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Reviews

Growth hormone signal transduction

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Abstract. Growth hormone (GH) promotes animal growth by stimulating bone and cartilage cell proliferation, and influences carbohydrate and lipid metabolism. Some of these effects are brought about indirectly via somatomedin induction in hepatocytes, others by acting directly on the target cells. In either case, GH first binds to specific receptors on cells to trigger a sequence of biochemical events culminating in a biological response. Recently much has been learnt about the molecular structure of GH receptor, its binding to ligand, and the ensuing signal transduction events.
Key words. Growth hormone; signal transduction; membrane receptor; cytokine receptors; inositol phosphates; diacylglycerol; primary response genes.

Introduction

Growth hormone (GH) is a peptide hormone produced by a specialized subset of pituitary cells known as acidophil cells. Despite its unique source GH is multifunctional, apparently influencing almost all tissue types including the immune cells. In addition to sustaining normal postnatal animal growth by promoting bone elongation, GH also influences carbohydrate and lipid metabolism. The lack of suitable in vitro models to study the direct effects of GH on target cells led in part to the 'somatomedin hypothesis' according to which GH acts on liver cells to stimulate the release of insulin-like growth factor 1 (IGF-1) which in turn is involved in bone and cartilage growth.

In recent years direct effects of GH on growth and differentiation of cartilage, bone and adipose cells and the presence of high affinity GH receptors (GHRs) in them have been demonstrated. However, reports that GH also induces IGF-1 in the above cells[23] have made it harder to understand the mechanisms of GH actions on different cell types. The establishment of in vitro models to study GH effects, and the cloning and in vitro transcription of GHR genes, combined with the recent use of recombinant GH in animal production[44] have sparked renewed interest in the mechanism of GH actions. Several reports on the early cellular and molecular events that follow GH-binding to its receptor have appeared. In the present article we have tried to unify these data on GH-signal transduction to understand the mechanisms underlying the multifunctionality of GH.

Structure of growth hormone

The human growth hormone (hGH) is a single chain polypeptide of about 191 amino acids, with two disulfide bridges spanning 110 and 6 residues respectively. It has a molec. wt of 22 K daltons and has no carbohydrate or prosthetic groups. Studies on secondary and tertiary structures have indicated it to have 4 helical bundles. The NH_2- and COOH-terminal helices (I and IV) are larger than the other two (II and III). In addition to these 4 helices in the core, 3 much shorter helices are found in the connecting loop between the major helices. Some of these minor helices might represent conformational changes of hGH occurring due to receptor binding, since these minor helices are not observed in porcine GH in the unbound state[22].

The biological effects of various fragments of GH on target cells in vivo and in vitro have been investigated. A fragment consisting of residues 5–117, containing a single aminoethyl cysteine, exhibits most of the biological properties of intact GH, except skeletal growth promotion. A 37 residue tryptic fragment obtained from the large disulfide loop of bovine GH displays growth-promoting activity and hence might include the active core of GH[1].

In addition to the commonly known 22 K GH, a 20 K variant hGH has been reported. It is 15 residues shorter than the normal peptide and retains a substantial growth-promoting activity but lacks insulin-like effect[43]. This 20 K GH variant molecule constitutes about 10–15% of human pituitary GH, but its physiological role is not known. Some smaller length human GH molecules with enhanced biological activities have also been observed. These are thought to be the proteolytic products of normal GH. Other variants include dimers and oligomers of GH and even the product of a second GH gene[53].

Structure of the growth hormone receptor

The molec. wt of rabbit liver GH receptor has been estimated to be 50–80 K[65,66,69,70] whereas receptors

from rat hepatocytes and adipocytes and human IM-9 lymphocytes are ~110 K[3,12,24]. A putative 130 K form of GHR purified from rabbit liver, gives rise to 50–60 K molecules on proteolysis, indicating that the 50–80 K rabbit liver receptor molecule is a proteolytic cleavage product of the whole receptor[42]. Ubiquitin, which copurifies with the liver GHR, seems to be covalently linked to it. Association of ubiquitin seems a common feature of cell-surface receptors because the PDGF receptor and the lymphocyte homing receptor are also ubiquitin-associated[54]. Ubiquitin is said to function as an intracellular relay for extracellular signals by its association with specific receptors[62]. Though experimental evidence is lacking, ubiquitin targetting might promote rapid turnover and degradation of GHR in vivo leading to receptor regulation and second messenger generation and thus signal transduction[42].

