GH improves spatial memory and reverses certain anabolic androgenic steroid-induced effects in intact rats

Alfhild Grönbladh, Jenny Johansson, Anatole Nösti1, Fred Nyberg and Mathias Hallberg

Division of Biological Research on Drug Dependence, Department of Pharmaceutical Biosciences, Uppsala University, PO Box 591, S-751 24 Uppsala, Sweden
1Department of Building, Energy and Environmental Engineering, University of Gävle, S-801 76 Gävle, Sweden

Abstract

GH has previously been shown to promote cognitive functions in GH-deficient rodents. In this study we report the effects of GH on learning and memory in intact rats pretreated with the anabolic androgenic steroid nandrolone. Male Wistar rats received nandrolone decanoate (15 mg/kg) or peanut oil every third day for 3 weeks and were subsequently treated with recombinant human GH (1.0 IU/kg) or saline for 10 consecutive days. During the GH/saline treatment spatial learning and memory were tested in the Morris water maze (MWM). Also, plasma levels of IGF1 were assessed and the gene expression of the GH receptors (Ghr), Igf1 and Igf2, in hippocampus and frontal cortex was analyzed. The results demonstrated a significant positive effect of GH on memory functions and increased gene expression of Igf1 in the hippocampus was found in the animals treated with GH. In addition, GH was demonstrated to increase the body weight gain and was able to attenuate the reduced body weight seen in nandrolone-treated animals. In general, the rats treated with nandrolone alone did not exhibit any pronounced alteration in memory compared with controls in the MWM, and in many cases GH did not induce any alteration. Regarding target zone crossings, considered to be associated with spatial memory, the difference between GH- and steroid-treated animals was significant and administration of GH improved this parameter in the latter group. In conclusion, GH improves spatial memory in intact rats and can reverse certain effects induced by anabolic androgenic steroid.

Key Words
- growth hormone (GH)
- anabolic androgenic steroids (AAS)
- Morris water maze (MWM)
- insulin-like growth factor 1 (IGF1)
- IGF2

Introduction

GH is a polypeptide produced in somatotrophs of the anterior pituitary. Over the past decades, the effects that GH and its mediator insulin-like growth factor 1 (IGF1) may exert on functions related to the CNS have received attention among many investigators (for review, see Nyberg (2000)). In particular, the effects of GH on cognitive functions have attracted attention (Maruff & Falleti 2005). Replacement therapy with recombinant human GH (rhGH) in patients suffering from GH deficiency (GHD) has been demonstrated to attenuate cognitive deficits and improve memory (Deijen et al. 1996, Falleti et al. 2006). In addition, GH replacement has been found to improve the overall quality of life in GHD patients (Burman et al. 1995). Recently, another mediator of GH effects, IGF2, has been implicated in memory functions (Chen et al. 2011). In addition, GH was shown to
Anabolic androgenic steroids (AASs), synthetic derivatives of testosterone, are known to induce profound effects in different peripheral organs as well as in a variety of regions in the CNS. A large number of studies have reported significant effects of AASs on CNS-related behaviors, such as aggression, anxiety, depression, and impaired cognitive functions (Su et al. 1993, Steensland et al. 2005, Hallberg 2011). In an animal model, treatment with supraphysiological doses of nandrolone decanoate was recently demonstrated to impair spatial memory in certain parameters of the MWM (Magnussen et al. 2009). On the contrary, Schneider-Rivas et al. (2007) demonstrated that the male steroid testosterone and GH administered alone or in combination improved long-term memory in young rats. A link between the GH/IGF1 axis and AAS-induced mechanisms has been discovered (Weissberger & Ho 1993, Veldhuis et al. 1997). For example a reduction of IGF1 in plasma has been reported after 24 months of AAS use (Bonetti et al. 2008) On the contrary, some studies have also reported testosterone to stimulate the production of IGF1 (Hobbs et al. 1993). Short-term treatment with testosterone has also been shown to inhibit GH release when stimulated with a GH secretagogue in dogs (Rigamonti et al. 2006), and in humans testosterone was shown to lower IGF1 bioavailability (Veldhuis et al. 2005). Interestingly, a recent study revealed that almost half of individuals abusing steroids combined these agents with GH (Skarberg et al. 2009). Fewer details are known of the association between GH and AAS in the CNS and on behavior, but it has been suggested that an interaction between testosterone and GH plays an important role in the modulation of memory (Schneider-Rivas et al. 2007). Hence, it would be of value to know if the steroid-induced impairment of cognitive function observed in man after AAS abuse could possibly be modified by administration of GH.

