DOI: 10.2436/20.1501.02.21

Endocrinologia molecular
(Jaume Reventós, ed.)

Treballs de la SCB. Vol. 56 (2005) 223-233

GROWTH HORMONE AND AGING

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RESUM

Les alteracions vasculars i degeneratives del sistema nerviós central (SNC) són dues de les causes més comunes de malaltia i de mort entre la gent gran; ambdues es correlacionen amb l'edat, amb la deficiència en GH, i poden afectar les funcions fisiològiques de la població d'edat avançada.

Amb la finalitat de clarificar els efectes de la GH en el metabolisme, en els vasos i en el SNC, hem dut a terme un estudi *in vivo* utilitzant rates velles Wistar tractades crònicament amb GH. Les rates velles varen presentar un augment en el pes de greix i una disminució de l'índex específic de gravetat (SGI) ($p < 0,05$) en comparar-les amb les rates adultes no tractades. La GH va reduir el pes en greix ($p < 0,05$), i va mostrar també una tendència a augmentar l'SGI. Es va analitzar també la resposta de diverses substàncies vasoactives en els anells aòrtics, i es va demostrar una disminució de la vasodilatació per acetilcolina i isoprenalina ($p < 0,05$) en els animals vells. La contracció induïda per acetilcolina+L-NAME era més alta en els animals vells que en els adults. L'administració de GH millorava les respostes vasodilatadores ($p < 0,05$) mentre que tendia a reduïr les respostes vasoconstrictores. L'àrea aòrtica mitja augmentava també en les rates velles, mentre que la GH reduïa aquest paràmetre ($p < 0,05$).

Les poblacions neuronals es reduïen en els hipocamps de les rates velles en comparar-les amb les joves. Aquesta reducció estava asociada a un augment dels nucleosomes i a una reducció de Bcl2 en el cervell. Les caspases 3 i 9 també varen augmentar. El tractament amb GH va augmentar significativament el nombre de neurones i va reduir els nucleosomes i les caspases i augmentar el Bcl2. En conclusió, el tractament per GH indueix l'aparició d'efectes beneficiosos en la composició del cos i ha restablert també les funcions cerebrals i vasculars en les rates velles.

Paraules clau: envelliment, GH, SNC, fisiologia vascular, neurones, metabolisme.

SUMMARY

Vascular and degenerative alterations of the central nervous system (CNS) are two of the most common reasons for illness and death in elderly people; they exhibit an age-related GH deficiency that can affect their physiological functions.

A study was conducted under chronic *in vivo* conditions using old Wistar rats, in order to clarify the effects of GH on the metabolism, vessels, and the CNS. The old rats showed an increased fat weight and a decreased Specific Gravity Index (SGI) ($p < 0.05$), as compared to the adult animals. GH reduced the fat weight ($p < 0.05$) and tended to increase the SGI (N.S.). The response to several vasoactive substances in aortic rings showed impaired vasodilatation to Acetylcholine and Isoprenaline ($p < 0.05$) in the old animals. Contraction, induced by Acetylcholine+L-NAME, was higher in the old rats than in the adults. GH administration improved the vasodilatory responses ($p < 0.05$) and tended to reduce the constrictory responses. The aortic media area was increased in the old rats, and GH reduced this parameter ($p < 0.05$).

The neuronal populations were reduced in the hippocampi of the old rats as compared to the young ones. This reduction was associated with an increase in nucleosomes and a reduction in Bcl2 in the brain. An increase was also detected in caspases 3 and 9. GH treatment was able to significantly enhance the number of neurons by reducing the nucleosomes and the caspases and by increasing Bcl2. In conclusion, GH treatment was able to show beneficial effects on body composition and was able to restore both vascular and brain functions in the old rats.

Keywords: Aging, GH, CNS, vascular physiology, neurons, metabolism.

INTRODUCTION

Growth hormone (GH) is the most abundant anterior pituitary hormone; it accounts for 4-10 % of the wet weight of the anterior pituitary in the human adult, amounting to about 5-10 mg per gland (Arce and Devesa, 2000).

GH of mammalian origin is active in many species, but humans are responsive only to human or primate GH (Hadley, 1992). GH is similar in structure to prolactin and placental lactogen.

