1α,25-Dihydroxyvitamin D₃ Inhibits GH-induced Expression of SOCS-3 and CIS and Prolongs Growth Hormone Signaling via the Janus Kinase (JAK2)/Signal Transducers and Activators of Transcription (STAT5) System in Osteoblast-like Cells

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Orlando Morales‡‡, Malin Hedengran Faulds¶, Urban J. Lindgren‡, and Lars-Arne Haldosen¶¶

From the Departments of Orthopedic Surgery and Medical Nutrition, Karolinska Institutet, Huddinge Hospital, S-141 86 Huddinge, Sweden

Growth hormone (GH) and 1α,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) are regulators of bone growth and bone metabolism. In target cells, GH activates several signaling pathways, among them the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. GH mainly activates JAK2 and STAT5α and β. The effects of 1,25-(OH)₂D₃ are mediated via a nuclear receptor, the vitamin D receptor, which, when bound by 1,25-(OH)₂D₃, activates the transcription of target genes. In earlier studies (Morel, G., Chavassieux, P., Barenton, B., Dubois, P. M., Meunier, P. J., and Boivin, G. (1993) Cell Tissue Res. 273, 279–286) synergetic interaction between 1,25-(OH)₂D₃ and GH regarding expression of osteoblastic markers has been described. The UMR 106 cell line is a rat osteosarcoma cell line with osteoblast-like properties. We have recently shown (Morales, O., Lindgren, U., and Haldosen, L. A. (2000) J. Bone Miner. Res. 15, 2284–2290) that UMR 106 cells express a GH-responsive JAK2/STAT5 signaling system. These cells also express the vitamin D receptor and respond to 1,25-(OH)₂D₃. In the present study we have investigated whether 1,25-(OH)₂D₃ influences GH signaling via the JAK2/STAT5 pathway in UMR 106 cells. We found that 1,25-(OH)₂D₃ prolonged GH signaling via the JAK2/STAT5 pathway. Pretreatment of cells with 1,25-(OH)₂D₃ was also necessary in order to detect GH-induced STAT5 transcriptional response. Furthermore, the pretreatment of cells with 1,25-(OH)₂D₃ rendered the cells the capacity to respond to repetitive GH-stimulation. In UMR 106 cells, GH induced the expression of the JAK/STAT negative regulatory proteins SOCS-3 and CIS. Interestingly, pretreatment with 1,25-(OH)₂D₃ inhibited GH-induced expression of these proteins. From these results we propose that 1,25-(OH)₂D₃ has an inhibitory effect on negative regulatory pathways acting on JAK2 and/or STAT5 in UMR 106 cells and that this, in all or partly, explains the effects of 1,25-(OH)₂D₃ on GH-signaling via the JAK/STAT pathway.

1α,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) is the most active metabolite of vitamin D₃ and is involved in several processes including Ca²⁺ transport, cell differentiation, immunological responses, and the regulation of gene expression (1). The effects of 1,25-(OH)₂D₃ are dependent upon the interaction of 1,25-(OH)₂D₃ with a cytosolic/nuclear receptor, the vitamin D receptor (VDR), followed by the interaction of the steroid receptor complex with selective regions of the promoter of genes, which are either activated or repressed (2). Vitamin D deficiency is associated with rickets in children and osteomalacia in adults. Earlier studies have shown that the effects of 1,25-(OH)₂D₃ on bone are mediated via the osteoblast (2). 1,25-(OH)₂D₃ is involved in differentiation of bone marrow stem cells into osteoclasts and osteoblast-like cells into osteoblasts. Differentiation into osteoclasts is accompanied by changes in biochemical properties of the cells such as, for example increased alkaline phosphatase activity and the increased synthesis of osteocalcin and type 1 collagen. VDR is present in normal osteoblast-like cells, osteosarcoma cells with osteoblast characteristics (2, 3), and osteoclasts (3, 4).

Growth hormone (GH) and insulin-like growth factor (IGF-1) are important regulators of longitudinal bone growth. GH regulates production of IGF-1 both in liver and bone cells. Recent data (5) indicate that the GH-regulated local production of IGF-1 in bone cells determines bone growth. Although many effects of GH on bone growth are mediated by IGF-1, experimental data (6) indicate that GH can directly influence bone cell function.