The aim of this study was to address this issue and to examine the impact of rhGH on learning and memory in male rats and to investigate the effects of this hormone on cognition in rats pretreated with supraphysiological doses of AAS. In addition, the effects on gene expression in hippocampus and frontal cortex as well as weight parameters were investigated.

### Materials and methods

#### Animal experiment

Forty-eight male Wistar rats of an age of 8 weeks were obtained from Taconic Farms (Ejeby, Denmark) and allowed to adapt to the new environment for 2 weeks. The animals, with a body weight of 316 ± 3 g at the start of the experiment, were housed together (three in each cage) in an air-conditioned room with controlled temperature (22–24 °C) and humidity (50–60%). They had free access to water and food and were kept under a reversed 12 h light:12 h darkness cycle with lights off at 0700 h. During the experiment rats were subjected to s.c. injections with nandrolone decanoate (Deca-Durabol, Organon, Oss, The Netherlands), 15 mg/kg, or peanut oil (Apoteket AB, Umeå, Sweden) every third day for 3 weeks (at 1600 h), from days 1 to 21. Following completed treatment with nandrolone decanoate or peanut oil, rats were injected s.c. with 1 IU/kg rhGH (Amersham) or saline for 10 consecutive days, days 22–31 of the experiment (see Table 1 and Fig. 1). The AAS nandrolone decanoate is a prodrug with an estimated half-life of at least 5 days (van der Vies 1985, Minto et al. 1997). The repeated injections will result in a depot effect with duration several weeks after the last administration (Minto et al. 1997). This means that the AAS-treated rats will have high concentrations of the steroid during GH treatment (including the water maze testing) and that the combined effects of the AAS and rhGH will be observed in the AAS+GH-treated animals.

### Table 1 Outline of the four treatment groups. Nandrolone decanoate/peanut oil was administered s.c. every third day for 3 weeks and GH/saline for 10 consecutive days

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Days 1–21</th>
<th>Days 22–31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Peanut oil</td>
<td>Saline</td>
</tr>
<tr>
<td>GH</td>
<td>Peanut oil</td>
<td>rhGH, 1 IU/kg</td>
</tr>
<tr>
<td>AAS</td>
<td>Nandrolone decanoate, 15 mg/kg</td>
<td>Saline</td>
</tr>
<tr>
<td>AAS+GH</td>
<td>Nandrolone decanoate, 15 mg/kg</td>
<td>rhGH, 1 IU/kg</td>
</tr>
</tbody>
</table>
Journal of Endocrinology was adapted from Karlsson platform in a pool filled with water. The MWM protocol ability of rodents to learn the spatial location of a hidden commonly used procedure for the assessments of the To study the effects of the hormone treatment on spatial allanimal experimental procedures followed the guidelines of the Swedish legislation on animal experimentation and were performed according to the protocol approved by the Uppsala Animal Care and Ethical Committee.

All rats were weighed eight times during the experiment. The animal experimental procedures followed the guidelines of the Swedish legislation on animal experimentation and were performed according to the protocol approved by the Uppsala Animal Care and Ethical Committee.

