Effects of growth hormone substitution therapy on cognitive functioning in growth hormone deficient patients: a functional MRI study

Arwert, L.I.; Veltman, D.J.; Deijen, J.B.; van Dam, P.S.; Drent, M.L.

published in
Neuroendocrinology
2006

DOI (link to publisher)
10.1159/000093337

document version
Publisher's PDF, also known as Version of record

Original Paper

Neuro
-endocrinology

Neuroendocrinology 2006;83:12–19
DOI: 10.1159/000093337

Received: January 23, 2006
Accepted after revision: April 13, 2006
Published online: May 15, 2006

Effects of Growth Hormone Substitution Therapy on Cognitive Functioning in Growth Hormone Deficient Patients: A Functional MRI Study

Lucia I. Arwert[a] Dick J. Veltman[b] Jan Berend Deijen[c] P. Sytze van Dam[d]
Madeleine L. Drent[a]

[a]Department of Endocrinology and [b]PET Center, VU University Medical Center, and
[c]Department of Clinical Neuropsychology, Free University, Amsterdam, and
[d]Department of Endocrinology, University Medical Center Utrecht, Utrecht, The Netherlands

Key Words
Growth hormone deficiency · Insulin-like growth factor I · Functional magnetic resonance imaging · Working memory · Cognition · Mood

Abstract
Patients with childhood-onset growth hormone (GH) deficiency (GHD) show impairments in mood and cognitive functioning which may resolve following GH substitution. Brain functional magnetic resonance imaging (fMRI) during performance of a memory task was used to assess the cerebral activity of such patients. Thirteen childhood-onset GHD patients (mean age 27.3 ± 6.9 years) were included in a double-blind, placebo-controlled study. The effects of 6 months of GH replacement or placebo therapy were studied using neuropsychological tests and fMRI. One patient was excluded from the study due to noncompliance with the protocol. Six months of GH substitution in these GHD patients resulted in improved memory functioning, both for long-term and working memory. fMRI showed activations during the working memory task in prefrontal, parietal, motor, and occipital cortices, as well as in the right thalamus and anterior cingulate cortex. Decreased activation in the ventrolateral prefrontal cortex was observed after GH treatment as compared with placebo treatment, indicating decreased effort and more efficient recruitment of the neural system involved. It can be concluded that GH treatment for 6 months improved the long-term as well as the working memory in patients with GHD, and this was associated with decreased brain activation in the ventrolateral prefrontal cortex. GH substitution in GHD patients is beneficial for cognitive functioning, the effects of which can be visualized by means of neuroimaging.

Introduction

The function of the growth hormone (GH)-insulin-like growth factor I (IGF-I) axis as well as the cognitive functioning decline with aging. In healthy elderly, mood and cognition are positively related to the level of IGF-I. More specifically, the IGF-I levels appear to be related to cognitive performance, short-term memory, and cognitive flexibility [1, 2]. Other studies have shown an association between GH deficiency (GHD) and cognition, especially memory performance. Patients with both adult-onset (AO) and childhood-onset (CO) GHD were found to have a depressed mood and an impaired cognitive functioning [2, 3]. GH substitution improved the cognitive function, as observed in attention and memory tasks [4], in a face

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0028–3835/06/0831–0012$23.50/0

Accessible online at:
www.karger.com/nen

Lucia I. Arwert
Department of Endocrinology, VU University Medical Center
PO Box 7057
NL–1007 MB Amsterdam (The Netherlands)
Tel. +31 20 444 2799, Fax +31 20 444 0502, E-Mail l.arwert@vumc.nl

recognition test [5], and in the symbol-number association subtest of the Wechsler Adult Intelligence Scale [6]. However, other studies in GHD patients reported no effect of GH substitution on the cognitive performance [7, 8]. The available data in a recent meta-analysis on GH and cognitive functioning in patients with GHD was too limited to draw conclusions with respect to the impact of GH treatment on cognition [9]. In a study on the effects of GH/IGF-I on the EEG activity, the importance of the GH-IGF-I axis in functioning of the anterior cingulate cortex was shown in patients with CO-GHD [10]. Impaired cognitive functioning and abnormal N-acetylaspartate/choline ratios in the brain, indicating a reduced neuronal integrity [11], were observed in patients with CO-GHD. Oertel et al. [12] performed a randomized double-blind, placebo-controlled trial over 6 months in 18 adults with GHD. Improvement of the attentional performance was observed after 3 and 6 months of GH substitution therapy, but there was no effect on verbal memory or verbal intelligence.

The mechanisms underlying the relation between GH and cognitive functioning are not yet fully understood. There are indications that GH can cross the blood-brain barrier [13], while choroid plexus, hypothalamus, putamen, and thalamus have been shown to possess binding sites for GH and IGF-I [14, 15]. Moreover, GH treatment has been found to reduce the concentration of homovanillic acid, a dopamine metabolite, in cerebrospinal fluid [13, 16, 17]. As the hippocampus is known to contain high levels of dopamine, particularly this structure may be affected by GH treatment which might explain the connection between GH treatment and cognitive functions, in particular memory.

