Growth Hormone Receptor/Binding Protein: Physiology and Function (43751)

ADRIAN C. HERINGTON,[1] SUSIE I. YMER, JANET L. STEVENSON, AND PETER ROUPAS

Centre for Child Growth and Hormone Research and Department of Clinical Biochemistry, Royal Children's Hospital, Melbourne, 3052, Australia

Abstract. **Soluble truncated forms of the growth hormone receptor (GHR) are present in the circulation of many species and are also produced by many tissues/cell types. The major high-affinity forms of these GH-binding proteins (GHBP) are derived by alternative splicing of GHR mRNA in rodents, but probably by proteolytic cleavage in other species. Questions still remain with respect to the origins, native molecular form(s), physiology, and function of the GHBPs, however. The observation that GH induces dimerization of the soluble GHBP and membrane GHR, and that dimerization of GHR appears to be critical for GH bioactivity suggests that the presentation of GH to target cells, in an unbound form or as a monomeric or dimeric complex with GHBP, may have significant implications for the ability of GH to activate specific postreceptor signaling pathways (tyrosine kinase, protein kinase C, G-protein pathways) known to be utilized by GH for its diverse biological effects. This minireview addresses some of these aspects and highlights several new questions which have arisen as a result of recent advances in our understanding of the structure, function, and signaling mechanisms of the membrane bound GHR.** [P.S.E.B.M. 1994, Vol 206]

Origins of the Growth Hormone-Binding Protein

Growth hormone-binding proteins (GHBP) have been identified in many species, both in the circulation (1–4) and, in some species and tissues, in soluble cytosolic fractions of cells (5). In rodents, which have been the most closely examined species, the GHBP (based on mRNA and/or immunohistochemical location) is expressed by and/or released from a wide range of tissues and cell types (6–8). In some species several forms of GHBP appear to be present. The primary GH-binding component of the native form(s) of GHBP is essentially the extracellular, GH-binding domain of the cell membrane GH receptor (GHR). The mechanism for generation of the GHBP appears to be species specific, with rodents being distinct from other species.

In the rat and mouse, the GHBP is generated primarily as a distinct protein product by expression and translation of an alternatively spliced variant of the GHR gene (9, 10). The alternative splicing substitutes a short sequence coding for a hydrophilic peptide in place of the GHR hydrophobic transmembrane domain and long cytoplasmic domain. It is argued that this hydrophilic sequence allows the expressed GHBP to be secreted from cells. Based on specific immunoprecipitation studies, at least some of the circulating GHBP in the rat is derived from the alternatively spliced GHBP mRNA (11). However, recent data from Goodman's laboratory (12, 13, and see elsewhere in this publication) have also indicated that, at least in rat adipocytes, the short form of the rat GHR remains associated with the cell membrane. This has potential implications for possible paracrine/autocrine physiological roles of the GHBP, not only in adipocytes but in many other tissues where the GHBP and GHR are co-expressed. Such a situation may be analogous to that for interleukin-6 (IL6) binding protein which can interact with a non-IL6-binding transmembrane protein (gp 130) which is involved in signal transduction (14). An additional observation, also with clear functional implications, is that expression of the rodent GHBP can be regulated, at least at the level of mRNA abundance, quite independently of GHR expression

[1] To whom requests for reprints should be addressed at Department of Clinical Biochemistry, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia.

0037-9727/94/2063-0238$10.50/0

(6, 15). This suggests, in rodents at least, that the GHBP has a biological role(s) that may be independent of the GHR.

The situation is less clear in other species. Although GHR mRNA transcripts smaller than the 4.0–4.5-kb mRNA coding for the membrane-bound full-length GHR have been reported in some species (e.g., 16, 17) none of these have yet been shown to code specifically for a truncated GHBP-like form. The weight of evidence in nonrodent species points to a quite distinct mechanism for GHBP generation—proteolytic cleavage. On the basis of partial amino acid sequence information for purified rabbit serum GHBP, such a cleavage would appear to occur close to the transmembrane domain (4, 18). Although two possible protease cleavage sites are present in this location in the extracellular domain of the rabbit GHR, the precise C-terminal end, and hence proteolytic cleavage site, of the purified native rabbit GHBP has not been determined. Recent evidence strongly supports such a mechanism, however. Sotiropoulos *et al.* (19) have shown that when COS-7 or CHO cells, which do not express endogenous GHR or GHBP, are transfected with full-length rabbit GHR cDNA they release soluble truncated forms of GH-binding activity into the culture medium. The soluble rabbit GHBP released had size and binding characteristics similar to those of native rabbit serum GHBP. Transfections with rat GHR cDNA did not yield soluble binding activity, consistent with knowledge that rat GHBP is primarily the product of specific mRNA translation. Although no studies were done with protease inhibitors, it seems most likely that release was effected via a proteolytic mechanism. No specific protease has been identified. The apparent cleavage of full-length GHR in a transfected cell model (CHO, COS-7) not normally expressing GHR suggests that a relatively nonspecific or ubiquitous protease might be involved. A common assumption is that cleavage occurs on the external surface of a cell following insertion of the GHR into the cell membrane. However, an equally likely explanation is that cleavage occurs within the cell without insertion into the membrane, with subsequent secretion or expulsion of the GH-binding domain. Previous studies (20, 21) have shown that a variety of pathways exist for intracellular processing, recycling, and trafficking of internalized GH-GHR complexes as well as, in all likelihood, for newly synthesized GHR.

