Growth Hormone & IGF Research 24 (2014) 42–46

Contents lists available at ScienceDirect

Growth Hormone & IGF Research

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

Effects of thyroid hormone on the GH signal transduction pathway☆

Paula Ocaranza *, Juan Javier Lammoglia [1], Germán Íñiguez, Rossana Román, Fernando Cassorla

Institute of Maternal and Child Research, School of Medicine, University of Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 18 July 2013
Received in revised form 30 October 2013
Accepted 6 January 2014
Available online 12 January 2014

Keywords:
Thyroid hormone
GH signal transduction
Skin fibroblasts

ABSTRACT

Background/aim: The importance of thyroid hormone on growth and development in children is well recognized. In addition, linear growth is highly dependent on the response of peripheral tissues to growth hormone, a process known as GH sensitivity, but little is known about the possible effects of T4 on this process.
Methods: We determined the effect of stimulation with recombinant human GH (rhGH; 200 ng/mL) alone or in combination with two different concentrations of T4 (250 nM and 500 nM for 24 h) on JAK2 and STAT5 activation in skin fibroblast cultures obtained from prepubertal boys with normal height.
Results: JAK2 and STAT5 were activated under co-incubation with T4 (at both concentrations) and rhGH in the non-nuclear fraction of the fibroblasts. In addition, after 24 h of co-incubation with rhGH and T4 (500 nM), we observed an increase in phospho-STAT5 in the nuclear fraction, when compared to GH and T4 stimulation alone. This effect was not observed when the fibroblasts were co-incubated with GH and the lower concentration of T4 (250 nM).
Conclusion: Combined stimulation with GH and T4 at a concentration of 500 nM increases synergistically nuclear phospho-STAT5 in skin fibroblasts, which may amplify tissue sensitivity to GH. These findings may help to explain the effect of T4 administration on growth velocity in some children with idiopathic short stature.
© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The importance of thyroid hormone (T4) on growth and development in children is well recognized [1–3]. Several studies have addressed the effects of supplementing thyroid hormone in short children with serum thyroid hormone levels in the low-normal range [3], but the possible mechanisms leading to the improvement in the growth velocity of some of these children during T4 administration have not been clarified. Linear growth is highly dependent on the response of peripheral tissues to growth hormone (GH) [4], a process known as GH sensitivity [5]. Several parameters such as nutritional status [6,7] as well as the circulating concentrations of sex steroids markedly influence GH sensitivity [8–10], but little is known about the possible effects of thyroid hormone on this process.

Responsiveness to GH in target cells is mediated by the GH receptor (GHR). The interaction of GH with its dimerized receptor causes the activation of multiple signaling pathways, among which the GHR/JAK/STAT is regarded as the main GH-activated signaling pathway. Janus kinase 2 (JAK2) constitutively associates with the GHR, which in turn induces tyrosine phosphorylation within itself and the GHR. Upon GH binding, members of the family of signal transducers and activators of transcription (STATs) are recruited to the GHR complex. These proteins undergo tyrosine phosphorylation by JAK kinases resulting in STAT dimerization and nuclear translocation, where they bind to specific DNA elements and stimulate gene transcription [11–14].

In this study, we have investigated the effects of thyroid hormone on GH sensitivity, by performing an *in vitro* study regarding the effects of co-incubation with levothyroxine over the response to GH in fibroblast cultures obtained from prepubertal boys with normal stature. We determined the activation of JAK2 and STAT5 after stimulation with GH alone, or in the presence of two different concentrations of T4 (250 nM and 500 nM) during 24 h. We documented that thyroid hormone supplementation at the higher concentration enhances synergistically the STAT5 response to GH stimulation in fibroblast cultures from children with normal height.

2. Subjects and methods

2.1. Study subjects

The study population consisted of 16 prepubertal boys (6.6 ± 0.6 years of age) with normal stature and weight for age and sex who underwent elective surgery for an unrelated condition. The subjects were recruited at the San Borja-Arriarán Hospital, and the study was approved by the Ethics Committee of the University of Chile. Informed consent was obtained from the parents of the children enrolled in the study.

☆ Footnote 1: Supported by Merck-Serono and FONDECYT 1095118.
* Corresponding author at: Casilla 226–3, Santiago, Chile. Tel.: +56 2 2977 0851; fax: +56 2 2424 7240.
E-mail address: pauocaranza@gmail.com (P. Ocaranza).
[1] Current address: Faculty of Medicine, University of Los Andes, Bogotá, Colombia.

