Signaling pathways activated by the growth hormone receptor

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In recent years, significant progress has been made in elucidating the signaling pathways activated by the growth hormone (GH) receptor. An initiating event is probably the activation of JAK2 (Janus kinase 2), a GH receptor-associated tyrosine kinase. Identification of the proteins recruited to the GH receptor-JAK2 complex and dissection of the signaling pathways that are subsequently activated will ultimately provide a basis for understanding GH action at the molecular level.

Growth hormone (GH), secreted by the anterior pituitary into the circulation, binds to membrane receptors in target tissues to stimulate body growth. Children who lack GH have short stature and GH therapy given before puberty increases their rate of growth and ultimate height. Animal studies further support an important role for GH in the promotion of growth. In addition to promoting growth, GH has important metabolic actions. In humans and other animals, exogenous GH decreases fat and increases lean body mass. New research continues to reveal other potential roles of GH, including regulation of cardiac and immune function, mental agility and aging.

The therapeutic use of GH continues to expand. In addition to being used to promote growth of short-statured children, GH is used in the USA for the treatment of adult GH deficiency and for human immunodeficiency virus-associated wasting. Potential future uses of GH include suppression of chronic hypercatabolism, acceleration of healing in pediatric burn patients and treatment of some forms of infertility. However, GH has also been linked to negative activities. In two large European studies, increased mortality was detected when critically ill patients with acute catabolism were placed on GH therapy. In addition, GH promotes insulin resistance and has been associated with retinal neovascularization and nephropathy, two debilitating complications of diabetes. As the therapeutic use of GH increases and as we become more cognizant of potential negative side effects of GH therapy, the need for a more complete understanding of GH receptor signaling becomes necessary. This review will highlight some of the recent advances made towards this goal.

The GH receptor

The cloning of the GH receptor in 1987 opened the door to the study of GH signaling at the molecular level. The cDNA for the human GH receptor encodes a 638 amino acid protein with single extracellular, transmembrane and cytoplasmic domains. Initially, the sequence of the GH receptor provided no clues as to how the receptor signals because the sequence bore no homology to receptors with known signaling mechanisms. However, the recognition that numerous receptors, including the GH receptor, have limited amino acid homology in a region of the extracellular domain prompted the classification of a new receptor superfamily, the cytokine/hematopoietin receptor superfamily. In addition to the GH receptor, members of this family include receptors for more than 25 ligands, including receptors for prolactin, leptin, erythropoietin, multiple interleukins (ILs), and the more distantly related receptors for interferon α, β and γ. In addition to homology in the extracellular domain, cytokine superfamily receptors contain one or more proline-rich motifs (termed ‘box 1’) in the membrane-proximal region of the cytoplasmic domain.

Determination of the structure of GH bound to the extracellular domain of the GH receptor has led to the model that a single GH molecule binds to two molecules of the GH receptor (Fig. 1). GH binding to the GH receptor–GH receptor complex is thought to be sequential. The initial step is high-affinity binding of GH to one GH receptor. A different face of GH then contacts the second GH receptor, stabilizing the GH receptor dimer. GH binding to a dimer of GH receptors is thought to be an initial and crucial event in GH signaling. Interestingly, the erythropoietin receptor, also a cytokine receptor, appears to exist as a preformed dimer and ligand binding induces conformational changes in both the extracellular and cytoplasmic domains. A conformational change in the extracellular domain of the GH receptor is also thought to be important for signaling. However, comparison of the structures of GH bound to a single GH receptor extracellular domain with the structure of GH bound to a dimer of receptors suggests that dimerization does not produce major conformational changes in the extracellular domain. At present, we know virtually nothing about the structure of the cytoplasmic domain of the GH receptor.

GH activates JAK2 tyrosine kinase

A clue to the mystery of the signaling mechanism of the GH receptor came with the finding that GH promotes the phosphorylation of tyrosines in the receptor and other cellular proteins. Furthermore, tyrosine kinase activity capable of phosphorylating...
JAK2 in GH receptor signal transduction

Many GH-activated signaling pathways involve JAK2, which is a member of the JAK family of tyrosine kinases. These pathways include the Ras–MAP (mitogen-activated protein) kinase pathway. GH activates this pathway probably by JAK2 phosphorylation of the protein SHC (Ref. 9). The Ras–MAP kinase pathway has been implicated in GH regulation of the fos gene by ternary complex factors (TCFs), which act in concert with the serum response factor (Ref. 21).

