Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 57; Numer/Number 5/2006
ISSN 0423–104X

Zmiany ekspresji mRNA dla hormonu wzrostu (GH) w przednim płacie przysadki mózgowej szczurów po pojedynczym podaniu interferonu (IFN) α

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Streszczenie

Wstęp: Interferon (IFN) α jest plejotropową cytokiną, której działanie następuje poprzez wpływ na wydzielanie innych cytokin oraz hormonów. Wielokierunkowe działanie wykazuje również hormon wzrostu (GH, *growth hormone*). W piśmiennictwie dostępne są doniesienia dotyczące wzajemnych oddziaływań między hormonami i cytokinami, dlatego celem pracy jest ocena wpływu pojedynczej dawki IFN α na ekspresję mRNA dla GH w przednim płacie przysadki mózgowej.

Materiał i metody: Badanie przeprowadzono na szczurach *Wistar*, którym po podzieleniu na odpowiednie grupy podano dootrzewnowo takie same objętości IFN α lub 0,9-proc. NaCl. Następnie zwierzęta uśmiercano przez dekapitację (w zależności od grupy), odpowiednio 2 i 4 godziny po podaniu IFN α/0,9-proc. NaCl, i pobierano przysadki mózgowe w celu oceny ekspresji mRNA dla GH z zastosowaniem metody hybrydyzacji *in situ*.

Wyniki: Pojedyncze podanie IFN α powoduje wzrost ekspresji mRNA dla GH w przednim płacie przysadki mózgowej, obserwowany 4 godziny po podaniu w porównaniu z ekspresją mRNA dla GH w grupie kontrolnej ($p < 0,01$).

Wnioski: Nie stwierdzono statystycznych różnic w ekspresji mRNA dla GH 2 godziny po podaniu IFN α w porównaniu z grupą kontrolną.

(Endokrynol Pol 2006; 5 (57): 482–486)

Słowa kluczowe: *GH mRNA, przysadka mózgowa, interferon* α

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Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 57; Numer/Number 5/2006
ISSN 0423–104X

Changes in growth hormone (GH) messenger RNA (GH mRNA) expression in the rat anterior pituitary after single interferon (IFN) α administration

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Abstract

Introduction: Interferon α (IFN-α) is a cytokine with pleiotropic effects which, via different pathways, influences the secretion of certain cytokines and hormones. Growth hormone (GH) secreted from the pituitary has physiological effects on various target tissues. The question is how IFN-α administered in various types of disease influences GH secretion. This study investigated the acute effect of IFN-α on GH mRNA expression in the rat anterior pituitary.

Objective: The aim of the study was to measure the cellular expression of GH mRNA by *in situ* hybridisation in the anterior pituitary after a single administration of IFN-α.

Material and methods: Rats were administered an intraperitoneal injection of IFN-α or saline. The rat pituitaries were taken 2 and 4 hours after IFN/saline administration and kept frozen until *in situ* hybridisation histochemistry. A 31–base ^{35}S-labelled oligonucleotide probe complementary to part of the exonic mRNA sequence coding for GH mRNA was used. All control and experimental sections were hybridised in the same hybridisation reaction.

Results: Acute administration of interferon α increased GH mRNA expression in the anterior pituitary in the 4-hour group in comparison with the control group, and there was no difference between the control group and the 2-hour rats.

Conclusion: A single IFN-α administration was found to exert an influence on anterior pituitary GH mRNA expression. These observations may pave the way for presenting a possible new action of IFN-α.

(Pol J Endocrinol 2006; 5 (57): 482–486)

Key words: *GH mRNA, anterior pituitary, interferon α*

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Introduction

The immune-neuroendocrine system plays an important role in maintaining homeostasis under a variety of conditions, including physiological and pathological status (caused by, for example, infection or neoplastic disease). Changes in the secretion of several immune mediators such as cytokines influence the secretion of different hormones and neuromediators [1–4].

It has been observed that hormonal changes induced by certain cytokines are important and occur at multiple levels. Indeed, immune and endocrine systems communicate with each other [1, 2]. Some hormones, such as adrenocorticotropic hormone (ACTH) [3] or melatonin (MLT) [4], are potent immunoregulatory factors, whereas the interactions of the immune system with some other hormones, including growth hormone (GH), are still a matter of debate.