The present study was designed to investigate the effect of GH substitution therapy in patients with CO-GHD on cognitive functioning, especially memory performance. To elucidate this issue, we used functional magnetic resonance imaging (fMRI). This technique provides a means to investigate the brain function in a noninvasive manner during performance of a memory task. fMRI provides information on the oxygenation state of the brain using the blood oxygenation level dependent response.

We performed a previous imaging study [18] using positron emission tomography to measure the brain activation in healthy elderly subjects with either high or low serum IGF-I levels. This positron emission tomography activation study revealed a difference in cerebral blood flow in subjects with higher IGF-I levels as compared with subjects with lower IGF-I levels [18]. We have recently shown [19] that both cognition and brain activity measured with fMRI during a working memory task were different in 13 patients with CO-GHD as compared with healthy matched controls. The GHD patients had a subnormal memory speed, but a normal quality of memory performance. The patients allocated more brain activity to areas involved in working memory, e.g., dorsolateral/ventrolateral prefrontal cortex, anterior cingulate cortex, and parietal cortex, than control subjects.

In the present study, we hypothesized that GH substitution in GHD patients would normalize working memory speed and/or performance and induce changes in associated brain activity. We expected that GH substitution would improve the working memory performance and alter the activity in brain areas associated with working memory (prefrontal and parietal cortices).

Patients and Methods

Patients

In the present study, 13 patients with CO-GHD were included, with a mean age of 27.3 ± 6.9 years. Inclusion criteria were diagnosis of CO-GHD confirmed with an insulin tolerance test or another GH stimulation test and optimal substitution for the other pituitary hormones. Exclusion criteria were neuropsychiatric diseases and noncompliance. All patients who were on GH therapy (n = 8) had to stop GH treatment for a 3-month washout period before entering the study.

Education levels were assessed, using a 5-point scale, defined in conformity with the levels of education specified by the European Community [20]. These levels ranged from (1) school education not completed, subject attended school for the mentally or physically handicapped, to (5) completed university/academy education. The mean educational level of the patient group, i.e., 3.8 ± 0.7, corresponds to at least 10 years of education and training, indicating an average-educated population.

All subjects gave written informed consent. The study was approved by the Medical Ethical Committee of the VU University Medical Center and was conducted according to the principles of the Helsinki Declaration.

Procedures

After a 3-month washout period following discontinuation of GH substitution, the IGF-I level was tested twice to confirm the GHD state. At the start of the study, neuropsychological and event-related fMRI scannings were performed. The baseline data from the 13 patients with CO-GHD as compared with healthy controls were described previously [19]. Thereafter, the patients were randomized either to active treatment with GH (Pfizer, Capelle a/d Ijssel, the Netherlands) or placebo for a period of 6 months. The treatment was given in a double-blind manner, and an endocrinologist not involved in the study determined the GH dosages according to the measured plasma IGF-I levels during treatment. Aim was to achieve IGF-I levels normal for age ± 0.5 SD. GH or placebo preparation of identical appearance was daily injected before nighttime. Both neuropsychological and fMRI scannings were repeated after 6 months.

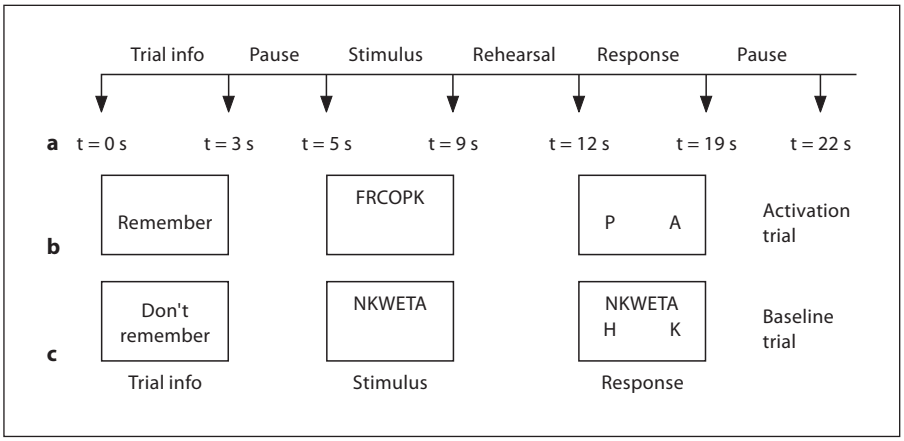

Fig. 1. a Flow chart of the time sequence (s) of presentation of the working memory task during fMRI scanning. **b** Graph of screens presented during an activation trial in the working memory task. Trial information (remember or do not remember) was presented on the screen for 3 s, thereafter the screen was blank for 2 s. A series of letters was presented for 4 s, after which the screen was blank for a 3-second delay interval. After this delay, one of the letters together with a novel letter appeared on the screen for 7 s. Thereafter the screen was blank for 3 s (constant pause), followed by the next series of letters. **c** Graph of screens presented during a baseline trial in the working memory task. In this condition, the series of letters was presented for 7 s, i.e., throughout the 4-second familiarization period and the 3-second delay interval.