The list of transcription factors implicated in GH regulation of gene transcription continues to grow. Recent additions include CCAAT/enhancer-binding protein β (C/EBPβ), C/EBPδ (Ref. 22), YY1, glucocorticoid receptor (Ref. 23), TCF factors Elk-1 and Sap-1a (Refs 21, 24), activating transcription factor (ATF)-2 and C/EBP homologous protein (CHOP) (Ref. 25), hepatocyte nuclear factor 1α (HNF-1α) (Ref. 26) and HNF-4 (Ref. 27). This list will undoubtedly grow ever more rapidly as new technologies are applied to the discovery of GH-regulated genes (Refs 20, 28).

The signal transducers and activators of transcription (STATs) family of transcription factors are key molecules in this process (Fig. 2). GH-activated JAK2 phosphorylates at least four members of this family (STATs 1, 3, 5A and 5B), leading to their dimerization, nuclear localization, DNA binding and activation of transcription (Ref. 17). Targeted disruption of the Stat5a and Stat5b genes in mice produces defects in liver gene expression and body growth, consistent with an important role of these STATs in GH action (Refs 18, 19). A second pathway that is important for GH regulation of gene transcription is the Ras–MAP (mitogen-activated protein) kinase pathway. GH activates this pathway probably by JAK2 phosphorylation of the protein SHC (Ref. 9). The Ras–MAP kinase pathway has been implicated in GH regulation of the fos gene by ternary complex factors (TCFs), which act in concert with the serum response factor (Ref. 21).

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An emerging theme from the work on GH regulation of transcription is that the coordinated activation of multiple transcription factors and signaling pathways will probably be required for the physiological regulation of genes by GH (Refs 20, 30).

**Fig. 1.** Model of GH activation of JAK2 tyrosine kinase. GH binding to two GH receptors increases the affinity of each receptor for JAK2. The two receptor-associated JAK2 molecules are in close proximity, so that each JAK2 can phosphorylate the activating tyrosine of the other JAK2 molecule (blue arrows), thereby activating it. Activated JAK2 then phosphorylates itself (red arrow) and the cytoplasmic domain of the GH receptor (purple arrows) on tyrosines. These phosphotyrosines within the GH receptor and JAK2 form binding sites for signaling proteins.

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; JAK2, Janus kinase 2; P, phosphate.
transport, lipogenesis and protein synthesis. Hence, GH and insulin might activate some common signaling pathways. Indeed, GH stimulates phosphorylation of the insulin receptor substrates 1, 2 and 3 (IRS-1, -2 and -3) (Fig. 2). Tyrosine phosphorylation of IRS proteins by JAK2 provides a binding site for the SH2 domain of the 85-kDa regulatory subunit of phosphatidylinositol (PI) 3′-kinase. Although PI 3′-kinase is required for insulin-stimulated glucose transport, GH-induced glucose transport has been reported not to require PI 3′-kinase. However, inhibition of PI 3′-kinase blocks GH-stimulated lipid synthesis and the anti-lipolytic action of GH (Ref. 33), suggesting that PI 3′-kinase activity is important for the insulin-like action of GH.

GH reduction of body fat involves both decreased lipogenesis and increased lipolysis. The signaling mechanisms by which GH regulates these processes are beginning to be explored. GH appears to inhibit adipocyte differentiation at a step before induction of genes required for terminal differentiation, such as the gene encoding peroxisome proliferator activated receptor γ (PPARγ) (Ref. 34). One signaling event that might be important in this process is GH activation of the cAMP-specific phosphodiesterase PDE4A5 by a PI 3′-kinase-dependent mechanism. GH and glucocorticoid stimulation of lipolysis is thought to involve an inhibitory action on the G protein G protein, thereby increasing cAMP accumulation and lipolysis. An involvement of STAT5 proteins in the lipolytic action of GH has also been reported.

The signaling pathways involved in GH stimulation of protein synthesis remain largely unknown. Specific tyrosines in the cytoplasmic domain of the GH receptor are required for GH stimulation of protein synthesis, suggesting that recruitment of molecules to the GH receptor is involved. GH is known to stimulate the activity of p70 S6 kinase, an enzyme thought to regulate translational activity.

