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Research report

Growth hormone reverses streptozotocin-induced cognitive impairments in male mice

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HIGHLIGHTS

- Growth hormone (GH) can reverse cognitive deficiencies in diabetic mice.
- GH treatment improves the learning ability of mice with cognitive impairments.
- In diabetic mice, GH promotes a more effective search strategy in the Barnes maze.

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ABSTRACT

In recent decades, growth hormone (GH) replacement therapy in human subjects deficient in the hormone has resulted in a number of beneficial effects on cognitive performance. Studies in hypophysectomised rats report similar effects of GH treatment on learning and memory tasks. The purpose of this study was to investigate the ability of GH to reverse learning impairments in mice with streptozotocin (STZ)-induced diabetes. Diabetic and control mice were given recombinant human GH (rhGH) 0.1 IU/kg/day for ten consecutive days. In the latter phase of the treatment the cognitive abilities of the mice were tested using the Barnes maze (BM). A profound hormonal effect was seen when analysing the search patterns used by the animals in the maze. rhGH treatment significantly counteracted the cognitive disabilities expressed as lack of direct search strategies on the last day in the BM. In addition, the number of primary errors made by diabetic mice during the acquisition phase was reduced by rhGH treatment, although the primary escape latency was unchanged in these animals when compared to saline-treated diabetic animals. These results suggest that specific cognitive impairments induced by STZ, i.e. the disabilities seen in strategic behaviour, could be reversed by exogenous hormone treatment. Our findings highlight the influence of GH on brain function and in particular on cognitive behaviour related to learning and memory.

1. Introduction

Growth hormone (GH) is an endogenous hormone that is released from the anterior pituitary. The beneficial effects of GH on cognitive function in animals and humans have been under investigation for several years. A number of preclinical and clinical studies have reported behavioural effects on the central nervous system (CNS) following treatment with recombinant human GH (rhGH) and binding studies have shown a high density of GH-specific receptors (GHR) in various brain regions [1–5]. In addition to the mostly desirable effects on body composition, rhGH treatment has also been associated with reduced psychiatric symptoms and improved wellbeing in adult GH-deficient (GHD) patients [6]. Furthermore, enhanced memory function has been reported in GHD subjects after long-term GH treatment [7,8]. Treatment with rhGH in hypophysectomised rats improves their performance of learning and memory tasks in the Morris Water maze and the Radial Arm maze [4,9]. In congruence with these results, GH has also been shown to prevent morphine-induced cell damage in hippocampal neurons [10].

For many years it has been under debate whether the observed CNS-related effects of GH are due to the actions of the hormone itself or those of its secondary mediator, insulin-like growth factor-1 (IGF-1). Whereas IGF-1 is known to reach the CNS from the circulatory system, it is still under discussion whether GH itself crosses the blood–brain barrier (BBB). Several theories, that strengthen the theory of GH passing over to the CNS, have been proposed. One suggests that the hormone is transported in to the CNS via the *median eminence*, an area in the hypothalamus where polypeptides and hormones can leave the CNS [11]. Another theory

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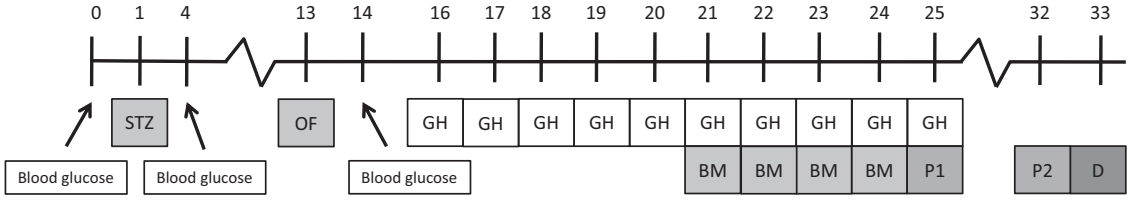

Fig. 1. Schematic timeline illustrating the experimental design. *Abbreviations*: Single high-dose streptozotocin injection (STZ), open field test (OF), intraperitoneal rhGH injection 0.1 IU/kg/day (GH), Barnes maze (BM), probe trial day 5 (P1), probe trial day 12 (P2), and decapitation (D). Days 21–24 in the timeline correspond to the acquisition phase in the BM, i.e. training days 1–4. Day 25 and day 32 correspond to the probe trials, referred to as day 5 and day 12.

suggests that GH reaches its brain targets via receptor-mediated transport through the endothelial cells of the *choroid plexus* [12]. The proposed theories are supported by findings reporting that the GH concentrations in cerebrospinal fluid (CSF) are profoundly increased by exogenous rhGH treatment [13] and correlate well with the administered dose of the hormone in GHD patients [14].

The mechanisms behind the actions of GH on brain function are still poorly understood. However, important signalling pathways regulating cognitive functioning, such as the *N*-methyl-D-aspartate (NMDA)-receptor system [15], are affected in rats treated with rhGH [4,15,16]. GH-induced effects seen on, for example, mRNA expression of the NR2B receptor subunit and the ratio of NR2B/NR2A subunits in the hippocampus of young rats [16] are compatible with improved cognitive function.