Neuropsychological Tests

The following tests were selected from the Neurobehavioral Education System (NES) [21] and were administered by means of a PC.

Mood: A shortened Dutch version of the Profile of Mood States (POMS) consisting of 32 items [22] was used for the assessment of depression, anger, fatigue, tension, and vigor. Higher scores for depression (8–40), anger (7–35), fatigue (6–30), and tension (6–30) reflect a negative mood; higher scores for vigor (5–25) reflect a better mood.

Memory: For the assessment of short-term memory, attention, and concentration, the participants were asked to repeat a string of digits by using the keyboard in the original order (digit span forward) or in the reverse order (digit span backward). Increasingly longer spans of digits were presented, until the subjects made two errors at the same span length. The lengths of the longest spans correctly repeated forwards and backwards were scored. For the associated learning task [23], nine word pairs consisting of a name and an occupation are one at a time displayed on a computer screen during 3 s. Immediately after the last combination, one of the names is shown together with a list of the nine occupations. The patients have to press a number on the keyboard that corresponds to the occupation applying to the name presented. Then, after feedback is given on the screen concerning the correctness of the response, the next name and the nine occupations are presented. In total nine trials are successively presented, until each of the nine names is paired with the nine occupations. The whole test was repeated twice, to allow the subject to learn as many paired names and occupations as possible. This recognition test measures short-term verbal learning (maximum score 27). For the assessment of the long-term memory, unexpectedly, after an interval of 60 min, the subjects had to match again each one of the nine names presented in the associated learning delayed recognition task with one of the nine occupations (maximum score 9). This time no feedback was given.

Imaging Paradigm

The task performed during neuroimaging was a parametric delayed non-match to sample (DNMTS) task, adapted in our laboratory. This is a working memory task specifically measuring verbal recognition memory. A detailed explanation of this task has been reported previously [19]. During the DNMTS task, a series of letters was presented on a computer screen for 4 s, and subjects were instructed to read the series of letters and memorize them. After a 3-second delay (blank screen), two cue letters, one left and one right on the screen, were shown during 7 s. The subjects were requested to select the letter not present in the previous string, using the button press compatible with the location of this letter on the screen (left or right). During baseline trials, the letter string was shown throughout the 3-second retention period and the 7-second cue letter presentation. Also during these trials, the subjects were requested to select the letter not present in the previous string by pressing the left or right button (fig. 1). Before the presentation of each series of letters, the subjects were informed (on the screen) on whether the upcoming set of letters had to be remembered (activation condition) or not (baseline condition). Trial information (remember or do not remember) was presented on the screen for 3 s, followed by a 2-second blank screen. Intertrial interval was 3 s. Different lengths of letter strings were presented in random order (3, 4, 5, 6, 7, or 8 letters), and activation and baseline trials were also presented in random order. To ensure that the participants were familiar with the task, the test was explained and practiced outside the scanner before MRI was started. To obtain an activation and baseline response, the subjects were instructed to perform as accurately and as fastly as possible within the given time limits. No feedback regarding correct

answers was provided during the task performance. A total of 48 series of letters were successively presented in random order (12 baseline trials, 36 activation trials). Main cognitive outcomes were number of correct responses and reaction time (RT) in milliseconds.

Neuroimaging

For fMRI an echo planar imaging (EPI) sequence (repetition time 3.045 s, echo time 45 ms, flip angle 90°) was used, creating transversal whole-brain acquisitions (35 slices, 3 × 3-mm in-plane resolution, slice thickness 2.5 mm with a 0.5-mm interslice gap). In total 350 EPI volumes per subject were scanned. A T_1-weighted structural MRI scan was acquired using a 1.5-tesla Magnetom Sonata MR scanner (Siemens, Erlangen, Germany; magnetization-prepared rapid acquisition gradient echo, inversion time 300 ms, repetition time 15 ms, echo time 7 ms, flip angle 8°, voxel size 1 × 1 × 1.5 mm). These structural MRI scans were used for correct anatomical interpretation of the results obtained by fMRI scanning.

Hormone Assays

Blood samples for IGF-I level determinations were drawn at the start of the study and after 3 and 6 months of treatment. Blood samples were also drawn for determination of the other pituitary functions. The serum concentrations of thyroid-stimulating hormone, free T_3, free T_4, cortisol, prolactin, luteinizing hormone, follicle-stimulating hormone, estradiol (females), and testosterone (males) were measured according to standardized techniques using commercially available radioimmunoassays. The plasma IGF-I levels were also measured using a commercially available assay (chemiluminescence; Nichols Institute Diagnostics, San Juan Capistrano, Calif., USA). The detection limit for IGF-I is 0.6 nmol/l. The intra-assay coefficient of variation is 3% at a serum IGF-I level of 20 nmol/l. The interassay coefficient of variation is 6% at a serum IGF-I level of 33 nmol/l and 8% at a serum IGF-I level of 7 nmol/l. The plasma IGF-binding protein 3 (IGFBP-3) levels were measured using a commercially available immunoradiometric assay (Diagnostic Systems Laboratories, Webster, Tex., USA) with a detection limit of 0.4 mg/l.