The somatotropic axis is also involved in the reaction to cytokine administration and several reports have given evidence that GH modulates immune function. GH secreted from the pituitary has physiological effects on various target tissues. The effects occur after GH binding to its receptor (GHR) and might be modulated by a circulating GH-binding protein [1, 2].

On the other hand, cytokines produced by activated immune cells interfere with the release of hormones from the pituitary gland [5]. Among these IFN-α has been shown to be a potent stimulator of the hypothalamic-pituitary-adrenal axis in animals and humans [6]. Its therapeutic efficacy for the treatment of cancer, lymphomas, leukaemias and infectious diseases (such as chronic hepatitis) is still a matter of investigation. Whereas the influence of IFN-α on cortisol secretion has been described [2], data concerning IFN-α impact on GH are rare and less conclusive.

The administration of endotoxin, which induces cytokine production, induces species-dependent effects on GH secretion increases in humans [7] but decreases in rats [8]. The mechanism of this action is not clearly understood. It may take place directly at the level of the pituitary gland or indirectly at the level of one or both hypothalamic neurohormones, GH-releasing hormone (GHRH) and somatostatin, which act as stimulator and inhibitor respectively of GH secretion [9–12]. IFN-α may exert a direct inhibiting effect on cell proliferation and on the production of different peptide hormones [12]. An inhibition of GH secretion has been observed after incubation with IFN-α in GH-secreting pituitary adenoma cultures. Additionally, an inhibition of hormone secretion by IFN-α was accompanied by an inhibition of intracellular hormone concentration [13].

The present investigation therefore set out to study the sub-acute effects of IFN-α on GH by measuring GH mRNA expression in the rat anterior pituitary.

Materials and methods

Animals

Sixty intact male Wistar rats (60 days of age; 250 ± 20 g) kept in a 12 h light- 12 h dark cycle (with lights switched on at 7.00 hours) were used for the experiment. These were divided into three groups as follows:

1. Experimental group — 2-hour animals injected with IFN-α;
2. Experimental group — 4-hour animals injected with IFN-α;
3. Control group — 2-hour animals injected with saline;
4. Control group — 4-hour animals injected with saline.

The animals were allowed laboratory chow and tap water *ad libidum* and were housed five in the cage.

Treatment

The experimental animals were injected at a dose of IFN-α 20 × 10^4 IU (2 ml) intraperitoneally, while the control animals were injected with the same volume of 0.9% NaCl.

The rats in groups 1 and 2 were subsequently decapitated at 2 and 4 hours after IFN-α administration and those in groups 3 and 4 were subsequently decapitated at 2 and 4 hours after saline administration. The pituitaries were removed and rapidly frozen on dry-ice and stored at -80°C until *in situ* hybridisation.

In situ *hybridisation*

Sections of 12 mm were taken through the pituitaries and riboprobe was used for *in situ* hybridisation to examine GH mRNA in these tissues. A 31-oligonucleotide probe (Presomatotropin) - 5′- CAg AgC gTC ATC gCT gCg CAT gTT ggC gTC A (TIP MOL BIOL, Poznań, Poland) was used in the experiment. The 3′ end of the probe was labelled with ^{35}S-αdATP (1200 Ci/mmol, NEN DuPont, UK).

Sections were fixed in 4% paraformaldehyde, washed in 1 × PBS and acetylated in a solution of 0.25% acetic anhydride, 1.4% triethanolamine in 0.6% sterile saline. After dehydration, delipidation and partial rehydration sections were allowed to air dry and were subsequently hybridised to probe overnight at 37°C. The slides were then washed four times over a one-hour period in 1 × SSC and incubated twice in 1 × SSC at room temperature for 15 min × 2. Finally, they were dipped briefly in water × 2 to remove salt deposits prior to drying and then dried complete in a warm air stream. All control and experimental sections were hybridised in the same hybridisation reaction.

Hybridised sections were exposed to autoradiography film for three days and the resulting images were analysed using a computer-aided image analysis system (Image 1.22 developed by Wayne Rasband, NIH Bethesda MD, USA) and run on an Apple MacIICi computer. The results obtained are presented as mean percentage changes from control ± standard error about the mean.