GH activates several signaling pathways, among them the Janus kinase (JAK)/the signal transducers and activators of transcription (STAT) pathway (7). When GH binds to its receptor (GHR), it induces receptor homodimerization and activation of the GHR-associated tyrosine kinase Janus kinase 2 (JAK2) (7). This leads to the phosphorylation of JAK2 and intracellular proteins, including the GH receptor and the STATs. Upon phosphorylation, the STAT proteins either homodimerize or heterodimerize, translocate to the nucleus, bind to their appropriate DNA response element, and stimulate transcription of GH-regulated genes (8). At present, there are seven known mammalian members of the STAT gene family. GH has been demonstrated to activate STATs 1, 3, 5α, and 5b (7). Signaling

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‡ To whom reprint requests should be addressed. Department of Orthopedic Surgery, Huddinge University Hospital K54, S-141 86 Huddinge, Stockholm, Sweden.

To whom correspondence should be addressed: Department of Medical Nutrition, Karolinska Institutet, Novum, S-141 86 Huddinge, Sweden. Tel.: 46-8-585-83703; Fax: 46-8-711-6659; E-mail: Lars-Arne.Haldosen@mednut.ki.se

1 The abbreviations used are: 1,25-(OH)₂D₃, 1α,25-dihydroxyvitamin D₃; GH, growth hormone; GHR, GH receptor; bGH, bovine GH; IGF-1, insulin-like growth factor; JAK, Janus kinase; STAT, signal transducers and activators of transcription; SH2, Src homology 2; SOCS, suppressors of cytokine signaling; CIS, cytokine-inducible SH2-containing protein; FBS, fetal bovine serum; PBS, phosphate-buffered saline; TBS, Tris-buffered saline; TTBS, TBS plus Tween 20; GEMS, gel electrophoresis mobility shift assay.
pathways involved in the GH and IGF-I effects on bone are, at present, not well understood, although it has been suggested that STAT5 mediates GH effects on bone growth (9, 10). Activation of the GHR by GH initiates also negative regulatory pathways important for termination of GH signaling (11). One negative regulatory pathway may involve tyrosine phosphatases such as SHP-1 and SHP-2, which bind to phosphorylated residues on GHR, JAK2, and STAT5 via their SH2 domains and dephosphorylate and inactivate these molecules. A second negative regulatory pathway requires protein synthesis. GH induces the expression of the suppressors of cytokine signaling (SOCS) and the cytokine-inducible SH2-containing protein (CIS). These proteins act as a negative feedback loop by binding via their SH2 domains to phosphorylated JAK2 or GHR and inhibiting signaling (7).

We and others (12, 13) have recently shown that the osteoblast-like osteosarcoma cell line UMR 106 expresses a GH-responsive JAK2/STAT5 signaling system. In UMR 106 cells, GH activates JAK2 and STAT5a and b. These cells also have shown to express VDR and to respond to 1,25-(OH)2D3 (New England Biolabs, Inc, Beverly, MA; diluted 1:1000), rabbit anti-JAK2 (Upstate Biotechnology, diluted 1:1000), goat anti-STAT5 (Oncogene Research Products, Calbiochem; diluted 1:400), goat anti-SOCS-3, or goat anti-CIS (Santa Cruz Biotechnology; diluted 1:500) in a buffer containing 1% milk protein in TBS plus 0.05% Tween 20 (TTBS). The secondary antibody was a goat anti-rabbit IgG or mouse-antigoat coupled with horseradish peroxidase and diluted 1:5000 in TTBS was applied for 1 h after washing the membrane three times with TTBS. The membrane was then analyzed with the enhanced chemiluminescence method (ECL, Amersham Biosciences).

Preparation of Whole Cell Extract and Immunoprecipitation—Before the addition of bovine growth hormone (bGH), UMR 106 cells were grown in 10-cm plates, starved for 24 h, and thereafter pretreated with 10 nM 1,25-(OH)2D3 for 2, 4, and 6 h. After treatment with bGH, the cells were rinsed with ice-cold PBS. Cells were then scraped into 2 ml of lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 10 mM NaF, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and incubated on ice for 15 min. The supernatant obtained after centrifugation was used as nuclear extract, nuclear extract, or immunoprecipitate, and samples were stored at −80°C.