The amino acid (AA) sequence studies on cDNA clones[42] indicate that the hGHR consists of 620 residues in addition to an 18-residue signal peptide. At the amino terminal, it has 246 residues representing the extracellular ligand-binding domain, followed by a 24-residue hydrophobic sequence, possibly the transmembrane domain. This is followed by a 350-residue sequence at the carboxyl terminal, representing the cytoplasmic domain that might be involved in the signal transduction process. 8 asparagine residues (5 in the extracellular and 3 in the cytoplasmic domain respectively) have been identified as potential glycosylation sites. GHR in vivo exists in glycosylated form because purified GHR from rabbit liver and IM-9 cells upon treatment with glycosidases shows a reduced molec. wt[3]. Glycosylation is a common feature of several growth factor receptors and is considered important for intracellular routing, proper insertion into the plasma membrane and turnover of the receptors. Inhibition of glycosylation by tunicamycin reduces receptor number on the membrane without affecting their ligand-binding capacity[25]. In contrast, in rat adipocytes and human lymphocyte IM-9, tunicamycin reduces the GHR affinity and in the latter cell type the number of GHR expressed on the membrane is also affected[16,39]. Furthermore, glycosylation is thought to be essential for GH binding and for normal rates of internalization and processing of bound GH[62].

GH receptor belongs to the cytokine receptor superfamily

On the basis of sequence analysis, the receptor for GH was first recognized to have considerable homology to the prolactin (Prl) receptor[11,42]. These two hormones are closely related and can even bind to each other's receptors. Later GHR was found to be a prototype of a new class of transmembrane receptors consisting of several cytokine receptors (CKR) including receptors for interleukin- (IL-) 2, 3, 4, 6 and 7, erythropoietin (EPO), granulocyte and granulocyte macrophage colony-stimulating factors (GM-CSF) and ciliary neurotrophic factor (CNTF) (fig. 1)[6,15,32].

Like the well-known receptor tyrosine kinases[67,72] the members of the CKR superfamily are organized into 3 domains comprising an extracellular ligand-binding domain, a single hydrophobic transmembrane domain, and an intracellular domain whose function is yet to be delineated. Extensive homology is observed between the extracellular domain of the GHR and that of the other members of the CKR superfamily. Within a stretch of 210 AA, four cysteine residues are present in the N-terminal half of this domain. This is a common feature of all the members of the CKR family except that of IL-7. Comparison of AA sequence by an Align program has shown a general homology of 15–24% between GHR and other members of this family and about 35% homology between GHR and PrlR. However, the WSXWS (Trp-Ser-X-Trp-Ser) motif commonly found in the extracellular domain 18–22 AAs upstream to the transmembrane domain of almost all the CKRs is not found in either the human or rabbit GHR. The functional roles of the cysteine residues and the WSXWS motif are not yet known. Despite extensive homologies the extracellular and transmembrane domains in the CKR superfamily (see below), several sequence and structural differences exist in the intracellular domain. The lengths of the intracellular domains range from 54 (GM-CSFR) to 568 AA (human IL-4 receptor). An extreme case seems to be the CNTFR, in which the cytoplasmic domain is completely absent[20]. The hydrophobic domain of this receptor is not in the usual site and does not function as transmembrane domain. Its carboxyl terminus does not possess a stop-transfer signal nor is followed by an intracytoplasmic domain. However, some limited sequence similarities are seen between the cytoplasmic domains of the CKR (except that of CNTFR). Whether such sequence similarities reflect a common signal transduction mechanism is to be explored. Even receptors having sequence homology at the extracellular domain need not always use similar signal transduction mechanisms. The PDGF and IL-1 receptors both have immunoglobulin-like extracellular domains, but the former has an intrinsic tyrosine kinase activity at the cytoplasmic domain and the latter has not (fig. 1)[55,71].

