Available online at www.sciencedirect.com
ScienceDirect
Growth Hormone & IGF Research 18 (2008) 148–156
www.elsevier.com/locate/ghir

Vitamin D3 cannot revert desensitization of growth hormone (GH)-induced STAT5-signaling in GH-overexpressing mice non-calcemic tissues ☆

A.I. Sotelo [a,*], J.G. Miquet [a], L. González [a], A. Bartke [b], D. Turyn [a]

[a] *Instituto de Química y Fisicoquímica Biológicas (UBA-CONICET), Facultad de Farmacia y Bioquímica, Junín 956, C1113AAD, Buenos Aires, Argentina*

[b] *Geriatrics Research, Department of Internal Medicine, School of Medicine, Southern Illinois University, Springfield, IL 62794, USA*

Received 12 February 2007; revised 27 July 2007
Available online 18 September 2007

Abstract

Growth hormone (GH) binding to a membrane receptor dimer triggers multiple intracellular signaling pathways. Signal transducers and activators of transcription are the most relevant of these pathways for GH action. GH also activates several inhibitory mechanisms, particularly suppressors of cytokine signaling (SOCS/CIS) proteins. GH-overexpressing mice exhibit hepatic desensitization of the JAK2/STAT5 GH-signaling pathway, associated with an increased abundance of CIS. Vitamin D3 has been shown to inhibit GH-induced expression of CIS and SOCS-3 and therefore prolong GH signaling in osteoblast-like cells. The purpose of the present study is to determine if vitamin D3 could attenuate CIS expression in GH-overexpressing mice, and consequently allow GH JAK2/STAT5 signaling in GH-responsive tissues in these animals. The abundance of CIS, SOCS-2, SOCS-3, STAT5b and GHR, as well as STAT5b tyrosine phosphorylation after a GH stimulus, were measured in liver and muscle of GHRH-transgenic mice treated with 1α,25-dihydroxyvitamin D3 for 7 days. This treatment did not diminish CIS expression in GH-overexpressing mice tissues, nor did the content of SOCS-2 and SOCS-3 significantly vary. GH-induced STAT5b phosphorylation levels were similar to basal values in transgenic mice liver treated with or without vitamin D; the refractoriness to GH was also present in muscle. Therefore, treatment with vitamin D was not sufficient to revert STAT5 GH signaling desensitization in non-calcemic tissues in GH-overexpressing mice.

Keywords: GH signaling; GH desensitization; Vitamin D; GH; GHR; STAT5; CIS; SOCS

1. Introduction

Growth hormone (GH) is an anabolic hormone that promotes postnatal body growth and bone longitudinal growth, among its most prominent actions. Secreted by the anterior pituitary, GH acts on distal tissues, including bone, liver, and muscle. Some of its actions are directly exerted by the hormone, while others are mediated by IGF-I [1]. GH signaling is initiated by ligand binding to a constitutive receptor dimer leading to the-*trans*-activation of the associated tyrosine kinase JAK2 [2]. Thus activated, the kinase phosphorylates itself and GHR on multiple tyrosine residues, which become docking sites for intracellular signaling mediators. GH promotes phosphorylation of signal transducers and activators of transcription (STATs) and adaptor proteins involved in the activation of mitogen activated

☆ Support for these studies was provided by UBA, CONICET, and ANPCYT (Argentina) to DT and AIS, and by NIH via grant AG 19899 and the Ellison Medical Foundation to AB.
* Corresponding author. Tel.: +54 11 4964 8290/8291; fax: + 54 11 4962 5457.
E-mail address: aisotelo@qb.ffyb.uba.ar (A.I. Sotelo).

1096-6374/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ghir.2007.08.001

protein (MAP) kinase and phosphatidylinositol 3′-kinase (PI-3K) among other signaling components [3,4]. STAT5b, the most relevant GH-mediator, is required for hepatic IGF-I gene transcription [5–8].

The activation of GH signal transduction also triggers negative mechanisms to modulate or terminate GH signaling, including phosphotyrosine phosphatases, suppressors of cytokine signaling (SOCS/CIS) and internalization/degradation of receptor complexes [9–12]. SOCS/CIS proteins are the only inducible inhibitors of the JAK/STAT signaling pathway, acting on a classical negative feedback loop [9,13,14]. Moreover, GH-induced transcriptional activation of SOCS-1, -2 and -3, as well as CIS, is dependent on activation of STAT proteins [8,11,15]. These suppressors also have a SOCS box motif that mediates targeting of associated proteins to proteasome degradation [9,14].

