Mechanism of Growth Hormone Action : Recent Developments - A Review

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ABSTRACT : The interaction of growth hormone with it's receptor results in dimerization of receptor, a feature known in action of certain cytokines. The interaction results in generation of number of signalling molecules. The involvement of Janus kinases, mitogen activated kinases, signal transduction and activator of transcription proteins, insulin like substrate, phosphatidylinositol 3-kinase, phospholipase C, protein kinase C is almost established in growth hormone action. There are still many missing links in explaining diversified activities of growth hormone. Amino acid sequence data for growth hormones and growth hormone receptors from a number of species have proved useful in understanding species specific effects of growth hormone. Complete understanding of growth hormone action can have implications in designing drugs for obtaining desired effects of growth hormone. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 12 : 1785-1793)*

Key Words : Growth Hormone, Somatotropin, Signalling Molecules, Growth Hormone Receptor

INTRODUCTION

Similar to other polypeptide hormone, growth hormone (GH) binds to it's receptor in cell membrane of target tissues. However, the observation that one molecule of GH binds to two molecules of growth hormone receptor (GHR) resulting in GHR dimerization, a common feature in action of certain cytokines, has created enormous interest to understand how GH acts. Further GH is known to exert diversified activities and this has put new challenges in understanding mechanism of GH action. In recent years, a beginning has been made to understand whole gamut of GH action and the present review focusses on these developments.

GROWTH HORMONE

Growth hormone, also referred as somatotropin, is a protein hormone produced in specific cells (somatotrophs) of pituitary gland (Wallis, 1988). In terms of weight, it is the most abundant hormone of the anterior pituitary accounting for about 10% of dry weight. Growth hormones from a number of species are sequenced. It comprises of about 191 amino acids with a molecular weight of 22 kDa (Havel et al., 1989). Two disulfide bridges, one connecting cystine residues at position 53 to 164 and other connecting cystine residues at position 181 to 189, configure the molecule into two loops (Havel et al., 1989). The crystal structure of growth hormone has been resolved. The hormone has an antiparallel four helix bundle core with a characteristic 'up-up-down-down' topology as shown in figure1 (Abdel-Meguid et al., 1987). There are two binding sites for GHR on each GH molecule. Site 1 and Site 2 are well apart in space. Site 1 is at the right hand side of the molecule, made up of surface of helices A and D as well as the AB loop. Site 2 is at the left and consists of helices A and C (Kossiakoff and de Vos, 1988). There are at least three conformational epitopes in bGH (Kumar and Rajput, 1999). The sequences of GH from Indian species of cattle, buffalo and goat are identical (Mukhopadhyay, 1999). The amino acid sequence for bovine growth hormone (bGH) and ovine growth hormone (oGH) differs only at a single position and thus it partly explains why bGH is biologically active in sheep (Johannson and Hart, 1986). However, GHs from other species differ moderately from each other. bGH and porcine growth hormone (pGH) share 90% sequence homology (Bauman and Vernon, 1993). Both bGH and pGH have only 65% homology with human growth hormone (hGH) (Bauman and Vernon, 1993). This partly explains the ineffectiveness of bGH and pGH on human growth (Lesniak and Roth, 1976; Moore et al., 1985).

GH is a powerful growth promoting and metabolic regulatory hormone (Isaksson et al., 1985). The range of biological effects of GH on growth and lactation is extraordinary. GH orchestrates many diverse physiological processes so that more nutrients are used for lean tissue accretion (during growth) referred as somatogenic effects or milk synthesis (during lactation) referred as lactogenic effect (Bauman and Vernon, 1993). These effects result from direct action of GH on carbohydrate and lipid metabolism, protein synthesis, specific protein synthesis, cell differentiation and proliferation (Wallis, 1988) (figure 2). These coordinated changes in tissue metabolism alter nutrient partitioning (Etherton and Bauman, 1998). The effect of GH on milk enhancement is well established in dairy animals including crossbred cattle and buffalo. The increase in yield ranging between 6 and 30% is dependent on dose of hormone, lactation period, genetic potential of animal and geographic location (Bauman et al., 1988; McBride et al., 1988; Burton and McBride, 1989; Chillard,