Image Processing

Imaging data were analyzed using Statistical Parametric Mapping (SPM2) software, developed by the Wellcome Department of Cognitive Neurology (London, UK). We discarded the first two EPI volumes of each fMRI time series to allow for magnetic saturation. Time series were corrected for differences in slice time acquisition and realigned to correct for subject movement. Thereafter, the images were spatially normalized to anatomical standard space, as defined by the SPM EPI template, and resampled to a voxel size of 3 × 3 × 3 mm. Data were smoothed using an 8-mm full-width half-maximum gaussian filter to increase the signal-to-noise ratio. Next, individual analyses were performed using the general linear model, with delta functions and a canonical hemodynamic response function to model responses of varying length to each stimulus. For each subject, weighted contrasts were computed for main effects (activation vs. implicit baseline) and for task load. Main effects are reported at $p < 0.005$, corrected for multiple comparisons using the false detection rate method [24], with a cluster threshold of 5 voxels, unless indicated otherwise. Group-by-task interactions were masked using the appropriate main effects. Regions determined by Talairach coordinates for peak effects were verified using anatomical localization.

Statistics

All analyses for demographic data, hormone values, mood scale scores, and task performance were carried out by means of the Statistical Package for the Social Sciences version 11.5. Variables were analyzed by means of multivariate analysis of (co)variance with group (GH or placebo treatment) as an independent factor and outcome measures as dependent variables. Baseline values were used as covariates to correct for differences in baseline values between treatment groups. DNMTS data comprising RT and number of correct responses for series with equal set size were averaged. This procedure resulted in six dependent variables for RT and for number of correct responses, i.e., regarding set 3, 4, 5, 6, 7, and 8. Statistical significance was set at the 0.05 level. Statistical tests for cognitive functioning were one-tailed, based on the hypothesis that GH substitution would enhance memory. Data are presented as mean values ± SD, unless indicated otherwise.

Results

fMRI scanning was performed in 13 patients; however, 1 patient was excluded from the study due to noncompliance with the treatment protocol. There were no changes in other medications across the 6-month study. Five patients received GH treatment, and 7 patients received placebo treatment. The patient characteristics are shown in table 1. Six months of GH therapy did result in significantly increased values of serum IGF-I and IGFBP-3 ($p < 0.005$), whereas no significant differences were observed in the placebo-treated group.

Neuropsychological Tests

After 6 months, there was a statistically significant difference in performance in the associate learning recognition task (measuring the long-term memory) in favor of the group that received GH therapy ($p = 0.004$). The increase in performance ratings in the GH-treated group was statistically significant ($p < 0.05$). In addition, there was a trend to a decrease in the RT on the DNMTS task during fMRI scanning in the group that received GH ($p = 0.055$). For all other neuropsychological scores, there were no significant differences between both groups. The mood scale scores improved in both groups, while no difference between the GH and placebo group was observed.

Imaging Data

Regions showing a significantly increased blood oxygenation level dependent signal for task-versus-baseline comparisons across groups (n = 12) are shown in table 2. We found an increased bilateral activity in dorsolateral

Table 1. Characteristics of the GHD patients at baseline (BL) and after 6 months (6 M)

	GH BL (n = 5)	Placebo BL (n = 7)	Treatment 6 M (n = 5)	Placebo 6 M (n = 7)	p
Mean age, years	26.6 ± 6.5	25.6 ± 7.7			
Male:female ratio	2:3	5:2			
I-GHD:MPHD	2:3	2:5			
Education level	3.8 ± 0.8	3.3 ± 0.5			
IGF-I, nmol/l	9.8 ± 4.4	7.6 ± 2.8	30.0 ± 6.6	6.5 ± 2.2	<0.005*
IGFBP-3, mg/l	2.9 ± 0.6	2.6 ± 0.5	4.3 ± 0.7	2.7 ± 0.6	<0.005*
Number of mistakes on DNMTS task (MRI)	1.2 ± 1.6	1.0 ± 1.3	0 ± 0	1.1 ± 1.4	0.045*
Mean RT on DNMTS task (MRI), s	1.5 ± 0.3	1.5 ± 0.4	1.2 ± 0.1	1.5 ± 0.3	0.055
Association learning total	22.4 ± 3.4	19.0 ± 2.9	23.2 ± 3.9	17.6 ± 5.8	n.s.
Association learning recall	8.4 ± 0.9	6.9 ± 2.2	9.0 ± 0	5.3 ± 2.2	0.004*
Digit span forward	7.2 ± 1.1	6.0 ± 1.0	7.8 ± 1.3	7.1 ± 1.1	n.s.
Digit span backward	6.4 ± 0.9	4.9 ± 1.7	6.6 ± 1.4	5.7 ± 1.6	n.s.
POMS depression	12.8 ± 5.8	9.0 ± 1.0	9.6 ± 2.3	8.6 ± 1.1	n.s.
POMS anger	10.6 ± 2.6	9.1 ± 2.4	7.8 ± 1.0	8.0 ± 1.5	n.s.
POMS fatigue	14.6 ± 7.3	10.4 ± 4.4	12.4 ± 8.4	9.0 ± 3.5	n.s.
POMS vigor	13.4 ± 1.5	16.1 ± 5.2	13.4 ± 4.0	17.7 ± 4.7	n.s.
POMS tension	8.0 ± 2.3	9.1 ± 4.2	7.6 ± 1.9	8.0 ± 2.4	n.s.