Circulating Forms of GHBP

Native forms of circulating GHBPs show significant heterogeneity in most species. Gel permeation chromatography and covalent cross-linking studies in humans, rabbits, rats, and mice (22–25) indicate species and specificity differences for multiple forms of GHBP activity, ranging in molecular weight from <50 kDa to >300 kDa. Some of these forms represent variants in post-translational modifications (e.g., glycosylation) of the GHBP derived from the GHR, others may represent dimerized complexes with GH (see below) or association with other circulating proteins. Some forms, as in the human, do not appear to be related at all to the membrane receptor (2, 25). Recent data in the rat (Ymer *et al.* unpublished) using gel filtration of serum alone, or [^{125}I]GH-serum complexes, have indicated the presence of at least four distinct regions of specific binding activity in rat serum, with some of the larger forms cross-reacting with a monoclonal antibody against the hydrophilic tail of the rat GHBP. This clearly suggests that these forms contain, as at least one component, the small (mol wt 35,000–50,000) GHBP derived from the alternatively spliced rat GHR mRNA. These studies also suggest that some of these higher molecular weight rat GHBP forms may dissociate to smaller GHBP complexes and that the smaller molecular weight species may dimerize to a larger complex. Further studies are necessary to properly define the relationship of these larger forms to the GHR-derived GHBP and to assess any possible functional significance.

Dimerization of the GHBP/GHR

It has recently been reported, on the basis of the crystal structure of a soluble complex between human GH and recombinant GHBP (26), that the GH-GHBP complex possesses the rather unusual stoichiometry of one GH molecule binding two GHBP molecules (27). The dimerization occurs through a single binding site on each of the GHBP molecules which bind in a sequential manner to two quite distinct sites within the GH molecule itself. The affinity for binding to GH Site 1 is slightly higher than for the subsequent binding of GHBP to GH Site 2. Although not shown directly, it has been postulated that the GH receptor, embedded in the cell membrane, also dimerizes in a comparable way in the presence of appropriate concentrations of GH.

The dimerization process appears to be important for GH action. GH analogs which have mutations in Binding Site 2, and are therefore unable to bind a second GHBP, are biologically inactive (27, 28). Furthermore, specific measurements with recombinant GHBP, as well as theoretical estimates, have shown a biphasic (bell-shaped) dependence of dimerization on the concentrations of both GH and GHBP. This relationship between GH and GHBP dimerization mirrors closely the pattern observed for GH-stimulated biological actions in several *in vitro* bioassay systems (29).

Computer modeling of the GH-$(GHBP)_2$ complex using the crystal coordinates determined by De Vos *et*

al. (26) have indicated that the GHBP C-terminal domains are brought close together following dimerization. Extrapolating this to the full-length GHR suggests that the cytoplasmic domains of the GHR would also be juxtaposed. This raises the possibility that the conformational changes invoked by dimerization allow subsequent interaction with one or more of the intracellular signaling pathways now known to be utilized by GH.

GHBP/GHR Interactions and Physiological Implications

The presence of multiple circulating forms of GH-GHBP complex, the ability of the GHBP to dimerize, and the relationship between GH action and GHR dimerization, raises important questions about (i) the stoichiometry of the native GHBP complex in serum and in extracellular fluid, (ii) possible functional sequelae of interactions between the GHR and GHBP, and (iii) the implications of this for a biological role for the GHBP, for which some evidence exists for endocrine, paracrine/autocrine and possibly intracrine (intracellular) roles.

