

Chronopharmacological effects of growth hormone on the executive function and oxidative stress response in rats

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ABSTRACT

Objective(s): To investigate the chronopharmacological effects of growth hormone on executive function and the oxidative stress response in rats.

Materials and Methods: Fifty male Wistar rats (36-40 weeks old) had ad libitum access to water and food and were separated into four groups: diurnal control, nocturnal control, diurnal GH-treated, and nocturnal GH-treated animals. Levels of Cu, Zn superoxide dismutase (Cu,Zn-SOD), and superoxide release by spleen macrophages were evaluated. For memory testing, adaptation and walking in an open field platform was used. GH-treated animals demonstrated better performance in exploratory and spatial open-field tests.

Results: The latency time in both GH-treated groups was significantly lower compared with the latency time of the control groups. The diurnal GH treatment did not stimulate superoxide release but increased the CuZn-SOD enzyme levels. The nocturnal GH treatment did not influence the superoxide release and CuZn-SOD concentration. GH treatment also resulted in heart atrophy and lung hypertrophy.

Conclusion: Growth hormone treatment improved the performance of executive functions at the cost of oxidative stress triggering, and this effect was dependent on the circadian period of hormone administration. However, GH treatment caused damaging effects such as lung hypertrophy and heart atrophy.

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Introduction

There is evidence in neuropsychology literature linking growth hormone (GH) deficiency with cognitive impairment. Patients with GH deficit are at risk of declining cognitive quality (1, 2); those with GH decline usually have an abnormal cognitive function (e.g. poor concentration and declining mental drive), tiredness, reduced physical stamina, irritability, decreased vitality, and problems in coping with stressful situations which can result in social isolation (1,2). The age-related spontaneous decrease in serum GH could, therefore, be related to the impairment of cognitive performance. These findings suggest that GH may play an important role in the age-related decline of cognitive performance (3-5).

Prospective studies should be performed in order to better explain and understand the effects of GH on cognitive function. Those studies can answer a lot of questions regarding case-control studies. Although there have been several prospective studies regard-

ing the effect of GH on cognitive function (6-11), no consensus could be established on whether GH treatment is really useful for cognitive function.

A meta-analysis by Arwert *et al* (10) considered the relationship between GH treatment and cognitive function, concluding that GH treatment had no beneficial effect. However, this conclusion was based on only four scientifically robust studies and did not consider all available data. On the other hand, another meta-analysis performed by Falletti *et al* (12) suggested that cognitive impairment caused by GH deficiency can be enhanced with GH replacement therapy. It is also possible that neurocognitive benefits of GH replacement could be applicable to other conditions (12).

It has been established that GH levels decline with age in both rodents and humans (13-15). It has been suggested that human GH secretion declines as much as 14% with each decade beyond 20 years of age (16). Additionally, it has been proposed that GH

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deficiency has been associated with progressive loss of body mass, especially of the lean body mass, with an increment of adiposity and metabolic risk events that are both age-related (17-18). GH treatment of elderly people improves body mass by decreasing both the adiposity and the bone density loss rate and increases the subjective well-being (19). However, other authors informed that treatment with GH induces smaller improvements in bone density but potentially enhances adverse effects such as carcinogenesis risk (16).

Several experimental models have been identified having elucidative roles of GH in aging animals (20), but the role of GH in the executive function is still controversial. The aim of this paper was determination of the chronopharmacological roles of GH in executive function and macrophage activation in rats.

Materials and Methods

Animals and operation procedures

Fifty male Wistar rats weighing (300–310 g) living in controlled light (photoperiod L: D 12:12) and temperature (25 °C) conditions with free access to water and food, were divided into four groups: diurnal control, nocturnal control, diurnal GH-treated, and nocturnal GH-treated. Diurnal and nocturnal GH groups received four intramuscular GH injections at 0.07 mg/kg of body weight (21), in the morning and night, respectively. The GH injection was administrated on days 1, 4, 7, and 14. Animals of the control group received a saline volume equivalent to 0.9%, following the same experimental protocol.

On the morning of the 14th day, diurnal GH-treated animals were submitted to the probe test. Nocturnal GH-treated animals were submitted to the same test at night periods. Before the test probe, both animal groups were deprived of food for a 12 hr period.