1096-6374/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ghir.2014.01.001

2.2. Chemicals

Recombinant human growth hormone (rhGH) was a gift from Dr. A. F. Parlow (National hormone and peptide program, NIH, USA) and thyroid hormone (T4) was donated by Merck-Serono. Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L of glucose, Dulbecco's phosphate buffered solution (DPBS), penicillin/streptomycin, and fungizone were purchased from Invitrogen (Grand Island, NY, USA). Heat inactivated fetal calf serum (FCS) was purchased from Biological Industries (Kibbutz Beit Haemek, Israel). Prestained molecular mass standard proteins were obtained from Fermentas (Burlington, Ontario, Canada), and all the other chemicals were purchased from Sigma (St. Louis, Mo, USA), unless stated otherwise.

2.3. Cell cultures

Primary fibroblast cultures were established from skin-biopsy specimens obtained from the healthy control children (at the time of surgery). The tissue (~1 mm^3) was cultured in DMEM supplemented with 10% FCS, 10 U/mL, penicillin/streptomycin and 0.25 μg/mL fungizone at 37 °C in a 5% CO_2 atmosphere. Fibroblast cultures were used between the third and sixth passage, in order to avoid the influence of plasma factors and senescent changes over the cellular response.

2.4. T4 dose–response and time–response assay

The dose and time chosen for the different assays were based on STAT5 phosphorylation. Skin fibroblast cultures seeded on 100-mm dishes at 80 to 90% confluence were washed with DPBS and serum deprived for 24 h and treated with the following stimuli: (i) a sole dose of rhGH (200 ng/mL), (ii) 4 concentrations of T4 (50 nM, 100 nM, 250 nM and 500 nM) and (iii) co-stimulation of GH plus T4 for 24 and 48 h. Higher concentrations of T4, including those employed in this study, have been used in different *in vitro* protocols [15–17]. Differences in STAT5 phosphorylation were observed at 24 h post-incubation and at the highest doses of T4 (250 nM and 500 nM), concentrations which were used in this study.

2.5. Protein extraction

Skin fibroblasts seeded on 100-mm dishes at 80 to 90% confluence were washed with DPBS and serum deprived for 24 h, and treated with two concentrations of T4, 250 mM and 500 mM in the presence of 200 ng/mL rhGH for 24 h. Protein extracts were prepared as described previously [18]. The protein content was determined by the Bio-Rad Bradford (Richmond, CA, USA) assay. Soluble fractions from fibroblasts were heated at 95 °C with 0.33 vol of SDS-PAGE sample buffer.

2.6. Analysis of the protein content by Western immunoblotting

Samples were resolved by SDS-PAGE under reducing conditions and transferred to nitrocellulose membranes (Bio-Rad, Richmond, CA, USA). To reduce non-specific antibody binding, membranes were incubated for 1 h at room temperature in TBS (pH 7.4) containing 0.1% (v/v) Tween-20 (TBST) blocking buffer with 3% w/v BSA. The membranes were then incubated overnight at 4 °C with antibodies anti-JAK2 1:2000 from Millipore (Billerica, MA, USA) and anti-TFIIB 1:400 purchased from B.D Transduction Laboratories (Franklin Lakes, NJ, USA); anti-phospho-JAK2 $Tyr^{1007/1008}$ 1:1000 purchased from Cell Signaling Technology (Danvers, MA, USA), anti-STAT5a/b 1:800 purchased from Abcam (Cambridge, MA, USA), anti-phospho-STAT5 $Tyr^{694/699}$ 1:1000 from Santa Cruz (Dallas, Texas, USA) and anti-β-actin 1:100.000 obtained from Sigma Aldrich (St. Louis, MO, USA).

After washing in TBST, membranes were incubated for 1 h at room temperature with horseradish peroxidase-linked secondary antibody (1:10.000 for peroxidase conjugated anti-rabbit IgG or peroxidase-conjugated anti-mouse IgG) purchased from Rockland (Gilbertsville, PA, USA). Immunoreactive proteins were visualized using the Pierce® ECL Western Blotting Substrate Kit (Thermo Scientific, Rockford, IL, USA), blots were captured by exposure using the Discovery 10gD Ultralum with Ultraquant software (6.0.0344 version). The OD of the protein bands relative to β-actin, TFII (nuclear protein) or the respective total content, were quantified by the Image J 1.38x (NIH, USA). For reblotting, membranes were stripped by incubation for 2 min at room temperature with Re-blot Mild Solution (Chemicon, Tamecula, CA, USA). Blots were washed, reblotted and immunolabeled as described above. Immunoreactive proteins were visualized, blots were captured and the OD of the protein bands relative to β-actin, TFII (nuclear protein) or the respective total content, were quantified by the Image J 1.38x (NIH, USA).