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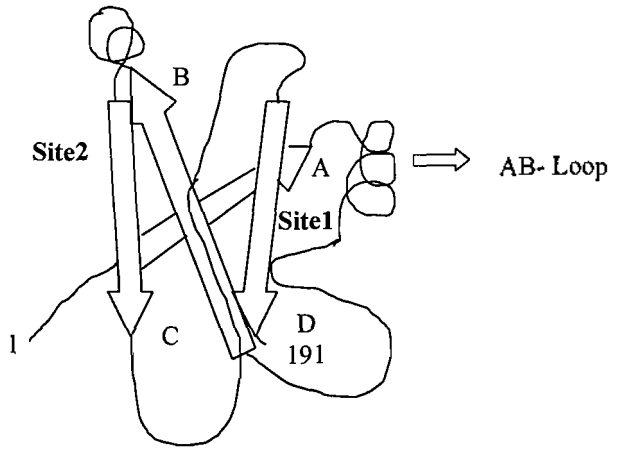

Figure 1. Structure of growth hormone

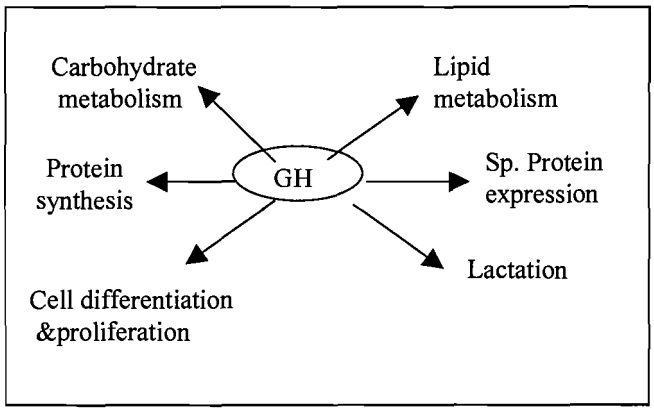

Figure 2. Pleiotropic effects of growth hormone

1989; Ludri et al., 1989; Prosser and Mepham, 1989). Recombinant bovine growth hormone (rbGH) (Burton and Mc Bride, 1989) is equally effective in milk enhancement in bovines and it's use is allowed in 25 countries (Spike, 1997) including US but still not permitted in Europe and Canada .

GROWTH HORMONE RECEPTOR (GHR)

GH produced in the pituitary is carried in the blood to the target tissues. The receptors for GH are present in abundant quantities in liver (Herington et al., 1976; Donner, 1980) and adipose tissues (Fagin et al., 1980; Gavin et al., 1982). However, their presence has also been reported in lymphocytes, fibroblasts, macrophages, chondrocytes, β - islet cells and osteoblasts (Waters et al., 1989). The wide distribution of receptors in tissues has created interest to define new role of GH in these tissues. GHR is a single transmembrane protein approximately 620 amino acids in length, the exact number of amino acids varies slightly from species to species. GHR is a glycoprotein and in most species, the molecular weight is in the range of 100-130 kDa (Carter-Su et al., 1984; Yamada et al., 1987; Spencer et al., 1994). The receptor consists of an extracellular domain of 246 amino acids towards N-terminal end, a short transmembrane domain of about 25 amino acids and a cytoplasmic domain of 350 residues towards C-terminal end (Leung et al., 1987; Kelly et al., 1991) (figure 3). GHR shares structural features with other cytokine receptors. The extracellular domain contains seven cysteine residues and five potential N- linked glycosylation sites. In it's cytoplasmic domain, GHR shares two motifs with other members of cytokine receptor superfamily. One is a membrane - proximal, proline rich motif referred as Box1. It is present in all members and consists of eight amino acids Ψ-X-X-X-Al-P-X-P where Ψ, Al, X and P represent hydrophobic residues, aliphatic residues, any amino acid and proline respectively. The second cytoplasmic motif