Statistical comparisons were made using the Fisher PLSD test and following one-way ANOVA. A $p < 0.05$ was considered significant.

Results

Effects on GH mRNA in the pituitary

At the pituitary level, GH mRNA was present in the anterior pituitary but not in the posterior pituitary. Single IFN-α (20 × 10^4 IU i.p.) administration did not influence GH mRNA expression in the group of rats decapitated at 2 hours after IFN administration in comparison with the control group (Fig. 1).

In the 4-hour group of rats a significant increase in GH mRNA expression was observed in the anterior pituitary (Fig. 2) in comparison with the control group ($p < 0.01$) and in comparison with the 2-hour group of rats ($p < 0.05$) (Fig. 3).

Discussion

The results of this study showed that intraperitoneal doses of IFN-α influence GH mRNA expression in the rat pituitary at the time point of 4 hours after injection, possibly suggesting a stimulatory effect of IFN-α on GH mRNA expression in the rat pituitary.

Our study focused on IFN-α influence on GH synthesis by estimating the expression of GH mRNA in the rat anterior pituitary. Little research has so far been performed into GH mRNA expression in the rat pituitary after IFN-α.

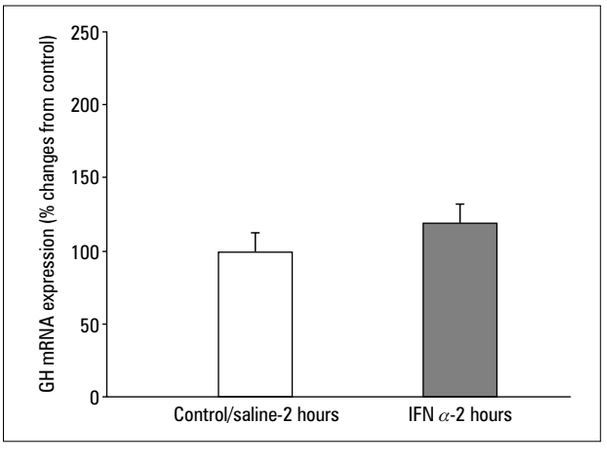

Figure 1. *GH mRNA expression in the anterior pituitary 2 hours after saline or interferon-α injection. The values represent the mean ± SEM shown as the percentage changes from control (saline)*

Rycina 1. *Ekspresja GH mRNA w przysadce mózgowej 2 godziny po podaniu odpowiednio 0,9-proc. NaCL/interferonu α. Wartość średnia ± SEM*

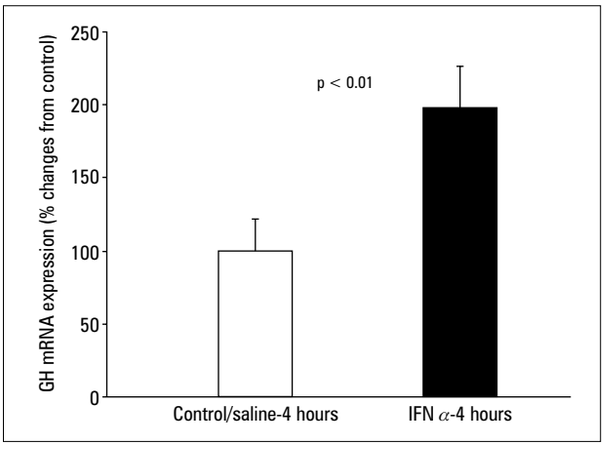

Figure 2. *GH mRNA expression in the anterior pituitary 4 hours after saline or interferon-α injection. The values represent the mean ± SEM; p < 0.01*

Rycina 2. *Ekspresja GH mRNA w przysadce mózgowej w 4 godziny po podaniu odpowiednio 0,9% NaCL/Interferon α. Wartość średnia ± SEM; p < 0,01*

In studying the effects of IFN-α on the secretion of GH the pulsatile release of GH should be considered. The occurrence of GH episodes seems to increase after INF-α when the drug is given acutely. We did not estimate the pulsatile secretion of GH but examined the effect of IFN-α at the cellular level (the folliculo-stellate cells), the source of GH, by estimating GH mRNA expression.