With respect to the relative stoichiometry and proportions of GH-GHBP monomers or dimers in the circulation, little if any direct evidence exists to our knowledge. Early data suggest that the GHBP in the human circulation binds up to 50% of secreted GH (2, 3, 30). More recently Veldhuis *et al.* (31), on the basis of a sophisticated mathematical analysis and computer simulation, reported that the percentage of GH bound can fluctuate quite rapidly between 10%–80% depending on the prevailing GH concentration. It seems likely, on the basis of gel filtration data, that much of the bound GH is probably in the form of monomeric complexes (2, 3). However, with acute increases and decreases in GH levels as a result of the pulsatile secretion of GH, the relative proportions of monomer: dimer may alter accordingly. Some of the higher molecular weight forms of GHBP complex found in some species (particularly rat and rabbit) may be due to GH-$(GHBP)_2$ homodimer formation. The relative ratios of free GH to monomeric to dimeric complexes may have significant implications for the *endocrine* roles of the GHBP. There is clear evidence that its endocrine role largely involves protection of GH from degradation and clearance, thereby leading to an increased plasma half-life (32, 33), as well as influencing the extravascular availability of GH (32) and hence its potential access and delivery to tissues and cellular GHR (31).

At the tissue level the complex interactions between GH, GHBP and GHR also provide a model in which it is likely that direct modulation of GH action will be encountered. At least three "dimerization" models are possible, each of which may have implications for GH action via initiation of signaling mechanisms. The three models are GH-$(GHBP)_2$, GH-$(GHR)_2$, and GH-(GHBP)-(GHR). It is highly unlikely that GH-induced dimerization of two soluble extracellular domains is going to activate, per se, an intracellular signaling pathway. Indeed, if local GHBP concentrations were sufficiently high, then GH-$(GHBP)_2$ formation may be favored over interaction of GH with cell-bound GHR and hence prevent, by simple competition, any cellular response to GH. This may be an explanation for the observation *in vitro* that exogenous addition of GHBP uniformly inhibits GH binding to cell surface GHR and GH action (e.g., 34). These earlier experiments and their interpretation may need reevaluation in the light of possible dimerization models discussed above. In particular, careful consideration must be given to the relative concentrations of GH and GHBP and the affinities of GHR and GHBP for GH.

There seems little evidence to suggest that the second model, dimerization of one GHBP with a single full-length GHR molecule, could not generate an intracellular signal. Although dimerization at the level of the extracellular domains appears to be a prerequisite for GH action (27) it is not clear whether juxtapositioning of two full-length cytoplasmic domains is also necessary. Indeed, the recent evidence from Colosi *et al.* (35) would suggest that as little as 54 amino acids (16%) of the cytoplasmic domain is sufficient to generate a limited proliferative response to GH in FDC-P1 cells. The studies from Goodman's laboratory (12, 13), which show that the GHBP associates with the cell membrane of the rat adipocyte and accounts for ~50% of detected membrane GH-binding sites, would also predict that formation of GH-GHBP-GHR complexes is likely.

It is clear from an increasing number of studies that the various "classes" of GH action (e.g., growth promoting, nuclear activation, metabolic, receptor internalization, etc.) require the involvement of different domains of the cytoplasmic portion of the GHR (36, 37). Activation of some of these domains may require a *trans* (two-chain) interaction, while others may only require a *cis* (one-chain) interaction. Whether GH is delivered to target cells in an unbound state or as a monomeric complex with GHBP (derived from the circulation or perhaps more likely from local GHBP production) may have a significant influence on the nature of any cell-associated dimerized complex subsequently formed and perhaps, therefore, on the nature of the signaling pathway(s) activated.

It is evident from the work of several groups that the biological actions of GH are mediated by, or involve changes in, several different signaling systems (38), including tyrosine phosphorylation pathways (the tyrosine kinase JAK-2 [39], the Mitogen Activated Protein Kinase [MAPK] pathways [40], and ribosomal S6 kinase [40]), protein kinase C (PKC) pathways (41)

including phospholipase C and D activation (Roupas *et al.*, unpublished), the family of GTP-binding proteins (G proteins) (42), and activation of early response cellular oncogenes such as c-*fos*, c-*myc* and c-*jun* (43). Given the involvement of all of these classical signaling mechanisms in GH action, it is possible that selectivity of the mechanism used may be dependent on the nature of the target cell and the components (GHBP and/or GHR) involved in formation of a dimerized complex.

The authors' own work reported here was supported by the National Health and Medical Research Council of Australia, the Diabetes Australia Research Trust, and the Royal Children's Hospital Research Foundation.