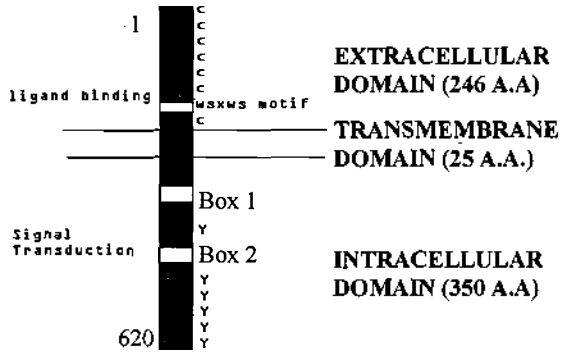

Figure 3. Structure of growth hormone receptor (GHR)

(Box2) begins with a cluster of hydrophobic amino acids, followed by negatively charged residues and ends with one or two positively charged amino acids (O'Neal et al., 1993) (figure 3). Mutation or deletion of Box1 and or Box2 in GHR and other cytokine receptor family members results in defective ligand mediated cellular growth in interleukin-3 dependent cell lines (DaSilva et al., 1994). A number of tyrosine residues are present towards C-terminal end of cytoplasmic domain. GHR is a member of class I of cytokine receptor, also called haematopoietic cytokine receptor family (Wells and deVos, 1996). Type I receptors are characterized by fibronectin type III modules, four conserved cysteine residues in N- terminal half, a cytokine receptor homology region (CRH) which contains the ligand binding determinants of the receptor and the conserved Try-Ser-X-Try-Ser (WSXWS) motif in the C-terminal part in the extracellular domain. This WS motif is not present in GHR where it is replaced by the amino acids Tyr-Gly-Glu-Phe-Ser and plays critical role in ligand binding (Moutousssamy et al., 1998).

The GHRs from a number of species have been sequenced (Kelly et al., 1993) and these are not identical. Percent homology / difference of GHRs from human, rabbit, sheep, cow, mouse, rat and chicken are presented in table 1

(Cramer and Talamantes, 1993). Cow and sheep shares 97% homology differing by only 3%. On the other hand, chicken GHR shares only 56% homology with mouse and with rat differing by 44%. The difference of 24% in cow GHR and human GHR (Cramer and Talamantes, 1993) and 35% in cow GH and human GH (Bauman and Vernon, 1993) is suggestive that bGH will not function in human (Cramer and Talamantes, 1993). Buffalo growth hormone receptor is not sequenced so far.

GHR is typically divided into extracellular domain, transmembrane region and intracellular domain. The sequence analysis of bGHR with respect to different domains of GHR from other species reveal that differences in bGHR and mouse GHR are more in extracellular domain as compared to other regions. Similar is the situation of bGHR and rat GHR (Hauser et al., 1990) (figure 4).

GROWTH HORMONE RECEPTOR DIMERIZATION

One molecule of GH binds with two molecules of GHRs leading to dimerization of the receptor, an essential step for expression of biological effects associated with GH (Cunningham et al., 1991). The GH has two sites referred as site 1 and site 2. Both sites bind to the same region of GHR. A sequential complex appears to form with the receptor first binding to site 1, after which a second receptor binds to site 2, followed by an interaction between receptor molecules themselves that maintain the dimer complex (deVos et al., 1992). A mutated GH that fails to induce growth hormone binding protein dimerization is biologically inactive when added to cells expressing GHR which suggests that GH induced dimerization of GHR is required for GH action. Also, GH variants that block binding at site 2 can form 1:1 complex (Fuh et al., 1992). At physiological relevant hormone concentrations (0.1 to 1nM), GHR binds with receptor in 1:2 ratio. But at superphysiologic hormone concentrations (>μM), the hormone saturates all the receptor molecules forming 1:1 complex, which prevents dimerization and signalling (Wells and deVos, 1996).

