The Significance of the Short Form of the Growth Hormone Receptor in Rat Adipocytes (43764)

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Abstract. **An isoform of the growth hormone (GH) receptor that lacks the transmembrane and intracellular domains is formed in adipocytes by alternate splicing of mRNA. This isoform is identical to the circulating GH-binding protein. The short isoform is about six times as abundant as the long isoform in rat adipocytes. It is located largely in the cytosolic compartment in association with intracellular membranes, but about 20% is present on the adipocyte surface as judged by the susceptibility to digestion by trypsin. In contrast, about 80% of the long isoform of the receptor is present on the cell surface. The two isoforms turn over at different rates and appear to be independently regulated. Both appear to contribute equally to GH binding. In preliminary studies, immunoneutralization of the short isoform decreased the magnitude of the effect of GH on glucose metabolism, suggesting that the short isoform may mediate some of the responses to GH.** [P.S.E.B.M. 1994, Vol 206]

Growth hormone (GH) produces multiple and complex physiological actions. It stimulates skeletal growth in adolescent animals and humans, and defends the lean body mass after growth is arrested. Stimulation of growth and overall protein synthesis depends upon the actions of the insulin-like growth factor, IGF-I, whose production it enhances. GH is also an important regulator of energy metabolism (1). Its metabolic effects temper the responses of muscle and adipose tissue to insulin and limit their rates of carbohydrate utilization. By decreasing the synthesis and deposition of triglycerides in adipose tissue while promoting fatty acid mobilization and utilization, GH reduces the proportion of fat in the body. Increased accessibility of fat for energy spares carbohydrate and protein reserves, and in conjunction with accelerated protein synthesis, produces a relative increase in lean body mass.

The adipocyte is an important target for metabolic actions of GH (2), which appear to require no intervention by IGF-I (3, 4). GH produces a sustained increase in lipolysis and a decrease in glucose metabolism. These actions, which are opposite to those of insulin, are seen after a delay of 1–2 hr, and require altered genomic expression (2, 5). The immediate effects of GH, paradoxically, are in the opposite direction. Acutely, GH is insulin-like; it stimulates glucose utilization and antagonizes catecholamine-induced lipolysis (6). Other apparently independent, but presumably related, actions of GH on the adipocyte include induction of unresponsiveness or refractoriness to its own insulin-like actions and maintenance of intracellular calcium concentrations (7).

In attempting to understand the nature of GH action in adipocytes, we became interested in the question of whether the number and diversity of biological responses results from a single unique interaction of GH with its receptor, or arises from the interaction of GH with more than one class of receptors, each with its own signal transduction pathway. On the one hand, linearity of Scatchard plots suggested a single class of receptors (8–12), while, on the other hand, selective diminution of insulin-like activity of chemically modified GH preparations was consistent with more than one class of receptors (12, 13). Experiments in which GH was covalently cross-linked to receptor proteins revealed the presence of multiple complexes and were consistent with the possibility that there may be more than one species of GH receptor (GHR).

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The availability of cDNA for the rabbit liver GHR (14) made it possible to address this issue directly. Smith *et al.* (15) reported that mouse liver and adipose tissue contain two mRNA transcripts that hybridize with the cDNA for the GHR and that the translation products of these mRNAs were of a size consistent with the apparent masses of cross-linked GH-receptor complexes formed by mouse liver membranes. Northern blot analysis of RNA extracted from mature adipocytes confirmed the presence of an abundant 1.2-kb species of mRNA in addition to the 4.5-kb transcript that encodes the full length GHR. When we isolated and sequenced the cDNA corresponding to the smaller mRNA transcript (16), we found that it encodes a polypeptide that is identical to the circulating GH binding protein described by Baumbach *et al.* (17). The mRNA for the GH binding protein is a transcript of the same gene that codes for the full-length GHR, and is formed by alternate splicing such that transcripts of exons that code for the transmembrane and cytosolic domains are replaced with an RNA segment that codes for a 17 amino acid hydrophilic carboxyl terminus. A similar alternative splicing event is seen in the mouse (18). The long and short isoforms of the GHR (GHR_L and GHR_S) thus share a common extracellular hormone binding domain, but have distinct carboxyl termini.

We immunized rabbits with a synthetic 17 residue peptide that corresponds to its unique carboxyl terminus and obtained an antiserum (AS1615) that specifically immunoprecipitates GHR_S. In studies of extracts of adipocytes that were incubated with [^{35}S]L-methionine to label cellular proteins, AS1615 immunoprecipitated a [^{35}S]labeled protein with a mass of ~50 kDa. This protein, which is considerably larger than the ~30 kDa predicted from its nucleotide sequence, probably contains considerable carbohydrate and apparently is subsequently processed to proteins with masses of 42 and 38 kDa.