1. Ymer SI, Herington AC. Evidence for the specific binding of growth hormone to a receptor-like protein in rabbit serum. Mol Cell Endocrinol **41**:153–161, 1985.
2. Herington AC, Ymer SI, Stevenson JL. Identification and characterization of specific binding proteins for growth hormone in normal human sera. J Clin Invest **77**:1817–1823, 1986.
3. Baumann G, Stolar MW, Amburn K, Barsano CP, De Vries BC. A specific growth hormone-binding protein in human plasma: Initial characterization. J Clin Endocrinol Metab **62**:134–141, 1986.
4. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. GH receptor and serum binding protein: Purification, cloning and expression. Nature **330**:537–543, 1987.
5. Herington AC, Ymer SI, Roupas P, Stevenson J. Growth hormone binding proteins in high-speed cytosols of multiple tissues of the rabbit. Biochim Biophys Acta **881**:236–240, 1986.
6. Tiong TS, Herington AC. Tissue distribution, characterisation and regulation of mRNA for growth hormone receptor and serum binding protein in the rat. Endocrinology **129**:1628–1634, 1991.
7. Carlsson B, Billig H, Rymo L, Isaksson OGP. Expression of the growth hormone-binding protein messenger RNA in the liver and extrahepatic tissues in the rat: Co-Expression with the growth hormone receptor. Mol Cell Endocrinol **73**:R1–R6, 1990.
8. Lobie PE, Garcia-Aragon J, Wang BS, Baumbach WR, Waters MJ. Cellular localization of the growth hormone binding protein in the rat. Endocrinology **130**:3057–3065, 1992.
9. Baumbach WR, Horner DL, Logan JS. The growth hormone-binding protein in rat serum is an alternatively spliced form of the rat growth hormone receptor. Genes Dev **3**:1199–1205, 1989.
10. Smith WC, Kuniyoshi J, Talamantes F. Mouse serum growth hormone binding protein has GH receptor extracellular and substituted transmembrane domains. Mol Endocrinol **3**:984–990, 1989.
11. Sadeghi H, Wang BS, Lumanglas AL, Logan JS, Baumbach WR. Identification of the origin of the growth hormone-binding protein in rat serum. Mol Endocrinol **4**:1799–1805, 1990.
12. Frick GP, Goodman HM. Characterization of the soluble isoform of the growth hormone receptor synthesized by rat adipocytes. Endocrinology **131**:3083–3090, 1992.
13. Frick PG, Tai L-R, Goodman HM. Subcellular distribution of the long and short isoforms of the growth hormone receptor in rat adipocytes: Both isoforms participate in specific binding of growth hormone. Endocrinology **134**:307–314, 1994.
14. Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T. Molecular cloning and expression of an IL-6 signal transducer, gp 130. Cell **63**:1149–1157, 1990.
15. Tiong TS, Herington AC. Ontogeny of hepatic mRNA for the growth hormone receptor and serum binding protein in the rat. Mol Cell Endocrinol **83**:133–141, 1992.
16. Ymer SI, Herington AC. Developmental expression of growth hormone receptor and insulin-like growth factor-1 mRNA in rabbit tissues. Mol Cell Endocrinol **83**:39–49, 1992.
17. Herington AC, Tiong TS, Ymer SI. Serum binding proteins for growth hormone: Origins, regulation of gene expression and possible roles. Acta Paediatr Scand **379**(Suppl):61–69, 1991.
18. Spencer SA, Hammonds RG, Henzel WJ, Rodriguez H, Waters MJ, Wood WI. Rabbit liver growth hormone receptor and serum binding protein. Purification, characterization and sequence. J Biol Chem **263**:7862–7867, 1988.
19. Sotiropoulos A, Goujon L, Simonin G, Kelly PA, Postel-Vinay M-C, Finidori J. Evidence for generation of the growth hormone-binding protein through proteolysis of the growth hormone membrane receptor. Endocrinology **132**:1863–1865, 1993.
20. Roupas P, Herington AC. Cellular mechanisms in the processing of growth hormone and its receptor. Mol Cell Endocrinol **61**:1–13, 1989.
21. Ilondo MM, Vanderschueren-Lodeweyckx M, Courtoy PJ, De Meyts P. Cellular processing of growth hormone in IM-9 cells: Evidence for exocytosis of internalized hormone. Endocrinology **130**:2037–2044, 1992.
22. Ymer SI, Herington AC. Structural studies on membrane-bound and soluble growth hormone binding proteins in rabbit liver. Biochem J **242**:713–720, 1987.
23. Smith WC, Talamantes F. Gestational profile and affinity cross-linking of the mouse serum growth hormone binding protein. Endocrinology **123**:1489–1494, 1988.
24. Baumann G, Shaw MA. A second lower affinity growth hormone binding protein in human plasma. J Clin Endocrinol Metab **70**:680–686, 1990.
25. Haldosen LA, Gustafsson JA. Detection of glycosylated GH-binding protein in rat serum. Mol Cell Endocrinol **68**:187–194, 1990.
26. De Vos AM, Ultsch M, Kossiakoff AA. Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. Science **25**:306–312, 1992.
27. Cunningham BC, Ultsch M, De Vos AM, Mulkerrin MG, Clauser KR, Wells JA. Dimerization of the extracellular domain of the human GH receptor by a single hormone molecule. Science **254**:821–825, 1991.
28. Fuh G, Cunningham BC, Fukunaga R, Nagata S, Goeddel DV, Wells JA. Rational design of potent antagonists to the human growth hormone receptor. Science **256**:1677–1680, 1992.
29. Ishizaka-Ikeda E, Fukunaga R, Wood WI, Goeddel DV, Nagata S. Signal transduction mediated by growth hormone receptor and its chimeric molecules with the granulocyte colony-stimulating factor receptor. Proc Natl Acad Sci USA **90**:123–127, 1993.
30. Baumann G, Amburn K, Shaw MA. The circulating growth hormone binding protein complex: A major constituent of plasma GH in man. Endocrinology **122**:976–983, 1988.
31. Veldhuis JD, Johnson ML, Faunt LM, Mercado M, Baumann G. Influence of the high-affinity growth hormone (GH)-binding protein on plasma profiles of free and bound GH and on the apparent half-life of GH. J Clin Invest **91**:629–641, 1993.
32. Baumann G, Amburn K, Buchanan TA. The effect of circulating growth hormone binding protein on metabolic clearance, distribution and degradation of human growth hormone. J Clin Endocrinol Metab **64**:657–660, 1987.
33. Baumann G, Shaw MA, Buchanan T. *In vivo* kinetics of a covalent growth hormone binding protein complex. Metabolism **38**:330–333, 1988.
34. Lim L, Spencer SA, McKay P, Waters MJ. Regulation of growth