2.7. Statistical analyses

The SDS for weight and height for our subjects were based on the National Center for Health Statistics (NCHS). The differences in JAK2 and STAT5 activation after incubation for 24 h with rhGH or T4 alone, or with the combination of both hormones with the two concentrations of T4 were compared by the Kruskal–Wallis test. Each protein was analyzed twice in each of the fibroblast cultures from the 16 subjects studied. Statistical analysis was performed with the GraphPad Prism program version 5.00, and P values less than 0.05 were considered significant.

3. Results

3.1. Clinical characteristics of the control prepubertal boys

The clinical characteristics of the boys studied are shown in Table 1. The 16 prepubertal boys had a mean age of 6.6 ± 0.6 years, a mean height of −0.20 ± 0.2 SDS, and a mean weight of −0.06 ± 0.3 SDS.

3.2. JAK2 activation

To determine whether co-incubation with T4 and hGH potentiates the intracellular growth hormone signaling pathway, we first analyzed the effect of these hormones on JAK2, an activator which is essential for this signaling pathway. For this purpose, we determined the changes in JAK2 phosphorylation after incubation with rhGH alone (200 ng/mL), or in combination with two different concentrations of T4 (250 nM and 500 nM).

In a previous report, we showed the time-course for phosphorylation of JAK2 after GH administration in fibroblasts from healthy control subjects. Briefly, it peaked within 15 min of the addition of rhGH (0.97 ± 0.08 vs 1.14 ± 0.08), and returned to basal levels after 60 min of GH stimulation [18]. Based upon this information, co-incubation studies showed that in the presence of 250 nM of T4, JAK2 activation was similar to rhGH stimulation alone (1.11 ± 0.1 vs 1.02 ± 0.03). Similar data was obtained when a higher dose of T4 (500 nM) was used (1.1 ± 0.1 vs 1.43 ± 0.31). These results shown in Fig. 1B suggest that co-incubation with hGH and T4 does not increase the activation of JAK2 over that obtained with rhGH alone, and phosphorylation levels are similar to those observed after 15 min of hormone stimulation.

Table 1
Clinical characteristics of the subjects enrolled in the study. Values are presented as mean ± SEM.

Subjects	Sex, male	Age, years (mean ± SEM)	Height, z score (mean ± SEM)	Weight, z score (mean ± SEM)
Prepubertal	16	6.6 ± 0.1	−0.2 ± 0.2	−0.06 ± 0.33

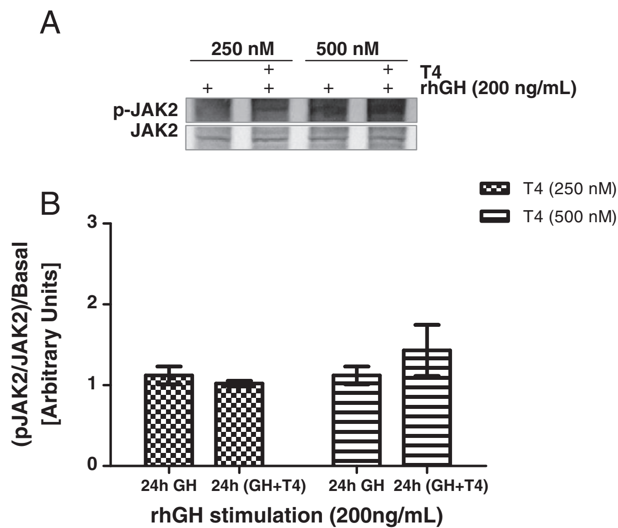

Fig. 1. Janus kinase (JAK)2 activation in skin fibroblasts after a 24 h GH incubation in the absence and presence of T4. Two concentrations of T4 were tested, 250 nM and 500 nM, and compared to rhGH (200 ng/mL). **(A)** Representative Western immunoblots raised against pJAK2 and JAK2. **(B)** Densitometric analysis of immunoblot data. Values are expressed as mean ± SEM.