**Water maze**

To study the effects of the hormone treatment on spatial learning and memory the rats were tested in the MWM, a commonly used procedure for the assessments of the ability of rodents to learn the spatial location of a hidden platform in a pool filled with water. The MWM protocol was adapted from Karlsson et al. (2011) and consisted of a black large circular pool with a diameter of 160 cm. The pool was filled with tap water, thermostatically controlled at 22±1 °C. The pool was divided into four different quadrants with a hidden platform submerged 1.5 cm underneath the water surface, placed in the southwest (target) quadrant. The pool was located in an experimental room with several cues and the location of the platform remained constant over the days of acquisition. The rats were started facing the pool wall and the starting position was randomized between the quadrants. The rats were allowed five acquisition trials during 5 consecutive days with four trials each day and were allowed to search for the platform for a maximum of 90 s. If the rat did not find the platform in 90 s, the experimenter gently guided it there. Before the start of the next trial the rats were allowed to stay at the platform for 30 s. Parameters such as escape latency, latency to target quadrant, swim distance, swim length and thigmotaxic swimming were recorded. The probe trial was performed 72 h after the last acquisition trial, as a single trial where the rats were allowed to swim for 90 s without the platform. The animals were started in the northeast quadrant. Latency in the first crossing of the former platform location (target zone), number of crossings of the target zone and the number and duration of visits to the different quadrants were analyzed in addition to the parameters scored in the acquisition trials. Also, the number of target zone crossings (TZCs) during the first 30 s of the probe trial was recorded.

**RNA extraction and cDNA synthesis**

Following the probe trial, the rats were decapitated and whole brains were removed and dissected using a rat brain matrix. All collected tissues were immediately frozen on dry ice and then stored at −80 °C until further analysis. RNA was extracted from hippocampus and frontal cortex using Qiagen’s RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer’s protocol. Briefly, 1000 μl Qiazol tissue lysis (Qiagen) was added to the frozen tissue sample and the samples were then quickly homogenized. Two hundred microliters of chloroform were added and the samples were then centrifuged at 4 °C (12 000 g, 15 min). A 1:1 volume of 70% ethanol was added to the supernatant and the samples were then eluted using Mini Spin columns. The RNA concentration was quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). The quality of the RNA was examined using the Xperion System for RNA analysis (Bio-Rad Laboratories, Solna, Sweden) and samples demonstrating clear 18S and 28S rRNA and RNA quality indicator between seven and ten were used for further analysis. cDNA synthesis was conducted using the High Capacity cDNA RT Archive kit (Applied Biosystems). The reactions were performed in a final volume of 100 μl including 250 ng of RNA, Multi-Scribe reverse transcriptase 50 U/μl, RT buffer, dNTP mixture, RT random primers and RNase-free water. The cycling parameters used were 25 °C for 10 min, 37 °C for 120 min and 85 °C for 5 min. A control reaction without reversed transcriptase was also performed.

**Quantitative real-time PCR**

The gene expression was analyzed with quantitative real-time PCR (qPCR) in 96-well plates with 2 μl cDNA (5 ng) and 23 μl master mix containing iQ SYBR Green Supermix (Bio-Rad Laboratories), 20 μM forward primer, 20 μM reverse primer and RNase-free water. Amplification was performed using a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories) with the following protocol: 95 °C for 3 min following 40 cycles of 95 °C for 15 s, 60 °C for 20 s and 72 °C for 40 s. To assure specific amplification, a melt curve was included in the end of each run. Each assay included samples, internal controls and negative controls in triplicates. A mean of the PCR
efficiency for each primer set was calculated using the software LinRegPCR, version 12.17, and used in the gene expression analysis (Ruijter et al. 2009). $C_q$ values were obtained from the CFX Manager Software (Bio-Rad Laboratories) and the calculation of normalized expression levels was performed using the software qBASEplus, version 2.0 (Biogazelle, Zwijnaarde, Belgium). GeNorm, a part of qBASEplus, was used to evaluate the stability of a set of reference gene candidates and three genes were chosen (Actb, Rpl19 and Arbp (Rplp0)) for normalization of the data. Primer sequences were designed using the Primer-BLAST tool (NCBI) and validated in silico using RTprimerDB primer evaluation, primers for Igf1 and Igf2 targeting several transcripts were based on previous studies (Chen et al. 2011, Garbayo et al. 2011). Primer sequences are presented in Table 2.