The circulating levels of this hormone decline during the first weeks after birth, but they reach adult levels after two or three weeks of life. A substantial increase in GH has been observed during puberty. Spontaneous episodes of GH secretion occur every 3-4 hours over a 24-hour period, with these secretory peaks being more frequent and smaller in females than in males (Tannenbaum, 1988). The highest secretion of GH occurs during the two first hours of nocturnal rest during the period of slow wave sleep.

Three hypothalamic hormones are involved in GH control: somatostatin (SS), GH Releasing Hormone (GHRH) and ghrelin, which is also synthesized in the stomach (Kojima *et al.*, 1999).

SS has an inhibitory effect on GH release in response to every known stimulus. The action of SS is exerted directly upon pituitary somatotrophs (Tannenbaum, 1988).

SS also inhibits TRH-induced TSH secretion, renin, parathormone, calcitonin, gastric HCl, acetylcholine and other neurotransmitters, blood platelet aggregation, brain cell electrical activity, and many other physiological processes.

GHRH was isolated in 1982 from two pancreatic tumors and was found to specifically stimulate GH secretion both *in vivo* and *in vitro* (Guillemin *et al.*, 1982; Rivier *et al.*, 1982). Hypothalamic GHRH has been found to be bound to specific receptors in the soma-

totrophic cells and to stimulate GH secretion, cell proliferation, and also the GH-gene transcription (Vance, 1990).

Each episodic secretion of GH is determined by the release of GHRH to the portal circulation, together with a decrease in somatostatin. This pulsatile pattern in the secretion of GH seems to be more important to the peripheral hormonal effects than to the total amount of secreted GH (Devesa *et al.*, 1992).

Ghrelin is a recently discovered peptide with 28 amino acids, synthesized mainly in the stomach mucosa, though it is also produced in the hypothalamus. This peptide is able to stimulate GH release both *in vivo* and *in vitro.* (Date *et al.*, 2000).

Other neurotransmitters, such as NA, DA, Ach, Serotonin, and/or GABA also play a role in GH secretion. The secretion of GH is also influenced by the endocrine system (García Barros and Devesa, 2000).

ACTIONS OF GH

GH acts on the tissue of a receptor (GHR) that consists of a transmembranous protein of 620 amino acids, codified by a gene located on chromosome 5 (García Barros and Devesa, 2000).

GH is an anabolic protein hormone that produces a positive nitrogen and phosphorus balance (Davidson, 1987). GH causes cells to grow and multiply by directly increasing the rate at which amino acids are used to synthesize proteins. Due to these effects, GH induces an increase in the growth rate of long bones and skeletal muscles during childhood and the teenage years.

GH stimulates lipolysis, which is the breakdown of triglycerides into fatty acids and glycerol. It provides substrates for glucose neosynthesis and, thus, has a sparing effect on glucose utilization. GH also promotes fat catabolism (García Barros and Devesa, 2000).

In normal subjects, GH stimulates insulin secretion, but at the same time, this hormone reduces the sensitivity of peripheral tissues, such as muscle and/or adipose tissue, to insulin. Elevated plasma glucose levels then stimulate insulin secretion and lead to a diabetogenic effect.

The absence of GH secretion in young children leads to dwarfism, whereas its overproduction during the postnatal period produces gigantism. In the adult, an excess of GH leads to acromegaly (Isaksson *et al.*, 1988).

A GH deficiency in an adult has been recently recognized as a specific clinical syndrome, characterized by a combination of metabolic and cardiovascular features that are more evident in women than in men (Hew, 1998). This syndrome includes a high prevalence of dyslipidaemia, glucose intolerance, central obesity, and hypertension. Early arteriosclerosis is found in this asymptomatic hypopituitary GH deficiency. All of these are important contributory factors to an increased cardiovascular risk (Rosen and Bengtsson, 1990).

A PHYSIOLOGICAL DECREASE OF GH SECRETION

The secretion of GH and IGF decrease with increased age; elderly people exhibit very low levels of GH and IGF I, as compared to young people (Todgood and Shalet, 1998). Metabolic changes also occur with aging, such as the diminution of muscular and osseous masses or the increment of adipose tissue. A relationship between these two circumstances has been detected and the term "somatopause" has been proposed to describe this situation (Hoffman *et al.*, 1993).

