Differential effects of STAT proteins on growth hormone-mediated IGF-I gene expression

Ben Varco-Merth and Peter Rotwein
Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, Oregon

Submitted 8 July 2014; accepted in final form 9 September 2014

GROWTH HORMONE (GH) and insulin-like growth factor-I (IGF-I) play central roles in multiple physiological processes in humans and other mammals. Both proteins are essential for somatic growth, are major contributors to normal tissue regeneration and repair, and are important regulators of intermediary metabolism (26, 34). Both GH and IGF-I also have been implicated in human aging and in disease, particularly in carcinogenesis (2, 20, 26, 45, 55), indicating that the activity of these proteins must be limited in scope and duration to maintain physiological homeostasis, and raising the need to devise strategies that enhance their positive physiological actions while limiting their negative impact.

GH and IGF-I have been intimately intertwined for nearly 60 years, ever since the somatomedin hypothesis of GH action postulated the existence of a factor [shown later to be IGF-I (11)] responsible for many of the physiological effects of GH (38). Subsequent studies have discerned the biochemical relationship between these two proteins. It is now known that GH, acting via its transmembrane receptor, rapidly stimulates IGF-I gene transcription, leading to sustained production of IGF-I mRNAs and synthesis of IGF-I (35).

Like related cytokine receptors, the GH receptor (GHR) engages the Jak-signal transducer and activator of transcription (Stat) signaling pathway (4). GH binding induces activation of the receptor-associated tyrosine kinase, Jak2 (3), which phosphorylates tyrosine residues on the intracellular part of GHR (26, 54), leading to the recruitment of Stat1, -3, -5a, and -5b, as well as other signaling molecules (26, 54).

The first Stats were characterized over 20 years ago as signaling agents for interferons α/β and γ (41, 42), and subsequent studies have broadened the biological importance of this protein family in physiology and disease (16, 27, 40, 59). Stats are typically found in the cytoplasm before cytokine stimulation and are recruited to phosphorylated tyrosine residues on intracellular segments of activated receptors, where they become phosphorylated on a tyrosine near the Stat COOH-terminus by a receptor-linked tyrosine protein kinase, usually Jak1–3 or Tyk2 (16, 27, 40). After dissociation from the receptor, Stats form dimers via reciprocal interactions of the Src homology 2 domain on one Stat molecule with the phosphotyrosine on another (27) and become translocated into the nucleus, where they bind as dimers to specific DNA sites in chromatin (16, 27, 40, 59). Most Stats recognize the palindromic DNA sequence, 5′-TTCx5-3′ [N is any deoxynucleotide, x = 2–4], but with distinct preferences depending on the individual Stat (12, 27). For Stat1, -3, and -5, x = 3, and their overall binding profiles are very similar (12).

Despite the demonstration that multiple signaling pathways act downstream of the GHR (26, 54), recently identified activating molecular lesions in the human STAT5B gene in individuals with impaired growth (19, 24), targeted knockouts of Stat5b (47, 49, 50) and the GHR in mice (1, 37), and biochemical and molecular studies (55), have defined Stat5b as the essential signaling molecule responsible for many of the critical biological actions of GH. For example, IGF-I gene transcription is rapidly and potently induced by GH via Stat5b in rodents (55), and humans lacking STAT5B have low levels of IGF-I (19). However, unlike several other genes whose transcription is stimulated by GH through Stat5b, in which critical Stat5b-binding sites are located within the proximal promoters (9, 57), there are no Stat5b transcriptional response elements near either of the two IGF1 gene promoters. Rather, several GH-inducible Stat5b-binding domains have been mapped throughout human IGF-I and rat and mouse IGF1 gene loci (7, 53, 56), and some of these elements have been found to possess chromatin properties of transcriptional enhancers (8, 10, 19, 35).

Here we have sought to determine whether other GH-activated Stats can promote IGF-I gene transcription, and thus...
potentially contribute to GH-regulated somatic growth. We find that human STAT5A is remarkably similar to STAT5B in its biochemical and functional responses to GH. In contrast, STAT1 and STAT3 show weaker profiles in vitro binding to Stat DNA elements from the rat Igf1 gene than STAT5B and are less potent than STAT5B in stimulating IGF-I gene transcription through these elements. Taken together, our results offer an explanation for why STAT5B is the key in vivo mediator of GH-activated IGF-I gene transcription and thus GH-regulated somatic growth.

