Hormones and Behavior 93 (2017) 18–30

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Spatial learning and memory in male mice with altered growth hormone action

Amrita Basu [a,b,1], Hewlet G. McFarlane [c,1], John J. Kopchick [a,d,*]

[a] Molecular and Cellular Biology Program, Edison Biotechnology Institute, Ohio University, Athens, OH, United States
[b] Department of Biological Sciences, Edison Biotechnology Insitute, Ohio University, Athens, OH, United States
[c] Department of Neuroscience, Kenyon College, Gambier, OH, United States
[d] Department of Biomedical Sciences, Heritage College of Osteopathic Medicine, Edison Biotechnology Institute, Ohio University, Athens, OH, United States

ARTICLE INFO

Article history:
Received 16 July 2016
Revised 13 February 2017
Accepted 3 April 2017
Available online xxxx

Keywords:
Growth hormone
Insulin-like growth factor 1
Spatial learning
Memory
Barnes maze
bGH
GHA
Mouse
Search strategy

ABSTRACT

Growth hormone (GH) has a significant influence on cognitive performance in humans and other mammals. To understand the influence of altered GH action on cognition, we assessed spatial learning and memory using a Barnes maze (BM) comparing twelve-month old, male, bovine GH (bGH) and GH receptor antagonist (GHA) transgenic mice and their corresponding wild type (WT) littermates. During the acquisition training period in the BM, bGH mice showed increased latency, traveled longer path lengths and made more errors to reach the target than WT mice, indicating significantly poorer learning. Short-term memory (STM) and long-term memory (LTM) trials showed significantly suppressed memory retention in bGH mice when compared to the WT group. Conversely, GHA mice showed significantly better learning parameters (latency, path length and errors) and increased use of an efficient search strategy than WT mice. Our study indicates a negative impact of GH excess and a beneficial effect of the inhibition of GH action on spatial learning and memory and, therefore, cognitive performance in male mice. Further research to elucidate GH's role in brain function will facilitate identifying therapeutic applications of GH or GHA for neuropathological and neurodegenerative conditions.

1. Introduction

Growth hormone (GH) is a peptide hormone (191 amino acids) secreted by somatotrophic cells of the anterior pituitary gland (Melmed, 2006). Secretion of GH is stimulated by GH releasing hormone (GHRH) and inhibited by somatostatin (SST), two hormones produced by different subsets of hypothalamic neurons (Morishita *et al.*, 2003). GH's actions are mediated upon binding with pre-dimerized, membrane-bound GH receptors (GHR) present on most mammalian cells. An exclusive combination of anabolic and catabolic functions makes GH a primary regulator of mammalian longitudinal growth, including effects on bone, muscle, liver, kidney and fat (Moller *et al.*, 2009). Many ofGH effects are mediated by insulin-like growth factor 1 (IGF-1) which is secreted primarily from the liver and most other cells in various tissues throughout the body (Bikle *et al.*, 2015; Le Roith, 2003).

Endogenous GH and GHR in the brain in various animals and human subjects suggest the possible involvement of GH/IGF-1 with structural and functional aspects of the central nervous system (CNS). Studies in our laboratory indicate altered brain specific GH gene transcription in mice (AB, JJK; *Manuscript in preparation*). Additionally, strong GH immunoreactivity has been observed in different areas of the rabbit and rat hypothalamus, cerebellum, cerebral cortex, thalamus, choroid plexus and brain stem (Lobie *et al.*, 1993), in rat cortical neurons (Scheepens *et al.*, 2001) and in the ventricular zone of the mouse brain (Turnley *et al.*, 2002). An endogenous GH/IGF-1 axis is suggested to be present in the brain, along with peripheral GH and IGF-1 accessing brain receptors after entering through the blood-brain barrier (BBB). In both male and female rats, GHR isoforms found in the liver are also detected in the cerebral cortex (Nogami *et al.*, 2010). GHR immunoreactivity is up-regulated in the subventricular zone (SVZ) of the hippocampus, where adult neurogenesis takes place, in hypoxic-ischemia mediated brain injury (Christophidis *et al.*, 2009). The presence of GHR mRNA in the brain stem and spinal cord has been detected by *in situ* hybridization in rats (Kastrup *et al.*, 2005). In humans, the highest GHR binding activity has been detected in the choroid plexus of the brain irrespective of sex (Lai *et al.*, 1991). These results have been nicely summarized by Nyberg (Nyberg and Hallberg, 2013; Nyberg, 2006).