Heterogeneity of the GH receptor

The existence of structural heterogeneity of GHR has been indicated by several lines of evidence based on the functional diversity of these receptors[65,69] and different classes of GHR seem to be associated with the different actions of GH[30]. DNA sequence studies indicate considerable sequence divergence at the 5′ region of the GHR cDNA clones. Among the 9 clones (6 human and 3 rabbit) studied by Leung et al.[42], 5 were unique and

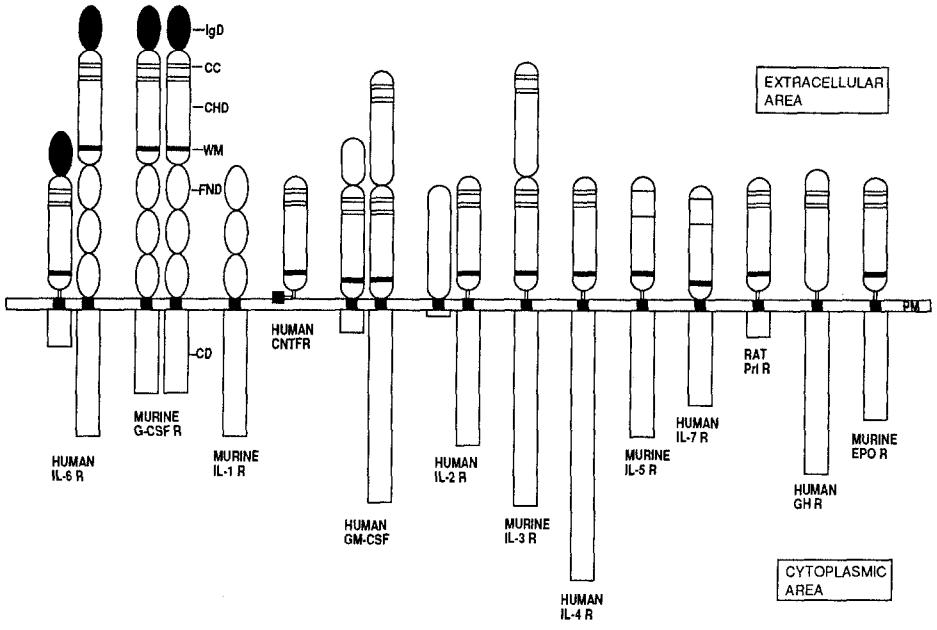

Figure 1. The cytokine receptor superfamily. The structural similarities between the members of the cytokine receptor (CKR) superfamily are schematically presented to show the CKR homology domain CHD (which is common to all the receptors of the family except IL-IR), immunoglobulin-like domain (IgD), and the fibronectin-like domain (FND). The conserved cysteines (CC) and the WSXWS motif (WM) are also represented. Note the absence of WM domain in the GH receptor. PM, plasma membrane; CD, cytoplasmic domain. Names of the receptors are abbreviated as in the text. The scheme models are based on reference 40.

their sequences diverged at the same point. Probably these clones were derived from multiple RNAs, differently spliced at the 5′ end. Thus, differential RNA splicing could be one of the processes leading to the heterogeneity of GHR. The synthesis of truncated receptors containing only the extracellular GH-binding domain, the hydrophobic transmembrane domain and a small (8 AA) cytoplasmic domain, has been proposed[42]. Certainly such a protein is likely to show altered intracellular signalling. We do know that the truncated Epidermal Growth Factor receptor-like protein is a well-known oncogene product *erb*-B and a truncated form of the human EPO receptor (product of alternatively spliced mRNA) exhibits signal transduction capacity, but differs functionally from its normal counterpart[16,46]. Thus molecular divergences of GHR cDNA sequences could be the major source of the structural and functional heterogeneity of this receptor[5,65], contributing to the functional specificity of different forms of GH[43,69].

GH binding leads to receptor dimerization

Though early studies reported the availability of a single receptor binding site in hGH[18], later work demonstrated the presence of 2 binding sites in the hGH molecule and indicated that hGH dimerizes its receptor through a sequential binding mechanism[17] (fig. 2). First the hormone-receptor complex is formed at site 1 of hGH and then an additional receptor molecule binds to site 2. This is demonstrated by the fact that, in spite of the existence of the site 1 intermediate (hGH.hGHR1), no site 2 complex intermediate (hGH.hGHR2) has been observed. In addition the 2nd receptor can bind to the GH only if the 1st receptor is already bound.