MATERIALS AND METHODS

Materials. Dulbecco’s Modified Eagle’s Medium (DMEM), fetal bovine serum (FBS), and phosphate-buffered saline were purchased from Mediatech-Cellogro (Herndon, VA). Trypsin-EDTA solution was from Invitrogen (Camarillo, CA). Cos-7 cells (ATCC CRL-1651) were obtained from American Type Cell Culture (Manassas, VA), and recombinant rat growth hormone (GH) was from the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD). Restriction enzymes were purchased from New England Biolabs (Ipswich, MA). Protease inhibitor tabs, polymericers, and ligases were from Roche Applied Sciences (Indianapolis, IN); the 660-nm protein assay reagent was from Pierce Biotechnologies (Rockford, IL); Immobilon-FL PVDF immunoblotting membranes were from Millipore (Billerica, MA); and Hoechst nuclear stain was from Polysciences (Warrington, PA). All primary antibodies were purchased from commercial vendors: Flag M2 and α-tubulin, Sigma (St. Louis, MO); Akt, phospho-(p)-tyrosine-100, pSTAT1, and pSTAT3, Cell Signaling (Danvers, MA); STAT1 and STAT3, Transduction Laboratories (Lexington, KY); STAT5A, Santa Cruz Biotecnology (Santa Cruz, CA); STAT5B, Zymed-Life Technologies (Grand Island, NY); and pSTAT5 and CREB, Millipore.

Recombinant plasmids. DNA encoding human STAT1α with an in-frame Flag epitope tag at its COOH-terminus was modified from pRC-CMV-STAT1α-Flag (Addgene, Cambridge, MA) by cloning the insert into pcDNA3 (Invitrogen) via Apal and NotI restriction sites. DNA for human STAT3 with an in-frame Flag epitope tag at its COOH terminus in pcDNA3 was modified from EF-STAT3DN.Ubc.GFP (Addgene). After the STAT3-Flag cDNA was cloned using HindIII sites into pBluescript (Stratagene, La Jolla, CA), the codon for phenylalanine-703 in STAT3DN was altered to wild-type tyrosine using site-directed mutagenesis (via Stratagene Quikchange kit), validated by DNA sequencing at the OHSU DNA Services Core. All other chemicals were reagent grade and were purchased from commercial suppliers.

Cos-7 cells were transiently transfected with expression plasmids for the mouse GHR or chimeras, for Jak2, and for wild-type STAT1, STAT3, STAT5A, or STAT5B. The next day cells were incubated in serum-free medium with 5 μg bovine serum albumin (BSA) for 16–18 h, followed by addition of rat GH (40 nM = 1,000 ng/ml) or vehicle in the same medium for various times, and whole cell and nuclear protein extracts were prepared, as described (52). Protein concentrations were determined with the Pierce A660 protein assay. For promoter-reporter assays, Cos-7 cells in 12-well dishes were cotransfected with different expression plasmids and individual promoter-reporter plasmids, as follows: GHR (75 ng), GHR-Jak2 fusion receptor (75 ng), Jak2 (75 ng), STAT1, STAT5A, or STAT5B (150 ng), STAT3 (75 ng plus 75 ng of pcDNA3), and luciferase reporter plasmids (125 ng). Later (24 h), cells were incubated for 16–18 h in serum-free medium and 1% BSA ± rat GH (40 nM). Cells were then harvested, and lysates were used for luciferase assays (10). Results were normalized to total cellular protein concentrations.