### IGF1 ELISA

Trunk blood was collected during decapitation in 0.1% EDTA, put on ice and then centrifuged at 1500 $g$ for 10 min in 4 °C. The plasma was collected and stored in a freezer, −80 °C, until further analysis. Secreted IGF1 in plasma was quantified using a commercial ELISA kit (mouse/rat IGF1 REF E25, Mediagnost, Reutlingen, Germany). Plasma was diluted in sample buffer (1:500) and further analyzed according to the manufacturer’s instructions.

### Statistical analysis

Values are presented as mean ± S.E.M. Statistical analyses were performed using GraphPad Prism, version 5.0d GraphPad Software Inc., La Jolla, CA, USA and SPSS. The normality of the data distribution was examined using the Shapiro–Wilks $W$ test. Results from the MWM acquisition trials, the time spent in the different quadrants during the probe trial, and the weight measurements were analyzed using two-way repeated measures ANOVA with Bonferroni’s multiple comparisons test. Data obtained from the MWM probe test were statistically analyzed using a univariate ANOVA using the mean rank (i.e. an average of the rank-transformed variables) as dependent variable and treatment as fixed factor and the nonparametric Kruskal–Wallis test and Dunn’s multiple comparisons test where appropriate. Results from the gene expression and the ELISA results were analyzed using one-way ANOVA and Tukey’s post hoc test where appropriate. The gene expression results were also analyzed using multivariate ANOVA to study a possible overall effect of treatment. Pearson’s correlation test was used for analysis of correlations. $P$ values <0.05 were considered significant.

### Results

#### Water maze

Spatial learning and memory was examined in the MWM and results from the 5 days of acquisition trials are presented in Fig. 2. No significant differences between the treatment groups in latency to target quadrant were observed, although a significant effect of day was seen ($F(4, 172) = 5.39$, MSE = 7.69, $P = 0.0004$, $\eta_p^2 = 0.066$). From the acquisition trials in the MWM it is obvious that data recorded did not demonstrate any alterations in latency to target zone between the groups, but an effect of day was found ($F(4, 172) = 48.72$, MSE = 143.68, $P < 0.0001$, $\eta_p^2 = 0.301$). In general, all groups learned to locate the platform, and as expected the escape latency decreased

<table>
<thead>
<tr>
<th>Gene names</th>
<th>Primer sequences</th>
<th>Accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actb</td>
<td>F: CGTCCACCGCGAGTACACCT R: ATCCATGGCAACTGTTGCG</td>
<td>NM_031144</td>
</tr>
<tr>
<td>Arbp(Rplp0)</td>
<td>F: GGCAATCCCTGACGACCG</td>
<td>NM_022402</td>
</tr>
<tr>
<td>Rpl19</td>
<td>F: GCGTCTGACGCAATGAGTGGTT</td>
<td>NM_031103</td>
</tr>
<tr>
<td>Igf1</td>
<td>F: GCTGAAGCCGTTCATTTAGC R: GAGGAGGCCAAATTCAACAA</td>
<td>NM_001082477, NM_001082478, NM_001082479, NM_178866 (transcript variant 1–4)</td>
</tr>
<tr>
<td>Igf2</td>
<td>F: CCCAGCGAGACTGCTGCGGA R: GGAAGTACGGCCTGAGAGGTA</td>
<td>NM_001190163, NM_001190162, NM_031511 (transcript variant 1–3)</td>
</tr>
<tr>
<td>Ghr</td>
<td>F: GAAATAGTGCAACCTGTACCATG</td>
<td>NM_031094</td>
</tr>
</tbody>
</table>

Actb, actin beta; Arbp, ribosomal protein, large, P0; Rpl19, ribosomal protein L19.
The number of TZCs was detected in the GH-treated animals receiving rhGH also had significantly more TZC than the controls. During the probe trial, the animals demonstrated that the rhGH-treated animals had a significantly decreased latency to the target zone compared with the controls. In order to investigate the existence of an overall effect of treatment the dependent variables were transformed using the ranks transformation method. A univariate ANOVA using the mean rank as dependent variable and treatment as fixed factor revealed a significant effect of treatment, \( F(3, 43) = 3.89, \ MSE = 67.79, \ p = 0.015, \ \eta^2 = 0.21 \). Follow-up comparisons using Bonferroni’s correction showed that rats treated with GH performed better overall than the controls, \( p = 0.013 \). There were no differences in thigmotaxis, swim speed or swim length during the probe trial.

