Discovery and mechanism of action of pegvisomant

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Abstract

Using a structure–function approach to the understanding of the molecular topology of the GH molecule, we discovered that glycine in the third α -helix of GH (G119 of bovine GH and G120 of human GH) was an important amino acid required for GH action. Substitution of this glycine residue with a variety of amino acids results in molecules that lack growth-promoting activity. More importantly, these molecules inhibit the actions of GH both *in vitro* and *in vivo*. These results, obtained more than a decade ago, were the basis for the discovery of GH antagonists. Since that time, efforts have been focused on establishing the mechanism by which these antagonists inhibit GH action. In this regard, *in vivo* expression of GH-antagonist genes in transgenic mice results in dwarf animals. The animals are fertile and possess no abnormal ‘phenotypes’. Dwarf mice have also been created by disrupting or ‘knocking out’ the GH receptor gene. Together, these results have laid the foundation for the clinical use of GH antagonists when endogenous GH levels are increased or when GH is known to be a factor in the progression of the disorder.

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Introduction

The journey of scientific discovery that led to the development of pegvisomant, a growth hormone (GH) receptor (GHR) antagonist, began approximately 15 years ago with a series of experiments aimed at elucidating the structure–function relationship of GH. This 22 kDa protein is secreted from the pituitary gland in an episodic pattern and is central to the regulation of growth (Fig. 1) (1, 2). It also has numerous other biological effects, including enhancement of milk production, nitrogen retention, lipolysis and diabetogenic-like effects (3). GH exerts its activity either directly on tissues such as liver, muscle, bone or fat to induce metabolic changes, or indirectly through the induction of insulin-like growth factor-I (IGF-I) expression, which also is a growth-promoting factor (Fig. 1) (1). This review outlines the experimental strategy that led to the discovery of pegvisomant, the first GHR antagonist to be used clinically.

Structure of GH and GHR

The 3-dimensional structure of GH was first determined in 1987 by X-ray crystallography, employing a genetically engineered variant of porcine (p) GH (4). The 191 amino acid pGH protein consists of four α -helices, ranging from 20 to 30 residues each, joined together by stretches of nonhelical polypeptide chains, which are tightly packed together in an antiparallel bundle

(Fig. 2) (4). There are two disulfide bridges in the protein, the first between Cys residues 53 and 164, the second between Cys residues 181 and 189 (4).

GHR is a transmembrane protein with an extracellular domain that binds to GH; the intracellular domain interacts with other proteins in a signal transduction cascade that controls cellular functions of the target cell (5). The binding relationship between GH and GHR was determined by X-ray crystallography, using the extracellular domain of the GHR bound to GH. In the crystals of the human (h) GH–receptor complex, a single hGH molecule is bound to the GHR in a ratio of 1:2, suggesting dimerisation of the receptor (6, 7). Further analysis shows that GH has two binding sites located on opposite sides of the bundle of α -helices (8). Binding of hGH to the two GHRs is thought to be sequential; site 1 on hGH binds one GHR first, followed by site 2 binding the second GHR (7, 8).

Structure–function relationship of GH

Mutagenic studies were conducted to determine the relationship between the structure and function of GH in parallel with the studies aimed at determining the 3-dimensional structure of GH. Identification of the appropriate amino acids within the sequence to modify is one of the difficulties encountered in this type of study. Considerable homology exists between GH amino acid sequences from different species, particularly in the α -helical regions. This suggests

Figure 1 Schematic depiction of the regulation of GH secretion and effects of GH within the body. GH is released in a pulsatile manner and then binds to GHRs on target tissue, including adipose, muscle, liver and bone. GH also promotes secretion of IGF-I that also stimulates growth processes. IGF-I inhibits further GH release via a long negative-feedback loop. (Reprinted, with permission, from (2).)

that these regions are important for the structural and functional integrity of GH (4). Alignment of the third α -helix into a 2-dimensional Edmundson wheel (axial) projection reveals an amphipathic segment of amino acids between residues 109 and 126 of bovine (b) GH (bGH (109–126)). An amphipathic segment of protein has hydrophilic amino acids clustered together and hydrophobic residues clustered separately in the axial projection of the helix. In bGH (109–126), however, amino acid residues 117, 119 and 122 are positioned so that an imperfect amphipathic α -helix is produced (Fig. 3A). Changing an imperfect amphipathic α -helix to a perfect α -helix had previously been shown to increase the potency of certain molecules such as GH-releasing factor (9). A series of studies was thus undertaken to determine whether modification of these three residues in the GH molecule that generate a perfect amphipathic α -helix would alter the growth-related properties of bGH. A mutated bGH gene was engineered in which the codon for Glu-117 was replaced with Leu (E117L), Gly-119 was replaced with Arg (G119R), and Ala-122 was replaced with Asp (A122D) (3^{Δ} bGH) (10). The resulting molecule possessed a perfect amphipathic α -helix with all

Figure 2 Schematic representation of the structure of GH. The four α -helices are drawn as cylindrical rods, while the regions of nonhelical polypeptide are shown as thin tape. The third α -helix is formed by residues 109–126. (Reprinted, with permission, from (2).)

hydrophobic residues below and hydrophilic residues above the midline of the helix (Fig. 3B).

