i>Gfap</i>				
	<i>r</i>	0.061	0.637**	-0.112	0.365
	<i>P</i>	0.791	0.003	0.628	0.104
G	<i>Alas1</i>				
	<i>r</i>	-0.007	0.712**	-0.207	0.233
	<i>P</i>	0.975	<0.001	0.381	0.322
G	<i>Hif1a</i>				
	<i>r</i>	-0.025	0.605**	-0.284	0.581**
	<i>P</i>	0.918	0.005	0.225	0.007

The two-tailed correlation matrix was calculated according to Pearson (see 'Materials and methods' section). The correlation coefficients (*r*) represent analysis of all animals (*n*=21), with significance levels (*P*). Significant correlations are shown in bold. Category of transcript is abbreviated 'Cat.', and the specific abbreviations of each category of transcript (N, N-Hbb, N and G) is found in [Tables 1 and 2](#). N/A, not applicable. **P*<0.05, ***P*<0.01.

between GH_i and GH_{x2} ([Table 4](#)). Still, the MMA confirmed that the relatively moderate responses are mostly in the same direction with respect to Hx and intact rats across the transcripts. With regard to category of transcript, MMA revealed many cases of significant differences between GH_i and GH_{x2}. Importantly, GH_i and GH_{x2} acted differently in the hippocampus and in the cortex. Correlation analysis between weight gain, GH-related transcripts which are local components of the GH-IGF1 system, and the specific transcripts revealed two types of associations. Overall, *Hbb* and *Alas2* were associated with weight gain whereas most other transcripts were associated with the local *Igf1r* expression. These findings are discussed below.

Differences between GH_i and GH_{x2}

There were moderate but conceptual differences in the response to the two different administration paradigms. Peripherally, this was reflected in an expected higher weight gain of GH_{x2} as compared to GH_i ([Table 3](#)). The responses in the hippocampus and in the cortex are sequentially discussed below.

The hippocampus showed a significant difference in response between GH_i and GH_{x2}. Specifically, GH_i suppressed glia- and neuron-related transcript abundance, whereas GH_{x2} restored abundance to intact levels ([Fig. 1A](#)). Thus, GH_{x2} administration could be more optimal in eliciting a response in the hippocampus regarding neuron- and glia-related transcripts, which is in agreements with reports on systemic responses to different modes of GH administration. For example, an administration frequency of 2-4 injections per day of GH optimises body growth in male Hx rats ([Jansson et al. 1982](#)). In agreement, the study by Isgaard ([Isgaard et al. 1988](#)) demonstrated that pulsatile treatment induces local *Igf1* in skeletal muscle and rib growth plate more effectively than continuous GH in male Hx rats. Our results would thus indicate that GH_{x2} is more effective to elicit responses not only in the male, but also in the female hippocampus.

In contrast, in the cortex, GH_i increased neuron-related transcripts, whereas GH_{x2} had no effect ([Fig. 1B](#)). The glia- and GH-related categories were unaffected by administration of either GH_i or GH_{x2} ([Fig. 1](#)). This discrepancy between the pattern of response to GH_i and GH_{x2} in the hippocampus and in the cortex could partly be explained by generally tighter associations between the *Igf1r* and the other transcripts in the cortex as compared to the hippocampus ([Table 5](#)).

Effects of GH administration with respect to neuron-Hbb neuroprotection and oxygenation-promoting properties

We have previously demonstrated that GH administration in female (Walser *et al.* 2011) and male (Walser *et al.* 2014) hypophysectomised rats robustly regulates the level of the *Hbb* transcript in the brain. In the present experiments, we also show that the transcript for the rate-controlling enzyme of haeme biosynthesis *Alas2* is similarly affected by GH. This gives support to that GH may be linked to neuroprotection against hypoxia.

There may be several explanations to this finding. To begin with, Hx may lower the basal metabolic rate and subsequent administration of GH may restore the basal metabolism accompanied by an increased oxygen consumption (Goodman & Grichting 1983). Indeed, an upregulation of brain Hbb has been shown after ischaemia-induced hypoxia (He *et al.* 2009). Furthermore, as the neuron-Hbb category responds to Hx by a considerable decrease in the levels of transcripts, in both sexes and in both brain regions, we conclude that this is likely driven by a mechanism unrelated to the effect on the other transcripts, whose generally tighter correlations to the *Igf1r*, instead suggests that the effects of GH may be more closely mediated to IGF-I signalling via the *Igf1r* (Table 5).