To determine the abundance of GHR_S and study its distribution in fat cells, we developed an immunofunctional assay that exploits the ability of GHR_S to bind GH specifically. In this assay, GHR_S is incubated with [^{125}I]hGH and the radioactive complex is immunoprecipitated with AS1615. Because the hormone binding site is near the amino terminus of GHR_S and the antigenic sites are at the carboxyl terminus, partially degraded or cleaved molecules of GHR_S are not detected in this assay. We prepared homogenates of adipocytes and, after centrifugation at 150,000*g* for 1 hr, examined the proteins that sedimented and those that remained in the supernatant fluid. Proteins in the pellet were extracted in mild detergent prior to assay. Somewhat surprisingly, more than 90% of the GHR_S recovered was present in the pellet. Furthermore, because it lacks a transmembrane domain, GHR_S was expected to be a secreted protein. However, virtually no GHR_S was detected in the medium in which a 1:3 suspension of cells had incubated for 3 hr.

To compare their abundance in adipocyte extracts, GHR_S and GHR_L were solubilized in mild detergent prior to incubation with various concentrations of [^{125}I]hGH. AS1615 was used to immunoprecipitate [^{125}I]hGH bound to GHR_S, and AS2941 (19), raised against the cytosolic domain of GHR_L (19), was used to immunoprecipitate [^{125}I]hGH bound to GHR_L (Fig. 1). Scatchard analysis revealed that GHR_S was almost six times as abundant as GHR_L. Each ml of adipocytes contained sufficient GHR_S to bind 137 ± 10 fmol of hGH and sufficient GHR_L to bind 24 ± 1 fmol of hGH. These data correspond to ~8300 copies of GHR_S and ~1400 copies of GHR_L per cell. Published estimates range from ~10,000 to ~25,000 GH binding sites per adipocyte (8–12). To the extent that solubilization and immunoprecipitation were less than 100% efficient, and that some receptor isoforms undoubtedly were damaged during extraction or assay, our analysis underestimates the actual abundance of the GHR isoforms. Control experiments, however, suggest that these factors have a similar impact on the estimates of GHR_S and GHR_L, so our assay provides a reliable assessment of the relative abundance of the two isoforms. The greater abundance of GHR_S than GHR_L parallels the greater abundance of the 1.2-kb mRNA as compared to the 4.5-kb mRNA.

These studies established that GHR_S is relatively abundant in adipocytes and is probably associated with cellular membranes. We sought next to determine whether any GHR_S is on the cell surface and therefore available to bind with extracellular GH. To do so, we

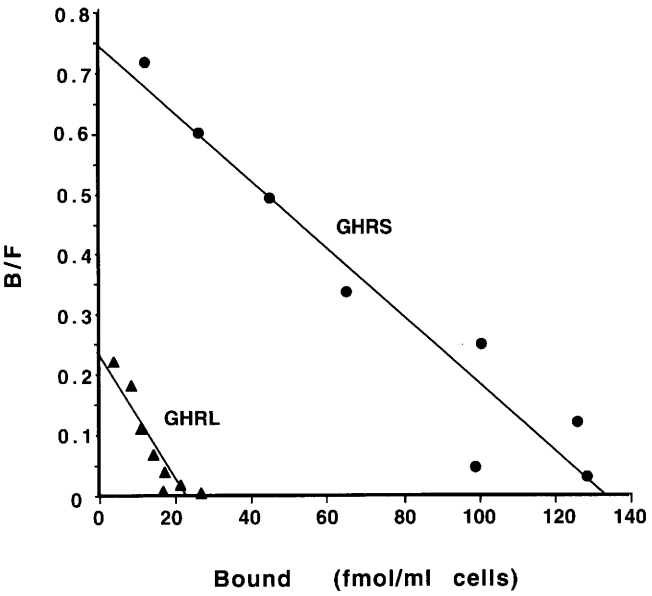

Figure 1. Scatchard analysis of [^{125}I]hGH binding by GHR and GHR_S in adipocyte extracts.