Aging is associated with several changes and alterations in the metabolism, body composition and organ functions (Ariznavarreta *et al.*, 2003). Elderly people show bone mineral density loss, lean body mass and muscular strength reduction, adipose tissue increase, in-

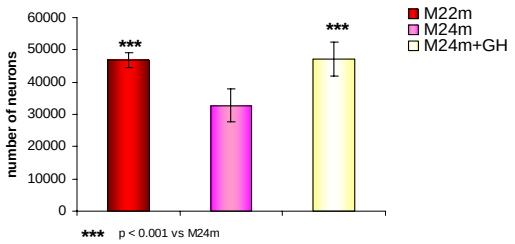

FIGURE 1. Effect of the treatment with GH on the number of neurons on the de hilus of the dentate gyrus of the hippocampus in 24 months old rats as compared with 22 and 24 months old untreated rats.

sulin resistance and glucose intolerance, etc. (Savine and Sönksen, 1999; Toogood *et al.*, 1996; Toogood and Shalet, 1998). Similarities have been detected in all of the consequences of GH deficiency (GHD) in adults and the changes observed in elderly people. These similarities point to a possible relationship between age-related physical impairment and the GH/IGF-1 axis decline that physiologically occurs with age (Toogood *et al.*, 1996; Toogood and Shalet, 1998; Ghigo *et al.*, 2001). Old age could be a physiological state of GH-deficiency.

Indeed, experimental evidence has demonstrated that GH treatment has beneficial effects on aged animals. It improves cerebral microvasculature (Sontag *et al.*, 1997), coronary blood flow, and heart capillary density in aging rats (Khan *et al.*, 2001).

In humans, GH treatment is able to enhance lean body mass and muscular strength, reduce body fat (Cuttica *et al.*, 1997; Holloway *et al.*, 1994; Rudman *et al.*, 1990), improve the plasma lipid profile (25), and increase bone mineral density (Rudman *et al.*, 1990). However, the effects of GH on vascular functions and structure in aged individuals are not well established.

In addition to their effects on somatic growth and metabolism, the hormones of the somatotrophic axis (i.e., Growth Hormone-Releasing Hormone (GHRH), Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1)) also exert some other actions on the cardiovascular system (Arce and Devesa, 2000).

EXPERIMENTAL DESIGNS

Male and female Wistar rats (22 months of age) were used in the study, and a group of young animals (3 months of age) was considered the control group. Ten animals per group were used.

Old rats were treated with saline solution or with GH twice a day at a dose of 1 mg/kg/day or 2/kg/kg/day for one month. The Specific Gravity Index (SGI), which represents the index: air weight / (air weight – water weight) × 0.9979 (specific gravity of water), was measured. Plasma levels and tissue concentrations of IGF-I in the liver and muscle were determined using a specific R.I.A. method after extraction with acid ethanol (Rol de Lama *et al.*, 2000).

Aortic rings (3 mm) were connected to a force transducer attached to a computer (Mc Lab 8E, AD Instruments) to evaluate vascular reactivity.

The CNS was immediately dissected to obtain discrete areas. Parts of them were immediately frozen at –80° C to be further submitted for homogenization, in order to be analyzed for Bcl2 and nucleosomes or for RNA extraction for Real Time PCR. The other areas of CNS were fixed in 4% paraformaldehyde in phosphate buffer, stained with Nissl staining and submitted to histological and histochemical studies, counting the number of neurons in the hilus of the dentate gyrus.

METABOLIC EFFECTS

Adults with GH-deficiency exhibit a diminution of their lean body mass and an increase in adipose tissue, which means a reduction in the muscular force capacity (Rosen *et al.*, 1993; Toogood and Shalet, 1998; Callum *et*

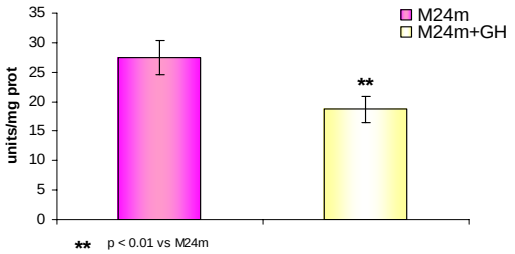

FIGURE 2. Effect of the treatment with GH on the levels of nucleosomes in cerebral homogenates of 24 months old rats as compared with 24 months untreated old rats.