Receptor dimerization is thought to be important in the signal transduction processes of several growth factors and hormones[14,52]. Monoclonal antibodies (Mabs), which oligomerize the rat Prl receptor, induce proliferation of rat lymphoma NL2 cells in culture[26] and promote weight gain in vivo. Mabs against human and bovine GH enhance in vivo GH actions such as growth promotion, cartilage sulphation and IGF-1 synthesis[4,69]. We suggest that the GH-Mab complex causes the above GH potentiation by facilitating the receptor dimerization rather than by improving GH half-life. Furthermore, hGH mutants which cannot induce receptor dimerization are biologically inactive[17]. These observations indicate that receptor dimerization is an essential feature of GH signal transduction. In the case of dimeric ligands like PDGF it is easy to understand the dimerization of a single chain receptor. But the two molecules of GHR use the same receptor residues to recognize two unrelated and distinctive epitopes on the ligand. This unusual formation of an hGH-GHR complex indicates an efficient way for monomeric ligands to use signal-producing receptor dimerization and thus presents a new model for ligand receptor binding[33].

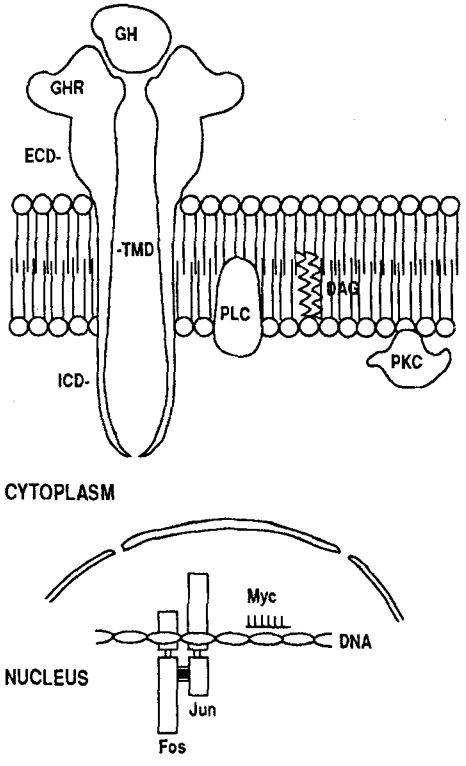

Figure 2. GH signal transduction pathway. GH brings about receptor dimerization by binding first to the extracellular domain (ECD) of one receptor molecule and then to that of another receptor molecule. The signal of GH-binding is probably transmitted via the transmembrane domain (TMD) to bring about conformational changes in the intracellular domain (ICD). Following this, several early biochemical events have been recorded including phospholipase C (PLC)-mediated DAG production and protein kinase C (PKC)-induced phosphorylation of the GHR and possibly some cellular proteins. Induction of *fos*, *jun* and *myc* genes are also observed. (Abbreviations are as in the text).

Receptor dimerization might lead to conformational changes in the cytoplasmic domain

The signal of GH binding and receptor dimerization needs to be conveyed within the cell. The transmembrane and intracellular domains of the growth factor receptors are known to play a key role in this. Unfortunately little is known of these processes in GHR or other members of the CKR family, though the importance of the transmembrane domain of the EPOR in the signal transduction process has recently been established[74]. Ligand binding alters the conformation of the intracellular domain, creating sites amenable to tyrosine kinase phosphorylation in receptors with kinase domains[67,72]. Other structural changes essential for signal transduction might equally be produced at the intracellular region of GHR. Receptor dimerization might be instrumental in both the above processes. These possibilities could be tested by introducing suitable mutations into the transmembrane domain that might change its flexibility. Furthermore, conformational changes in the cytoplasmic portion of GHR upon GH binding could be analyzed using antibodies directed at peptides from different regions of GHR. Complexes of GH-GHR from GH-activated cells could be prepared in non-denaturing conditions and their responses to antibody binding could be studied in comparison with receptors from nonactivated cells[21].