I-GHD = Isolated GHD; MPHD = multiple hormone deficiencies. For explanation of the other abbreviations see text.
* $p < 0.05$. The p values concern comparisons at month 6 between GH and placebo groups correcte for baseline differences.

Table 2. Regions showing significantly increased brain activation in the total group (n = 12) during fMRI scanning (task versus implicit baseline)

Region (cortex)	Coordinates of peak voxel			Z-value	BA
	X	Y	Z		
VLPFC left	42	3	3	5.89	45
VLPFC right	–45	12	9	4.24	45
DLPFC anterior	42	33	15	4.69	46
DLPFC posterior	60	9	21	4.63	46
Anterior cingulate	9	9	36	4.49	24
Parietal cortex left	54	–30	45	6.30	40
Parietal cortex right	–42	–45	45	3.95	40
Thalamus right	–6	–9	–3	3.87	
Motor cortex	45	–21	–60	5.91	4
	–24	–21	57	3.57	4
Occipital	27	–69	–6	5.18	18/19
	–6	–93	0	3.85	17

BA = Brodmann area. For explanation of the other abbreviations see text. Cluster size threshold 5 voxels.

Table 3. Regions showing significantly increased brain activation in the total group (n = 12) during fMRI scanning (for task load effect)

Region (cortex)	Coordinates of peak voxel			Z-value	BA
	X	Y	Z		
VLPFC left	36	24	0	5.89	45
DLPFC left	54	12	33	4.85	9
DLPFC right	–42	30	30	5.3	9
Anterior cingulate	–6	12	48	5.02	32
Anterior prefrontal	–36	54	15	4.43	10
Parietal cortex left	30	–60	39	5.74	40
Parietal cortex right	–39	–51	39	4.04	40
Temporal-parietal	45	–60	–12	4.07	7
Occipital	24	–87	3	4.47	37/19
	–24	–69	48	3.78	17

BA = Brodmann area. For explanation of the other abbreviations see text. Cluster size threshold 5 voxels.

prefrontal cortex (DLPFC) and ventrolateral prefrontal cortex (VLPFC), as well as in anterior cingulate, parietal, occipital, and motor cortices and right thalamus (fig. 2). No significant group interactions were found for the task-versus-baseline comparison. Task load contrasts are presented in table 3. An increased activation linearly associated with task load was found in parietal cortex and occipital cortex, as well as bilaterally in DLPFC, anterior

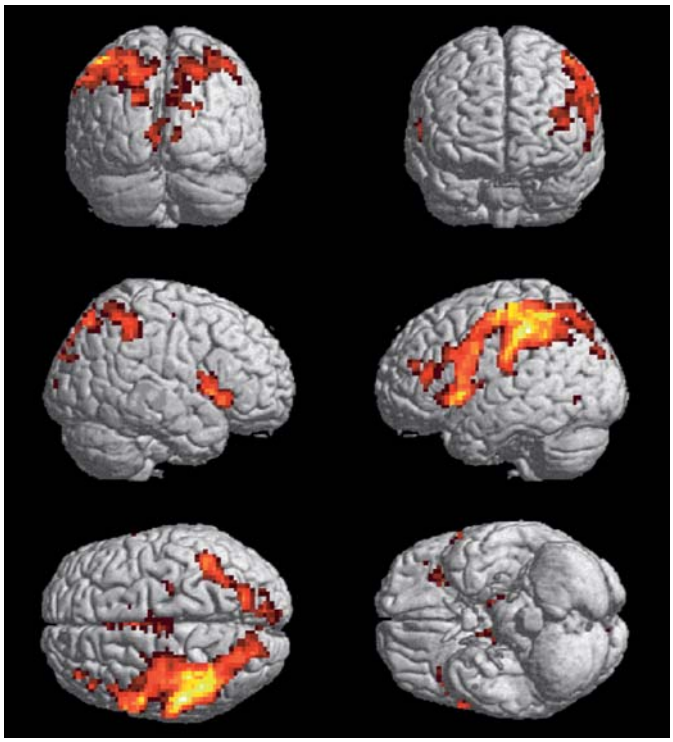

Fig. 2. Brain activation across sessions for all patients (n = 12) for task versus baseline (thresholded at $p < 0.001$, extent >5 voxels). Yellow color expresses more brain activation (on a scale of red, orange, and yellow).