Preparation of Nuclear Extract—Before the addition of bGH, UMR 106 cells were grown in 10-cm plates,starved of FBS, and thereafter pretreated with 10 nM 1,25-(OH)2D3 for 24, 48, and 72 h. After treatment with bGH, the cells were rinsed with ice-cold PBS. Cells were then scraped into lysis buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 6 mM MgCl2, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and disrupted with a Dounce homogenizer (Göteborgs Termometerfabrik, Gothenburg, Sweden). The nuclear pellet obtained after centrifugation was resuspended and maintained in 3 volumes of extraction buffer (20% glycerol, 20 mM HEPS, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and incubated on ice for 30 min. The supernatant obtained after centrifugation was used as nuclear extract. Protein concentration was measured by the Bradford method.

Preparation of Whole Cell Extract—Before the addition of bGH, UMR 106 cells were grown in 10-cm plates, starved for 24 h, and thereafter pretreated with 10 nM 1,25-(OH)2D3 for 2, 4, and 6 h. After treatment with bGH, the cells were rinsed with ice-cold PBS. Cells were then scraped into 2 ml of lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 10 mM NaF, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and incubated on ice for 15 min. The supernatant obtained after centrifugation was used as whole cell extract. Protein concentration was measured by the Bradford method.

Western Blotting—SDS-solubilizing buffer was added to whole cell extract, nuclear extract, or immunoprecipitate, and samples were boiled. Proteins were separated on 7.5 or 12% SDS-PAGE gels and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA) by semidyed blotting. The membranes were blocked for 1 h with a buffer containing 5% milk protein in TBS. After washing, the membrane was incubated overnight with rabbit anti-phospho-STAT5 (Santa Cruz Biotechnology, diluted 1:1000), rabbit anti-JAK2 (Upstate Biotechnology, diluted 1:1000), goat anti-SOCS-3, or goat anti-CIS (Santa Cruz Biotechnology; diluted 1:500) in a buffer containing 1% milk protein in TBS plus 0.05% Tween 20 (TTBS). The secondary antibody was a goat anti-rabbit IgG or mouse-antigoat coupled with horseradish peroxidase and diluted 1:5000 in TTBS was applied for 1 h after washing the membrane three times with TTBS. The membrane was then analyzed with the enhanced chemiluminescence method (ECL, Amersham Biosciences).

RESULTS

1,25-(OH)2D3 Enhances and Prolongs GH signaling via the JAK2/STAT5 System in UMR 106 Osteoblast-like Cells—UMR 106 cells, grown for 48 h in the absence or presence of 100 nM 1,25-(OH)2D3, were stimulated with 30 nM bGH for 10, 60, or 120 min. Whole cell extracts were immunoprecipitated with anti-JAK2 antibody and the immunoprecipitates were analyzed by Western blotting using anti-phosphotyrosine antibody (Santa Cruz Biotechnology). Protein concentration was measured by the Bradford method.

Preparation of Nuclear Extract—Before the addition of bGH, UMR 106 cells were grown in 10-cm plates, starved of FBS, and thereafter pretreated with 10 nM 1,25-(OH)2D3 for 24, 48, and 72 h. After treatment with bGH, the cells were rinsed with ice-cold PBS. Cells were then scraped into lysis buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 6 mM MgCl2, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and disrupted with a Dounce homogenizer (Göteborgs Termometerfabrik, Gothenburg, Sweden). The nuclear pellet obtained after centrifugation was resuspended and maintained in 3 volumes of extraction buffer (20% glycerol, 20 mM HEPS, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and incubated on ice for 30 min. The supernatant obtained after centrifugation was used as nuclear extract. Protein concentration was measured by the Bradford method.

Preparation of Whole Cell Extract—Before the addition of bGH, UMR 106 cells were grown in 10-cm plates, starved of FBS, and thereafter pretreated with 100 nM 1,25-(OH)2D3 for 24, 48, and 72 h. After treatment with bGH, the cells were rinsed with ice-cold PBS. Cells were then scraped into lysis buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 6 mM MgCl2, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and disrupted with a Dounce homogenizer (Göteborgs Termometerfabrik, Gothenburg, Sweden). The nuclear pellet obtained after centrifugation was resuspended in 3 volumes of extraction buffer (20% glycerol, 20 mM HEPS, pH 7.9, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and incubated on ice for 30 min. The supernatant obtained after centrifugation was used as nuclear extract. Protein concentration was measured by the Bradford method.