Early events in GH signal transduction

When GH binds to its receptor, it rapidly activates a set of early responses including phosphorylation of the cytoplasmic region of the receptor itself, production of diacylglycerol (DAG) through phospholipase C action, induction of some primary response genes like c-*fos*, c-*jun*, *jun*-B and c-*myc*, and internalization and down-regulation of the receptor. These events ultimately lead to the synthesis of DNA in proliferating cells or the synthesis of specific functional and structural proteins in differentiating cells. What are the important early responses in GH signal transduction and how are they interrelated?

GHR has no kinase activity but it activates phosphorylation

The GHR does not possess an intrinsic tyrosine kinase (TRK) domain and accordingly no TRK activity is found in GHR isolated from IM-9 and rat liver cells[39]. However in murine 3T3-F442A fibroblasts which differentiate into adipocytes, GHR undergoes ligand-promoted tyrosine phosphorylation. Probably GH-GHR binding causes the activation of a cellular TRK which phosphorylates the tyrosyl residue on the receptor[29]. We do not know whether this tyrosyl phosphorylation is involved in GH-induced cellular growth, differentiation and metabolism. But a role for protein kinase C (PKC) activity in the GH-induced differentiated cell function has been demonstrated in fresh preparations of rat adipocytes[60]. Pretreatment of these cells with phorbol ester PMA, a known stimulator of lipogenesis through PKC activation, inhibits GH-induced lipogenesis. This might mean that GH-induced lipogenesis is also mediated through PKC activation and that down-regulation of PKC by the phorbol ester renders the GH ineffective. An essential role of PKC in GH signal transduction process has been confirmed in mouse osteoblasts by studying the GH-induced expression of some primary response proto-oncogenes like c-*fos*, c-*jun* and *jun*-B[13,60]. Protein synthesis is not needed for the activation of these proto-oncogenes in the osteoblasts[58,59], but Ca^{+2}/calmodulin-independent PKC activity is essential because very low concentrations of PKC inhibitors, H7 (reference 3b) and staurosporine[64], suppress the above proto-oncogene activation. As a wide spectrum of PKC are affected by these inhibitors, the specific types involved could not be defined. The serine residues of GHR in the above cells are also phosphorylated. Since a regulatory role for serine phosphorylation has

earlier been suggested for insulin receptors[10,47], such a role in GHR is also possible.

Diacylglycerol (DAG) is important in early response gene activation

Induction of DAG is assumed to play an important role in the GH signal transduction process. In preadipocyte ob1771 cells prostaglandin F2α induces the receptor-mediated hydrolysis of inositol phospholipids to yield inositol phosphates (IPs) and DAG. However, in the same cells GH-treatment is followed by formation of DAG but no IPs are detectable, implying that DAG in this case is produced in response to a phospholipase-C action[8] that does not involve IPs.

Osteoblasts also produce DAG but no IPs in response to GH[58,59,60]. Hence it is evident that in the above cases DAG is generated from sources other than inositol phospholipid. Though several other candidates[9,41,48] could be suggested, the exact source of DAG in GH signal transduction needs to be investigated. In addition, it is also worth studying the above phenomenon in other members of the CKR family.

Induction of primary response genes

Early in the signal transduction process of several growth factors, some primary response genes are activated; most of their products are nuclear transcription-related factors or DNA-binding proteins which are also proto-oncogenes[35]. Some primary response genes so far studied in GH signal transduction process are the proto-oncogenes: c-*fos*, c-*jun*, *jun*-B and c-*myc*.

The c-*fos* and c-*jun* proto-oncogenes are expressed at low basal levels in most cell types. Upon diverse extracellular stimuli, these genes are rapidly and transiently induced. Fos (the protein product of c-*fos*) is a nuclear phosphoprotein that forms stable heterodimeric complexes with Jun (the product of c-*jun*), contributing to the DNA-binding activity associated with the regulatory AP-1 site. Thus c-*fos* and c-*jun* are implicated in the transcriptional regulation of several genes containing the AP-1 binding site. Moreover, several Fos-related proteins, such as Fra-1 and Fos-B, and Jun-related proteins like Jun-B and -D, are thought to form homo- or heterodimeric complexes in various combinations to give transcriptional selectivity when binding to the DNA sequence (see ref. 19). The c-*myc* gene, which codes for a labile nuclear phosphoprotein, is thought to influence cell proliferation by modulating transcription and by affecting the initiation of DNA replication directly (table)[49].