3.3. STAT5 activation

Following the growth hormone signaling pathway, our next step was to study the time-course activation of STAT5b. For this part of the study, the fibroblasts were incubated with 200 ng/mL of rhGH over short periods of time (minutes), and the cytoplasmic lysates were analyzed by SDS-PAGE and immunoblotting. As shown in Fig. 2B, STAT5 phosphorylation increased after 15 min (0.91 ± 0.24 vs 1.67 ± 0.3), peaked within 30 min of the addition of rhGH (0.91 ± 0.24 vs 2.01 ± 0.52), and returned to basal levels after 60 min. (See Fig. 2B).

Since the STAT5 response was confirmed after GH incubation in this *in vitro* cell model, we analyzed the effect of the 24 h co-incubation with rhGH and T4 on STAT5b activation in the non-nuclear fraction. As shown in Fig. 2C, we observed similar phosphorylation levels with the 250 nM (1.108 ± 0.1 vs 1.106 ± 0.03) and 500 nM concentrations of T4 (1.11 ± 0.1 vs 0.99 ± 0.04).

3.4. STAT5 nuclear translocation

An important feature of STAT5 concerns its translocation to the nucleus following activation and dimerization in the cytoplasm. Our first approach was to study how nuclear phospho-STAT5 varies among GH stimulation over short periods of time (minutes). As shown in Fig. 3B, an increase in phospho-STAT5 was observed after 15 min (0.77 ± 0.15 vs 1.09 ± 0.04) of stimulation, which was maintained for 30 min (0.77 ± 0.15 vs 1.08 ± 0.14), and returned to basal levels after 60 min of GH stimulation.

Co-incubation with the lower concentrations of T4 (250 nM; Fig. 4B), showed similar levels of nuclear phospho-STAT5 when compared to the 24 h GH treatment (0.98 ± 0.1 vs 1.25 ± 0.2). However, when we compared the phosphorylation levels with cells treated solely with T4 (250 nM) and rhGH (200 ng/mL) for 24 h, phosphorylation levels were increased only in the presence of GH. When a higher concentration of T4 (500 nM) was used, skin fibroblasts co-incubated with both hormones showed a significant increase in nuclear phospho-STAT5, when compared to the levels observed with rhGH (1.25 ± 0.2 vs 2.11 ± 0.2), or T4 (500 nM) alone (0.65 ± 0.18 vs 2.11 ± 0.2). Phosphorylation levels observed with both doses of T4 were similar to those obtained under basal conditions, suggesting that co-incubation with rhGH and the higher concentration of T4 increases nuclear phospho-STAT5 synergically in human skin fibroblasts (Fig. 4B).

4. Discussion

The possible effects of thyroid hormone over the growth hormone signaling pathway have not been studied adequately. This signal transduction cascade induced by the growth hormone receptor triggers a complex array of biochemical events, and involves a large number of distinct molecules, mainly GHR/JAK2/STAT5.

The use of skin fibroblast cultures has gained importance in the study of the GHR and its signaling pathway, since it maintains the intrinsic characteristics after being removed from its natural *in vivo* environment [19]. These cultures have been used to study GH action in children with short stature caused by complete GH insensitivity (GHI) [20,21], and in children with idiopathic short stature [22–24]. It has been demonstrated that in fibroblasts obtained from normal children, IGFBP-3 mRNA and secretion increase when stimulated with 200 ng/mL rhGH and compared to fibroblasts obtained from children with GHI [20].