GHR dimerization results in activation of signalling molecules/pathways and the diverse effects of GH are mediated through these signalling molecules (figure 5).

Table 1. Homology/ differences in GHR

	% Difference in aminoacids						
	1	2	3	4	5	6	7
1 Human		16	19	24	30	31	41
2 Rabbit	84		23	18	24	26	40
3 Sheep	81	77		3	29	29	42
4 Cow	76	82	97		29	29	43
5 Mouse	70	76	71	71		15	44
6 Rat	69	74	71	71	85		44
7 Chicken	59	60	58	57	56	56	
	% Amino acid homology						

(Adapted from Cramer and Talamantes., 1993)

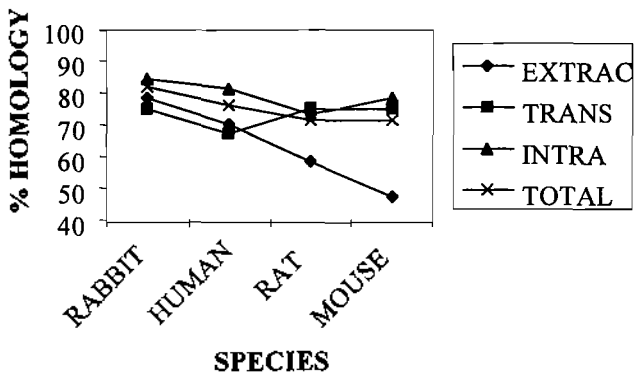

Figure 4. Amino acid sequence homology of bovine GHR with different domains of GHR

SIGNALLING MOLECULES / PATHWAYS

A number of signalling molecules have been recently identified in different pathways as shown in fig 6. These pathways are not completely elucidated (Carter Su et al., 1996).