* Corresponding author at: Edison Biotechnology Institute, Ohio University, Athens, OH 45701, United States.
E-mail addresses: amrita.basu0502@gmail.com (A. Basu), mcfarlaneh@kenyon.edu (H.G. McFarlane), kopchick@ohio.edu (J.J. Kopchick).
[1] AB and HGM contributed equally to this work.

http://dx.doi.org/10.1016/j.yhbeh.2017.04.001
0018-506X/

The effect of GH/IGF-1 in the brain has been shown to be neuroprotective and neuroproliferative. Rats treated with GH or GH releasing peptide-6 (GHRP-6) show up-regulation of anti-apoptotic/pro-survival pathways in the brain (increased Akt activation, upregulated BCL-2 expression and inactivation of pro-apoptotic BAD pathways) (Frago *et al.*, 2002). Additionally, GH treatment has been shown to protect age-related decline in the ratio of ionotropic glutamatergic NMDA receptor subunits NR1 and NR2B in both young and old rat (at transcript and protein levels) (Le Grevès *et al.*, 2002). Also, reduced spinal cord growth is detected in transgenic mice expressing a GHR antagonist (GHA), thus mimicking human GHD (Lund, 2004).

Neurocognitive assessment in patients with acromegaly, a condition of GH hyperactivity due to increased GH secretion by a pituitary adenoma, and dwarfism due to GH deficiency (GHD), has established a role of GH/IGF-1 axis in higher order human CNS functions such as cognition, memory, behavior, quality of life (QoL) and appetite (Arwert *et al.*, 2006; Rowles *et al.*, 2005; Sievers *et al.*, 2012; Webb and Badia, 2007). Studies with several cohorts of patients with acromegaly using a variety of neuropsychological tests and questionnaires indicate an effect of elevated GH levels on cognitive performance, QoL and behavior. Short and long term memory, executive functioning, attention, and verbal fluency are negatively affected in naïve acromegaly patients compared to their age- and sex-matched controls (Leon-Carrion *et al.*, 2010). Also, a significant correlation was detected between physiologic and cognitive changes in naïve untreated acromegalic patients and cognitive dysfunction in the area of memory, recall, concentration and mental agility has been reported in subjects with controlled or active acromegaly. Difficulties with concentration and learning, and severe impairment in verbal recall, mental agility and memory in patients with active acromegaly indicate that abnormally high levels of GH (or IGF-1) may negatively influence behavior and cognition (Yedinak and Fleseriu, 2014).

Changes in brain structure and functional capacities with a significant impact on QoL have been reported in GHD patients. Poorer QoL has been reported due to social isolation, fatigue and sexual inactivity (Rosén *et al.*, 1994). Neuropsychological assessment in adults with childhood onset (CO) GHD, treated with recombinant human GH (rhGH), demonstrates a poor performance score in tests for verbal memory, planning of behavior and attention. Magnetic resonance spectroscopy in these GHD subjects demonstrates increased choline (a metabolite marker of increased synthesis or breakdown of neuronal membrane), reduced NAA (*N*-acetylaspartate, a marker for neuronal integrity) and a decreased NAA/Choline ratio (a measure of neuronal integrity and viability) compared to the controls (van Dam *et al.*, 2005).

Animal studies, however, reveal a different outcome of the effect of altered GH levels on behavior and learning. The presence of GHR in the rat hippocampus and amygdala indicates a possible effect of GH on memory processing, cognition and behavior. In a study with Sprague-Dawley rats, GH mRNA was notably up-regulated in the hippocampal cell layers 24 h after training of associative learning (Donahue *et al.*, 2002). Contrary to the cognitive difficulties reported in human GHD individuals, Ames dwarf mice (a model of GHD due to genetic pituitary dysfunction) show delayed signs of aging with respect to memory performances (Kinney *et al.*, 2001b). These studies signify a potential role of GH in modulating memory, cognition and behavior in normal and abnormal GH states. However, conflicting results in animals and human subjects with respect to memory and behavior warrant further studies into the role of GH in behavior and cognition.