Taken together, it appears that endogenous neuronal (non-erythrocyte) haemoglobin in neurons is involved in neuroprotection although its function is still not fully clear.

Differences between the sexes Interestingly, when comparing the results from our previous investigations in male rats (Walser *et al.* 2014) with the results in the present study of female rats, there is a marked difference in response between the sexes. In both sexes, in the hippocampus, the transcripts were generally increased by GHx2 whereas this effect was small or absent after GH_i. However, in the cortex, these transcripts were unresponsive to GHx2 but responded in opposite directions to GH_i; in females with an increase and in males with a decrease (Fig. 1A and B) (Walser *et al.* 2014). This indicates that the two brain regions are divergently receptive to the two different administration strategies. Other reasons put forward for sex differences in expression are that oestradiol treatment increases local GH expression in the cerebellum and in the hippocampus with only a marginal effect in the hypothalamus, and that the sex chromosome regulates GH expression within the hypothalamus (Quinnies *et al.* 2015). Indeed, this suggests

that the two brain regions, the hippocampus and the cortex may be differentially affected by the two administration paradigms of GH in the sexes. Future studies on GH administration should preferably also include a group of sex-hormone substituted rats, in addition to the cortisol and thyroxine-substituted Hx rats.

Clinical relevance

By administration of GH in two different modes, followed by attaining the expression of sixteen transcripts we tried to provide a more comprehensive picture of the effects of GH in the hippocampus and in the cortex. Although, the mode of administration did not have a decisive importance in our female rats, GHx2 in spite of being more male-like than GH_i, elicited a somewhat greater effect on all transcripts in the hippocampus and in particular on *Hbb* and *Alas2*.

Neuronal-Hbb may have neuroprotective properties, as discussed above for hypoxic neuroprotection (He *et al.* 2009), but, in addition, there are also indications that Hbb is involved in multiple sclerosis (Brown *et al.* 2016) and in Parkinson's disease (Shephard *et al.* 2014). Furthermore, a link between *Hbb/Alas2* and dopamine signalling malfunction in the restless legs syndrome has been shown (Jellen *et al.* 2013). The fact that GHx2 decreased expression of most transcripts except *Hbb* and *Alas2* in the cortex, suggests that GH_i may be favourable to induce these plasticity-related transcripts in this brain region. However, such a conclusion would need confirmation in a study that also includes substitution of sex hormones.

Neuroprotective effects are most often considered to involve the acute phase of an injury, while the ensuing recovery phase involves more of effects on long-acting plasticity. Although the significant differences between modes of GH administration in the neuron- and glia-categories of transcripts were small, they may nevertheless be important for long-term plasticity after brain injuries. Specifically, chronic central treatment of a unilateral stroke with GH in adult rats was associated with slightly more rapid recovery of motor functions and with better spatial memory (Pathipati *et al.* 2009). Moreover, the consequences of TBI can be improved in humans by an acute/subacute and delayed administration of GH several years after the TBI (Maric *et al.* 2010). Therefore, GH administration paradigms may have significance for both acute neuroprotection and long-term plasticity and thereby the outcomes of brain injuries.

Summary

Hbb and *Alas2* where considerably decreased by Hx and robustly restored by both administration paradigms, GHx2 more efficiently than GHi, in the hippocampus and in the cortex of the female rat, which may have consequences for neuroprotective actions of GH. Regarding the other transcripts, the effects of GH were smaller. However, using the MMA, we showed that the administration of GHx2 was more effective in increasing or restoring transcript levels in the hippocampus whereas GHi was more effective in the cortex. Even so, it should be stated that these moderate differences may have consequences for neuroprotection against ischaemic injuries, perhaps with different profiles in different brain regions.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/JOE-16-0656>.

Declaration of interest

Jan Oscarsson is employed by AstraZeneca. The other authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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