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The Effect of Growth Hormone on mRNA Expression of the $GABA_{B1}$ Receptor Subunit and GH/IGF Axis Genes in a Mouse Model of Prader-Willi Syndrome

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Purpose: Growth hormone (GH) therapy substantially improves several cognitive functions in PWS. However, the molecular mechanisms underlying the beneficial effects of GH on cognition remain unclear in PWS. In this study, we investigated the effects of recombinant human GH on the gene expression of GABAB receptor subunits and GH/insulin-like growth factor (IGF) axis genes in the brain regions of PWS-mimicking mice (*Snord116del*).
Methods: *Snord116del* mice were injected subcutaneously with 1.0 mg/kg GH or saline, once daily for 7 days. The collected brain tissues were analyzed for mRNA content using quantitative PCR (qPCR) in the cerebellum, hippocampus, and cerebral cortex.
Results: GH increased the mRNA expression level of the $GABA_{B1}$ receptor subunit ($GABA_{BR1}$) and IGF-1R in the cerebellum. Furthermore, a significant positive correlation was found between the level of $GABA_{BR1}$ mRNA and the expression of the IGF-1R transcript. GH also induced an increase in the mRNA expression of IGF-2 and IGF-2R in the cerebellum.
Conclusion: These data indicate that GH may provide beneficial effects on cognitive function through its influences on the expression of $GABA_{BR1}$ and GH/IGF-1 axis genes in PWS patients.

Keywords: Prader-Willi syndrome, *Snord116del* mice, Cognitive impairment, Growth hormone, $GABA_B$ receptor subunit

Introduction

Prader-Willi syndrome (PWS) is a complex neurogenetic disorder caused by loss of paternally expressed imprinted genes on human chromosome 15q11-q13[1)]. PWS is characterized by neurobehavioral abnormalities, cognitive impairment, and hypothalamic dysfunction including growth hormone (GH) deficiency (GHD) with short stature[2)]. GH replacement therapy has been reported to improve cognitive development in infants and adults[3-5)], and to prevent cognitive deterioration and improve cognitive skills in children with PWS[6)]. However, the physiological and molecular mechanisms underlying the improvements in cognitive function after GH treatment remain unclear in PWS.

The GH/insulin-like growth factor (IGF)-1 axis is important for the growth, development, and function of the central nervous system (CNS)[7)]. GH deficiency in adults is characterized by cognitive impairment, which can be ameliorated by GH treatment[8)]. GH administration attenuates cognitive deficits and improves memory in hypophysectomised rodents[9)]. Another mediator of GH effects, IGF-2, has been proposed as a novel cognitive enhancer[10)]. The presence of binding sites for GH and IGF-1 in the brain has been suggested as evidence that GH crosses the blood–brain barrier[11,12)], although the mechanisms behind the actions of GH on brain function remain unclear.

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS, acts via $GABA_A$ and $GABA_B$ recep-

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tors. The functional $GABA_B$ receptors consist of two subunits, $GABA_{BR1}$ and $GABA_{BR2}$[13], which are responsible for the neuromodulatory effect of GABA[14,15]. Recently, exogenous GH has been reported to increase the abundance of the $GABA_B$ receptor in rat brain[16] and $GABA_{BR1}$ gene expression in hypophysectomised rat[17]. These findings suggest a possible correlation between GH-induced cognitive function and the $GABA_B$ receptor. The Snord116 deletion (*Snord116del*) mouse, a PWS-mimicking model, is a dwarf strain caused by the deletion of the Snord116 C/D box snoRNA cluster. Since GH is an important regulator of developmental and cognitive functions in the CNS, we investigated the effects of GH on the expressions of $GABA_B$ receptor subunits as well as the GH/IGF axis gene in specific brain regions known to be affected by GH treatment[18,19] in *Snord116del* mice.