hormone bioactivity by a recombinant human GH-binding protein. Endocrinology **127**:1287–1291, 1990.
35. Colosi P, Wong K, Leong SR, Wood WI. Mutational analysis of the intracellular domain of the human growth hormone receptor. J Biol Chem **268**:12617–12623, 1993.
36. Moldrup A, Allevato G, Dyrberg T, Nielsen JH, Billestrup N. Growth hormone action in rat insulinoma cells expressing truncated growth hormone receptors. J Biol Chem **266**:17441–17445, 1991.
37. Moller C, Hansson A, Enberg B, Lobie PE, Norstedt G. Growth hormone (GH) induction of tyrosine phosphorylation and activation of mitogen-activated protein kinases in cells transfected with rat GH receptor cDNA. J Biol Chem **267**:23403–23408, 1992.
38. Roupas P, Herington AC. Post-receptor signalling mechanisms for growth hormone. Trends Endocrinol Metab (in press).
39. Argetsinger LS, Campbell GS, Yang X, Witthuhn BA, Silvennoinen O, Ihle JN, Carter-Su C. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. Cell **74**:237–244, 1993.
40. Anderson NG. Growth hormone activates mitogen-activated protein kinase and S6 kinase and promotes intracellular tyrosine phosphorylation in 3T3-F422A preadipocytes. Biochem J **284**:649–652, 1992.
41. Smal J, De Meyts P. Sphingosine, an inhibitor of protein kinase C, suppresses the insulin-like effects of growth hormone in rat adipocytes. Proc Natl Acad Sci USA **86**:4705–4709, 1989.
42. Roupas P, Chou SY, Towns RJ, Kostyo JL. Growth hormone inhibits activation of phosphatidylinositol phospholipase C in adipose plasma membranes: Evidence for a growth hormone-induced change in G protein function. Proc Natl Acad Sci USA **88**:1691–1695, 1991.
43. Gurland G, Ashcom G, Cochran BH, Schwartz J. Rapid events in growth hormone action. Induction of *c-fos* and *c-jun* transcription in 3T3-F422A preadipocytes. Endocrinology **127**: 3187–3195, 1990.