Preparation of Whole Cell Extract—Before the addition of bGH, UMR 106 cells were grown in 10-cm plates, starved of FBS, and thereafter pretreated with 10 nM 1,25-(OH)2D3 for 2, 4, and 6 h. After treatment with bGH, the cells were rinsed with ice-cold PBS. Cells were then scraped into lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 10 mM NaF, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 0.1 mM Na3VO4) and incubated on ice for 15 min. The supernatant obtained after centrifugation was used as whole cell extract. Protein concentration was measured by the Bradford method.
The influence of 1,25-(OH)₂D₃ pretreatment on the DNA binding activity of GH-activated STAT5 was analyzed with GEMSA using a radioactive probe containing a STAT5 binding site. Cells were first treated with or without 1,25-(OH)₂D₃ for 24, 48, or 72 h, after which bGH was added for 10 min and nuclear extracts were prepared. GEMSA analysis showed increased GH-induced STAT5 DNA binding activity in cells pretreated with 1,25-(OH)₂D₃ for 24, 48, and 72 h as compared with untreated cells (Fig. 4, compare lanes 1–3 with 4–6). The highest STAT5 DNA binding activity was detected in cells pretreated for 48 h (Fig. 4, lane 5). Next, we studied the time course of GH-induced STAT5 DNA binding activity in cells treated with or without 1,25-(OH)₂D₃ for 48 h. In Fig. 5 it can be seen that pretreatment with 1,25-(OH)₂D₃ significantly prolonged GH-induced STAT5 DNA binding activity. Furthermore, the quantitation of radioactivity with a phosphorimaging device (Fuji) showed 20, 30, and 80% increased STAT5 DNA binding activity in pretreated cells as compared with 1,25-
(OH)$_2$D$_3$-untreated cells, at time points 10 min, 1, and 6 h, respectively (data not shown). To analyze the influence of 1,25-(OH)$_2$D$_3$ on GH-induced STAT5 functional capacity, UMR 106 cells, treated with or without 1,25-(OH)$_2$D$_3$ for 48 h, were transfected with the STAT5-responsive reporter gene at two different concentrations. Cells were then stimulated with 50 or 100 nM GH for 24 h, after which reporter gene activity was analyzed. In 1,25-(OH)$_2$D$_3$-untreated cells, no significant reporter gene activity was evoked by GH stimulation regardless of the concentration of either the reporter gene or GH (Fig. 6). On the other hand, in 1,25-(OH)$_2$D$_3$-pretreated cells, GH clearly increased reporter gene activity (Fig. 6). In conclusion, the above presented data show that 1,25-(OH)$_2$D$_3$ pretreatment of UMR 106 cells resulted in slightly increased and markedly prolonged activation of STAT5 after a single stimulation with GH. Furthermore, this pretreatment was also shown to be necessary for inducing the functional response of GH-activated STAT5.

**Pretreatment with 1,25-(OH)$_2$D$_3$ Renders UMR 106 Osteoblast-like Cells Responsive to Repeated Stimulation with GH**—It is known that the secretory pattern of GH is important for optimum growth (19). Longitudinal bone growth has been shown to be enhanced in animals receiving GH intermittently. This regimen is similar to the secretory pattern of male animals. A constant low level of GH, typical of the female GH secretory pattern, is less efficient in promoting growth. Furthermore, GH given in intervals and with low levels in between peaks also seems to allow target tissues to become responsive to the next GH exposure. This is the opposite of the situation with constant low levels of GH in which target tissues show a decreased response or partial refractoriness to further stimulation of GH (19).