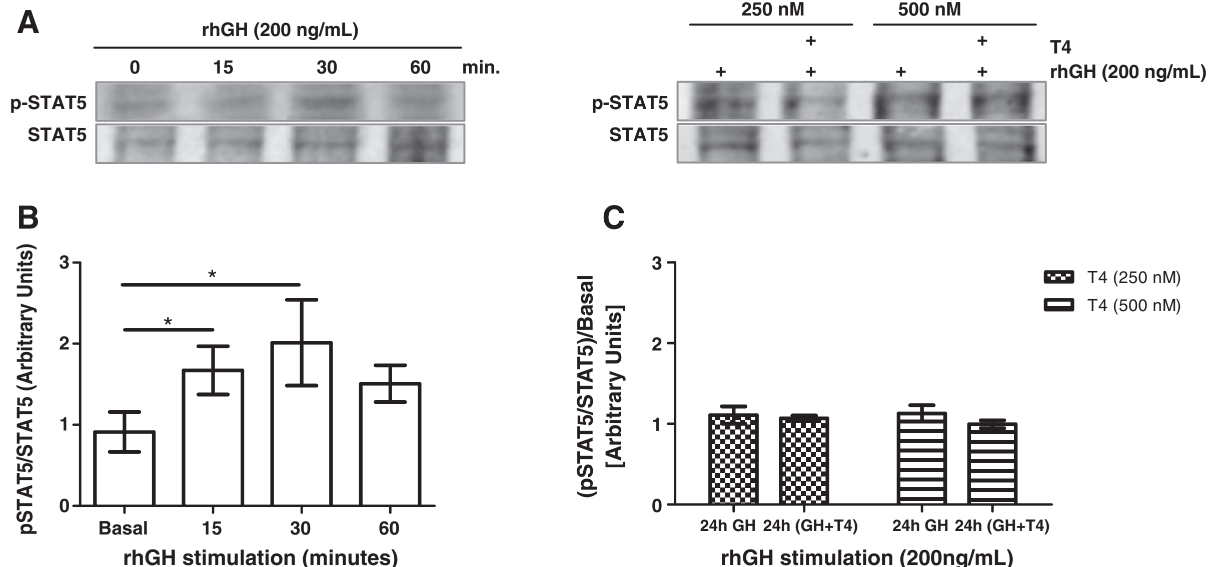

Fig. 2. Signal transducers and activators of transcription (STAT)5 activation in the non-nuclear fraction of skin fibroblasts. **(A)** Representative Western immunoblots raised against pSTAT5 and STAT5 for time-course and co-incubation studies. **(B)** Time-course of STAT5 activation in skin fibroblasts after recombinant human growth hormone (rhGH) stimuli. **(C)** STAT5 activation after a 24 h rhGH (200 ng/mL) incubation in the absence and presence of T4 (250 nM and 500 nM). Values are expressed as mean ± SEM. *$p < 0.05$.

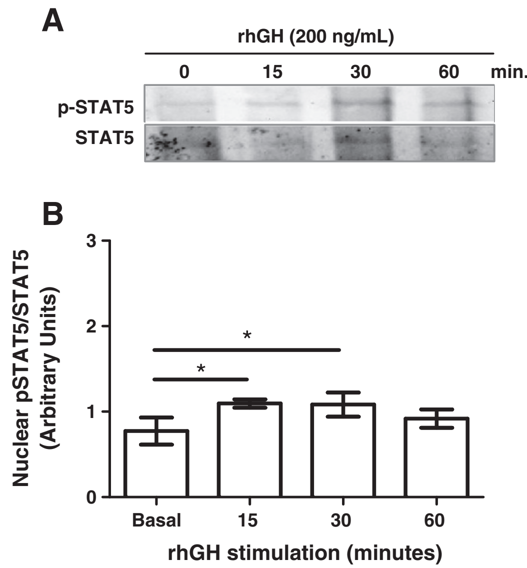

Fig. 3. Time course STAT5 phosphorylation in the nuclear fraction of skin fibroblasts cultures. **(A)** Representative Western immunoblots raised against pSTAT5 and STAT5. **(B)** Nuclear time-course of STAT5 phosphorylation in skin fibroblasts after recombinant human growth hormone (rhGH) stimuli. Values are expressed as mean ± SEM. *$p < 0.05$.

The activation of the JAK/STAT signaling pathway is also activated in these cell cultures when a GH concentration of 200 ng/mL is used. GH rapidly activates STAT signaling in normal skin fibroblasts, where STATs1 and 5 appear to bind DNA [21]. Local IGF-I production in these cells has also been reported [25], however expression of GHR mRNA and protein is relatively low [19,26].

In this study, we investigated the effects of rhGH alone, or in combination with two different concentrations of T4 over JAK and STAT5 activation in fibroblast cultures from prepubertal children with normal stature. Our first approach was to establish the activation level of these mediators on the GH signaling pathway following solely GH stimulation. JAK2 and STAT5 activation was observed in the cytoplasm following 15 and 30 min of rhGH administration, respectively. The information provided by these assays, gave us valuable information which was used for comparison in the co-incubation studies.