Materials and Methods

1. Animals and drug treatment

All animal experiments were carried out in accordance with a protocol approved by the Institutional Animal Care and Use Committee, Laboratory Animal Research Center, Samsung Biomedical Research Institute (Seoul, Korea). *Snord116del* mice (B6(Cg)-Snord116tm1.1Uta/J) were obtained from The Jackson Laboratory (Bar Harbor, 352 Maine, USA). At the start of the experiment, the mice were 3 weeks old (n=6–8 for each group). Male *Snord116del* mice were injected subcutaneously with 1.0 mg/kg GH (Growtropin, provided from Dong-A Pharmaceutical Co., Yongin-si, Korea) or saline, once daily for 7 days. All animals were weighed every day to monitor their biological response in weight gain. On day 8 of the experiment, the mice were sacrificed, and the cerebellum, hippocampus, and cerebral cortex were dissected using a brain matrix.

Table 1. List of genes and assays for real-time PCR

Gene name	Gene ID	ABI assay number
Insulin-like growth factor 1	*Igf-1*	Mm00439560_m1
Insulin-like growth factor 1 receptor	*Igf-1r*	Mm00802831_m1
Insulin-like growth factor 2	*Igf-2*	Mm00439564_m1
Insulin-like growth factor 2 receptor	*Igf-2r*	Mm00439576_m1
Gamma-aminobutyric acid B receptor 1	*Gabbr1*	Mm00444578_m1
Gamma-aminobutyric acid B receptor 2	*Gabbr2*	Mm01352554_m1
Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*	Mm99999915_g1

2. RNA extraction and cDNA synthesis

Brain tissues were prepared for RNA extraction using the RNeasy Lipid Tissue Mini Kit (QIAGEN, MD, USA), according to the protocol provided by the manufacturer. The conversion of total RNA to cDNA was performed using the High Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA).

3. Quantitative polymerase chain reaction

The expression of six genes (*Gabbr1*, *Gabbr2*, *Igf-1*, *Igf-1r*, *Igf-2*, and *Igf-2r*) was quantified using a TaqMan® Gene Expression Assay (Applied Biosystems), which included a TaqMan® real-time quantitative polymerase chain reaction (qPCR) in the cerebellum, hippocampus, and cerebral cortex. Predesigned gene-specific primers and probes were used to detect each gene (Applied Biosystems), as presented in Table 1. The amount of each transcript was normalized to the amount of GAPDH expressed in the same sample.

4. Statistical analysis

All statistical analyses were performed using GraphPad Prism

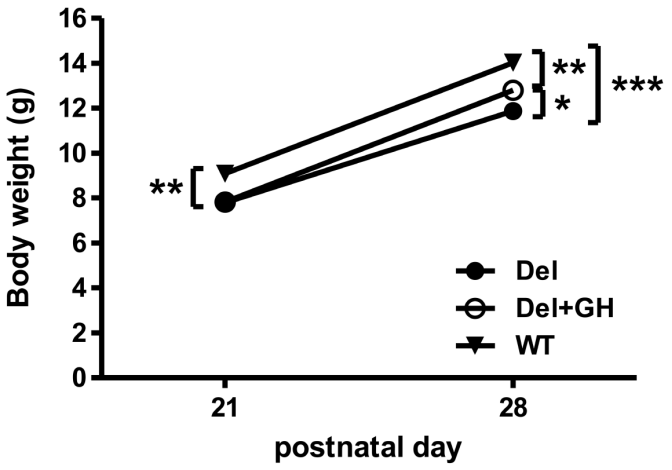

Fig. 1. Effects of GH treatment on body weight. Mice received daily subcutaneous injections of GH (1.0 mg/kg) or saline injection for 7 days. Body weights were measured over 8 days. Data represent mean ± S.E.M. n=7–8/group. **P*<0.05, ***P*<0.01, ****P*<0.001. GH, growth hormone; Del, *Snord116del* mice injected with saline; Del+GH, *Snord116del* mice injected with recombinant growth hormone; WT, wild-type mice injected with saline.

5.0b (GraphPad Software, Inc., La Jolla, USA). The weight measurements were analyzed using two-way repeated ANOVA. The results from the qPCR were analyzed using one-way ANOVA with a *post hoc* Student–Newman–Keuls test for the statistical analysis of the differences between the groups. The correlations were tested by simple regression analysis. Values are presented as mean±SEM, and *P*-values less than 0.05 were considered significant.