We investigated whether 1,25-(OH)$_2$D$_3$ pretreatment of UMR 106 cells could possibly influence responsiveness to GH when given intermittently. Several dishes of UMR 106 cells were grown in the absence or presence of 1,25-(OH)$_2$D$_3$ for 48 h. Cells were then treated with or without GH. Nuclear extracts were made from two dishes after 10 and 50 min of GH exposure. The remaining cells were washed with PBS (37°C) and incubated in fresh GH-free medium. Nuclear extracts were made at time points 60 min and 2.5 h after washing with PBS. Cells were then stimulated again with GH, and nuclear extracts were made at time points similar to those after the first exposure to GH. After an additional 2.5 h in GH-free medium, the cells were again stimulated with GH, and nuclear extracts were made. Nuclear extracts were then analyzed with GEMSA (Fig. 7, A and B). In 1,25-(OH)$_2$D$_3$-untreated cells, GH-activated STAT5 DNA binding was detectable up to 50 min after the first GH exposure but not 1 h after a PBS wash (Fig. 7A, lanes 2, 3, and 4). Only weakly detectable STAT5 DNA binding activity was seen 10 min after the second GH exposure but not after 50 min (Fig. 7A, lanes 6 and 7). In 1,25-(OH)$_2$D$_3$-untreated cells the third GH impulse did not result in detectable STAT5 DNA binding activity (Fig. 7A, lanes 10 and 11). In cells pretreated with 1,25-(OH)$_2$D$_3$, the first impulse of GH activated STAT5 DNA binding activity. In addition, STAT5 DNA binding activity was still detected 1 h after a PBS wash (Fig. 7B, lanes 2, 3, and 4). Interestingly, in cells exposed for a
B1,25-(OH)2D3 delayed and reduced the GH-induced expression of SOCS-3 and CIS in UMR 106 cells. In conclusion, these results show that pretreatment with 1,25-(OH)2D3 rendered UMR 106 osteoblast-like cells responsive to repeated stimulation with GH.

Treatment of UMR 106 Cells with 1,25-(OH)2D3 Decreases and Delays GH-induced Expression of SOCS-3 and CIS—The magnitude and/or duration of JAK/STAT signaling is influenced by several factors, one of them being negative regulatory proteins, e.g., members of the SOCS family. We investigated whether 1,25-(OH)2D3 could influence the expression of SOCS proteins in UMR 106 cells. Cells grown for 48 h in the absence or presence of 10 nM 1,25-(OH)2D3 were stimulated with 30 nM GH for 2, 4, and 6 h. Whole cell extracts were analyzed by Western blotting using antibodies recognizing SOCS-3 (Socs 3) (A) and CIS (Cis) (B).

In a recent study by Gerland et al. (13) it was shown that GH could activate JAK2 and STAT5, but not STAT5 transcribing activity in UMR 106 cells. They speculated that the expression levels of signaling components probably were too low for GH-induced activation of gene expression. They could show that it was only in STAT5 or GH receptor-transfected UMR 106 cells that GH could induce a transcriptional response. In our study we could detect GH-induced STAT5 DNA binding activity in both untreated and 1,25-(OH)2D3-pretreated UMR 106 cells (Figs. 4 and 5), but it was only in 1,25-(OH)2D3-pretreated cells that GH could induce a functional STAT5 response (Fig. 6). 1,25-(OH)2D3 pretreatment did not increase the expression of JAK2 or STAT5 (Figs. 1B and 2B). Thus, the expression levels of these two proteins cannot explain the effects of 1,25-(OH)2D3 on STAT5 functional capacity.

The only differences from 1,25-(OH)2D3-untreated cells that we could detect in 1,25-(OH)2D3-pretreated cells were a slightly increased and significantly prolonged GH-induced phosphorylation of JAK2 and a slightly increased and significantly prolonged presence of GH-activated STAT5 with DNA binding capacity in the nuclear compartment. The latter could be due to the prolonged influx of activated STAT5 into the nuclear compartment because of extended JAK2 activation. Another explanation could be the decreased nuclear deactivation of STAT5 (i.e., dephosphorylation) either in combination with extended JAK2 activation or as a sole cause. From these results we propose that 1,25-(OH)2D3 has an inhibitory action on negative regulatory pathways acting on JAK2 and STAT5 in UMR 106 cells.