Chronically elevated GH is anti-insulin-like, promoting insulin resistance and diabetes. GH is thought to have this effect at least in part by interfering with the ability of insulin to stimulate carbohydrate metabolism. Potential underlying mechanisms for this action of GH are emerging. Decreased insulin receptor, IRS-1 and IRS-2 tyrosyl phosphorylation in response to insulin have been reported in rodent models of chronic GH excess. In contrast, GH excess can lead to chronic activation of the IRS–PI 3′-kinase pathway in liver, reducing the degree of insulin-induced activation. In addition, GH inhibits the expression of the gene encoding glucose transporter 1 (GLUT1). The relative contributions of these events to the GH-induced insulin resistance seen in humans and other animals remains to be determined.

**GH regulation of the cytoskeleton**

Given that GH is a positive regulator of cell growth and differentiation, and these processes require changes in cell shape and/or location, it is not surprising that GH stimulates cell motility and spreading. The cytoskeleton is intimately involved in the regulation of cell morphology and movement. GH is known to stimulate actin rearrangement and microtubule polymerization. GH is reported to stimulate the assembly of a multiprotein complex including JAK2, focal adhesion kinase, paxillin, tensin and several other proteins involved in cell adhesion and/or movement. A dominant negative form of the JAK2-binding protein SH2-B blocks GH-induced membrane ruffling and overexpression of wild-type SH2-B enhances it, implicating SH2-B in GH regulation of the actin cytoskeleton. Exactly how activated JAK2 transmits a signal to the cytoskeleton remains unclear.

**Regulation of GH receptor–JAK2 signaling**

The pattern of GH secretion into the bloodstream is thought to be an important factor in determining sex-specific gene regulation and body growth rate. Thus, the intensity and kinetics of signals emanating from the GH receptor–JAK2 complexes is expected to shape the action of GH. Recent studies have revealed that the activity of the GH receptor–JAK2 complex can be regulated at several levels.

**SOCS**

A family of cytokine-inducible genes, termed suppressors of cytokine signaling or SOCS, are important in the regulation of GH receptor–JAK2 signaling. Of the eight known members of the SOCS family, GH induces expression of...
Fig. 3. Regulation of GH receptor–JAK2 signaling. SH2-B enhances GH receptor signaling by increasing the activity of JAK2. GH-induced expression of SOCS proteins inhibits further GH signaling by decreasing the activity of JAK2. Tyrosine phosphatases, such as SHP-2, might also contribute to inhibiting GH receptor signaling by dephosphorylating tyrosines in the GH receptor and/or JAK2. Abbreviations: GH, growth hormone; GHR, growth hormone receptor; JAK2, Janus kinase 2; P, phosphate; SHP-2, src homology 2 domain-containing protein tyrosine phosphatase 2; SOCS, suppressor of cytokine signaling.

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SOCS-1, -2, -3 and CIS in rat liver to varying degrees and with different kinetics53–55. How SOCS transcription is induced by GH is not fully understood, but STAT proteins are thought to be involved56,57.

SOCS proteins are thought to inhibit signaling by inhibiting the kinase activity of JAKs. SOCS-1 appears to inhibit JAK2 kinase activity by binding to JAK2 directly56,58. SOCS-1 inhibition of JAK2 activity involves interactions between SOCS-1 and the kinase activation loop of JAK2 (Ref. 58). It is thought that a region N-terminal to the SH2 domain of SOCS-1 acts as an inhibitory pseudosubstrate of JAK2 (Refs 58,59). Consistent with a direct action of SOCS-1 on JAK2 activity, SOCS-1 inhibits tyrosyl phosphorylation of overexpressed JAK2 even when the GH receptor is not present55,56. CIS and SOCS-3, two SOCS proteins that are prominently induced in liver by GH (Refs 53–55), appear to require the GH receptor to inhibit JAK2 (Refs 55,60). Thus, CIS and SOCS-3 might gain access to JAK2 by first binding to the GH receptor. Transgenic mice constitutively expressing CIS have reduced body weight61, presumably because of decreased GH-induced activation of JAK2. SOCS-2 might also have a role in GH signaling. Mice deficient in SOCS-2 are giant, suggesting that SOCS-2 might be important for terminating signaling by GH or IGF-1 (Ref. 62).