Results

Compared to the WT mice, the *Snord116del* mice with GHD exhibited reduced body weight, and GH treatment significantly increased gains in body weight (Fig. 1). This indicates that the administered GH was physiologically active and had an expected systemic effect on body growth.

The expression of six genes (*Gabbr1*, *Gabbr2*, *Igf-1*, *Igf-1r*, *Igf-2*, and *Igf-2r*) in the cerebellum, hippocampus, and cerebral cortex was analyzed in Snord116del mice treated with GH (Del+GH) or saline (Del) and wild-type (WT) mice with saline. The results from the gene expression analysis of *Gabbr1* and *Gabbr2* in the cerebellum, hippocampus, and cerebral cortex are displayed in Fig. 2. In the cerebellum, there were significant differences between the treatment groups regarding the mRNA expression of *Gabbr1* ($P<0.05$) where both the Del+GH and WT groups showed increased *Gabbr1* mRNA expression compared with the Del group, but no effect on the *Gabbr2* expression was observed. However, the administration of GH did not alter the expression of *Gabbr1* or *Gabbr2* in the hippocampus and cerebral cortex.

The results from the gene expression analysis of *Igf-1*, *Igf-1r*, *Igf-2*, and *Igf-2r* in the cerebellum, hippocampus, and cerebral cortex are shown in Figs. 3 and 4. There was a significant difference between the treatment groups regarding the *Igf-1r*, *Igf-2*, and *Igf-2r* expression in the cerebellum. A significant decrease of

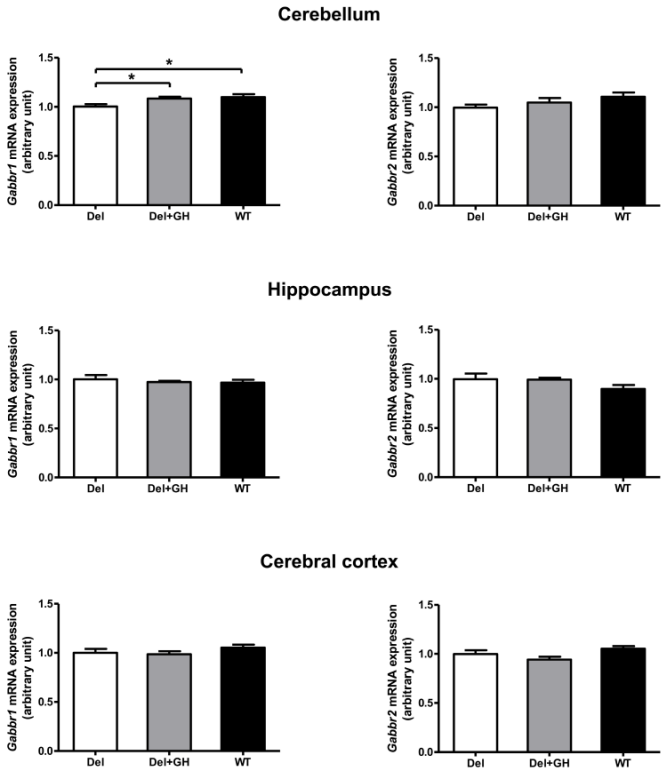

Fig. 2. Effects of GH treatment on mRNA expression of $GABA_{BR1}$ and $GABA_{BR2}$ in the cerebellum, hippocampus, and cerebral cortex. Values are expressed as mean±SEM, n=7–8/group. **P*<0.05, ***P*<0.01. GH, growth hormone; *Gabbr1*, $GABA_{B1}$ receptor subunit; *Gabbr2*, $GABA_{B2}$ receptor subunit; Del, *Snord116del* mice injected with saline; Del+GH, *Snord116del* mice injected with recombinant growth hormone; WT, wild-type mice injected with saline.