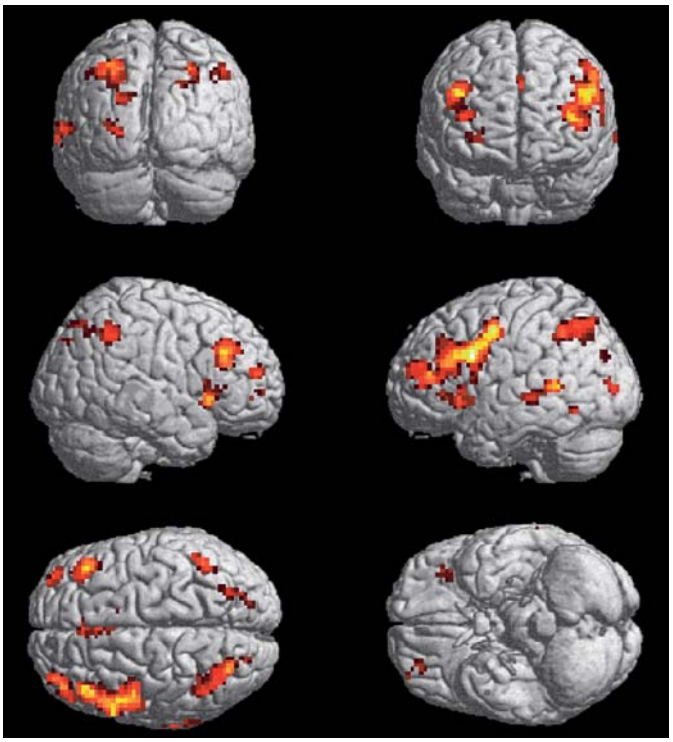

Fig. 3. Brain activation across sessions for all patients (n = 12) for task load (thresholded at $p < 0.001$, extent >5 voxels). Yellow color expresses more brain activation (on a scale of red, orange, and yellow).

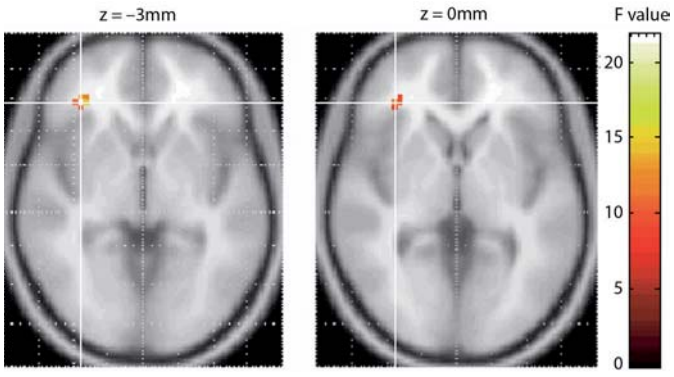

Fig. 4. Task load by group (placebo vs. GH therapy) interaction in the left ventrolateral cortex.

cingulate cortex, anterior prefrontal cortex, and left VLPFC (fig. 3). Task-by-group interactions in favor of the group that received placebo were found in the left VLPFC (fig. 4). The brain activity in this region decreased in patients who received GH therapy as compared with those who received placebo.

Discussion

With regard to neuropsychological functioning, this double-blind, placebo-controlled study performed in 12 patients with CO-GHD showed that GH therapy improved the long-term memory, as measured with the associate learning recall task. We also observed an increased speed of working memory performance during fMRI scanning, as the RTs decreased after GH treatment as compared with placebo treatment. These results are in line with those of previous studies which showed that the activity of the somatotropic axis is associated with neurocognitive functioning. However, the number of intervention studies on this topic is limited. Deijen et al. [4] showed normalization in memory performance during 2 years of GH substitution in CO-GHD men. The first 6 months of this study were placebo controlled which showed that the associate learning task improved after 6 months of GH therapy as compared with placebo. During a 10-year follow-up period of these patients, improvement in memory performance as compared with baseline was still found after 10 years of GH therapy [25]. Another intervention study on cognitive

functioning [26] did not report any beneficial effects of GH substitution as compared with placebo. In this randomized, double-blind, placebo-controlled study, 40 men with AO-GHD received GH or placebo for 18 months. Following 18 months of GH replacement therapy, there were no significant changes in the cognitive function. These authors concluded that chronic low-dose GH replacement therapy did not result in significant beneficial effects on the cognitive function. A recent placebo-controlled study done in 18 patients with AO-GHD showed that 6 months of GH therapy improved attentional functioning as compared with placebo. However, verbal memory and nonverbal intelligence did not improve [12].

In the present study, the mood scale scores improved after therapy in all patients, and the scores were not different between treatment groups. This can be explained by a placebo effect on mood which is frequently seen after GH treatment when evaluating the quality of life. In a recently conducted meta-analysis [9], we found 6 months of GH treatment to be as effective as placebo treatment in improving the quality of life.