took advantage of an earlier observation that mild treatment of adipocytes with trypsin for 10 min reversibly depleted fat cells of binding sites for GH (20). We prepared adipocytes and divided them into three groups. The first group was studied immediately after cell isolation. We measured GH binding to intact cells and to GHR_S and GHR_L in cell-free extracts. Cells in the remaining two groups were incubated for 10 min with 0.1 mg/ml trypsin. Binding to cells, GHR_S and GHR_L was measured immediately (Group 2) or after a 2-hr recovery period (Group 3) in which the cells were treated with soybean trypsin inhibitor and incubated in trypsin-free medium. Treatment with trypsin decreased binding of [^{125}I]hGH to intact cells by about 75%, to GHR_S by about 20%, and to GHR_L by about 70%, suggesting that most of GHR_L is on the cell surface, while most of GHR_S is inaccessible to the enzyme, and presumably is intracellular. Within 2 hr, the cells completely recovered their capacity to bind to GH, and there was a parallel recovery of GHR_L. In contrast, binding of GH to GHR_S not only failed to recover, but declined further during the post-trypsin incubation period.

The decline in GHR_S that occurred in the course of a 2-hr incubation *in vitro* apparently was unrelated to trypsin treatment, since simply incubating cells for 2 hr produced a similar decline in GHR_S. Selective loss of GHR_S, while maintaining GHR_L constant, suggested that synthesis and/or degradation of the two receptor isoforms are independently regulated. To estimate the rates of turnover of the two isoforms, we measured their rates of decline after inhibition of protein synthesis with cycloheximide (20 μg/ml). Because duration of incubation is a confounding factor, all cells were incubated for 2 hr, and cycloheximide was added to separate aliquots at 30-min intervals. As reported previously (20), binding of [^{125}I]hGH declined with a half-time of 30–45 min. This finding is consistent with the observed full recovery of surface binding sites within 2 hr after trypsin. GHR_L decreased at a similar rate, also in accord with its full recovery 2 hr after trypsin. In contrast, inhibition of protein synthesis had little effect on GHR_S, which decreased by only about 25% in 2 hr in cells that incubated with cycloheximide compared to its abundance in cells that incubated without the inhibitor. From these results we conclude that cells synthesize little GHR_S under the conditions of *in vitro* incubation, and that the decrease in total GHR_S results from inadequate replenishment of the GHR_S that is degraded. Since ~50% of the total GHR_S that was present in freshly isolated cells remained after 2 hr of incubation in the presence of cycloheximide, it appears that, not only is GHR_S synthesized more slowly than GHR_L, but it is also degraded at a slower rate.

In contrast to the rapid decline in surface binding sites seen when protein synthesis was inhibited, inhibition of RNA synthesis with actinomycin D for 4 hr had no effect on GH binding to intact adipocytes (20). In harmony with this finding, Northern analysis of RNA extracted from cells incubated *in vitro* in the presence or absence of actinomycin D, revealed no changes in abundance of either the 4.5 or the 1.2-kb mRNA. These observations indicate that the messages for both GHR_L and GHR_S are considerably longer lived than the proteins they encode, and suggest that the different rates of synthesis of GHR_L and GHR_S result from regulation at the level of translation. Whether such translational control of GHR_S also occurs *in vivo* or is simply an artifact of *in vitro* conditions remains to be determined.

Despite the decline in total GHR_S in cells incubated *in vitro* for 2 hr, GHR_S on the cell surface was maintained at a constant level. When trypsin sensitivity of GHR_S was measured in cells that had incubated *in vitro* for 2 hr prior to addition of trypsin, the loss of GHR_S was as great as in freshly isolated cells. Figure 2 summarizes data from nine experiments with freshly isolated cells and three experiments with cells that preincubated for 2 hr. Total GHR_S decreased during incubation *in vitro,* but the decrease was limited to the intracellular (trypsin-insensitive) pool with the amount on the surface remaining constant. GHR_L remained constant with respect to the total amount and distribution between trypsin-sensitive and insensitive compartments. Figure 2 further shows that GHR_S and GHR_L are not only maintained on the cell surface in constant amounts, but also that they are present on the cell surface in nearly equal abundance.

