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GROWTH HORMONE PROMOTES THE EXPRESSION OF ZIF268 GENE IN THE HIPPOCAMPUS

LA HORMONA DE CRECIMIENTO PROMUEVE LA EXPRESIÓN DEL GEN ZIF268 EN EL HIPOCAMPO

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Abstract

Growth hormone (GH) plays an important role in cognitive processes such as memory consolidation, and it has been suggested that could be involved in processes of brain plasticity. These changes may occur by promoting gene expression in the brain. In this study we showed that GH administration (5ng/kg) for 7 days improves spatial learning and promotes expression of zif268 gene in the rat hippocampus. In addition, a Morris Water Maze (MWM) learning test induced a strong expression of zif268 gene in the hippocampus of adult rats. The effect was even greater when a MWM test and GH were applied together. Our results suggest that GH might be favoring plasticity processes in the hippocampus, by promoting gene expression.
Key words: Brain plasticity, memory, hippocampus, Morris water maze.

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Resumen

La hormona de crecimiento (HC) juega un papel importante en los procesos cognitivos como la consolidación de la memoria, y se ha sugerido que podría participar en los procesos de plasticidad cerebral. Estos cambios podrían ocurrir a través de la expresión de genes en el cerebro. En este estudio mostramos que la administración de hormona de crecimiento (5ng/kg) por 7 días promueve la expresión del gen zif268 en el hipocampo de la rata. Además, la ejecución de una prueba de aprendizaje como el Laberinto Acuático de Morris (LAM) induce una fuerte expresión del gen zif268 en el hipocampo de la rata adulta. El efecto fue mayor cuando la HC y el LAM se aplicaron juntos. Nuestros resultados sugieren que la HC podría favorecer los procesos de plasticidad en el hipocampo a través de la expresión de genes.
Palabras clave: Plasticidad cerebral, memoria, hipocampo, laberinto acuático de Morris.

Introduction

Growth hormone (GH) plays an important role in cognitive processes such as other neurotransmitter (Perez, Liy & Meneses, 2006) in a memory consolidation and learning (Schneider, Rivas, Vázquez-P, Vázquez-S & Borgonio, 1995) (Le Grevés et al., 2006), and it has been suggested that it could be involved in processes of brain plasticity (Le Grevés et al., 2006). GH receptors have been described throughout the entire brain, but more abundant in the hippocampus, putamen, and thalamus (Nyberg & Burman, 1996) (Nyberg, 2000). Some studies have reported that zif268 gene expression (also called Egr-1, NGF-1A, Krox-24 or TiS8) is induced in animals trained in different learning paradigms, suggesting its involvement in memory processes (Da Costa, Broad & Kendrick, 1997) (Chaudhuri, 1997). Zif268 is a zinc-finger transcriptional factor belonging to the family of immediate-early genes (IEG) (Milbrandt, 1987), which is expressed in various areas of the brain including the amygdala, cortex, thalamus, and hippocampus (Herdegen et al., 1995). Since GH favors memory consolidation and brain plasticity, one may ask if these changes occur by promoting gene expression in the brain. The promoters of zif268, c-fos, and junB genes contain a serum response element, which mediate GH-promoted expression (Hodge et al., 1998). The goal of this study was to determine if GH improves spatial learning and promotes the expression of zif268 gene in the hippocampus of the adult rat.

Methods

Subjects

Male Wistar rats (250-300 g) were housed at 22ºC ± 1ºC in a 12:12 light: dark cycle, with food and water ad libitum. All experimental procedures were approved and conducted according to the Institutional Ethical Committee, in agreement with the national (Norma Oficial Mexicana, NOM-062-ZOO-2003) and international guide-

lines (Society for Neuroscience) for the production, care and use of laboratory animals. All precautions were taken to minimize pain or discomfort of the animals. The number of animals used was also minimized.

Apparatus

For the MWM, a dark circular pool (diameter 140 cm) was filled with clear water and the dark escape platform (painted was the same color than pool with purpose not visible to animals) (11x11 cm) was placed 1 cm below the surface. A dark pool was located in a room with distal visual cues on the walls. The time taken for the swimming rat to reach the platform (escape latency in seconds) was measured for each trial (Gary, 2007).