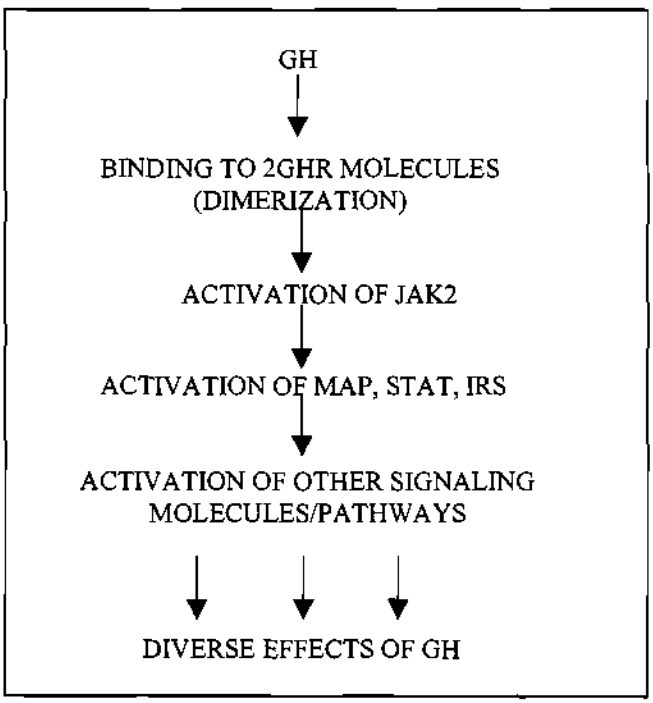

Figure 5. An overview of growth hormone action

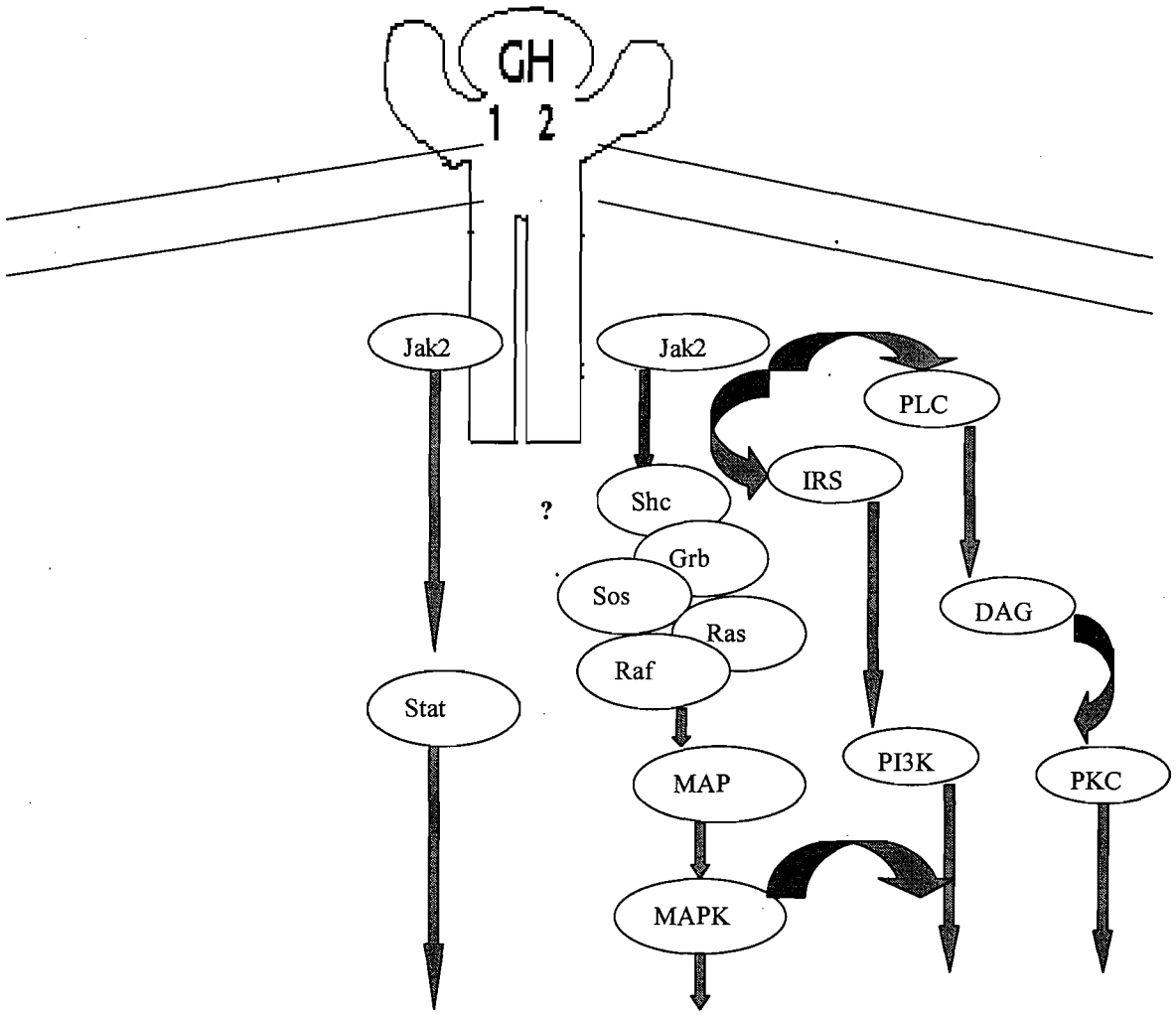

Figure 6. Model of growth hormone signalling

Jak2 tyrosine kinase

The dimerization of receptor is followed by activation of Janus kinases (Jak) in which Jak proteins are phosphorylated (Argetsinger et al., 1993; Campbell et al., 1993; Wells and deVos, 1996). The Janus kinases represent a family of soluble tyrosine kinases strongly implicated in signal transduction of cytokine family. The Jak proteins contain two tyrosine kinase domains and thus named after two faced Roman God 'Janus'. There are four members of the Jak family, called Jak1, Jak2, Jak3 and Tyk2 with molecular weight ranging from 125-135 kDa. These kinases are characterized by the presence of two kinase domains and absence of Src homology 2 (SH2), SH3 and membrane spanning domains (Ihle et al., 1994).