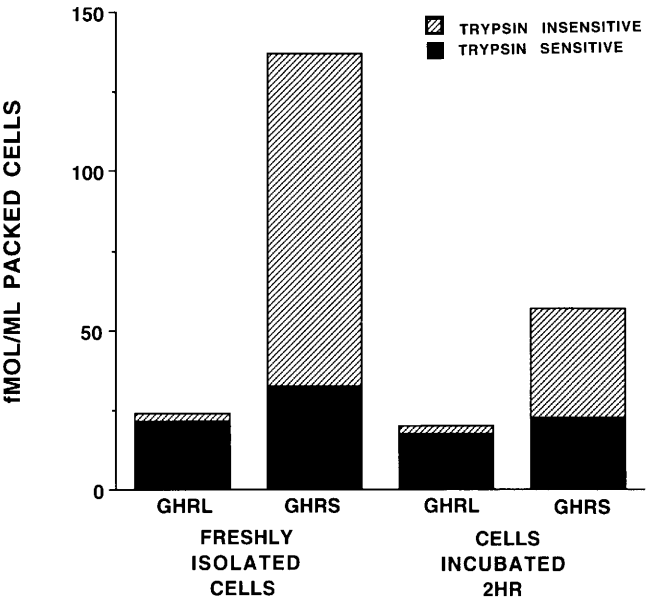

Figure 2. Trypsin sensitivity of GHR_L and GHR_S in extracts of freshly isolated adipocytes and in extracts prepared after 2 hr of incubation.

Because both GHR_S and GHR_L are present on the adipocyte surface, it is likely that both contribute to cellular binding of GH. Indeed, over-expression of GHR_S in 3T3-L1 adipocytes resulted in as much as a 4-fold increase in the capacity of these cells to bind [^{125}I]hGH, even though GHR_L was unchanged (21). Furthermore, in experiments in which [^{125}I]hGH was covalently cross-linked to surface binding sites, AS1615 specifically immunoprecipitated a labeled complex with a mass of ~60 kDa that is consistent with the combined mass of GH (22 kDa) and the mature GHR_S (38 or 42 kDa). This observation suggests that GHR_S is held on the adipocyte surface at a location and in a configuration that is favorable for binding GH.

If, indeed, GHR_S binds GH at the adipocyte surface, we may next ask what role, if any, it plays in expression of GH actions. In an earlier study (10), we found that, whereas the delayed responses to GH are maximum when fewer than 50% of the surface receptors are occupied, full expression of insulin-like responses requires saturation of binding sites (Fig. 3). Smal *et al.* made a similar observation (22). Thus, if occupation of all of the binding sites is required for full expression of the insulin-like response, and GHR_S comprises some, and perhaps as many as half, of the binding sites, it is reasonable to propose that GHR_S may contribute to expression of the response. To test this proposition, we examined the effects of AS1615 on the ability of GH to produce an insulin-like stimulation of lipogenesis in adipocytes. At a dilution of 1:20, AS1615 had no effect on GH binding to adipocytes but nevertheless reduced the response to GH by about 50%. While these preliminary findings await confirmation and expansion, they provide the first evidence that GHR_S may have a role in signal transduction.

These studies strongly suggest that GHR_S is a component of a signal transducing apparatus on the surface of rodent adipocytes. Although we have yet to establish how GHR_S is tethered to the adipocyte surface or intracellular membranes and how it interacts with intrinsic membrane proteins to generate a signal, a precedent for similar behavior was established for IL-6 and its receptor which belongs to the same family of the GH-cytokine receptors (23). In the presence of its ligand, a mutant form of the IL-6 receptor which lacked a transmembrane domain nevertheless participated in signal transduction by associating with a specific membrane spanning transducer, gp 130 (24). It is possible that a similar mechanism is operative for GH and GHR_S. Our findings raise additional questions. Is participation of GHR_S in expression of the GH response an anomaly found only in rodents, or does it represent a general phenomenon? If so, does the GHBP that arises in other species by post-translational cleavage of GHR_L instead of alternative splicing of RNA, play the same role as GHR_S, or has an alternatively spliced product of the GHR gene somehow escaped detection in the nonrodent species studied to date? Answers to these questions will go a long way toward elucidating the significance of the GHR_S.

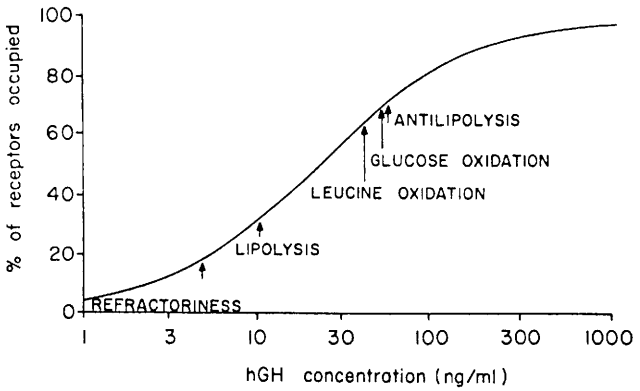

Figure 3. Theoretical relationship between receptor occupancy and biological responses of adipocytes. The arrows indicate the points on the curve that correspond to a half-maximal response for the indicated effects of GH. (Reprinted from Ref. 10 with permission.)