Vitamin D3 is normally synthesized in the skin from a cholesterol-derived precursor as a prohormone. It must then be modified to 25-hydroxyvitamin D3 in the liver and subsequently transformed to 1α,25-dihydroxyvitamin D3 [1α,25-$(OH)_2D_3$] in the kidney, before becoming functionally active [16,17]. Although calcium mobilization is the principal action of vitamin D3, it is also involved in many other functions, such as terminal cell differentiation, modulation of immune response and regulation of gene expression [16,17]. In the osteosarcoma cell line UMR 106, vitamin D3 prolonged GH signaling via the JAK2/STAT5 pathway. This effect was associated with the inhibition of GH induction of the JAK/STATs signaling suppressors SOCS-3 and CIS in cells pre-treated with vitamin D3, suggesting an inhibitory effect of 1α,25-$(OH)_2D_3$ on pathways regulating JAK2/STAT5 signaling in these osteoblast-like cells [18].

The JAK2/STAT5 signaling pathway is desensitized in liver of transgenic mice overexpressing GH. This lack of response to GH was associated with an important increase of CIS hepatic levels [19,20]. The influence of 1α,25-$(OH)_2D_3$ treatment on GH-induced STAT5 phosphorylation and on CIS/SOCS expression in liver and skeletal muscle of GHRH-transgenic mice were studied to investigate the effect of vitamin D3 on GH signaling modulation in GH target tissues other than those related to bone metabolism. GHRH-transgenic mice were used because they exhibit GH signaling desensitization and CIS overexpression, and thus can serve as a model to evaluate relevant changes in GH signaling on non-calcemic tissues. The aim of this study was therefore to assess if treatment with 1α,25-$(OH)_2D_3$ could allow GH signaling resensitization in these GH-overexpressing animals. If 1α,25-$(OH)_2D_3$ were able to reverse the desensitization caused by prolonged treatment with continuous doses of GH, this steroid could also be considered as a coadjuvant for chronic GH therapies other than those restricted to the promotion of longitudinal growth in GH-deficient children.

2. Materials and methods

2.1. Animals

Transgenic Mt-hGHRH animals were derived from animals originally produced by Dr. K. Mayo [21] and kindly provided by Dr. J. Hyde. Adult transgenic mice and their normal siblings were produced by mating hemizygous male carriers of the Mt-hGHRH gene with normal C57BL/6JxC3H/J F_1 females. The mice were housed 3–5 per cage in a room with controlled light (12 h light/day) and temperature (22 ± 2 °C). The animals had free access to food (Lab Diet Formula 5008, containing a minimum of 23% protein, 6.5% fat, and a maximum of 4% fiber; PMI Inc., St. Louis, MO, USA) and tap water. The protocol of these studies complied with applicable laws and regulations and was approved by institutional committee.

3. Hormone treatments

Female animals (3–5-month-old) received vitamin D3 i.p. (2.5 pmol/g body weight) for 7 days in 0.2 mL/100 g BW propyleneglycol or vehicle [22], during the last hour of the light-cycle [23].

Mice were fasted overnight, and injected i.p. with 5 μg of oGH per g of body weight (BW) in normal saline (0.9% NaCl). Control animals were injected with saline to evaluate basal conditions. The animals were killed 7.5 min after injection, and the tissues were removed and frozen in liquid nitrogen.

In order to confirm vitamin D preparation was active, phosphatemia was determined, which increased in normal mice upon treatment. Since transgenic mice present higher basal phosphate levels, these animals did not exhibit a significant rise in phosphate values after treatment with vitamin D. Results were 4.03 ± 0.48 mg/dL vs. 5.69 ± 0.44 for normal (non-transgenic mice) and 6.84 ± 1.03 vs. 7.29 ± 1.18 for GH-overexpressing animals, for vehicle or vitamin D treated mice respectively. Calcemia could not be determined in these samples due to the presence of EDTA used to avoid clotting during blood extraction.

3.1. Protein solubilization of tissues

Tissues were homogenized in 10 volumes of solubilization buffer (1% Triton X-100, 100 mM Hepes pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 0.035 trypsin inhibitory units/mL aprotinin) at 4 °C and then centrifuged at 100,000*g* at 4 °C in a Beckman 90 Ti rotor (Palo Alto, CA, USA) for 50 min to remove insoluble material. Muscle homogenates were incubated 30 min at 4 °C prior