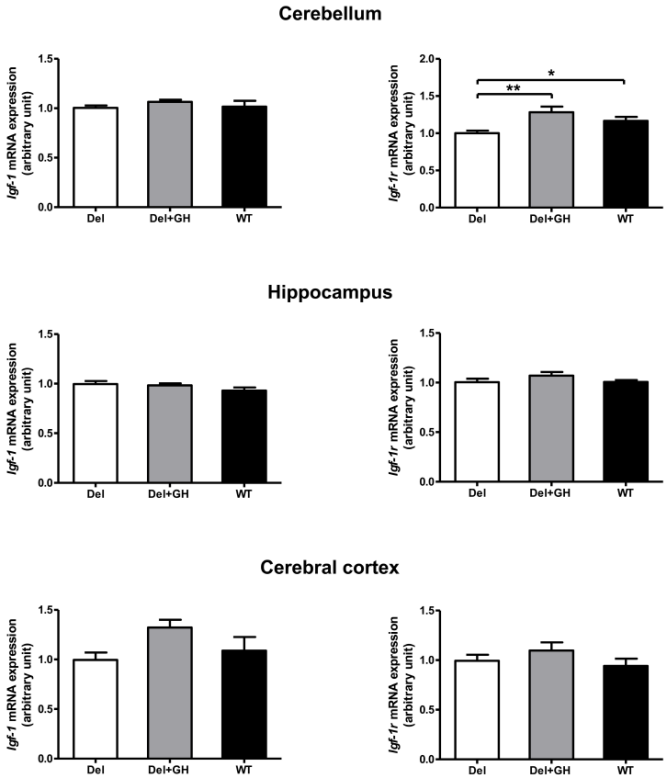

Fig. 3. Effects of GH treatment on mRNA expression of IGF-1 and IGF-1R in the cerebellum, hippocampus, and cerebral cortex. Values are expressed as mean±SEM, n=7–8/group. **P*<0.05. GH, growth hormone; *Igf-1*, insulin-like growth factor 1; *Igf-1r*, insulin-like growth factor 1 receptor; Del, *Snord116del* mice injected with saline; Del +GH, *Snord-116del* mice injected with recombinant growth hormone; WT, wild-type mice injected with saline.

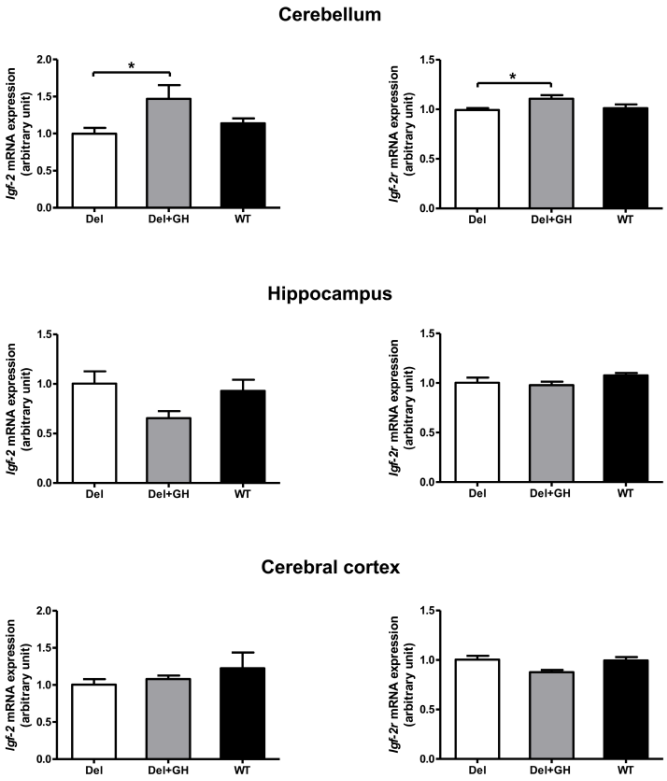

Fig. 4. Effects of GH treatment on mRNA expression of IGF-2 and IGF-2R in the cerebellum, hippocampus, and cerebral cortex. Values are expressed as mean±SEM, n=7–8/group. **P*<0.05. GH, growth hormone; *Igf-2*, insulin-like growth factor 2; *Igf-2r*, insulin-like growth factor 2 receptor; Del, *Snord116del* mice injected with saline; Del +GH, *Snord-116del* mice injected with recombinant growth hormone; WT, wild-type mice injected with saline.