On the 16th day, animals were anesthetized with pentobarbital (50 mg/Kg body weight, IP) and submitted to the surgical procedure, which removed the organs for biometric analyses. The spleen was placed in phosphate buffered saline (PBS) solution for macrophage isolation and superoxide release determination. The blood was collected for superoxide dismutase (SOD) determination. All experimental animal procedures followed ethical standards and the study was institutionally approved.

Obtaining the plasma

Animal blood was collected using an anticoagulant and centrifuged for 15 min at 160 g with separation of the plasma from the red blood cells. Plasma samples were stored in individual tubes (1 ml) at -20 °C for determination of the Cu,Zn-SOD enzyme.

Isolation, purification, and identification of macrophages

After maceration of the spleens in PBS, cells were fractioned by centrifugation (160 g, 30 min) by gradient density (1,077 g/liter) using Ficoll-Paque

(Pharmacia, Sweden). Then, the isolated macro-phages were independently re-suspended in serum-free medium 199 (Gibco, USA) which was subsequently washed two times. Using a Newbauer hemocytometer, the macrophage levels were adjusted to a final concentration of 1×10^6 cells/ml (21, 22) and used in superoxide release test. Light microscopy was used to characterize macrophages populations and the cell viability was checked using the trypan blue exclusion assay (21).

Superoxide anion releasing

As previously described in the literature (21-23) the macrophage release of superoxide anion was measured by the cytochrome C (Sigma) reduction assay. Briefly, macrophages were centrifuged twice at 160 g, for 10 min at 4 °C and 500 ml of phagocytic cells was resuspended in PBS buffer containing 2.6 mM CaCl₂, 2.0 mM MgCl₂ and cytochrome C (2 mg/ml). Macrophages were stimulated with 10 µl Phorbol Myristate Acetate (PMA, Sigma) at a concentration of 10⁻⁷M (21, 22). 100 µl cell suspensions were incubated for 60 min at 37 °C on cell culture plates. The rates of reactions were measured by absorbance at 450 nm, and the results expressed as nmol/O₂⁻. Duplicate or triplicate experiments were done.

CuZn-Superoxide Dismutase Determination (CuZn-SOD)

SOD enzyme was measured in plasma samples using the nitroblue tetrazolium test (NBT, Sigma) and was spectrophotometrically read at 560 nm (24).

0.5 ml aliquots of plasma were placed in test tubes; and 0.5 ml aliquots of standard hydro-alcoholic solutions (1:1; v/v) were prepared in other pipes. The experimental tubes and standard solutions were added to 0.5 ml of chloroform-ethanol solution (1:1 ratio) followed by 0.5 ml of NBT and EDTA (diaminoethane tetra acetic acid) solution at a 1.0:1.5 ratio (v/v). pH was raised to 10.2 using 2.0 ml of carbonate buffer plus hydroxylamine (25-26).

The test pipes stayed at room temperature for 15 min and were read using spectrophotometry at 560 nm. Superoxide dismutase was calculated as follows: $SOD = (Abs\ standard - Abs\ sample / Abs\ standard) \times 100 = \% \text{ of NBT reduction} / SOD$. The results were expressed in international units (IU) of CuZn-SOD (22, 24).

Open-field test

For the analysis of exploratory and spatial activities, animals were placed in the left rear quadrant of a 50x50x39cm open field with wood color walls and the floor divided into 12 equal rectangles. The first test was performed during a 10 min observation session. The performance of the animal in the maze was assessed by measuring the time latency since its entry into the left rear until the opposite departure. Three hours later, animals were

brought to explore the apparatus again for another 10 min and the same measures were recorded to evaluate their performance in the maze (27-28).

Statistical analysis

Weight, SOD release, SOD values, and latency time were analyzed by the variance analysis (ANOVA), the statistical F and significant differences minimum were calculated by the Tukey method (29). Results were expressed as mean±standard deviation (SD). The statistical significance was considered when *P*-value was less than 0.05.

Results

Effects of GH on biometrical evaluation

There was no difference in body weight of the GH-treated group compared to the control group. Biometrical measurement of organs showed that GH effects were not observed in the kidney, independently of administration periods. Heart weight was significantly decreased in both groups treated with GH, whereas the lung and liver weights of nocturnal GH-treated rats were significantly (*P*<0.05) enhanced as compared with the weights of those of the nocturnal control rats (Table 1).