Jak2, a 130kDa tyrosine kinase is identified as first signalling molecule activated as a result of binding of a GH molecule to it's receptor (Silvennoinen et al., 1993). The activation of Jak2 occurs from autophosphorylation at tyrosine side chain. The activated Jak2 subsequently phosphorylates GHR tyrosine residues present in cytoplasmic domain. The binding of activated Jak2 with GHR is the first interactive event in the cytoplasm that activates other signalling molecules such as Src homology containing proteins, insulin receptor substrate-1 (IRS-1 and IRS-2), mitogen activated protein (MAP) kinases (also referred as extracellular signal regulated kinase or ERK) and signal transducers and activator of transcription (Stat) proteins (Frank et al., 1994).

The association of GHR with Jak2 and tyrosyl phosphorylation of both Jak2 and GHR requires proline rich motif of GHR (Wang and Wood, 1995). GHR mutants with Box1 mutated or deleted fails to show GH-dependent tyrosyl phosphorylation of SHC proteins, IRS-1 and IRS-2, ERK-1 and 2 and Stat proteins (Goujon et al., 1994).

MAP kinase pathway

Once GH binds to the GHR, it activates the GHR-associated tyrosine kinase Jak2, whereupon both GHR and Jak2 become tyrosine phosphorylated (Argetsinger et al., 1993). Various signalling pathways are activated by GH which leads to the GH dependent changes in gene expression, metabolism, cellular differentiation and body growth (Davidson, 1987). One class of signalling molecules known to be activated by GH (Anderson, 1992; Campbell et al., 1992) is the mitogen activated protein or microtubule-asociated protein (MAP) kinases or extracellular signal regulated kinases (ERK) (Gronowski et al., 1994).

The MAP kinases are a group of serine/threonine /tyrosine kinases that play a pivotal role in the regulation of cellular growth and differentiation (Cobb et al., 1991). The first two GH-dependent tyrosyl phosphorylated proteins identified were ERK1 and ERK2 (Anderson, 1992; Campbell et al., 1992; Winston and Bertics, 1992). Activation of MAP kinases requires proline rich Box1 of GHR, the same region involved in Jak2 activation (VanderKurr et al., 1994).

One potential signalling pathway leading to MAP kinase activation is the SHC-Grb2-Sos-Ras-Raf-MEK-ERK pathway. SHC proteins contain a Src homology SH2 and a collagen like domain. SHC binds to phosphorylated tyrosines on activated receptor tyrosine kinases. Subsequently tyrosyl phosphorylation of SHC provides a binding site for SH2 domain of growth factor receptor bound 2 (Grb2) protein (VanderKuur et al., 1995a). This Grb2 binds via it's SH3 domain to the mammalian homolog of the Drosophilla gene product, son of sevenless (Sos), a guanine nucleotide exchange factor, which in turns activates the small GTP binding protein, Ras. Ras, in turn activates the mixed function the serine/threonine/tyrosine kinase (MEK) which then phosphorylates and activates the MAP kinases designated ERK1 and ERK2 (Anderson, 1992; Campbell et al., 1992; VanderKuur et al., 1997).