In quiescent ob1771 and 3T3-F442A preadipocytes GH induces c-*fos* expression within 15 min[23,31]. In the latter cell type the GH-induction occurs in the complete absence of other hormones and serum factors. The c-*jun* gene is also induced by GH, but with a slightly delayed time course compared to that for c-*fos*. Though the above cells do synthesize IGF-1, the induction of c-*fos* and c-*jun* appears to be a direct effect of GH because it is rapid and cycloheximide-resistant[31]. Furthermore, inhibition of IGF-1 induction hardly affects the activation of the above primary response genes. Similarly, in mouse osteoblasts GH activates c-*fos*, c-*jun* and *jun*-B independent of protein synthesis[60]. In addition, the binding of the Fos-Jun complex to TPA-responsive element recognition-sequence increases in the nuclear extracts of osteoblasts exposed to GH. The Fos-Jun complex is known to be involved in the regulation of aP2 gene transcription in 3T3-F442A cells[34]. Rapid induction of these proto-oncogenes might be an important early step in GH signal transduction, where Fos might function as a nuclear switch linking the early events associated with GH-GHR binding to late events such as DNA synthesis and gene expression. GH-induced cell proliferation also involves early induction of c-*myc* gene as demonstrated in rat liver, kidney and lymphoid cells (fig. 2)[7,45].

Early effects of GH on preadipocytes

Time after GH addition	Cellular response
>15 min	Tyrosine phosphorylation of GHR and possibly other proteins[29]
>15 min	DAG production[13,28,60]
5–15 min	Proto-oncogene (c-*fos*, c-*jun* and *jun*-D) activation[23,31]
3–48 h	c-*myc* induction[7,45] IGF-1 induction[8]
8–18 h	aP2 and glycerol-3-phosphate dehydrogenase synthesis[34]

Concluding remarks and summary

The recent reports on several features of GHR structure and its ligand binding have provided insight into the molecular mechanisms of GH signal transduction.

– 1) The GHR has been recognized as a member of the CKR superfamily based on its amino acid sequence and structural homology to other family members including receptors for IL-2, 3, 4, 6 and 7, EPO, GM-CSF and CNTF. These receptors generally have a high homology between their extracellular domains and a relatively high homology in the transmembrane and cytoplasmic domains, suggesting involvement of common signal transduction mechanisms[15,32].

– 2) The structural and functional heterogeneity of GHR within a given cell type or among different cell types might arise by differential splicing of the mRNA[42], in addition to other processes like posttranslational modifications. Receptor heterogeneity could be one of the mechanisms of differential signal transduction underlying different biological activities of GH.

– 3) In spite of a single receptor-binding site in GH predicted by the AA sequence analysis, crystallographic studies have revealed two binding sites which induce

receptor dimerization in a sequential fashion[17]. It is also evident that receptor dimerization might generate conformational changes in the cytoplasmic domain, an essential feature of signal transduction.

– 4) Several early responses of the target cells following GH treatment have been identified. Though GHR itself has no kinase-like sequences, its cytoplasmic domain undergoes PKC-activated phosphorylation. More important, PKC-mediated phosphorylation is essential for GH-effected lipogenesis in adipocytes[2] and activation of proto-oncogenes in adipocytes and osteoblasts[13, 28, 58, 59]. However, the types of PKC and substrate proteins involved in these phosphorylation processes and how they transduce the GH signal are yet to be defined.

– 5) Production of DAG through phospholipase-C action has been shown to be essential in GH cellular actions[58, 59]. However, since no production of inositol phosphates has been detected in the above system[23], the source of DAG needs to be clarified.

– 6) Rapid and transient activation of nuclear transcription factors like c-*fos*, c-*jun*, *jun*-D occurs in adipocytes, osteoblasts and other cell types exposed to GH[23, 58, 59, 60]. The induction of these oncogenes has been identified as part of the signal transduction process in cellular activation by several growth factors[35] and is considered to be the nuclear switch that links ligand-receptor binding to later events such as gene expression[19].

Future studies on GHR should provide additional insights into the molecular mechanism of signal transduction not only in cytokine receptor superfamily but also by receptors of peptide hormones and growth factors in general.

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