Following the GH signaling pathway, we determined the activation of JAK2 after 24 h stimulation with both hormones, either alone or in combination. Our results showed that JAK2 activation, is similar in fibroblasts cultured with rhGH alone, or in combination with the two concentrations of T4. These findings suggest that the activation of JAK2 appears to be independent of the concentrations of T4 used in our study, and appears to be due solely to GH stimulation.

Our next step was to evaluate whether co-incubation with rhGH and T4 had any differential effect over STAT5 activation when compared to incubation with GH alone. The results of these studies indicate that the activation of STAT5 was similar in the non-nuclear fraction of the fibroblasts when they were incubated with rhGH alone, or in combination with any of the two concentrations of T4. This suggests that the activation for STAT5 in the non-nuclear fraction is mostly due to GH.

An important issue for activated STAT5 is related to its translocation to the nucleus, where it acts as a transcription factor for specific genes. Our study documented a different pattern for nuclear STAT5 phosphorylation after co-incubation with rhGH and the higher concentration of T4. At a T4 concentration of 250 nM, nuclear phospho-STAT5 was similar to that observed with rhGH alone showing only significant phosphorylation levels when compared to T4 250 nM alone, which are similar to basal levels. The phosphorylation behavior was different when we increased the T4 concentration to 500 nM in our cultures. Nuclear phospho-STAT5 was significantly higher when compared to incubation with GH alone, and to T4 500 nM alone. This 1.6 fold increase in nuclear phospho-STAT5 compared to GH stimulation, or 3 fold increase compared to basal levels, suggests a synergistic effect of T4 on GH, which was exclusively observed in the presence of higher concentrations of T4.

The mechanisms which may explain the increase in STAT5 nuclear translocation after co-incubation with 200 ng/mL rhGH and 500 nM concentrations of T4 are not known. Both isoforms of STAT5, STAT5a and STAT5b, translocate to the nucleus even in the absence of phosphorylation, and this process may be GH-independent [27]. An important issue is that phospho-STAT5 is dephosphorylated in the nucleus, prior to being recycled to the cytoplasm.

The model proposed by Zeng R *et al.*, (2002) for the nucleo-cytoplasmic transport of STAT5b indicates that in the absence of cytokine-stimulation, the non-phosphorylated momoneric form of STAT5b shuttles continuously between the nucleus and the cytoplasm. This non-phosphorylated form is mainly located in the cytoplasm, so the nuclear export rate may be much faster than the nuclear import

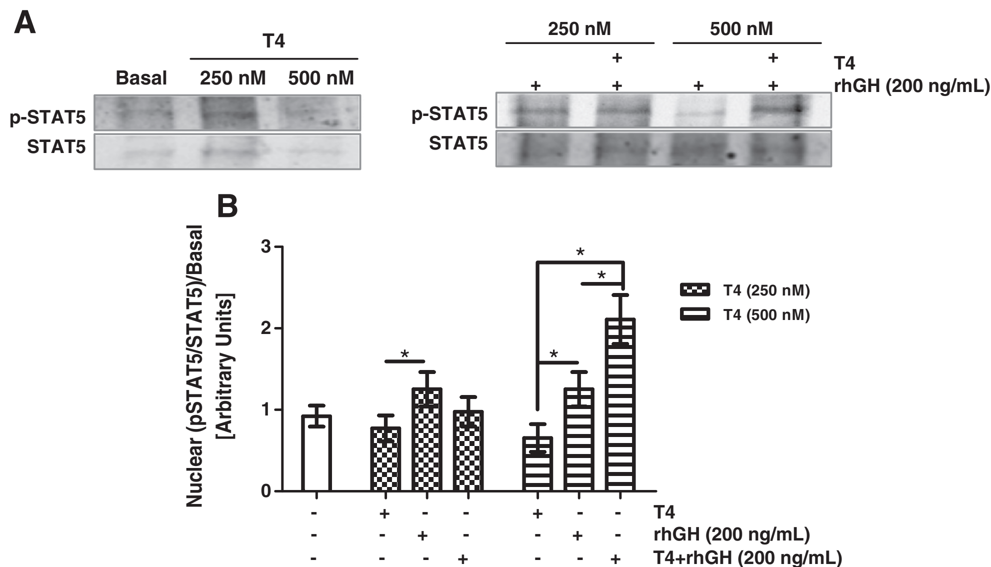

Fig. 4. STAT5 phosphorylation in the nuclear fraction of skin fibroblasts cultures. **(A)** Representative Western immunoblots raised against pSTAT5 and STAT5 in co-incubation studies. **(B)** STAT5 phosphorylation after a 24 h rhGH (200 ng/mL) incubation in the absence and presence of T4 (200 nM and 500 nM). Basal phosphorylation levels (white bar) are also shown. Values are expressed as mean ± SEM. *$p < 0.05$.

rate. Upon stimulation, STAT5 dimerizes and is phosphorylated masking the region responsible for nuclear export, leading to nuclear translocation. After stimulation is terminated, STAT5b dimers are tyrosine dephosphorylated, and the monomers are sent back to the cytoplasm.