GH promotes rapid tyrosyl phosphorylation of SHC proteins in 3T3-F442A fibroblasts (VanderKuur et al., 1995a) and SHC association with Grb2 (VanderKuur et al., 1995b) with the subsequent formation of Grb2-Sos complex that serves to activate Ras and thereby engage Raf-MEK-ERK pathway. MAP kinase substrates include other protein kinases (e.g c-Raf1, the S6 kinases designated ribosomal S6 kinases $p70^{rsk}$ and $p90^{rsk}$), phospholipase A2 and transcription proteins (Davis, 1993). GH activates the S6 kinase, $p90^{rsk}$ in 3T3-F442A Fibroblasts (Anderson, 1992).

IRS-1 and 2 and phosphatidylinositol 3- kinase

The acute effects of GH on fat cells include the stimulation of glucose transport, increased conversion of glucose to glycogen, increased lipid synthesis and CO_2 production from glucose, and increased oxidation of leucine and pyruvate. These effects are usually summarized as being insulin like (Wiederer and Loffler, 1987) which suggests that GH may share some signalling molecules viz. the substrate of insulin receptor IRS-1 & 2 and PI3-kinase (Davidson, 1987; Goodman, 1968). GH phosphorylates IRS-1 in primary culture of rat adipocytes, 3T3-F442A fibroblasts or CHO cells expressing GHR (Souza et al., 1994; Argetsinger et al., 1995; Ridderstrale et al., 1995).

The region of GHR required for tyrosyl phosphorylation of IRS-1 & 2 is the same as required for Jak2 activation. Phosphorylated tyrosine residues in IRS-1 & 2 inturn provide binding sites for SH2 containing proteins viz. 85-kDa regulatory subunit of PI-3 kinase (Sun et al., 1991; Sun et al., 1993). Consistent with a role for PI-3 kinase in insulin like metabolic effects of GH, the PI3-kinase inhibitor wortamannin blocks the ability of GH to stimulate lipid synthesis in rat adipocytes (Ridderstrale and Tornquist, 1994).

The Phospholipase C /protein kinase C / Ca^{2+} pathways

The GH mediates diacylglycerol production by means of phosphatidylcholine breakdown involving a phospholipase C coupled to GHR via a pertusis toxin-sensitive G protein in Ob1771 preadipocytes (Catalioto et al., 1990). In contrast, in kidney proximal tubule membranes, the change in DAG is accompanied by rapid, transient increase in level of inositol triphosphate (IP3) (Rogers and Hammerman, 1989). This DAG is a known activator of protein kinase C (Doglio et al., 1989; Johnsson et al., 1990).

The GH loses it's ability to stimulate lipogenesis (Smal and deMeyts, 1987), induce c-fos expression (Gurland et al., 1990; Billestrup et al., 1995) and increased Ca^{2+} uptake upon treatment of cells with inhibitors of PKC or phorbal esters to deplete PKC or by inhibitors of PKC, suggesting a role of PKC in this process. This observation suggests the prsence of another pathway referred to as PLC/ PKC/ Ca^{2+} pathway.

GH causes an increase in intracellular free Ca^{2+}concentration in freshly isolated rat adipocytes (Smal and deMeyts, 1987), IM-9 lymphocytes (Gurland et al., 1990) and CHO cells expressing GHR (Billestrup et al., 1995). The GH- induced increase in Ca^{2+} in rat adipocytes is mimicked by DAG and blocked by the PKC inhibitor suggesting that GH activation of Ca^{2+} channels involves phospholipid hydrolysis and activation of PKC. Ca^{2+} seems

Table 2. Different signalling molecules identified in different cell lines/species

Signalling mol./ Species	*Jak1*	*Jak2*	*SHC*	*Grb2*	*STAT1*	*STAT3*	*STAT5*	*IRS1*	*IRS2*	*MAP*	Ca^{2+}	References
3T3-F442A[a]	+	+	+	+	+	+	+	+	+	+	N.D	Argetsinger et al.,1995;Smit et al., 1997;Argetsinger et al;1993.
HT1080[b]	+	+	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	N.D	Han et al., 1996.
IM-9[c]	-	+	-	-	-	-	+	N.D	N.D	-	+	Frank et al.,1994; Gurland et al.,1990; Finbloom et al., 1994; Silva et al.,1994.
CHO[d]	-	+	+	+	N.D	N.D	N.D	+	+	+	+	Argetsinger et al., 1995; Billestrup et al., 1995;Vanderkuur et al., 1995b
In vivo Rat	N.D	+	+	+	?	?	?	+	+	N.D	N.D	Thirone et al.,1999;Ram et al., 1996; Chow et al.,1996.