Most of the animal studies on memory and cognition focus on GHD due to pituitary dysfunction and fewer studies have investigated learning and memory in mice with inhibited GH action due to expression of a GHR antagonist (Kopchick *et al.*, 2014). Similarly, no studies of spatial learning and memory have been reported in a transgenic mouse model of acromegaly. Therefore, in the present study we assessed spatial learning and memory in two different mouse models of altered GH action; transgenic male bovine GH (bGH) mice which mimic human acromegaly and dwarf male transgenic GHR antagonist (GHA) mice with suppressed GH-GHR induced signaling. We hypothesized that changes in GH action in mice will significantly alter learning and memory retention. Specifically, we hypothesized that transgenic GH expression in male bGH mice will negatively interfere with their spatial learning and memory retention, while antagonism of GHR in male GHA mice will have a beneficial effect on spatial cognition compared to their respective wild type (WT) controls.

2. Materials & methods

2.1. Animals

Male C57BL/6J mice were housed in Edison Biotechnology Institute (Ohio University, OH, USA) animal facility with 14 h/10 h light/dark cycle and ad libitum access to food and water. The mice used in the study belong to two genetic groups: 1) male, 12–14 months old transgenic bovine bGH and their age- and sex-matched WT littermates and 2) male, 12–14 months old dwarf transgenic bovine GHA mice and their age- and sex-matched WT controls ($n = 8$–12 in each group). At the age of ten months, the mice were transported to the animal facility of Kenyon College in collaboration with Dr. Hewlet McFarlane (Kenyon College, Gambier, OH, USA) where they were housed (four animals/cage, housing conditions the same as that of the Edison Biotechnology Institute animal facility) during the time of experiment.

Transgenic bGH mice have been generated by designing plasmids with the bGH gene fused with various promoter/enhancers such as the mouse metallothionein-1 or rat phosphoenol pyruvate carboxykinase (PEPCK) promoters. Transgenic giant bGH mice display a phenotype similar to human acromegalic patients and have been extensively studied in relation to GH function. The bGH mice in our laboratory has been generated in a C57BL/6J strain via embryonic pronuclear microinjection of a plasmid containing bGH cDNA under direction of the mouse metallothionein I transcription regulatory element(Berryman *et al.*, 2004; Chen *et al.*, 1994; Wen Y. Chen *et al.*, 1991a). These mice have increased plasma bGH, GHR, GH binding protein (GHBP), IGF-1 and are hyperinsulinemic (high blood insulin) with normoglycemia (normal blood glucose) (Ding *et al.*, 2011). Due to lipolytic action of GH, bGH mice have lower percentage of body fat, plasma leptin and adipokine levels and increased organ weights compared to control mice (Berryman *et al.*, 2004). Enhanced GH induced intracellular signaling and associated complications result in a shorter lifespan (12–18 months) (which is ~30–40% of normal lifespan of ~24 months) due to cardiovascular disease, glomerulosclerosis, hepatocellularmegaly and co-morbidities of T2DM (Berryman *et al.*, 2011, 2004; Ding *et al.*, 2013; Junnila *et al.*, 2014).

A dwarf mouse strain due to GHR antagonism has been generated in our laboratory by designing a plasmid containing bGH gene with a site-directed mutation that encodes a GHR antagonist, and microinjection of the plasmid into the male pronucleus of fertilized eggs in C57BL/6J-SJL mice (which were then backcrossed more than twenty generations into pure C57BL/6J background) (Berryman *et al.*, 2004; Chen *et al.*, 1994; Wen Y. Chen *et al.*, 1991b; Chen *et al.*, 1990). Decreased GH action in these mice causes dwarfism with reduced body weight (72% of control mice) and increased percentage of fat mass, unaltered lean tissue weights (normalized to body weight), decreased absolute tissue weight, reduced IGF-1 (50% of normal) and higher food consumption compared to age and sex matched control mice (Berryman *et al.*, 2004; Wen Y. Chen *et al.*, 1991a; Wen Y. Chen *et al.*, 1991b; Junnila *et al.*, 2014; Sackmann-Sala *et al.*, 2014).

The Barnes Maze (BM) is a dry land maze for assessment of spatial learning and memory in rodents (rats, mice) (Barnes and Barnes, 1979). The maze is an excellent alternative for Morris Water Maze (MWM), a swimming test of spatial memory in rodents (Morris, 1984). The maze consists of a circular platform with equally spaced, same diameter holes around the periphery, elevated above the ground and one of the holes attached to an escape box. BM employs natural