Effect of GH on oxidative stress

Neither diurnal nor nocturnal GH treatment significantly changed the quantity or quality (viability) of the macrophages (Table 1). GH treatment, independently of administration phases, did not significantly stimulate superoxide release by spleen macrophages. However, when stimulated with PMA macrophages, released more superoxide in both groups (Figure 1).

The diurnal GH-treatment influenced serum CuZn-SOD concentration in rats. A significant rise was observed in SOD levels in diurnal GH-treated rats (83.5±13.1) compared with the diurnal control rats

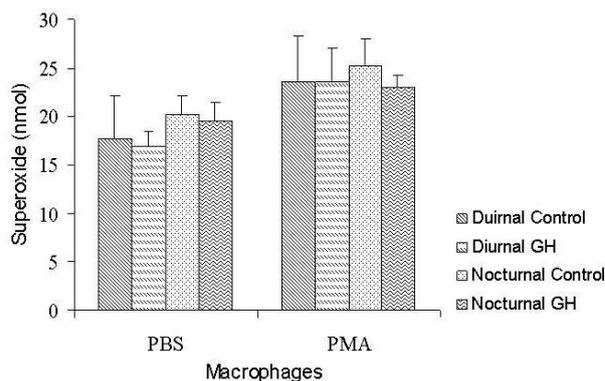


Figure 1. Effects of GH on macrophages superoxide release of control and diurnal GH-treated rats. PBS (unstimulated cells) and PMA (10⁷M -stimulated cells). Results represent the mean and SD of 10 experiment with different cells. **P*<0.05 comparing the superoxide release PMA cells stimulated with PBS, considering the same experimental group

(16.2±4.3) (Table 1). Nocturnal GH administration did not significantly change the CuZn-SOD concentrations when compared to the control group (29.0±10.5 x 26.4±12.5, respectively) (Figure 2).

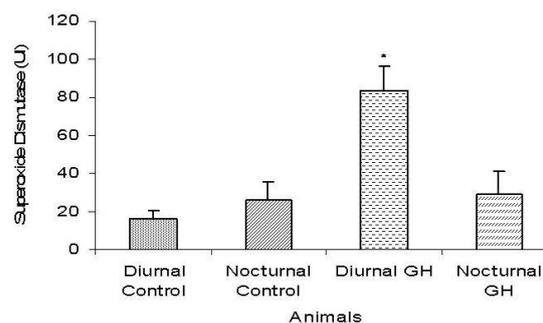


Figure 2. CuZn-Superoxide Dismutase Serum of control and GH-treated rats in different phases (diurnal and nocturnal). The results represent the mean and SD of 10 experiments of animals. **P*<0.05 comparing the controls groups with the GH-treated rats groups

Table 1. General characteristics of GH-treated rats and control rats

Parameter measured	Diurnal control rats	Nocturnal control rats	Diurnal GH-treated rats	Nocturnal GH-treated rats
Body weight	333±30	325±23	328±19	335±14
Heart weight	1,5±0,12	1,42±0,11	1,03±0,18*	1,17±0,30*
Kidney weight	1,35±0,04	1,33±0,06	1,34±0,07	1,41±0,07
Lung weight	1,80±0,12	1,60±0,10	1,72±0,30	2,10±0,20*
Liver weight	9,0±0,20	8,10±0,40	11,8±0,8*	10,3±0,50*
Number of macrophages (x10 ⁶ cells/ml)	4.2±0.5	4.6±0.6	3.2±0.5	3.8±0.4
Viability of macrophages (%)	89±4.7	88.5±4.3	88.9±5.7	85.8±5.9

The results represent mean±SD of experiments with 5 different animals

* *P*<0.05 (ANOVA) comparing the control group with the GH-treated group in the same periods

Table 2. Open-field test performance of GH-treated and control rat groups

Groups	First probe test (minutes)	Second probe test (minutes)
Diurnal control rats	6,10 ± 0,22	3,02 ± 0,02†
Nocturnal control rats	5,10 ± 0,77	2,83 ± 0,01†
Diurnal GH-treated rats	4,05 ± 0,40 *	3,01 ± 0,01
Nocturnal GH-treated rats	4,40 ± 0,51*	2,63 ± 0,44†

The results represent mean±SD of experiments with 5 different animals

* $P < 0.05$ (ANOVA) comparison of control groups with GH-treated groups, considering the same periods' probe test

† $P < 0.05$ comparing the first test probe with the second test probe, considering the same group

Effects of GH-treatment on executive function

In the first test, it was observed that GH-treated groups had a significant decrease in latency times when compared with the control groups (Table 2).