during the acquisition days. An effect of day was seen in thigmotaxis, i.e. the time spent for swimming 15 cm from the border of the pool, \( F(4, 172) = 13.19, \ MSE = 70.80, \ p < 0.0001, \ \eta^2 = 0.13 \); swim speed \( F(4, 172) = 13.93, \ MSE = 5.43, \ p < 0.0001, \ \eta^2 = 0.13 \); and swim distance \( F(4, 172) = 53.62, \ MSE = 76.240.21, \ p < 0.0001, \ \eta^2 = 0.32 \), during the acquisition trials. No effect of treatment was seen in these parameters.

The results from the probe trial demonstrated an overall improved behavior in the rhGH-treated animals. Results from the 90 s probe trial, performed 72 h after the last acquisition trial, are presented in Fig. 3. Data obtained demonstrated that the rhGH-treated animals had a significantly decreased latency to the target zone compared with the controls. During the probe trial, the animals receiving rhGH also had significantly more TZC than the AAS-treated animals. Furthermore, a significant increase in the number of TZCs was detected in the GH-treated animals compared with controls also from the data recorded during the first 30 s of the probe trial (Fig. 3C). The rhGH-treated animals had more visits (percentage of total visits) to the target quadrant than the AAS+GH-treated animals (Fig. 3D). Using a two-way repeated measures ANOVA the probe trial results also demonstrated an effect of quadrant \( F(3, 129) = 15.76, \ MSE = 80.01, \ p < 0.0001, \ \eta^2 = 0.20 \), in the parameter time spent in quadrants (percentage of total time); however, no effect of treatment was found. In order to investigate the existence of an overall effect of treatment the dependent variables were transformed using the ranks transformation method. A univariate ANOVA using the mean rank as dependent variable and treatment as fixed factor revealed a significant effect of treatment, \( F(3, 43) = 3.89, \ MSE = 67.79, \ p = 0.015, \ \eta^2 = 0.21 \). Follow-up comparisons using Bonferroni’s correction showed that rats treated with GH performed better overall than the controls, \( p = 0.013 \). There were no differences in thigmotaxis, swim speed or swim length during the probe trial.

Quantitative real-time PCR
The expression of the gene transcripts of Igf1, Igf2, and Ghr from frontal cortex and hippocampus was analyzed, and the results are presented in Fig. 4A and Table 3. As shown, an increase of the Igf1 gene transcript in hippocampus in the GH-treated animals compared with the group treated with AAS was found. Furthermore, a multivariate ANOVA revealed a trend toward a significant overall effect of treatment on the gene transcripts from the hippocampus, $F(9, 126)=1.90$, $P=0.058$. No such trend was found regarding the gene transcripts from the frontal cortex.

IGF1 ELISA
The IGF1 concentration in plasma was determined using ELISA. The results demonstrated a significant decrease of the IGF1 plasma concentration in the AAS-treated group compared with controls and rhGH-treated animals (Fig. 4B). This decrease in IGF1 plasma levels was also seen in the AAS+GH group compared with the rhGH-treated animals, although the animals receiving AAS+GH had a slightly higher mean (1410+141 ng/ml) compared with the AAS-treated animals (1196+105 ng/ml). Correlation test with behavioral parameters from MWM did not demonstrate any strong correlations.

Weight parameters
The animals were weighed eight times during the experiment and the results from the body weight measurements are presented in Fig. 5. The two-way repeated measures ANOVA demonstrated a significant interaction between day and treatment, $F(21, 301)=51.63$, MSE = 56.23, $P<0.0001$, $\eta^2=0.12$. An effect of both day $F(7, 301)=553.8$, MSE = 3.66, $P<0.0001$, $\eta^2=0.33$ and treatment $F(21, 301)=51.63$, MSE = 56.23, $P<0.0001$, $\eta^2=0.20$ was found. Bonferroni’s post hoc test revealed several differences between the treatment groups, for example, from day 12 the AAS and AAS+GH-treated animals had a slower weight development compared with the controls. On day 22 the rhGH treatment was initiated, and on days 28 and 31 the AAS+GH-treated animals gained significantly more weight than the animals receiving AAS. On days 28 and 31 there were significant differences between all four treatment groups, where the rhGH-treated animals had gained most weight since day 1 of the experiment demonstrating an overall effect of the GH treatment.