[a] rat fibroblast cell line, [b] human cell line, [c] human lymphocyte cell line, [d] chinese hamster ovary cell line, N.D. - not determined, ? - controversial reports, + present, - absent.

to be important for activation of some GH- induced genes such as serine protease inhibitor gene Spi2.1 and refractory effects of GH on metabolism in adipocytes since verapamil blocks these GH effects (Waxman et al., 1995). Mutagenesis studies in CHO cells suggest that GH-dependent increase in Ca^{2+} requires the C-terminal half of GHR, but may not require the prolines in Box 1, thus raising the possibility that calcium signalling may be independent of Jak2 activation (Billestrup et al., 1995).

Stat proteins

The Signal transducer and activator of transcription (Stat) proteins are recognized for their dual function in signal transduction in the cytoplasm and activation of transcription in the nucleus, hence the name Stat (Wang and Wood, 1995). Stat proteins are latent cytoplasmic factors that possess a common organization: a conserved block of 50 amino acid in the N-terminal region and SH3 like domain, a highly conserved SH2 domain, a conserved tyrosine residue and a divergent C-terminal domain (Ihle, 1996; Han et al., 1996). As a result of binding of GH to its receptor, and Jak2 activation, Stat proteins are phosphorylated by Jak2 kinase on their conserved C-terminal Tyr (Ihle, 1996). Subsequently, homo or hetero-dimers of Stat proteins are formed which translocate to the nucleus and bind to DNA and activate transcription of target genes (Pellagrin and Dusenter-Fourt, 1997).

There are seven Stat proteins which have been identified by cDNA cloning in mammals including the two isoforms of Stat5. The Stat activation requires Jak2 activation since Jak2 deficient cell lines fail to activate Stat proteins (Smit et al., 1997). The activation of different Stat proteins is found to depend on cell type, state of cell differentiation. For instance, GH activates Stat 1, Stat 3 and both Stat 5 isoforms in 3T3-F442A fibroblasts but it does not activate Stat1 in IM9 cells (Finbloom et al., 1994). Further, activation of Stat 5 isoforms also differs among tissues or cell lines. In fibroblasts, GH activates both isoforms of Stat 5, while in liver Stat 5b is the isoform essentially expressed (Moutoussamy et al., 1998).

Signalling molecules in different cell lines / species

It appears that presence or absence of signalling molecules generated in GH action is dependent on cell line / species (table 2). Jak2 signalling molecule is generated in all cell lines / species tested so far. In IM-9 cell line, many signalling molecules identified in rat fibroblast cell line are found to be absent. It is not still absolutely clear whether these signalling molecules are really absent or methodology adopted for detection of these molecules was not sensitive enough. Since, GH exhibits many diverse functions, it is very likely that some of these signalling molecules are generated in abundant quantities while others in negligible quantities or are absent. Once signalling pathway for each function is elucidated, it could be possible to design drugs for desirable effects.

CONCLUSION

Trends in research suggests that knowledge gained in understanding of molecular events in cytokine action has helped in elucidation of events in growth hormone action.

Still, little is known how GH exerts diverse biological effects. Complete understanding of growth hormone action can have implications in designing drugs for obtaining desired effects of growth hormone. Since each IgG molecule is bivalent, it could be very interesting to see whether anti-idiotypic antibodies against bGH can result in signal transduction. The presence of hinge region in the antibody molecule provides flexibility to Fab regions and thus it perhaps can help in better way in dimerization of GHRs.

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