Considering that this model explains STAT5 translocation and activation under GH-stimulation, one possible mechanism for T4 signal amplification might be to sustain the dimer in its active-phosphorylated form inside the nucleus. This mechanism might explain the increase in phosphorylated nuclear STAT5 levels that we observed with the higher concentrations of T4.

In conclusion, our results indicate that co-incubation with rhGH and T4 at a concentration of 500 nM during 24 h increases synergistically nuclear phospho-STAT5 in cultured skin fibroblasts from prepubertal children with normal stature. These findings suggest that T4 may enhance GH sensitivity, and may help to explain the beneficial effect of T4 administration on linear growth in short children with hypothyroidism and in some children with idiopathic short stature.

References

[1] E. Cetinkaya, A. Aslan, S. Vidinlisan, G. Ocal, Height improvement by L-thyroxine treatment in subclinical hypothyroidism, Pediatr. Int. 45 (2003) 534–537.
[2] S.M. Shahani, P.K. Varthakavi, P.A. Bhate, P.C. Merchant, K.L. Patel, Hypothyroidism in children/adolescents. Clinical and hormonal profiles, J. Assoc. Physicians India 37 (1989) 566–570.
[3] O. Eyal, S. Blum, R. Mueller, F.O. Smith, S.R. Rose, Improved growth velocity during thyroid hormone therapy in children with Fanconi anemia and borderline thyroid function, Pediatr. Blood Cancer 51 (2008) 652–656.
[4] C. Ohlsson, S. Mohan, K. Sjögren, A. Tivesten, J. Isgaard, O. Isaksson, J.O. Jansson, J. Svensson, The role of liver-derived insulin-like growth factor-I, Endocr. Rev. 30 (2009) 494–535.
[5] Y. Kasukawa, D.J. Baylink, R. Guo, S. Mohan, Evidence that sensitivity to growth hormone (GH) is growth period and tissue type dependent: studies in GH-deficient lit/lit mice, Endocrinology 144 (2003) 3950–3957.
[6] L.A. Norbeck, J.D. Kittilson, M.A. Sheridan, Resolving the growth-promoting and metabolic effects of growth hormone: differential regulation of GH-IGF-I system components, Gen. Comp. Endocrinol. 151 (2007) 332–341.
[7] R. Roman, G. Íñiguez, J.J. Lammoglia, A. Ávila, T. Salazar, F. Cassorla, The IGF-I response to growth hormone is related to body mass index in short children with normal weight, Horm. Res. 72 (2009) 10–14.
[8] K.K. Ho, J. Gibney, G. Johannsson, T. Wolthers, Regulating of growth hormone sensitivity by sex steroids: implications for therapy, Front. Horm. Res. 35 (2006) 115–128.
[9] J.O. Jørgensen, J.J. Christensen, E. Vestergaard, S. Fisker, P. Ovesen, J.S. Christiannsen, Sex steroids and the growth hormone/insulin-like growth factor-I axis in adults, Horm. Res. 64 (Suppl. 2) (2005) 37–40.
[10] G. Guercio, M.A. Rivarola, E. Chaler, M. Maceiras, A. Belgorosky, Relationship between the growth hormone/insulin-like growth factor-I axis, insulin sensitivity, and adrenal androgens in normal prepubertal and pubertal girls, J. Clin. Endocrinol. Metab. 88 (2003) 1389–1393.
[11] R.H. Hackett, Y.D. Wang, S. Sweitzer, G. Feldman, W.I. Wood, A.C. Larner, Mapping of a cytoplasmic domain of the human growth hormone receptor that regulates rates of inactivation of JAK2 and STAT proteins, J. Biol. Chem. 272 (1997) 11128–11132.
[12] S.O. Kim, J. Jiang, W. Yi, G.S. Feng, S.J. Frank, Involvement of the Src homology 2-containing tyrosine phosphatase SHP-2 in growth hormone signaling, J. Biol. Chem. 273 (1998) 2344–2354.