Immunocytochemistry. Cos-7 cells in six-well tissue culture dishes were transiently transfected with expression plasmids for the mouse GH receptor or chimeras, for Jak2, and for wild-type STAT1, STAT3, STAT5A, or STAT5B. The next day cells were incubated in serum-free medium with 2% BSA for 16–18 h, followed by addition of the same medium ± rat GH (40 nM) for 2 h. Cells then were fixed in 4% paraformaldehyde for 15 min at 20°C and permeabilized with a 50:50 mixture of methanol and acetone for 2 min followed by blocking for 2 h at 20°C in 0.25% normal goat serum. After addition of primary antibodies (1:2,000 dilution) in blocking buffer overnight, followed by a washing step, and incubation in Alexa Fluor 488 goat anti-mouse IgG (1:2,000 dilution) in blocking buffer for 2 h, images were obtained with a Nikon Eclipse E800 compound microscope with CCD camera, using Nikon analysis software. Nuclei were stained with Hoechst dye.

DNA-protein binding studies. Electrophoretic gel-mobility shift assays and DNA competition experiments were performed as described (10, 52) with Cos-7 nuclear protein extracts (2 μg of protein) and Cy5.5-labeled double-stranded oligonucleotides (5–10 nM). Sequences for the top strand for all double-stranded DNA probes used are listed in Table 1 and, except for the high-affinity SIE element (14), have been described previously (52), as has the preparation of double-stranded probe DNA (10). After incubation of proteins and DNA for 60 min at 4°C, products were separated by electrophoresis through non-denaturing 5% polyacrylamide gels in 1× Tris borate/EDTA (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.3) at 160 volts for 40 min at 4°C. After electrophoresis, bands representing protein-bound DNA and free probe were visualized using the LiCoR Odyssey and version 3.0 analysis software.
Table 1. DNA sequences of oligonucleotide probes

<table>
<thead>
<tr>
<th>Probe</th>
<th>Top Strand (5’ to 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td>CACCAATTATGTGAAATTAAAC</td>
</tr>
<tr>
<td>R3</td>
<td>AAAATATTTTTTGGATTAAGCA</td>
</tr>
<tr>
<td>R4</td>
<td>GAAAGATCTTGTCTCAGAATTTGCTGTTTC</td>
</tr>
<tr>
<td>R8</td>
<td>AGTCGAGGAACACTGAAGTCTG</td>
</tr>
<tr>
<td>R9</td>
<td>GTCCTTTTTTTTTTTTGAAAGTAGAGTTAAA</td>
</tr>
<tr>
<td>R13</td>
<td>CTTCCCTCTTGGAAACTC</td>
</tr>
<tr>
<td>R34</td>
<td>GCCCGCTTCTGGAGAAAAGAG</td>
</tr>
<tr>
<td>R35</td>
<td>TCTGCTCTCTGAAATGAGAA</td>
</tr>
<tr>
<td>R53</td>
<td>TGATCTCTTTGACAATGTAAC</td>
</tr>
<tr>
<td>R54</td>
<td>GAATTATTTGCATCTGAAACAT</td>
</tr>
<tr>
<td>R57</td>
<td>AAGTTTTCTCGAAATGGGAA</td>
</tr>
<tr>
<td>R58</td>
<td>TCCAGTTCCTGAGAAAGGA</td>
</tr>
<tr>
<td>R59</td>
<td>GGAAAATTCCGAAAGTGAG</td>
</tr>
<tr>
<td>R60</td>
<td>CCAATCTCTCTGAAAGATGT</td>
</tr>
<tr>
<td>R61</td>
<td>CATAGGTTCAGAAGAGAG</td>
</tr>
<tr>
<td>SIE</td>
<td>TGGACATCTTTCTGGATTAAC</td>
</tr>
</tbody>
</table>

Core signal transducer and activator of transcription-binding sites are in italics. SIE, c-sis-inducible element.

Data analysis. Paired and unpaired Student’s t-tests were performed using Graphpad Prism 5.0 with Bonferroni corrections used for multiple comparisons.