Discussion
The results from this study demonstrated an improved performance of the rhGH-treated animals in the MWM, suggesting an impact of GH on memory. The effects of rhGH on the animals treated with AAS were not pronounced indicating that certain AAS-induced behaviors related to spatial performance and underlying mechanisms seem unaffected by GH. However, administration of rhGH showed prominent effects on weight gain and was able to increase body weight also in the animals pretreated with AAS.

The results from the MWM probe trial demonstrated improved spatial memory functions in the rhGH-treated rats. This is in accordance with previous studies using experimental rats with depleted endogenous GH production due to removal of the hypophysis, where the hormone was found to improve performance in the MWM as well as in the radial maze (Le Grevès et al. 2006, 2011). GHD in both rats and humans has been associated with memory impairments, which is attenuated by GH treatment (Burman & Deijen 1998, Nieves-Martinez et al. 2010, Li et al. 2011a). The results from the MWM also suggest that rhGH may improve the performance of the AAS-treated animals in certain parameters, e.g. TZC. Positive effects from GH on drug-induced effects have been reported earlier from studies on opioid-induced apoptosis in hippocampal cells (Svensson et al. 2008). Several studies suggest that not only GH but also IGF1 is involved in cognition (Markowska et al. 1998, Sonntag et al. 2005, Gong et al. 2012). Hence, both these growth factors may possibly be able to act as cognitive enhancers on drug-induced cognitive impairments.

The mechanism by which GH and IGF1 elicit their positive effects on cognition is still not clarified in all its detail. The somatotropic axis is believed to have
an important impact on neuroprotection (Nyberg 2000, Nyberg & Sharma 2002) and neurogenesis (Aberg et al. 2006). GH treatment has been shown to promote neurogenesis in hippocampal areas both in intact and hypophysectomized animals (Aberg et al. 2009, David Aberg et al. 2010). In addition, treatment with GH may attenuate age-related alterations of hippocampal plasticity (Ramsey et al. 2004). The rhGH treatment in the present study increased the IGFI gene expression in hippocampus compared with the AAS-treated animals. In alignment with this is a recent demonstration of increased hippocampal Igf1 gene expression in pituitary-intact animals, after GH treatment (Li et al. 2011b). Since hippocampus represents a brain area essential for learning and memory it is tempting to suggest that an upregulation of the Igf1 gene transcript in the hippocampus may be associated with improved spatial behavior as seen in the rhGH-treated animals. However, in a GH-deficient rat model no alterations of IGFI were found after 7 and 30 days of GH treatment (Yan et al. 2011). Thus, it seems that there may be differences in the effects exerted by GH when comparing intact and hypophysectomized animals.

Previous studies have shown that both GH and IGFI interact with the NMDA receptor system. Both hormones were shown to affect the organization of the NMDA receptor subunits in a way that is consistent with increased long-term potentiation (LTP) and thereby improved cognitive capabilities (Le Grevès et al. 2002, 2005). Increased LTP in hippocampal structures has been suggested to activate a cascade of enzymatic reactions to increase CREB leading to the formation of new synapses and improved cognition (Abel & Nguyen 2008, Gould 2010).

Gene expression of Ghr in the hippocampus of young rats has earlier been observed to increase after treatment with GH, although no difference in older rats was observed (Le Grevès et al. 2002). Interestingly, the Ghr gene expression levels in hippocampus showed a trend toward being lower in the AAS treatment group. A recent study demonstrated a negative correlation between GHR expression levels and learning in diabetic mice where higher Ghr gene transcript levels were associated with an increased learning ability (Enhamre et al. 2012). In the present study no effect from the hormone treatments was seen on Igf2 gene expression levels. IGF2 is believed to have a significant role in memory and has recently been proposed as a novel cognitive enhancer (Agis-Balboa et al. 2011, Chen et al. 2011). In addition, this growth factor is highly expressed in the hippocampus (Rotwein et al. 1988).