Procedure

The animals were randomly divided in 4 groups, n=6 per group. Group 1 received a daily injection of recombinant human GH (rhGH) 5ng/kg (Humatrope, Eli Lilly, USA) during 7 consecutive days, and group 2 (Control) received a daily injection of saline solution for the same period (Figure 1A). On day eighth, animals from both groups were decapitated at 09:00 AM, brains were obtained and processed as describe below (Figure 1A). Group 3 received a daily injection of saline and was subjected to a MWM test (Control-MWM); whereas group 4 received a daily injection of rhGH and was subjected to a MWM (rhGH-MWM) (Figure 1B). All injections were intraperiotonealy, performed daily at 09:00 AM. Body weight from all animals was also daily recorded. The dose of rhGH administered was taken from previous experiments conducted in our laboratory (García-García et al., 2011).

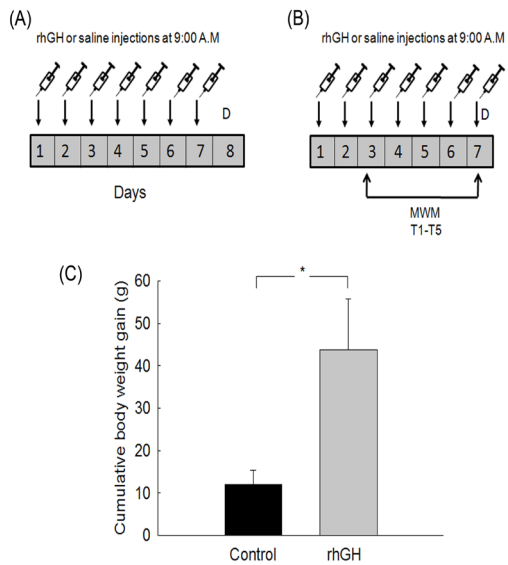

Figure 1. Experimental design and effect of GH administration on cumulative body weight.

The water maze training began on experimental day 3 for groups 3 and 4 (corresponding to T1 on figure 1B), followed by eight training trials on days 3 to 7 (T1-T5 on figure 1B). The platform remained all the time in the same location in the tank. All MWM tests were performed at the beginning of the dark period, using a soft light. Immediately after the last trial of the MWM test on day 7 (T5), animals were decapitated and brains harvested (Figure 1B).

Brains from all groups were immediately obtained, kept on ice and cut sagitally through the midbrain line. The hippocampus was dissected from the left and right hemispheres, immediately frozen in dry ice, and stored at -80ºC for RNA isolation.

RNA isolation and quantitative PCR (qPCR)

RNA was isolated from rat hippocampus with the Trizol reagent, following the manufacturer's recommendations (Invitrogen, USA). RNA concentration was determined from OD values at 260 nm, and RNA integrity was determined by agarose gel electrophoresis. Reverse transcription (RT) reactions were carried out with 2.5 µg of total RNA in a final volume of 20 µl, using M-MLV reverse transcriptase, according to the manufacturer's recommendations (Invitrogen, USA). qPCR reactions were performed by triplicate, containing 1 µl of the corresponding RT reaction, 5 pmoles of each primer, 10 µl of 2x SYBR®Green qPCR mix (Invitrogen, USA), and water up to 20 µl. Reactions were run in an ICycler (BioRad, USA), according to the protocol recommended by the manufacturer of the reaction mix (Invitrogen, USA): 2 min at 50ºC, 10 min at 95ºC, 40 cycles of 30 sec at 95ºC and 1 min at 60ºC, followed by a melting curve analysis. Triplicates of each RT sample containing primers for β-actin were also included to normalize the data. Primer sequences (from 5′ to 3′) were: zif268 CAGCAGTGATGAACGCAAGA forward, AGCCCGGAGAGGAGTAAGAG reverse; β-actin AGGCTGTGCTGTCCCTGTAT forward, GCTGTGGTGGTGAAGCTGTA reverse. Amplification efficiencies were obtained with the LinReg program, and changes in relative gene expression were calculated as reported by Pfaffl (2001), using β-actin as internal control.