Some factors, such as lipopolysaccharide (LPS), can stimulate the cells of the immune system, mainly monocytes and lymphocytes, which further induces mul-

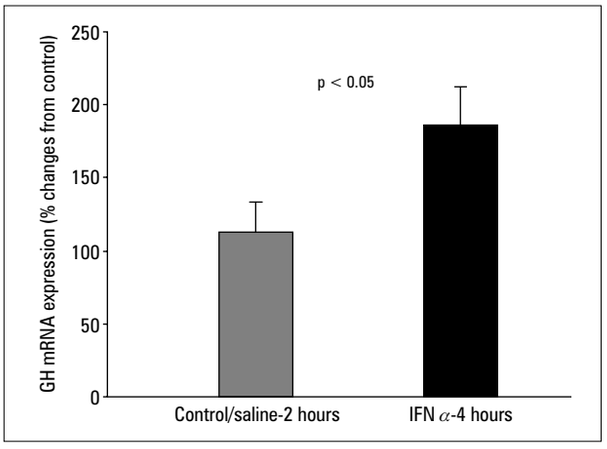

Figure 3. *GH mRNA expression in the anterior pituitary 2 and 4 hours after interferon-α injection. The values represent the mean ± SEM; p < 0.05*

Rycina 3. *Ekspresja GH mRNA w przysadce mózgowej 2 i 4 godziny po podaniu interferonu α. Wartość średnia ± SEM; p < 0,05*

tiple changes in the organism, including stimulation of cytokine (lymphokine and monokine) production and release from macrophages and lymphocytes, and, in particular, a rapid increase in cytokine secretion, including TNF-α, IFN-α, interleukin (IL)-1 and IL-6. Consequently, these circulating cytokines could interfere with both synthesis and secretion of several hormones from the pituitary [8–12].

Some data concern changes in pituitary hormones and in cytokine concentration after administration of IFN-α in patients with chronic hepatitis type C. The results observed were an increase in ACTH, GH and cortisol concentration 60–120 min after injection. In contrast, serum levels of TSH, PRL, LH and FSH did not change significantly [14].

When IFN-α is used therapeutically, it is given over a long period, sometimes for several months. It is both interesting and important to elucidate the possible impact of IFN-α administration on GH synthesis and secretion, especially in view of the IFN-α treatment of children suffering from chronic hepatitis types B and C.

To our knowledge, the hormonal effects of IFN-α administration have not been reported to date, with the exception of the contributions by Muller et al. [2], who observed that sub-acute administration of IFN-α stimulates GH secretion on the following day, and D'Urso et al. [15], who found a significant stimulatory effect of IFN-α on GH release with a peak value 6 hours after injection. In apparent confirmation of this hypothesis the results of an experiment by Nash et al. [16] show that TNF-α increases GH mRNA expression in ovine pituitaries.

Our preliminary results are in agreement with the observations of Muller at al. [2], which show that

sub-acute administration of IFN-α reported in patients with hepatitis B or C infection stimulated the release of GH. An opposite effect was observed after chronic administration (on the 28th day after the start of IFN-α therapy).

In contrast to these results, Vankelecom et al. [17] described the inhibiting effect of IFN-α administration on stimulated GH release in normal rat anterior pituitary cell cultures.

This discrepancy in comparison with our results may be explained by the differences in the experimental schedule as this study was performed on monolayer cultures of pituitary cells with IFN-γ.

To summarise all available data it may be concluded that the possible mechanism of IFN-α action on pituitary cells could result from the following types of activity:

- direct — by stimulating synthesis of GH in the anterior pituitary cells;
- indirect — through the stimulation of synthesis of other cytokines (IL-1, IL-6, TNF-α), further stimulating cells synthesising GH [9, 17];
- a mixture of both these [2, 17].

This preliminary observation may pave the way for presenting a possible new IFN-α action. To complete these data and to draw final conclusions it is necessary to extend the experiment to include the chronic model of interferon a administration, which can mirror that used with patients with chronic hepatitis.

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