al., 2002). An increase in force and exercise capacity has been reported in elderly people, when GH therapy has been instituted (Cuttica *et al.*, 1997; Juul, 1996; Salomon *et al.*, 1989). In our study, the results indicated that GH treatment in the old rats decreased fat tissue and increased muscular mass, as reflected by the increment in the SGI (see figure 1). The higher this index was, the higher the muscular mass and the smaller the quantity of fat. Treatment with GH also increased plasmatic, hepatic and muscular IGF-I in the old rats. An increase in weight gain was also observed.

In our study, the old rats showed an increase in epididimary and periuterine fat, together with a reduction in the SGI of the male carcasses, which meant that adiposity was augmented and the lean body mass was reduced. GH administration reduced epididimary and periuterine fat and increased SGI in the old rats of both sexes, which meant that this treatment was able to increase muscle mass and reduce fat (Castillo *et al.*, 2003).

As regards body composition, our data were consistent with previous studies obtained in our laboratory (Pérez Romero *et al.*, 1999). SGI is an index that relates lean body mass and fat mass; the higher it is, the less fat the animal has. In our study, rats showed an increase in epididimary and periuterine fat, together with a reduction in the SGI of the male and female carcasses, which meant that adiposity was augmented and lean body mass was reduced. GH administration reduced epididimary and periuterine fat and increased SGI in the old rats of both sexes, which meant that this treatment was able to increase muscle mass and reduce fat. It has been demonstrated that GH treatment in both GHD adults and elderly people is able to improve several parameters related to body composition (Rudman *et al.*, 1990; Mc Callum *et al.*, 2002; Bengston *et al.*, 1993). A good example of this is reducing abdominal obesity, which is a strong predictor of cardiovascular risk (Despres *et al.*, 1998). The increment in SGI in old GH-treated rats is associated with an increase in body weight gain, as compared to the weight loss observed in old untreated animals. This confirms the preponderance of the anabolic properties of GH in the old animals over the lypolitic effects on fat tissue.

It has been previously reported that there is a decrease in GH and IGF-1 production with age (Castillo *et al.*, 2003). However, in the present study, reduced plasma IGF-1 levels were only seen in males, whereas hepatic IGF 1 content was significantly reduced in both sexes (Castillo *et al.*, 2005). GH administration was able to significantly increase the hepatic content and plasma IGF-1 levels.

VASCULAR EFFECTS

Aging is associated with both structural and functional changes that take place in the vascular wall (Matz *et al.*, 2000; Maeso *et al.*, 1999). Aging reduces endothelium-dependent relaxation, in response to different agonists, and it increases endothelium-dependent contraction. There is also an increase in the media-intima thickness, as well as changes in the cellular and extra-cellular composition of the vessel wall (Maeso *et al.*, 1999).

GH-deficient patients show a greater risk of cardiovascular alterations (Rosen, 1990) and endothelial dysfunctions, including a re-

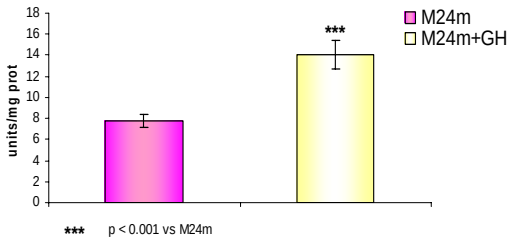

FIGURE 3. Effect of the treatment with GH on the levels of Bcl-2 on cerebral homogenate of 24 months old rats as compared with 24 months old untreated rats.

duced vascular endothelial-dependent relaxation (Evans *et al.*, 1999).

Aging is associated with an impaired endothelium-dependent vasodilatation. This is suggested by the reduced response to both acetylcholine and isoprenaline in old rats, as compared to young ones, without changes in the endothelium-independent relaxation, as shown by the absence of differences in the response to sodium nitroprusside (Maeso *et al.*, 1999; Koga *et al.*, 1998; Tominaga *et al.*, 1994). Similar results have been obtained in experiments carried out on humans, measured as responses to the brachial arterial infusion of acetilcholine by pletismography (Andrawis *et al.*, 2000). This endothelial dysfunction seems to parallel the general deterioration of the animals, as shown by the correlation found between the maximal relaxation to acetylcholine and the body composition parameters.