1,25-(OH)2D3 influence on negative regulatory pathways can also be proposed from the second part of our study in which we analyzed the effect of 1,25-(OH)2D3 on the JAK/STAT signaling in UMR 106 cells repetitively stimulated with GH. In rats, mice, and certain other species the growth hormone secretory pattern is sexually dimorphic (20). In adult male rats, plasma GH profiles are characterized by plasma GH pulsing every 1 h (21). Females exhibit more continuous plasma GH levels of about 20–40 ng/ml. Pulsatile GH secretion, which begins at puberty, is more effective than continuous GH in promoting weight gain and longitudinal bone growth (22). The mechanism by which a temporal plasma profile of GH regulates body growth is not well understood. The GH secretory pattern also regulates the sexually dimorphic expression of certain genes in liver (23). Waxman et al. (24) studied the intracellular mediator of the effects of plasma GH pulses on sexually dimorphic liver-expressed genes. Pulsatile but not continuous GH exposure activates liver STAT5b. Disruption of the STAT5b gene leads to an apparent GH pulse insensitivity of liver tissue associated with a loss of male-specific liver gene expression (9). Furthermore, these mice exhibit a decrease in male characteristic pubertal body growth rate. Using a rat hepatocyte-derived cell line, Waxman and co-workers (25) have studied in detail the molecular mechanisms regulating activation/deactivation and repetitive stimulation of JAK2/STAT5 pathways in liver cells. It was shown that a minimum 2.5-h GH-free period was required in between repetitive GH-pulses for STAT5 activation to occur. They could also show that a tyrosine phosphatase and a serine/threonine kinase, both of unknown identity, took part in resetting JAK/STAT signaling for subsequent rounds of GH activation. As described under “Results,” UMR 106 cells, not pretreated with 1,25-(OH)2D3, responded to the first GH pulse but only weakly to the second and not at all to the third GH pulse.
pulse, despite 2.5-h GH-free period in between pulses (Fig. 7A). Thus, the first pulse induced a GH refractory state. This is in contrast to the study of GH-pulsed liver cells described above, wherein cells responded to GH pulses with 2.5-h GH-free periods between pulses. Interestingly, in 1,25-(OH)₂D₃-pretreated UMR 106 cells the second and third GH pulse induced STAT5 DNA binding activity that was lower than after the first pulse but clearly detectable (Fig. 7B).

The GHR/JAK2/STAT5-pathway is negatively regulated by deactivation mechanisms. These systems are activated as GH activates GHR and are important for the termination of GH signaling (7). One negative regulatory pathway involved in GH signaling includes certain members of a family of eight cytokine-inducible proteins named suppressors of cytokine signaling or cytokine-inducible SH2 containing protein (7). GH has, in different experimental systems, been shown to induce the expression of different combinations of SOCS 1–3 and CIS genes, whose products act as a negative feedback loop by binding via their SH2 domains to tyrosine-phosphorylated JAK2 or GHR and thus inhibiting signaling (7). Interestingly, transgenic mice constitutively expressing CIS have reduced body weight (26) and, conversely, mice deficient in SOCS-2 exhibit a giant or high growth phenotype (27, 28). In different studies it has been shown that glucocorticoids strongly inhibit both basal and hormone-induced expression of SOCS-3 (29, 30). We show here that pretreatment with 1,25-(OH)₂D₃, in UMR 106 cells the second and third GH pulse induced STAT5 transcrip
tional activity (11, 31). Thus, it is possible that the down-regulation of SOCS-3 and CIS proteins (Fig. 8, A and B). Both SOCS-3 and CIS have been shown to inhibit or decrease GH activation of STAT5 and STAT5-dependent transcriptional activity (11, 31–33). Thus, it is possible that the down-regulation of SOCS-3 and CIS by 1,25-(OH)₂D₃ renders to UMR 106 cells the capacity to transcriptionally respond to GH and may also explain the prolonged DNA binding activity of STAT5 and the increased responsiveness to GH when given intermittently.

A second negative regulatory pathway is thought to involve tyrosine phosphatases such as SHP-1 and SHP-2, which bind to and dephosphorylate GHR, JAK2, and STAT5. In the case of STAT5, this dephosphorylation has been reported to occur both in the cytosol and in the nucleus (34, 35). Reduced expression or activity of SHP-1 and/or SHP-2 by 1,25-(OH)₂D₃ could be one explanation for its effect on GH-induced JAK2/STAT5 tyrosine phosphorylation. To our knowledge, no report has demonstrated regulated expression of SHP-1 or SHP-2 genes and, thus, these phosphatases seem to be constitutively expressed. Still, it remains to be studied if 1,25-(OH)₂D₃ can inhibit or reduce the expression and/or activity of two these two tyrosine phosphatases.

The physiological connection to our findings is not known at present. In some earlier in vitro cell studies it has been shown that co-administration as compared with separate administration of 1,25-(OH)₂D₃ and GH was more effective in increasing the expression of osteoblastic marker genes (15, 16). If this increased expression of marker genes also was due to 1,25-(OH)₂D₃-enhanced GH signaling via the JAK2/STAT5 pathway remains to be studied. Furthermore, it can also be speculated whether the growth retardation seen in children with vitamin D₃ deficiency is partly due to relative GH refractoriness of bone cells.

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