All data are expressed as mean ± SEM. Body weight was analyzed using a Student's t-test (SigmaPlot 10.0). For zif268 mRNA expression, one way analysis of variance (ANOVA) was used to determine whether there were differences across different groups, followed by a Tukey´s post hoc test (SigmaPlot vers. 10.0), probability levels of $P<0.05$ were considered significant.

Results

rhGH-treated rats showed more gain of cumulative body weight than saline-treated rats (Control group), values were 43.8 ± 11.9 g and 12.03 ± 3.3 g, respectively (Figure 1C, $P < 0.01$). This increase in body weight confirmed the physiological effects of this dose of rhGH. On the other hand, rhGH-treated rats showed a significant reduction of the mean value of escape latency in the MWM test on day 6 (T4) and

7 (T5) of training (Control group T4 = 5.28 ± 0.41 s; T5 = 5.00 ± 0.53 s; rhGH group T4 = 3.40 ± 0.25 s; T5 = 3.34 ± 0.37 s, $F_{1,4}$=35.3, P < 0.001 Figure 2A). Escape latency values on day 3 and 5 (T1 and T3) did not show significant differences between control and rhGH treated rats (Control group T1 = 23.85 ± 2.98 s; T3 =8.75 ± 1.10 s; rhGH group T1 = 22.80 ± 3.50 s; T3= 6.89 ± 0.74 s, $F_{1,4}$=3.43, P= 0.68 Figure 2A).

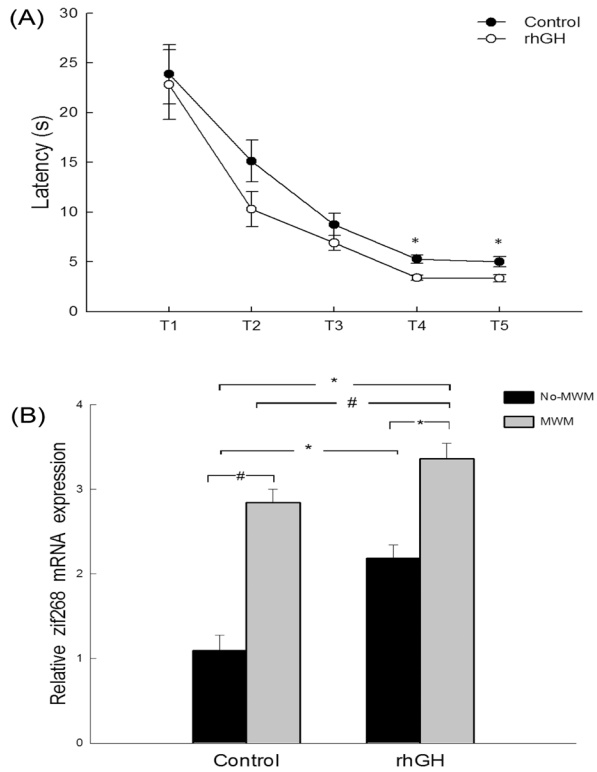

Figure 2. GH and a MWM learning test induce the expression of zif268 in the rat hippocampus

In addition, the results showed that rhGH administration significantly increases zif268 mRNA expression levels in the hippocampus of adult rats compared to control (No-MWM) animals. Relative zif268 mRNA expression levels were 2.18 ± 0.10 for rhGH No-MWM treated animals, and 1.09 ± 0.05 for Control No-MWM group (P < 0.001, Figure 2B).

On the other hand, control animals subjected to MWM (Control-MWM) showed increased zif268 mRNA expression levels compared to control animals without MWM

training. Relative zif268 mRNA expression levels were 1.09 ± 0.05 for Control No-MWM group, and 2.84 ± 0.12 for Control-MWM ($P< 0.05$, Figure 2B).

Interestingly animals subjected to MWM test in combination with rhGH administration showed even higher zif268 mRNA expression levels; relative zif268 mRNA expression levels were 3.36 ± 0.3 for rhGH-MWM rats, and 2.84 ± 0.12 for Control-MWM animals ($P < 0.05$, Figure 2B).