In the second test, both control groups (diurnal and nocturnal) had a significant decreased on the latency time. The second latency time in the diurnal GH-treated group tended to decrease, but this difference was not statistically significant. Comparing with the first latency time the nocturnal GH-treated group had a significantly decreased second latency time.

Discussion

The present study experimentally assessed the chronopharmacological roles of GH in executive function and oxidative stress response, which could be implicated in pathophysiological changes of rats.

Several studies have suggested that GH affects neuronal networks and psychological processes. GH hormone deficiency in children may cause sleep disturbances, immaturity and deviant personality development (30-31). In adults GH deficiency has been associated with subjects who are single and unemployed (32), and who had lowered subjective well-being (33, 34).

Our data shows that GH-treated rats displayed improved performance in a task that requires spatial orientation, implementation of a strategy, and working memory, all these capacities are included in the executive function category. The GH treated animals spent less time to accomplish their exploratory and spatial tasks in the open-field test. The time latency in both GH-treated groups was significantly lower compared to the control groups.

Several studies have suggested that GH also affects pathophysiological processes.

In patients with heart failure, it was suggested that GH-treatment restored heart contraction and relaxation (35). In GH-deficient patients, GH treatment improved both heart muscle mass and heart function (36). Studies regarding GH and heart failure have reported that hormone effect is dose-dependent. Lower doses exert beneficial effects, enhancing antioxidant defense and reducing oxidative stress, whereas higher GH doses exert detrimental effects related to mitochondrial energy

metabolism and oxidative stress (37). GH overproduction reduces life span and enzymatic antioxidant defenses, and increases the production of pro-inflammatory cytokines, tissue inflammatory markers, cardiac apoptotic rates, and also increases the burden of oxidative stress, which decisively contributes to aging, cardiac dysfunction, and neurodegeneration (38-45).

In the current work, GH-treated rats presented a decrease in heart weight and an increase in lung weight which reinforces the pathological effects of supraphysiological levels of GH. In addition, another model of excessive GH production is represented by acromegaly patients. In acromegaly, a condition in which the patient is exposed to higher levels of GH, mortality due to diabetes, hypertension, stroke, congestive cardiac failure, and ischemic heart disease were associated with the excess of blood GH values (46). This data confirms findings from acromegalic patients that had decreased cardiac performance and heart vascular impairment despite left ventricular hypertrophy (47).

In our experiment, GH treatment exerted beneficial effects by enhancing antioxidant defenses in the nocturnal phase. Antioxidant defense system includes both enzymatic and non-enzymatic pathways. Among the enzymatic antioxidant mechanisms, various studies have reported that CuZn-SOD has an important role (48, 49), whereas it has been previously reported that SOD activity displays no difference in GH-treated animals (37). The rise of the SOD concentration occurred in the nocturnal GH-treated group showing that GH may be both dose- and phase-dependent.

On contrary, there was no change detected in the quantity or viability of spleen macrophages in either GH-treated or control groups. The capacity of activation of those cells evidenced by superoxide release, showed no significant differences either, indicating that GH-treatment did not alter those cells. When those cells were stimulated by PMA we observed an increase in superoxide release in both GH-treated and control groups.

Our results indicate an association between GH and cognitive function in rats, confirming the possibility that GH replacement may be accompanied by an improvement in executive functioning in

human patients as proposed by many authors (5, 11, 12, 50, 51).

Conclusion

We suggest that GH administration may enhance executive functions according to the period of the day, at the cost of increasing the risk of oxidative stress. Administration in the diurnal period increased oxidative burst, while in the nocturnal period GH is more likely to contribute to the reduction of oxidative stress.

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Conflict of interest

There is no conflict of interest since this study was not supported by third parties.

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