RESULTS

GH promotes the tyrosine phosphorylation, nuclear translocation, and transcriptional activity of human STAT1, -3, and -5B. Prior studies have shown that ligand binding to the GHR leads to the activation of multiple intracellular signaling pathways, including those mediated by several Stats in various tissues and cultured cell lines (5, 14, 15, 29, 43). We have previously reconstituted GH-regulated signaling in Cos-7 cells transiently expressing the mouse GHR and rat Stat5b and have used this approach to dissect the functional and biochemical features of seven distinct regions in the rat Igf1 locus that exhibited acute GH-stimulated binding of Stat5b in hepatic chromatin (10, 52). Here we use a similar experimental design to assess the properties of human STAT1, STAT3, STAT5A, and STAT5B as mediators of GH action. Coexpression of mouse GHR and STAT5B in Cos-7 cells led to its acute GH-mediated tyrosine phosphorylation (Fig. 1A). In contrast, GH-stimulated tyrosine phosphorylation of STAT1 or STAT3 was not observed, despite similar levels of production of all three Stats (Fig. 1A). However, coexpression of mouse Jak2 was able to promote GH-activated tyrosine phosphorylation of STAT5B, even though the Jak2 kinase domain was fused to the proximal intracellular part

Fig. 1. Distinct pathways for activation by growth hormone (GH) of signal transducer and activator of transcription (STAT) 5B and STAT1 and -3. A: detection of tyrosine phosphorylated (p) STAT1, -3, and -5, the Flag epitope, and α-tubulin by immunoblotting in whole cell protein extracts of Cos-7 cells after transfection of expression plasmids for the mouse GH receptor (GHR) and/or Jak2, as indicated, and either human STAT1, STAT3, or STAT5B, and treatment with recombinant rat GH (40 nM) for 60 min. STAT1, -3, and -5B are all Flag tagged. B: immunocytochemistry of Cos-7 cells transfected with expression plasmids for mouse GHR, mouse Jak2, and either human STAT1, STAT3, or STAT5B, and treatment ± GH (40 nM) for 120 min. Nuclei were stained with Hoescht dye (blue).
of the rabbit GHR (58). When coexpressed with individual STATs into Cos-7 cells, the GHR-Jak2 kinase fusion receptor promoted the robust tyrosine phosphorylation of STAT1, -3, and -5B in the absence of GH (Fig. 3A) and stimulated their DNA-binding activity toward multiple double-stranded probes, as measured by in vitro electrophoretic mobility shift experiments. However, as assessed by competition studies, for most probes tested, the relative DNA-binding affinities of STAT1 and STAT3 were lower than STAT5B (Fig. 3, B and C). In contrast, a chimeric GHR containing the pseudokinase domain of Jak2 was inactive (Fig. 3B).

When tested in reporter gene assays, the GHR-Jak2 kinase fusion receptor potently stimulated the ability of STAT1 and -3 to enhance the transcriptional activity of pGAS-TA-Luc compared with GHR plus Jak2 and GH treatment, but had little additional effect on STAT5B (compare Fig. 4 with Fig. 2B). The chimeric receptor also increased the activity of STAT1 and STAT3 toward other reporter genes containing Igf1 promoter 2, including those encoding Stat5b-binding elements R2–4, R34–35, R57–59, R60–61, and 2× R34–35 (Fig. 4). For several of these luciferase reporter genes (R2–4, R57–59, R60–61), the transcriptional potency of STAT1 or STAT3 was equivalent to that of STAT5B (Fig. 4). Under the same experimental conditions, the GHR-Jak2 pseudokinase domain fusion receptor was ineffective in all cases (Fig. 4). Taken together, the results in Figs. 3 and 4 suggest that, under the right conditions of sustained activation, STAT1 or STAT3 could possibly stimulate IGF-I gene transcription.

STAT5A and STAT5B are comparable mediators of GH actions. STAT5A and -5B are the most similar of the seven Stats (16, 27). The human proteins are ~96% identical in amino acid sequence and appear to be interchangeable in a number of biological contexts (16). To test the ability of STAT5A to be activated by GH, we cotransfected it with mouse GHR in Cos-7 cells. Following addition of GH, STAT5A was as rapidly and potently tyrosine phosphorylated as was STAT5B (Fig. 5A), was detected in the nucleus to an equivalent degree (Fig. 5, B and C), and was able to bind similarly to several double-stranded DNA probes, as assessed by in vitro electrophoretic mobility shift assays (Fig. 5D). STAT5A also mediated the robust activation of several reporter genes containing rat Igf1 promoter 2 and individual Stat-binding elements from the rat Igf1 gene, with results being identical to those seen with STAT5B (Fig. 5E). Thus, STAT5A appears to be as potent a mediator of specific biological effects of GH as STAT5B.