[13] M.R. Stofega, J. Herrington, N. Billestrup, C. Carter-Su, Mutation of the SHP-2 binding site in growth hormone (GH) receptor prolongs GH-promoted tyrosyl phosphorylation of GH receptor, JAK2, and STAT5B, Mol. Endocrinol. 14 (2000) 1338–1350.
[14] J. Iyer, N.C. Reich, Constitutive nuclear import of latent and activated STAT5a by its coiled coil domain, FASEB J. 22 (2008) 391–400.
[15] A. Zamoner, C. Funchal, M.C. Jacques-Silva, C. Gottfried, F.R. Barreto Silva, R. Pessoa-Pureur, Thyroid hormones reorganize the cytoskeleton of glial cells through Gfap phosphorylation and Rhoa-dependent mechanisms, Cell. Mol. Neurobiol. 27 (2007) 845–865.
[16] G. Wongbandue, K. Jewgenow, K. Chatdarong, Effects of thyroxin (T4) and activin A on in vitro growth of preantral follicles in domestic cats, Theriogenology 15 (79) (2013) 824–832.
[17] H. Ishibashi, M.R. Witt, J. Nabekura, M. Nielsen, Modulation of diazepam-insensitive GABA(A) receptors by micromolar concentrations of thyroxine and related compounds in vitro, Brain Res. 15 (1490) (2013) 1–8.
[18] P. Ocaranza, X. Gaete, R. Román, F. Morales, G. Íñiguez, F. Cassorla, Phosphotyrosine phosphatases in GH-stimulated skin fibroblasts from children with idiopathic short stature, J. Pediatr. Endocrinol. Metab. 26 (2013) 833–840.
[19] S.R. Oakes, K.M. Haynes, M.J. Waters, A.C. Herington, Demonstration and localization of growth hormone receptor in human skin and skin fibroblasts, J. Clin. Endocrinol. Metab. 75 (1992) 1368–1373.
[20] J. Freeth, R. Ayling, A. Whatmore, P. Towner, A. Price, M. Norman, P. Clayton, Human skin fibroblasts as a model of growth hormone action in GH receptor-positive Laron's Syndrome, Endocrinology 138 (1997) 55–61.
[21] J.S. Freeth, C.M. Silva, A.J. Whatmore, P.E. Clayton, Activation of the signal transducers and activators of transcription signaling pathway bt growth hormone (GH) in skin fibroblasts from normal and GH binding protein-positive Laron Syndrome children, Endocrinology 139 (1998) 20–28.
[22] J. Martinez Pinto, T. Salazar, P. Ocaranza, A. Fuentes, R. Román, F. Cassorla, Cytoplasmic and nuclear STAT3 in GH-stimulated fibroblasts of children with idiopathic short stature, Horm. Res. Paediatr. 74 (2010) 251–258.
[23] A.P. Rojas-Gil, E. Kostopoulou, I. Karageorgou, K. Kamzelas, B.E. Spiliotis, Increased growth hormone receptor (GHR) degradation due to over-expression of cytokine inducible SH2 domain-containing protein (CIS) as a cause of GH transduction defect (GHTD), J. Pediatr. Endocrinol. Metab. 25 (2012) 897–908.
[24] P. Ocaranza, F. Morales, R. Román, G. Íñiguez, F. Cassorla, Expression of SOCS1, SOCS2, and SOCS3 in growth hormone-stimulated skin fibroblasts from children with idiopathic short stature, J. Pediatr. Endocrinol. Metab. 25 (2012) 273–278.
[25] J.J. Cook, K.M. Haynes, G.A. Werther, Mitogenic effects of growth hormone in cultured human fibroblasts, J. Clin. Invest. 81 (1988) 206–212.
[26] J. Murphy, E. Vrhovsek, L. Lazarus, Identification and characterization of specific growth hormone receptors in cultured human fibroblasts, J. Clin. Endocrinol. Metab. 57 (1983) 1117–1124.
[27] R. Zeng, Y. Aoki, M. Yoshida, K. Arai, S. Watanabe, STAT5B shuttles between cytoplasm and nucleus in a cytokine-dependent and -independent manner, J. Immunol. 168 (2002) 4567–4575.