The present imaging data showed brain activation in the parietal and prefrontal areas during performance of the working memory task. Specifically, we found activations in DLPFC, VLPFC, and anterior cingulate and anterior prefrontal cortices. These areas are well-known working memory areas and have been found in numerous other studies on working memory [for a review, see ref. 27]. The VLPFC activity may reflect maintenance (storage) and subvocal rehearsal. DLPFC recruitment has been observed during manipulation working memory tasks, but also during maintenance tasks, in particular at higher memory loads. In addition, the anterior cingulate cortex is involved in response selection and error monitoring [28], whereas posterior parietal activity has been explained as being due to storage as well as attentional processes. We observed robust activation during the working memory task, as well as for task-versus-baseline comparisons and for task load, the latter presumably being more specific [29, 30]. Interaction effects were found in the VLPFC for the task load contrast. After GH substitution, we observed a decreased activity in the VLPFC as compared with placebo treatment. These results are in line with those of our previous study [19], in which we found an increased brain activation in GHD patients as compared with healthy matched controls. The most likely explanation is that the decreased activity in a working-memory-associated region reflects decreased effort and more efficient recruitment of the neural system associated with the working memory. An abnormally increased dorsolateral and/or anterior cingulate activity during working memory tasks has been observed in various neuropsychiatric disorders such as schizophrenia [31] and obsessive-compulsive disorders [32]. In these studies, the patient performance was usually, but not invariably, impaired relative to control subjects. For example, in schizophrenia patients, aberrant patterns of brain activity have been found despite near-normal performance [33]. In the present study, interaction effects were observed only in the left VLPFC, whereas other differences failed to reach significance which may have been due to our small sample size. Alternatively, the treatment duration (6 months) may have been too short.

In addition, it could be argued that our design was not balanced with regard to the number of activation trials (36) and baseline trials (12) presented in our DNMTS task. However, in view of the length of each trial (approximately 9 s), the number of observations (scans) for each condition appears to be sufficient. Finally, the trend found towards improvement in the speed of the working memory performance might have reached significance with an increased number of patients included in this study.

From other brain imaging studies, there is some evidence that female sex steroid hormones modulate brain metabolism and blood flow both during cognitive activity and during the baseline period [for a review, see ref. 34]. Estrogen replacement therapy in postmenopausal women increased the regional cerebral blood flow in the temporal lobe during the resting state. Testosterone replacement has been shown to enhance brain perfusion in the midbrain in hypogonadal men [35].

In summary, in line with previous neuropsychological findings, data from the present study demonstrate that GH substitution in GHD patients improves long-term and working memory functions. In addition, the present fMRI data indicate increased efficiency of working-memory-associated cortical regions after GH substitution as compared with placebo. However, this interpretation should be made with caution in view of our small sample size. Future research should aim at replicating these findings in a larger group and at determining whether GH substitution also leads to improved memory function in other patients groups, such as patients with AO-GHD, or in healthy elderly subjects with decreased IGF-I levels.

Acknowledgments

The authors would like to thank P.F.C. Groot, MSc, for developing of the DNMTS task. This work was supported by a grant from Pfizer (The Netherlands).