The 3^{Δ} bGH analogue was then characterized *in vitro* and *in vivo*. Data from *in vitro* GHR binding studies showed no significant difference between wild-type bGH and 3^{Δ} bGH in their GHR interactions, indicating that 3^{Δ} bGH had a binding affinity similar to wild-type GH. Surprisingly, however, when the gene encoding 3^{Δ} bGH analogue was expressed in transgenic mice, it resulted in growth retardation; the transgenic mice that expressed the mutated GH were significantly smaller compared with nontransgenic littermates (10). This result was contrary to the expectation that the 3^{Δ} bGH transgenic mice would be larger than normal. When pituitary levels of mouse (m) GH were determined in these transgenic mice, GH levels were relatively high but serum IGF-I levels were low (11). The expression of mGH in the pituitary gland is controlled by IGF-I as part of a classic negative-feedback system. When GH is released, it interacts with the GHR, stimulating production of IGF-I in the target cells. If GH interaction with its receptor is inhibited and IGF-I is not expressed, then the negative feedback on GH production in the pituitary gland does not occur, resulting in increased GH concentrations. Thus, in the 3^{Δ} bGH transgenic mice, 3^{Δ} bGH was acting as an *in vivo* antagonist to mGH activity, the first such functional GH antagonist to be identified.

The explanation as to why the mutated GH was unable to elicit a growth response was unknown. It bound the receptor with a high affinity and with similar kinetics to the wild-type GH *in vitro*, but the three amino acid substitutions in GH appeared to prevent

Figure 3 Edmundson wheel projection of the third α -helix of (A) wild-type bGH, and (B) the mutated GH (3^{Δ} bGH). Amino acids are depicted with their position in the polypeptide chain and the corresponding hydrophilicity values. Shaded residues are hydrophobic. The Gly residue is nonpolar and is depicted with dots. (Reprinted, with permission, from (10).)

activation of the receptor (10). Structural studies then demonstrated that dual binding sites on hGH to two GHRs are required for receptor dimerisation and activation, which results in growth-promoting activities (7, 8). Thus, one GH molecule interacting with two GHRs are required for subsequent intracellular signalling that ultimately results in the various phenotypes associated with GH action. One should note that the GH antagonist does not inhibit GHR dimerisation but prevents proper dimerisation (12, 13).

Although the structure of 3^{Δ} bGH had been characterised, it was not clear which one or more of the three amino acid changes was required for the antagonistic activity. Unique GH analogues were generated for each of the amino acid residues in question. As with the triple-mutation analogue, all three of the single mutated analogues bound to the GHR with similar affinities. However, it was only when the G119 residue was substituted (bGH-G119R) that a dwarf transgenic mouse was produced (14). Thus functional antagonism of the GHR in the 3^{Δ} bGH analogue was determined by the amino acid in the G119R mutation. Similar studies were performed with hGH where altering hGH Gly-120 to Arg resulted in an hGH antagonist (15).

GH as an antagonist: the role of Gly-119 substitution

Gly-119, which corresponds to Gly-120 in hGH, is invariant in the amino acid sequences of GH family members (16), suggesting that this residue is crucial

for the functioning of GH. Gly is the smallest amino acid and lacks a bulky side chain. In a space-filling model of hGH, Gly-120 is positioned alongside an Ala residue (the second-smallest amino acid), producing a substantial cleft in the 3-dimensional model of the protein (Fig. 4). Mutational studies showed that replacing this Gly with several amino acids other than Ala resulted in an hGH antagonist (14). A subsequent report confirmed that replacing hGH G120 with arginine resulted in a GH antagonist (17). In the co-crystal

Figure 4 Space-filling models of GH and the mutated GH antagonist, showing the cleft that occurs naturally in the third α -helix of GH because of the presence of Gly, and the same cleft filled in the GH antagonist by an amino acid with a large side group. (Reprinted, with permission, from (14).)

structure of the GH–receptor complex, the cleft in the third α -helix interacts with a Trp residue in the GHR molecule (Trp-104). Any residue larger than an Ala or Gly residue at residue 120 of hGH results in disruption of the proper binding of the second receptor; thus no functional receptor dimerisation or subsequent intracellular signalling (12, 13).

The first GHR antagonist: pegvisomant

Like wild-type hGH, genetically engineered variants of GH are rapidly cleared by the kidney and have very short elimination half-lives. This potentially limits their usefulness as GH antagonists. To overcome these problems, the GH antagonist (hGH G120K) was pegylated, that is, four or five polyethylene glycol (PEG) residues were added to the molecule. These PEG molecules are covalently attached to Lys residues producing a molecule with a mass of 42–46 kDa (18, 19).

In addition to the single amino acid modification of binding site 2 (G120R) discussed earlier, the pegvisomant molecule has modifications at binding site 1 that increase binding affinity and give it a competitive binding advantage over wild-type hGH (reviewed by Wells and colleagues (3)). The eight substitutions in binding site 1 are H18D, H21N, R167N, K168A, D171S, K172R, E174S, I179T (20, 21). It has recently been suggested that the crucial changes are K168A and K172R, both Lys residues. Substitution of these Lys residues with other amino acid removed potential PEG addition sites (13). The resulting GHR antagonist has been termed pegvisomant.

An additional concern is that the introduction of a modified GH protein into patients could trigger the formation of antibodies. In long-term clinical studies, antibodies were detected in 27 of 160 patients treated with pegvisomant (16.9%); however, tachyphylaxis was not seen (23).

The results of the above-described studies laid the foundation for the clinical use of GHR antagonists in acromegaly. Pegvisomant, the first GHR antagonist available for clinical development, has a prolonged half-life, does not induce antibodies to itself, and acts as an antagonist at the GHR *in vitro* and *in vivo* (1, 19, 21). Pegvisomant has been shown to effectively reduce serum IGF-I concentrations in both short- and long-term clinical studies, demonstrating its efficacy in greater than 97% of patients in the treatment of acromegaly (22, 23). A recent review concerning the discovery and use of GH antagonists has been presented (24).

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