A decrease in endothelial NO availability, due to reduced synthesis and/or major degradation by oxidative stress, has been suggested as an important mechanism, underlying the altered response to endothelium-dependent agents during aging (Matz *et al.*, 2000; Maeso *et al.*, 1999; Tachudi. *et al.*, 1996; Loo *et al.*, 2000). In addition, an increase in contracting factors, which can counteract the effects of relaxing ones, could also be involved in this altered endothelial function. This notion is supported by the fact that senescent animals show a higher response to acetylcholine+LNAME than young ones, as shown in the present study. A similar increase in endothelium-dependent contraction has been previously reported (Koga *et al.*, 1998; Küng and Lüscher, 1995). Thromboxane A_2 or PGH_2 have been proposed as the agents accounting for the increase in endothelium-dependent contraction with aging (Matz *et al.*, 2000). Moreover, the changes observed in the structure of the vascular wall could be an additional mechanism involved in the impairment of endothelial function, since there is a negative correlation between the maximal relaxation to acetylcholine and the media cross-sectional area. In fact, these changes could partially explain the reduction in phenylephrine contraction observed in the old rats, due to alterations in the contractile machinery of the smooth muscle cells. In our study, the administration of GH to old rats produced an expected increase in the plasma levels of IGF-1, which was accompanied by an improvement in endothelial function and vessel structure (Castillo *et al.*, 2003, 2005).

The possible mechanisms, underlying the beneficial effects exerted by GH administration, could involve an increase in endothelial NO availability. This affirmation is based on the fact that several studies have shown that GH-induced IGF-1 is not only able to stimulate NO synthesis in endothelial cells (Boger, 1999; Boger *et al.*, 1996), but it can also decrease NO degradation by reducing oxidative stress production (Evans *et al.*, 2000; Castillo *et al.*, 2004). This enhanced NO availability could positively influence vascular function and structure. Another possible mechanism could involve the decrease in the vasoconstrictor agents previously mentioned, such as Thromboxane A_2 or PGH_2, which could counteract NO actions; this has been shown by the tendency towards a reduction of endothelim-dependent contraction by GH. These data confirm previous studies, which have shown that GH can exert beneficial effects on the cardiovascular system of aged animals (Sontag *et al.*, 1997; Khan *et al.*, 2001).

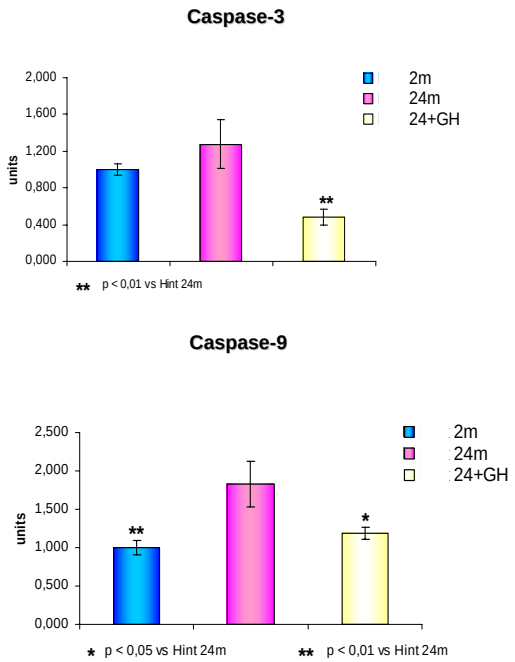

FIGURE 4. Effect of the treatment with GH on the expresion of Caspase-3 and Caspase-9 in the hypothalamus of 24 months old rats as compare with 24 months old untreated rats.

EFFECTS ON CNS

The hippocampus, a brain region involved in spatial and episodic memory (may significantly contribute to the age-associated decline in cognitive abilities. Although in most brain areas there is no massive neuronal loss with aging, a significant reduction in the number of neurons has been reported in the hilus of the dentate gyrus of the hippocampal formation in aged humans and in 24 month-old Fischer 344 male rats.