Administration of GH is known to decrease the endogenous release of the hormone through an auto-regulatory feedback mechanism (Andersson et al. 1983) and this may result in less prominent increases of IGFI

### Table 3

Results from the qPCR analysis presented as fold of controls (mean ± S.E.M.). A multivariate ANOVA revealed a trend towards a significant overall effect of treatment on the gene transcripts from the hippocampus but not in the frontal cortex. For details regarding the statistical analysis, see text. *n* = 11–12/group. The data for Igf1 mRNA in hippocampus is shown in Fig. 4A.

<table>
<thead>
<tr>
<th></th>
<th>Frontal cortex mRNA (fold of control)</th>
<th>Hippocampus mRNA (fold of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Igf1</td>
<td>Ghr</td>
</tr>
<tr>
<td>Controls</td>
<td>1.00 ± 0.03</td>
<td>1.00 ± 0.02</td>
</tr>
<tr>
<td>GH</td>
<td>1.05 ± 0.04</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td>AAS</td>
<td>1.12 ± 0.10</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>AAS + GH</td>
<td>1.21 ± 0.20</td>
<td>0.97 ± 0.02</td>
</tr>
</tbody>
</table>

**Bonferroni’s post hoc test where appropriate.** A significant interaction between day and treatment was found as well as a main effect of both day and treatment (for further details, see text). *P < 0.05 compared with controls; *P < 0.05 compared with the AAS-treated group.

### Figure 5

Body weight gain expressed as percentage of initial body weight. Values are expressed as mean ± S.E.M., *n* = 11–12/group. Statistical testing was performed with two-way ANOVA for repeated measurements and Bonferroni’s post hoc test where appropriate. A significant interaction between day and treatment was found as well as a main effect of both day and treatment (for further details, see text). *P < 0.05 compared with controls; *P < 0.05 compared with the AAS-treated group.
levels in intact animals than expected (Tannenbaum 1980). In the present study no significant increase in the IGF1 plasma levels in the animals receiving rhGH could be seen however, no decrease in IGF1 levels were seen either. A limited number of studies have reported the effects of GH administration on IGF1 levels in intact animals, although a previous study demonstrated that rhGH treatment in Wistar rats did not affect the plasma levels of IGF1 and in this case the authors speculated that the IGF1 release is maximized in these intact animals and cannot be enhanced further (Bielohuby et al. 2011). Furthermore, the same authors state that IGF1 is not a reliable biomarker in wild-type mice and other parameters such as total body weight and liver weight may be more suitable as markers of GH administration (Bielohuby et al. 2011). However, in hypophysectomized rats alterations in the IGF1 levels in plasma following GH treatment have been reported (Maiter et al. 1988, Le Greve’s et al. 2011). Also in acromegaly elevated levels of IGF1 have been reported although the relationship between GH and IGF1 levels in plasma is not always straightforward (Neggers et al. 2011). In the present study, the IGF1 levels in plasma was measured more than 12 h after the last rhGH injection; perhaps a significant increase would have been detected if plasma had been collected earlier after GH administration. Stress-related increases of cortisol secretion have also been demonstrated to decrease plasma IGF1 levels (Rosmond et al. 1998). Further studies are needed to fully elucidate this mechanism in intact animals. Several studies have demonstrated a relationship between AAS and the GH/IGF1 axis, although an association between AAS administration and IGF1 plasma levels has not yet been verified. In this study the IGF1 plasma level was significantly attenuated in the AAS-treated animals, clearly demonstrating a connection between these two hormone systems. The observed reduction of IGF1 plasma concentration in the AAS-treated animals might depend on a negative feedback inhibition induced by steroid treatment. In humans, testosterone administration to older men was demonstrated to improve spatial cognition; however, no change in IGF1 or IGF2 serum levels was found and the authors suggested that the effects of AAS and GH on cognition may be independent from each other (Cherrier et al. 2004). However, serum levels of IGFI and IGF2 might also depend on both dose and treatment length and may not in all cases be suitable markers for CNS effects.