Phosphatases
In addition to SOCS proteins, termination of GH-activated STAT signaling is thought to involve activation and/or recruitment of one or more protein tyrosine phosphatases to GH receptor–JAK2 signaling complexes. Dephosphorylation of the GH receptor would terminate recruitment of signaling molecules to the complex and might also signal receptor internalization and degradation56. Dephosphorylation of the crucial activating tyrosine within the kinase domain of JAK2 would be expected to deactivate JAK2. The phosphatase(s) responsible are unknown. Two candidate phosphatases are the SH2 domain-containing phosphatases SHP-1 and SHP-2. SHP-1 and SHP-2 have been implicated in dephosphorylation of tyrosines on JAK2 or the GH receptor64–68. Other phosphatases are also probably involved63,69.

SH2-B
In addition to negative regulation by SOCS proteins and phosphatases, GH signaling can be positively regulated. Activated JAK2 recruits the SH2 domain-containing protein SH2-B (Ref. 70). SH2-B increases the kinase activity of JAK2, leading to enhanced activation of downstream signaling proteins such as STAT5b (Ref. 71). Enhancement of JAK2 activity by SH2-B might be an important mechanism for boosting GH receptor–JAK2 complex signaling.

Conclusions
Our current model of GH signaling includes GH binding to two GH receptors followed by activation of JAK2 kinase. JAK2 then autophosphorylates and phosphorylates the GH receptor, providing binding sites for signaling molecules and leading to the activation of multiple pathways that control gene transcription and metabolism. Studies on tissues isolated from animals treated with GH have confirmed the physiological nature of many of these signaling events53,72. Identification of the entire repertoire of signaling pathways and genes regulated by GH and understanding how the activity of GH receptor–JAK2 complexes is regulated in physiological contexts will undoubtedly reveal new insights into the mechanism of action and therapeutic potential of GH.

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Endothelial dysfunction in endocrine disease

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In addition to diabetes mellitus and obesity, acromegaly, Cushing’s syndrome, hypopituitarism, hyper- and hypothyroidism, hyperparathyroidism and polycystic ovary syndrome are associated with either increased mortality from, or increased prevalence of, cardiovascular disease (CVD). Recently, endothelial dysfunction has been identified as an early marker of CVD and has been shown to predict future coronary artery disease, before atherosclerotic changes appear in arteries. Thus, measurement of endothelial function might identify at-risk individuals early and be a useful means of assessing response to treatment aimed at reducing long-term morbidity and/or mortality from CVD. Such studies are being undertaken in hypopituitarism and other endocrinopathies, and are reviewed herein. Endothelial function in large vessels can be measured noninvasively by ultrasound measurement of flow-mediated endothelium-dependent dilation (FMD). Serum markers of endothelial function, such as von Willebrand’s factor, thrombomodulin, E-selectin and intercellular adhesion molecule 1, could be increased and be useful for evaluation of treatment, because they correlate inversely with FMD.

Atherosclerosis is not only a major cause of morbidity and mortality in the general population but its prevalence is significantly increased in certain endocrine disorders. Although atherosclerosis is primarily a disease of large conduit arteries, it also causes functional changes in the microcirculation. Major cell types contributing to atherosclerosis are endothelial cells, vascular smooth muscle cells (VSMC), fibroblasts, white blood cells (particularly monocytes and neutrophils) and platelets. The endothelium is of primary importance because of its strategic location between the blood and underlying smooth muscle. Furthermore, atherosclerosis primarily involves the intima, the compartment immediately beneath the endothelium, whose cells play a major role in regulating the composition of the intima.

The endothelium is highly active metabolically and plays a key role in vascular homeostasis through the release of a variety of autocrine and paracrine substances. The healthy endothelium, particularly endothelium-derived nitric oxide (NO), not only modulates the tone of the underlying vascular smooth muscle but also inhibits several proatherogenic processes, including monocyte and platelet adhesion, oxidation of low density lipoproteins, synthesis of inflammatory cytokines, smooth muscle proliferation and migration, and platelet aggregation, thus exhibiting important anti-atherogenic effects. It also has an antithrombotic and fibrinolytic function. The key mediator of these functions is NO (reviewed in Ref. 2). Endothelial cell dysfunction is the initiating event in the development of atherosclerosis and assessment of endothelial function by different methods has emerged as a tool for detection of evidence of preclinical cardiovascular disease (CVD).

Several endocrine diseases are known to be associated with increased incidence or prevalence of cardiovascular or cerebro-vascular morbidity or mortality, namely diabetes mellitus (DM), obesity,