It is well known that GH exerts important effects on the CNS (Nyberg, 2000), increasing the psychological capacity of adults, memory, concentration, alertness, and the capacity for work (Burman *et al.*, 1995). Some neurotransmitters also change under GH treatment (Johanson *et al.*, 1995; Segovia *et al.*, 2001). Receptors for GH exist in the CNS at different levels: neurons, glia, and endothelial cells in the vessels (Lobil *et al.*, 1993). Under GH stimulation IGF-I is produced in the cerebral tissue (López Fernández *et al.*, 1996), probably playing a local trophic role (Torres Aleman, et al,. 1994). The emergence of new neurons in the brain is a well- documented phenomenon, especially in young animals, though the real significance of this fact is still unknown (Trejo *et al.*, 2001).

Using an unbiased stereological estimation of the total number of neurons in the hilus of the dentate gyrus of the rat hippocampus, we detected the following: there was a significant sex dimorphism in neuronal content, a preservation of neuronal content until 22 months of age, and a significant neuronal loss between 22 and 24 months of age in both sexes (Azcoitia *et al.*, 2005). Our present findings indicated that the number of hilar neurons was also sexually dimorphic in the rat, with males exhibiting more neurons than females. The sex difference was detected in young adult rats and was maintained until 24 months of age. The sex differences in hippocampal structure and function may be a consequence of the perinatal effects of testosterone on male rats. In addition, sex steroids affect the adult hippocampus and may protect adult hilar neurons from excitotoxic cell death. The neuronal counts were not significantly different at 3 months of age compared to 22 months, an advanced age for rats. However, between 22 and 24 months of age there was a clear deterioration in the structure of the hilus. Our findings were also in agreement with a previous report, showing a significant neuronal loss in the hili of 24 month-old male Fischer 344 rats, compared to 12 month-old animals.

The stereological estimation of the total number of hilar neurons revealed that 24 month-old rats that were treated with GH had more neurons in the hilus than control animals, treated with vehicle (see figure 1). The neuroprotective effect of GH was observed in

both sexes. GH is a neuroprotective factor for the brain and spinal cord of young animals, and it prevents hippocampal neuronal cell loss after unilateral hypoxic-ischaemic brain injury. In young animals, the neuroprotective effects of GH appear to be mediated, at least in part, by the activation of GH receptors. Furthermore, it is known that the administration of GH to old rats increases IGF-I expression in the brain, including the hippocampus. Since IGF-I is a neuroprotective factor for hilar neurons, the local production of this molecule may mediate the neuroprotective effects of GH. Our present findings indicated that GH could protect hilar hippocampal neurons from degeneration, associated with aging. The measurements of nucleosomes in brain homogenate showed an age related increase that was accompanied by a reduction in Bcl2, an apoptosis protective protein. The increase in nucleosomes was also associated with an increase in caspases 3 and 9. After GH treatment, a clear inhibition of apoptosis was observed, which was accompanied by a decrease in nucleosome levels (see figure 2) and an increase in Bcl-2 levels (see figure 3). These changes could have been due to the activation of intracellular signals, either directly by GH or by IGF I activation, the levels of which were increased after the systemic GH administration (Frago, 2002). A possible mechanism is the inhibition that GH and/or IGF I exert on Pl3-quinase, which mediates the phosphorylation of Akt/protein-quinase (Akt/PKB) (Frago, 2002). The result is that Bad is phosphorylated (Datta *et al.*, 1997), since it is unable to bind anti-apoptotic proteins, such as Bcl-2, so that this protein increases. On the other hand, Akt/PKB is able to activate the transcription factor CREB, which also stimulates Bcl-2 expression. Increased Bcl-2 could be responsible, first, for decreased caspase-9 and subsequently, for a decrease in caspase-3 (see figure 4).

In conclusion, GH treatment for 10 weeks in old male rats was able to restore vascular functions and structure and prevent neuronal apoptosis in the CNS.

ACKNOWLEDGEMENTS

This work was made possible through a grant from the GHESME Ministry of labor and social affairs. IMSERSO.

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