In certain MWM parameters, e.g. TZC, a tendency toward impaired performance in the AAS-treated animals was recorded, although no pronounced differences between controls and AAS-treated animals could be detected. The effect of rhGH treatment in the AAS-pretreated rats appears to be relatively weak although the rhGH treatment induced memory improvements in intact nonsteroid-treated rats. Thus, an apparent interaction between the two hormones may in some parameters result in a weaker effect of GH on cognitive function. Changes in spatial learning have earlier been demonstrated following 2 weeks of treatment with nandrolone decanoate (Magnusson et al. 2009). Other studies have not observed any effects on spatial memory in male rats after treatment with AAS compounds (Clark et al. 1995, Smith et al. 1996). Interestingly, a study examining the combined effect of testosterone and GH demonstrated improved long-term memory assessing the extinction response using a passive avoidance test (Schneider-Rivas et al. 2007). This research group also reported that testosterone enanthate facilitates long-term memory applying passive avoidance conditioning in male rats (Vazquez-Pereyra et al. 1995). However, in these experiments all steroids were applied 45 min before the training session. Thus, alterations in spatial learning and memory induced by the AAS may be acute or short term, and in this experiment the MWM probe trial was performed 10 days after the last dose of AAS. It should also be noted that the setups for memory testing and animal strains as well as other conditions in all these experiments are different. We have previously observed strain differences in the sensitivity toward AAS between Wistar and Sprague–Dawley rats regarding their response in a test measuring aggressive behavior (Johansson et al. 2000b, Steensland et al. 2005). Overall, we believe Wistar rats to be more suitable for behavioral studies although behavioral differences within Wistar rats from different suppliers also have been shown (Palm et al. 2011).

The results from weight measurements demonstrated that the weight gain (percentage of initial weight) significantly increased in the animals receiving rhGH. This is in line with a previous study demonstrating increased body weight gain in rats with intact pituitaries receiving GH, where an increase in food intake in the GH-treated rats also was observed (Azain et al. 1995). Also in hypophysectomized rats, GH treatment has been demonstrated to increase weight gain compared with untreated controls (Le Greve’s et al. 2006, 2011). Increased lean body mass and reduction of fat mass have been associated with GH treatment and changes of body composition or increased food intake could be an explanation for the weight gain. The reduction in gain of weight observed in the AAS and AAS+GH-treated
animals was expected since earlier studies of rats injected with nandrolone decanoate have demonstrated reduced gain of weight in these animals (Johansson et al. 2000a, Lindblom et al. 2003). In addition, a reduction of food intake in animals treated with nandrolone decanoate has been demonstrated (Lindblom et al. 2003). Interestingly, after day 22 when the AAS administration ended and the rhGH treatment was initiated, the AAS+GH group started to increase in weight again as compared with the AAS-treated rats that received saline instead of rhGH. Thus, GH can partly compensate for the effect on body weight induced by AAS, although the underlying mechanisms for the weight increase need further investigations.

To conclude, the results presented here demonstrate that rhGH and AAS both have an impact on body weight gain and that rhGH was able to attenuate the reduction of weight caused by the AAS administration. Furthermore, rhGH induces a significant improvement on memory in pituitary-intact male rats. The spatial performance of the AAS-treated rats was, however, not significantly altered by administration of rhGH, suggesting that the effects induced by AAS and examined in this study are not affected by the pituitary hormone. Although the present study does not fully clarify the mechanism by which GH and AAS affect memory, the ability of GH to enhance spatial memory in rats is impressive. The experiments presented here were conducted with rhGH in rats but nevertheless the knowledge of the significant improvement of memory observed should be considered in the search for effective strategies to treat drug-induced impairments of cognitive function.

References


Received in final form 13 October 2012

Accepted 22 October 2012

Accepted Preprint published online 22 October 2012