Igf-1r mRNA expression was found in the Del group compared to the WT group, and GH administration induced an increase of *Igf-1r* expression (P<0.05). In addition, alterations of *Igf-2* and *Igf-2r* mRNA expression were found; the Del+GH group showed increased *Igf-2* and *Igf-2r* expression (P<0.05). However, GH administration did not alter the expression of *Igf-1*, *Igf-1r*, *Igf-2*, or *Igf-2r* in the hippocampus or cerebral cortex (Figs. 3 and 4).

Discussion

We demonstrated both that the expression of $GABA_{BR1}$ and IGF-1R transcripts are markedly decreased in the cerebellum of *Snord116del* mice compared to WT mice and that GH increases the expression of $GABA_{BR1}$ and IGF-1R transcripts. $GABA_B$ receptor has been reported to be important for neuronal excitability and plasticity and is suggested to be involved in the regulation of long-term potentiation, which is the cellular mechanism for learning and memory[15,20]. GH treatment affects the functionality and density of $GABA_B$ receptors in the area of the brain associated with cognition[16]. Other studies have revealed that GH treatment up-regulated the expression of $GABA_{BR1}$ transcript in rat brain, suggesting a connection between GH and the $GABA_B$ system[17,21].

The present study showed a significant positive correlation between the mRNA level of IGF-1R and $GABA_{BR1}$ in the cerebellum. This finding is in agreement with a recent observation that the activation of the $GABA_B$ receptor induces IGF-1R transactivation leading to survival signaling in the cerebellum[22]. Moreover, several studies have suggested that the $GABA_B$ receptor protects the brain from ischemic damage and improves memory[23-25], providing evidence that stimulation of the $GABA_B$ receptor may be involved in a mechanism by which GH regulates brain function, including a cognitive and neuroprotective effect.

In addition, GH increased the gene expression for IGF-2 and IGF-2R in the cerebellum. IGF-2, another mediator of GH action, is known to be important for brain development and to have neurotrophic or neuroprotective properties[26,27]. IGF-2 signaling has been implicated in cognitive function, and it has been suggested that the effect of IGF-2 as a memory enhancer is selectively mediated by IGF-2R. IGF-2 has been shown to promote IGF-2R-dependent, persistent long-term potentiation, demonstrated by memory improvement[10]. Our data suggest the possibility that IGF-2/IGF-2R signaling could have an important role in GH-induced cognitive function in *Snord116del* mice.

However, the expression of $GABA_{BR1}$, $GABA_{BR2}$, IGF-1, IGF-1R, IGF-2, and IGF-2R in the hippocampus and cerebral cortex was unaffected by GH administration. The GH activity may be different regionally, because the brain is highly heterogeneously functional. Several potential mechanisms, such as differences in blood–brain barrier permeability and the distribution of GH receptor (GHR) and GH binding protein (GHBP), may account for differences in the effects of GH on $GABA_B$ receptor subunits and GH/IGF axis expression in specific brain regions of *Snord116del* mice. The local expression of GH and the presence of GHR in the cerebellum indicate that the cerebellum is an autocrine and/or paracrine site of GH action[28]. As it is known that GH and IGF-I increase brain growth, myelination, and has neuroprotective properties[29-31], we could speculate that if the GH treatment had any effect of the brain, it would have a positive effect in terms of brain normalization.

Overall, the findings of the present indicated that GH restores the gene expression of $GABA_{BR1}$ and IGF-1R and increases IGF-2 and IGF-2R in the cerebellum of *Snord116del* mice. The alterations of $GABA_{BR1}$ and IGF-1R observed in *Snord116del* mice

could, at least partly, account for cognitive impairment. Because GHD during early life can impair proper brain development, thereby leading to cognitive deficits, it is suggested from the our study that GH may provide beneficial effects on cognitive function through its influences on the expression of GABABR1 and GH/IGF-1 axis genes in PWS patients.

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