**DISCUSSION**

The identification of a number of natural mutations in STAT5B in humans with short stature associated with reduced serum IGF-I levels has established that STAT5B is a critical mediator of somatic growth via the GH-IGF-I axis (19, 24). In parallel, biochemical studies have demonstrated that the hormone-bound GH receptor activates STAT5B via Jak2 (43, 58), a key receptor-associated tyrosine kinase (26, 54), leading to induction of IGF-I gene transcription (55, 56), and molecular experiments have shown that STAT5B is both necessary and sufficient for GH-regulated IGF-I gene activation in vivo (55). Unlike other GH- and STAT5B-dependent genes, IGF-I does not contain any STAT5B response elements in its promoters (9, 10), but, rather, as shown by studies evaluating rat and mouse Igf1 chromatin, it appears that hormone-activated IGF-I gene transcription occurs through recruitment of multiple distinct GH-regulated STAT5B-binding elements with characteristics of long-range transcriptional enhancers (10). Here we have sought to determine whether other GH-activated STATs can induce IGF-I gene expression, and thus potentially contribute to the biological effects of GH. Using a combination of biochemical and functional studies, we find that human STAT5A and STAT5B comparably activate GH-dependent IGF-I gene transcription, and thus postulate that
STAT5A could potentially substitute for STAT5B in mediating GH-regulated somatic growth if it were expressed at levels similar to STAT5B in GH-responsive tissues. In contrast, STAT1 and STAT3 are less potent than either STAT5A or STAT5B in stimulating reporter gene activity through STAT DNA elements from the rat Igf1 gene, in part because they show an overall weaker profile of in vitro binding to these DNA segments than STAT5B. Taken together, our results offer a molecular explanation for why STAT5B is a key in vivo mediator of GH-activated IGF-I gene transcription and thus GH-regulated somatic growth.

**GHR, Jak2, and STAT activation.** Previous experiments in which intracellular domains of the GHR were functionally dissected have suggested that tyrosine residues found in the COOH-terminal part of the receptor are needed for maximal GH-stimulated activation of STAT5B in cultured cells (43–45), and analogous gene replacement studies in mice have shown that the same distal segment of the GHR mediates maximal GH-induced tyrosine phosphorylation of Stat5b in the liver and is required to promote normal somatic growth (37). In contrast, more membrane-proximal regions of the GHR were found to mediate maximal hormone-induced activation of STAT1 and -3 (43–45), with Jak2 in all cases being the essential tyrosine kinase for these biological actions of the GHR (43).

Additional cell-based studies, using the same chimeric GHR-Jak2 kinase receptors employed here, had found previously that the fusion receptor is a weaker activator of STAT1 and -3 and a far weaker inducer of STAT5B than the intact GHR (58). Our data support a different conclusion, since we find that the chimeric receptor is at least as potent as GH-activated GHR plus Jak2 in stimulating tyrosine phosphorylation of STAT1 and -3, and far more potent in promoting STAT1- and STAT3-dependent gene activation. We also show that the chimeric GHR-Jak2 kinase receptor is a strong stimulator of STAT5B. Possible reasons for the substantial divergence between our results and those of Yi et al. (58) may include technical differences in experimental design, possible distinctions between STATs from different species [we used human STATs, Yi et al. (58) studied mouse Stats], although individual members of this family are highly conserved among mammals (27, 40), or other more subtle differences. Nevertheless, our data support the idea that the GHR, acting in concert with Jak2, functions as a multivalent signaling transducer, and that multiple STATs can participate in the biological effects of GH.