References

1 van Dam PS, Aleman A: Insulin-like growth factor-I, cognition and brain aging. Eur J Pharmacol 2004;490:87–95.
2 Deijen JB, de Boer H, Blok GJ, van der Veen EA: Cognitive impairments and mood disturbances in growth hormone deficient men. Psychoneuroendocrinology 1996; 21: 313–322.
3 Soares CN, Musolino NR, Cunha NM, Caires MA, Rosenthal MC, Camargo CP, Bronstein MD: Impact of recombinant human growth hormone (RH-GH) treatment on psychiatric, neuropsychological and clinical profiles of GH deficient adults: a placebo-controlled trial. Arq Neuropsiquiatr 1999;57:182–189.
4 Deijen JB, de Boer H, van der Veen EA: Cognitive changes during growth hormone replacement in adult men. Psychoneuroendocrinology 1998;23:45–55.
5 Almqvist O, Thoren M, Saaf M, Eriksson O: Effects of growth hormone substitution on mental performance in adults with growth hormone deficiency: a pilot study. Psychoneuroendocrinology 1986;11:347–352.
6 Sartorio A, Molinari E, Riva G, Conti A, Morabito F, Faglia G: Growth hormone treatment in adults with childhood-onset growth hormone deficiency: effects on psychological capabilities. Horm Res 1995;44:6–11.
7 Abbott D, Rotnem D, Genel M, Cohen DJ: Cognitive and emotional functioning in hypopituitary short-statured children. Schizophr Bull 1982;8:310–319.
8 Degerblad M, Almkvist O, Grunditz R, Hall K, Kaijser L, Knutsson E, Ringertz H, Thoren M: Physical and psychological capabilities during substitution therapy with recombinant growth hormone in adults with growth hormone deficiency. Acta Endocrinol (Copenh) 1990;123: 185–193.
9 Arwert LI, Deijen JB, Witlox J, Drent ML: The influence of growth hormone (GH) substitution on patient-reported outcomes and cognitive functions in GH-deficient patients: a meta-analysis. Growth Horm IGF Res 2005; 15:47–54.
10 Lijffijt M, van Dam PS, Kenemans JL, Koppeschaar HP, de Vries WR, Drent ML, Wittenberg A, Kemner C: Somatotropic axis deficiency affects brain substrates of selective attention in childhood-onset growth hormone deficient patients. Neurosci Lett 2003; 353:123–126.
11 van Dam PS, de Winter CF, Vries R, Grond J, Drent ML, Lijffijt M, Leon KJ, Aleman A, de Haan EH, Koppeschaar HP: Childhood-onset growth hormone deficiency, cognitive function and brain N-acetylaspartate. Psychoneuroendocrinology 2005;30:357–363.
12 Oertel H, Schneider HJ, Stalla GK, Holsboer F, Zihl J: The effect of growth hormone substitution on cognitive performance in adult patients with hypopituitarism. Psychoneuroendocrinology 2004;29:839–850.
13 Burman P, Hetta J, Karlsson A: Effect of growth hormone on brain neurotransmitters. Lancet 1993;342:1492–1493.
14 Adem A, Jossan SS, d'Argy R, Gillberg PG, Nordberg A, Winblad B, Sara V: Insulin-like growth factor 1 (IGF-1) receptors in the human brain: quantitative autoradiographic localization. Brain Res 1989;503:299–303.
15 Nyberg F: Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance. Front Neuroendocrinol 2000;21:330–348.
16 Burman P, Hetta J, Wide L, Mansson JE, Ekman R, Karlsson FA: Growth hormone treatment affects brain neurotransmitters and thyroxine [see comment]. Clin Endocrinol (Oxf) 1996;44:319–324.
17 Johansson JO, Larson G, Andersson M, Elmgren A, Hynsjö L, Lindahl A, Lundberg PA, Isaksson OG, Lindstedt S, Bengtsson BA: Treatment of growth hormone-deficient adults with recombinant human growth hormone increases the concentration of growth hormone in the cerebrospinal fluid and affects neurotransmitters. Neuroendocrinology 1995; 61:57–66.
18 Arwert LI, Veltman DJ, Deijen JB, Lammertsma AA, Jonker C, Drent ML: Memory performance and the growth hormone/insulin-like growth factor axis in elderly: a positron emission tomography study. Neuroendocrinology 2005;81:31–40.
19 Arwert LI, Veltman DJ, Deijen JB, van Dam PS, Delemarre-van de Waal HA, Drent ML: Growth hormone (GH) deficiency and memory functioning in adults visualized by fMRI. Neuroendocrinology 2005;82:32–40.
20 Schufried G: The PC/S Vienna Test System: Test Management Program, Mödling 1992.
21 Letz R: NES2 User's Manual, version 4.6. Albany, Neurobehavioral Systems, 1993.
22 Shacham S: A shortened version of the Profile of Mood States. J Pers Assess 1983;47:305–306.
23 Emmen HH, Hogendijk EM, Hooisma J, Orlebeke JF, Uitdehaage SH: Adaptation of Two Standardized International Test Batteries for Use in The Netherlands for Detection of Exposure to Neurotoxic Compounds. Rijswijk, Medisch Biologisch Laboratorium – TNO, 1988.
24 Genovese CR, Lazar NA, Nichols T: Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 2002;15:870–878.
25 Arwert LI, Deijen JB, Muller M, Drent ML: Long-term growth hormone treatment preserves GH-induced memory and mood improvements: a 10-year follow-up study in GH-deficient adult men. Horm Behav 2005;47: 343–349.
26 Baum HB, Katznelson L, Sherman JC, Biller BM, Hayden DL, Schoenfeld DA, Cannistraro KE, Klibanski A: Effects of physiological growth hormone (GH) therapy on cognition and quality of life in patients with adult-onset GH deficiency. J Clin Endocrinol Metab 1998; 83:3184–3189.
27 Fletcher PC, Henson RN: Frontal lobes and human memory: insights from functional neuroimaging. Brain 2001;124:849–881.
28 Weissman DH, Giesbrecht B, Song AW, Mangun GR, Woldorff MG: Conflict monitoring in the human anterior cingulate cortex during selective attention to global and local object features. Neuroimage 2003;19:1361–1368.
29 Jansma JM, Ramsey NF, Coppola R, Kahn RS: Specific versus nonspecific brain activity in a parametric N-back task. Neuroimage 2000; 12:688–697.
30 Veltman DJ, Rombouts SA, Dolan RJ: Maintenance versus manipulation in verbal working memory revisited: an fMRI study. Neuroimage 2003;18:247–256.
31 Manoach DS, Gollub RL, Benson ES, Searl MM, Goff DC, Halpern E, Saper CB, Rauch SL: Schizophrenic subjects show aberrant fMRI activation of dorsolateral prefrontal cortex and basal ganglia during working memory performance. Biol Psychiatry 2000;48:99–109.
32 van der Wee NJ, Ramsey NF, Jansma JM, Denys DA, van Megen HJ, Westenberg HM, Kahn RS: Spatial working memory deficits in obsessive compulsive disorder are associated with excessive engagement of the medial frontal cortex. Neuroimage 2003;20:2271–2280.
33 Kindermann SS, Brown GG, Zorrilla LE, Olsen RK, Jeste DV: Spatial working memory among middle-aged and older patients with schizophrenia and volunteers using fMRI. Schizophr Res 2004;68:203–216.
34 Maki PM, Resnick SM: Effects of estrogen on patterns of brain activity at rest and during cognitive activity: a review of neuroimaging studies. Neuroimage 2001;14:789–801.
35 Azad N, Pitale S, Barnes WE, Friedman N: Testosterone treatment enhances regional brain perfusion in hypogonadal men. J Clin Endocrinol Metab 2003;88:3064–3068.