Hypofysectomised rats are often used to study the effect of GH on memory and cognition. Interestingly, cognitive impairments and morphological alterations in brain regions associated with learning and memory have also been observed in diabetic rodents [17–20]. Recently, we used the Barnes maze (BM) to investigate cognitive function in diabetic C57BL/6J mice with learning impairments. We found that diabetic mice displayed pronounced cognitive impairments during the acquisition phase of the BM compared to controls [21]. In addition, the gene expression of GHR was altered in the diabetic mouse prefrontal cortex (PFC) [21], a brain region associated with working memory and learning and planning processes [22,23], which could influence brain circuits involved in cognitive functioning. Thus, our findings clearly suggest that the diabetic state affects the GH/IGF-1 axis. The aim of this study was to investigate the effect of rhGH treatment on the cognitive performance of diabetic male mice in the BM. Specifically, we focused on the influence of rhGH treatment on the search patterns used by the mice in the acquisition phase.

2. Materials and methods

2.1. Animals and induction of diabetes

C57BL/6J male mice (Taconic, Denmark) weighing approximately 22 g (aged 7–9 weeks) were kept under a reversed 12 h dark/light cycle with lights on at 19:00 h. The animals were housed under standardised conditions (20–24 °C and a humidity of 45–65%), 2–3 mice per cage. They were monitored daily and weighed three times weekly. Animals losing >20% of their initial weight were immediately excluded from the study and euthanised. Food and water were provided ad libitum. The local ethical Committee in Uppsala, Sweden, approved the experiments.

Animals were randomly divided into four groups: saline (sal)/sal ($n = 9$), streptozotocin (STZ)/sal ($n = 11$), sal/GH ($n = 10$) and STZ/GH ($n = 9$). The mice were injected i.v. with a single dose of either STZ 150 mg/kg (Sigma Aldrich, Schnelldorf, Germany) or saline in a corresponding volume. Starting 5 days prior to the BM tests, the mice received daily i.p. injections, at 17:00 h, of rhGH 0.1 IU/kg (Genotropin®, Pfizer, Sweden) or saline for 10 days. Further details regarding the experimental design are presented schematically in Fig. 1. Blood glucose levels were measured, in the tail blood, using an Accu Chek Aviva blood glucose meter (Roche Diagnostics, Germany). All animals with a blood glucose level >16.7 mmol/L three days after the induction were considered diabetic and included in the study.

2.2. Behavioural testing

2.2.1. Open field (OF)

The spontaneous activity of the animals was studied using an open field (OF) paradigm. The apparatus used in this study, with minor modifications, has been described elsewhere [24]. Briefly, the arena consisted of a white circular platform (diameter 90 cm) with dark walls. The open area was divided into three zones [24]. Each animal was placed in the outer circle by the experimenter and was allowed to freely explore the area for 10 min. During this period, the time before entry into the middle and central zones (latency), the number of visits into each zone (frequency) and the time spent in each zone (duration) were evaluated. The testing sessions were recorded using a video camera and behavioural scoring was conducted manually using SCORE 3.4 software. Behavioural testing in the OF was conducted under light conditions (ca 25 lx), during the dark period of the dark/light cycle.

2.2.2. Barnes maze (BM)

The BM protocol used in this study was adapted with minor modifications from Enhamre et al. [21]. The maze consisted of a white circular platform (diameter 92 cm) with 20 holes around the outer border. A rectangular box (11 cm × 13 cm × 5.5 cm) was attached under one of the holes; this was called the *target hole*. The experimenter placed the mouse in the centre of the maze, using a dark start box, and left the room immediately after removing the start box. During the training or acquisition phase, each mouse underwent a total of 4 trials per day for 4 consecutive days. On day 5 (D5) and day 12 (D12), representing short-term and long-term memory retention, respectively, probe trials were carried out to evaluate the ability of the mice to remember the position of the target hole. In the probe trial the hole leading to the target box was closed. As a reinforcer to motivate the animals to search for the target hole, all behavioural tests in the BM were conducted under bright light (420 lx) during the dark period of the dark/light cycle. A video tracking system was connected to a computer outside the experimental room in order to record the animals' behaviour. The main outcomes measured were the *primary escape latency* (time to locate the target hole) and the *primary number of errors* made in finding the target hole (see the Nature protocol for full definitions (http://dx.doi.org/10.1038/nprot.2007.390)). In the probe trials, the time spent in each quadrant of the maze while searching was also measured. Viewer II software (Biobserve) was used to analyse the video files. All results are presented as the mean values from the four trials carried out each day. The behaviour of the mice in each BM trial was also manually classified as *direct* (where the mouse moved directly to the target hole or to an adjacent hole before visiting the target hole), *serial* (where the first visit to the target hole was preceded by visits to at least two adjacent holes in a serial manner with a maximum of one centre crossing allowed) or *mixed* (where hole searches were separated by crossing through the centre of the maze or involved unorganised search).

2.3. Statistical analysis

The normality of the data distribution was analysed using the Shapiro–Wilks test. The behavioural data did not pass the normality test, and were subsequently analysed using Kruskal–Wallis one-way ANOVA followed by Dunn's multiple comparison test. Repeated measurements (over the training days) were analysed with a non-parametric Friedman test followed by Dunn's post hoc test where appropriate. All results are expressed as medians plus minimal and maximal values. For all statistical analyses, a probability value $p < 0.05$ was considered statistically significant. Graphpad software (Prism 5.0) was used to assess the statistical analyses.

3. Results and discussion

Overall, the results of this study indicate that GH has the ability to counteract several cognitive impairments observed in mice with STZ-induced diabetes. Interestingly, the search strategies used by the animals in their attempts to locate the target hole in the BM were clearly affected by GH treatment.