**STAT DNA binding and transcriptional activity.** The seven mammalian STATs serve as mediators for overlapping but distinguishable cytokine and growth factor signaling pathways (27, 40). In addition to preferences for individual receptors and Jaks (27, 40), STATs also have been found to have distinct DNA-binding profiles (12). Although all STATs recognize the palindromic double-stranded DNA sequence, 5’-TTCN3-3’ [top strand shown, where N is any deoxynucleotide, and $x = 2–4$ (27, 40)], individual STAT proteins have dramatically different affinities for the same DNA segment, as defined by results of both biochemical and genomic studies (12, 17, 18, 22, 23, 28, 33, 39, 60, 61). For example, in vitro binding-site selection experiments found small differences between the overall preferences of STAT1 and STAT5 (12). Both proteins recognized DNA sequences of the type, 5’-TTCN1/N2/N3GAA-3’ (top strand), with STAT1 highly favoring and STAT5 moderately preferring a C in the $N_1$ position (12) [STAT5A and STAT5B had identical

![Fig. 3. Distinct DNA-binding preferences of STAT1, -3, and -5B are maintained after maximal activation by a GH receptor-Jak2 kinase fusion protein.](http://ajpendo.physiology.org/DownloadedFrom/10.1152/ajpendo.00324.2014)
binding profiles (12)). In contrast, STAT6 strongly preferred 5′-TTCNN\textsubscript{1}N\textsubscript{2}N\textsubscript{3}N\textsubscript{4}GAA-3′ (top strand) (12). More recent genomic analyses of STAT DNA binding in chromatin, using ChIP-DNA sequencing approaches, revealed at most minimal differences among STAT1, STAT3, and STAT5 (17, 18, 22, 23, 28, 33, 39, 60, 61), with slightly higher statistical preferences for STAT1 and STAT3 to bind to DNA with a C in the N\textsubscript{1} and a G in the N\textsubscript{3} position (17, 18, 22, 28, 33, 39). Importantly, none of these studies have identified a way to accurately predict which DNA elements would bind STAT1 or -3 rather than STAT5A or -5B.

Our results, which have examined the STAT-binding elements identified by ChIP within the rat IGF1 gene (10), agree with these published data, since they do not reveal any clear preferences for specific types of DNA sequences that distinguish among STAT1, -3, or -5B, either by direct in vitro binding studies or indirectly through promoter-reporter gene assays. Moreover, we find at best a modest correlation between in vitro binding affinity of a specific STAT for a DNA sequence and transcriptional potency in cells. For example, after activation by the chimeric GHR-Jak2 receptor, STAT1 and STAT3 were as effective as STAT5B in stimulating the transcriptional activity of a reporter gene containing rat IGF1 promoter 2 fused to either R2–4, R34–35, or R57–59, or R60–61 STAT DNA elements, despite the lower in vitro DNA-binding affinity of STAT1 and STAT3 compared with STAT5B for each STAT site within each of the three elements. Similarly, STAT5B was more potent than STAT1 or STAT3 in promoting transcription via the R34–35 element, despite nearly identical binding by all three proteins to R34 DNA (although binding was weaker for STAT1 or STAT3 than STAT5B at R35).

Identical GH-induced activity of human STAT5A and STAT5B. STAT5A and STAT5B are highly conserved genes that are adjacent to each other on human chromosome 17, and are 96% identical in their amino acid sequences (16). We find that the ligand-bound GHR promotes a nearly superimposable activation of both proteins in cotransfected Cos-7 cells, leading to comparable high-affinity binding to the same DNA elements, and nearly identical transcriptional activation of reporter genes containing rat IGF1 promoter 2 and any of six different Stat-binding elements from the rat IGF1 gene. Our results extend previous cell-based studies that examined the biochemical features of rat Stat5a and Stat5b and their potential roles as mediators of GH actions (44), and parallel other published data illustrating that STAT5A and STAT5B play comparable signaling roles for other cytokines (6, 16, 47). Our observations further suggest that hormones, such as prolactin and leptin, which activate multiple STATs, including STAT5A (13, 21, 25, 31), could promote IGF-I gene transcription under specific circumstances in selected tissues.