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1. Goodman HM. Growth hormone and metabolism. In: Schreibman MP, Scanes CG, Pang PKT, Eds. The Endocrinology of Growth, Development, and Metabolism in Vertebrates. San Diego: Academic Press, pp93–115, 1993.
2. Goodman HM. Growth hormone and the metabolism of carbohydrate and lipid in adipose tissue. Ann NY Acad Sci **148**:419–440, 1968.
3. Schwartz J, Goodman HM. Comparison of the delayed actions of growth hormone and somatomedin on adipose tissue metabolism. Endocrinology **98**:730–737, 1976.
4. Schwartz J, Foster CM, Satin MS. Growth hormone and insulin-like factors I and II produce distinct alterations in glucose metabolism in 3T3-F422A adipocytes. Proc Natl Acad Sci USA **82**:8724–8728, 1985.
5. Goodman HM. Effects of growth hormone on isolated adipose tissue. In: Pecile E, Müller EE, Eds. Growth Hormone, Proceedings of the International Symposium. Amsterdam: Excerpta Medica, pp153–171, 1968.
6. Birnbaum RS, Goodman HM. Comparison of several insulin-like effects of growth hormone. Horm Metab Res **11**:136–142, 1979.
7. Schwartz Y, Goodman HM, Yamaguchi H. Refractoriness to growth hormone is associated with increased intracellular calcium in rat adipocytes. Proc Natl Acad Sci USA **88**:6790–6794, 1991.
8. Fagin KD, Lackey SL, Reagan CR, DiGirolamo M. Specific binding of growth hormone by rat adipocytes. Endocrinology **107**:608–615, 1980.
9. Gavin JR, Saltman RJ, Tollefsen SE. Growth hormone receptors in isolated rat adipocytes. Endocrinology **110**:637–643, 1982.
10. Grichting G, Levy LK, Goodman HM. Relationship between

binding and biological effects of hGH in rat adipocytes. Endocrinology **113**:1697–1702, 1983.
11. Grichting G, Goodman HM. Growth hormone maintains its own receptors in rat adipocytes. Endocrinology **119**:847–854, 1986.
12. Chipkin SR, Tai L-R, Kostyo JL, Goodman HM. Different growth hormone receptor interactions mediate insulin-like and lipolytic responses of rat adipose tissue. Endocrinology **125**: 450–458, 1989.
13. Goodman HM, Kostyo JL. Altered profiles of biological activity of growth hormone fragments on adipocyte metabolism. Endocrinology **108**:553–558, 1981.
14. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. Growth hormone receptor and serum binding protein: Purification, cloning and expression. Nature **330**:537–543, 1987.
15. Smith WC, Linzer DIH, Talamantes F. Detection of two growth hormone receptor mRNAs and primary translation products in the mouse. Proc Natl Acad Sci USA **85**:9576–9579, 1988.
16. Frick GP, Goodman HM. Characterization of the short isoform of the growth hormone receptor synthesized by rat adipocytes. Endocrinology **131**:3083–3090, 1989.
17. 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.
18. Smith WC, Kuniyoshi J, Talamantes F. Mouse serum growth hormone (GH) binding protein has GH receptor extracellular and substituted transmembrane domains. Mol Endocrinol **3**:984–990, 1989.
19. Frick GP, Tai L-R, Goodman HM. Subcellular distribution of the long and short isoforms of the GH receptor in rat adipocytes: Both isoforms participate in specific binding of growth hormone. Endocrinology **134**:307–314, 1994
20. Gorin E, Goodman HM. Turnover of growth hormone receptors in rat adipocytes. Endocrinology **116**:1796–1805, 1985.
21. Bick T, Frick GP, Leonard JL, Goodman HM. Overexpression of the rat GH binding protein (GHBP) increases cellular binding of GH and produces functional changes in 3T3-L1 preadipocytes. Program and Abstracts, The Endocrine Society, p329, 1993.
22. Smal J, Closset J, Hennen G, De Meyts P. Receptor binding properties and insulin-like effects of human growth hormone and its 20 kDa-variant in rat adipocytes. J Biol Chem **262**:11071–11079, 1987.
23. Kelly PA, Dijiane J, Postel-Vinay M-C, Edery M. The prolactin/growth hormone receptor family. Endocr Rev **12**:235–251, 1991.
24. Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T, Kishimoto T. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp 130. Cell **58**:573–581, 1989.