Significant differences were also observed between rhGH-MWM rats and rhGH (No-MWM), relative zif268 mRNA expression was 3.36 ± 0.33 and 2.18 ± 0.10, respectively ($P < 0.001$, Figure 2B). Finally, significant differences between rhGH-MWM and control (no-MWM) were also observed, relative zif268 mRNA expression was 3.36 ± 0.33 and 1.09 ± 0.05, respectively; $P < 0.001$, Figure 2B).

Discussion

In the present study we have shown that rhGH administration improves memory acquisition and promotes the expression of the zif268 gene in the hippocampus of adult rats. The cognitive effects of GH treatment have been studied extensively. For example, it has been reported that GH administration improves memory in several species (Le Grevés et al., 2006). In addition, GH administration increases protein synthesis in the brain (Ohsumi, Tujioka, Hayase, Nagata & Yokogoshi, 2008) and memory consolidation (Le Grevés et al., 2006). GH also influences the N-methyl-D-aspartate (NMDA) receptor system in the hippocampus (Le Grevés et al., 2006), (Thomas, Hall & Everitt, 2002) an essential component of long-term potentiation (LTP), which is highly involved in memory acquisition (Thomas, Hall & Everitt, 2002). Furthermore, plasma levels of GH normally decline with age and treatment with recombinant GH reduce age-related impairment in cognitive function. Several studies have demonstrated the existence of GH receptors in the hippocampus (Nyberg & Burman, 1996) (Anderson, Jeftinija & Scanes, 2004), and studies with ^{125}I have shown that GH crosses the blood-brain barrier in rodents (Pan et al., 2005). Therefore, GH has a direct effect on brain functions, for instance improving memory and inducing gene expression in neurons (Le Grevés M, Steensland, Le Grevés P & Nyberg, 2002). Thus, it can be concluded that rhGH-improved memory acquisition may be by inducing gene expression such as zif268.

Recently, in situ hybridization revealed that zif268 is expressed within specific regions of the hippocampus during fear memory retrieval (Thomas, Hall & Everitt, 2002), suggesting that zif268 gene may contribute to plasticity and reconsolidation accompanying the retrieval process. Furthermore, other studies have reported a close association of zif268 expression with hippocampal LTP (Cole, Saffen, Baraban & Worley, 1989) (Worley et al., 1993), a major form of protein synthesis-dependent plasticity in the adult brain. Thereby, our results suggest that GH might be favoring plasticity processes in the hippocampus by promoting zif268 gene expression, similar to previous *in vitro* experiments (Hodge et al., 1998). However, expression of

zif268 was more significant in the hippocampus of animals exposed only to MWM test; suggesting that zif268 is related to individual learning capability. It has been reported that zif268 induction occurs immediately after learning associated with objects, suggesting an important role in memory formation (Guzowski, Setlow, Wagner & McGaugh, 2001). Although, GH administration and spatial learning induce the expression of NMDA receptors in the hippocampus through a common signaling pathway such as MAP/ERK (Davis, Vanhoutte, Pages, Caboche & Laroche, 2000); our results showed that there is not a synergistic effect between GH and the MWM on promoting the expression of zif268 gene.

Although, the molecular process underlying this plasticity has not yet been elucidated, our results suggest that zif268, which is expressed after GH administration, may contribute to brain plasticity increase, perhaps by inducing expression of other genes such as synapsin I, a peripheral protein of the small synaptic vesicles involved in the regulation of neurotransmitter release (Thiel, Schoch & Petersohn, 1994). Several data indicate that long term plasticity mechanisms involve increased synthesis of the synaptic vesicle components, as well as the induction of IEGs (Nedivi, Hevroni, Naot, Israeli & Citri, 1993).

Conclusion

This study shows that the expression of plasticity-associated zif268 is stimulated in the rat hippocampus after GH administration, and also by a MWM test as previously showed. GH administration in combination to a MWM test, induce higher zif268 mRNA expression levels. Thereby, our results suggest that GH might be favoring plasticity processes in the hippocampus, by promoting early gene expression, which may be implicate in memory acquisition.

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