Perspective. GH and IGF-I are pivotal agents in human physiology and disease, and a key scientific and medical challenge is to develop strategies that enhance their positive biological actions on growth, metabolism, and tissue repair (26, 34, 36) while limiting their negative effects on aging and cancer (2, 20, 32, 46). This will require a fuller understanding of the fundamental mechanisms by which GH controls IGF-I biosynthesis. Here we have defined some of the biochemical features that distinguish how STAT5B (or potentially STAT5A) can function as a potent mediator of GH-induced IGF-I gene transcription while STAT1 or STAT3 cannot. Further studies employing chromatin-based transcriptional systems and other experimental models should help pinpoint the unique aspects of STAT5B that are responsible for its critical and distinctive role in the biology of GH and IGF-I.
Fig. 5. Comparable activation by GH and similar transcriptional activity profiles for STAT5A and STAT5B. A: immunoblotting of Cos-7 whole cell protein extracts for pSTAT5, STAT5A, STAT5B, the Flag epitope, and α-tubulin illustrate comparable stimulation of tyrosine phosphorylation of STAT5A and STAT5B at different times after exposure of cells to GH (40 nM). Both STAT5A and STAT5B are Flag tagged. B: immunoblotting of Cos-7 nuclear protein extracts for the Flag epitope, pSTAT5, and CREB reveal similar nuclear accumulation of tyrosine-phosphorylated STAT5A and STAT5B after incubation of cells with GH (40 nM) for 60 min. The pTyr and pSTAT5 bands comigrated with each other, and with the bands detected with the anti-Flag antibody. C: comparable nuclear accumulation of tyrosine-phosphorylated STAT5A and STAT5B as revealed by immunocytochemistry of Cos-7 cells transfected with expression plasmids for GHR and either STAT5A or STAT5B, and treated with GH (40 nM) for 120 min. The nuclei were stained with Hoescht dye (blue). D: analogous DNA-protein binding of STAT5A and STAT5B as assessed by gel-mobility shift experiments using Cy5.5-labeled double-stranded oligonucleotides for R3 or R60 (see Table 1 for DNA sequences), and 2 μg of nuclear protein from Cos-7 cells transfected with expression plasmids for GHR and either STAT5A or STAT5B, and incubated ± rat GH (40 nM) for 60 min. The arrow indicates a protein-DNA complex. E: results of luciferase assays in Cos-7 cells transiently transfected with expression plasmids for GHR and either STAT5A or STAT5B, and reporter plasmids containing rat Igf1 promoter and exon 2 without (—) or with individual Stat-binding elements. Cells were incubated with vehicle or rat GH (40 nM) for 18 h. The graph depicts results of 4 independent experiments performed in duplicate for each promoter plasmid cotransfected with STAT5A or STAT5B, and treated ± GH. Except where indicated, all reporter gene results were statistically significant when comparing cells incubated with GH with those treated with vehicle (P < 0.05 to P < 0.0001, paired t-test). There was no difference in results between cells expressing STAT5A or STAT5B. Raw luciferase values for the Igf1 promoter 2 reporter plasmid ± GH ranged from 250 to 1,000 light units/10 s.

ACKNOWLEDGMENTS

We appreciate the advice and assistance from our colleagues throughout the course of these studies and thank Drs. Stuart Frank, Vivian Hwa, and Olga Timofeeva for gifts of reagents.

Current address for P. Rotwein: Texas Tech University Health Sciences Center at El Paso, Department of Biomedical Sciences, Medical Sciences Building I, Rm. 1012, 5001 El Paso Drive, El Paso, TX 79905 (e-mail: peter.rotwein@ttuhsc.edu).

GRANTS

These studies were supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-069703 (to P. Rotwein).


4. E854 GH, STATS, AND IGF-I

5. Gronowski AM, Rotwein P. 15.


7. Daughaday WH, Rotwein P. 56.


13. In vivo growth hormone treatment rapidly stimulates the kinase JAK2 by the growth hormone receptor (Abstract).

14. Interpretation of cytokine signaling


17. In vivo targeting

18. Interpretation of cytokine signaling


43. Smith JS, Meyer DJ, Billestrup N, Norstedt G, Schwartz J, Carter-Su C. The role of the growth hormone (GH) receptor and JAK1 and JAK2 kinases in the activation of Stats 1, 3, and 5 by GH. Mol Endocrinol 10: 519–533, 1996.


