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Human Growth Hormone: 45-kDa Isoform with Extraordinarily Stable Interchain Disulfide Links has Attenuated Receptor-Binding and Cell-Proliferative Activities

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Abstract

Background—Human growth hormone (hGH) is a complex mixture of molecular isoforms. Gaps in our knowledge exist regarding the structures and biological significances of the uncharacterized hGH molecular variants. Mercaptoethanol-resistant 45-kDa human growth hormone (MER-45kDa hGH) is an extraordinarily stable disulfide-linked hGH homodimer whose biological significance is unknown.

Objectives—To elucidate the pharmacokinetic abilities of dimeric MER-45-kDa hGH to bind to GH and prolactin (PRL) receptors and to elucidate its abilities to stimulate cell-proliferation in lactogen-induced and somatogen-induced in vitro cell proliferation bioassays.

Design—The binding of MER-45-kDa hGH to GH and PRL receptors was tested in radioreceptorassays (RRAs). Competitive displacements of [^{125}I]-bovine GH from bovine liver membranes, [^{125}I]-ovine PRL from lactating rabbit mammary gland membranes and [^{125}I]-hGH from human IM-9 lymphocytes by unlabelled GHs, PRLs or dimeric MER-45-kDa hGH were evaluated. The abilities of dimeric MER-45-kDa hGH to stimulate proliferation of lactogen-responsive Nb2 lymphoma cells and to stimulate proliferation of somatogen-responsive T47-D

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human breast cancer cells was assessed by incubation of cells with GHs or PRLs and subsequently measuring growth using the MTS cell proliferation assay.

Results—Dimeric MER-45-kDa hGH, compared to monomeric hGH, had reduced binding affinities to both GH and prolactin receptors. In a bovine liver GH radioreceptorassay its ED_{50} (197.5 pM) was 40.8% that of monomeric hGH. In a human IM-9 lymphocyte hGH RRA its ED_{50} (2.96 nM) was 26.2% that of monomeric hGH. In a lactating rabbit mammary gland prolactin RRA its ED_{50} (3.56 nM) was 16.8% that of a monomeric hGH. Dimeric MER-45-kDa hGH, compared to monomeric hGH, had a diminished capacity to stimulate proliferation of cells in vitro. In a dose-response relationship assessing proliferation of Nb2 lymphoma cells its ED_{50} (191 pM) was 18.0% that of monomeric hGH. While monomeric hGH stimulated a 2.2-fold proliferation of T47-D human breast cancer cells above vehicle control, dimeric MER-45-kDa hGH was unable to stimulate the cells to proliferate and slightly inhibited their proliferation to 77.6% that of control.

Conclusions—The topological arrangement of monomeric hGHs to form an unusually stable disulfide-linked dimer markedly diminishes hGH's binding affinities to both GH and PRL receptors and also drastically attenuates its ability to stimulate proliferation of cells in vitro.

Keywords

Dimeric human growth hormone; somatotropin; isoform; radioreceptorassay; molecular variant

1. Introduction

Pituitary GHs are important regulators of growth, metabolism and development [1–3]. The hGHs are a complex mixture of molecular isoforms that constitute approximately 10% of the dry weight of the pituitary gland [4–10]. Although a 22-kDa hGH is the predicted protein product of the hGH-N gene, posttranscriptional and posttranslational processing produce different hGH isoforms, as shown in a western blot of human pituitary extract separated by 2-D IEF/SDS-PAGE [7]. Small hGH isoforms (5-kDa and 17-kDa) are produced by fragmentation of the 22-kDa hGH and a slightly smaller alternatively spliced 20-kDa hGH is also produced [4–8,10]. Other molecular variants of hGH include the 24-kDa and 12-kDa glycosylated hGHs [11–13], deamidated hGHs [14] and phosphorylated hGHs [15,16]. In addition, large hGH isoforms such as a 35-kDa hGH, 45-kDa hGH dimers and higher oligomers of hGH have been reported [4–8,10]. The molecular variants are present in serum to varying degrees [8,17]. A few isoforms of hGH have enhanced biological activities when compared to the 22-kDa hGH, others have reduced bioactivity, several are completely bioinactive and some can only regulate a limited number of metabolic and physiological processes [4–8,10]. The hGH isoforms can thus act as full or partial agonists and antagonists of monomeric 22-kDa hGH. Many of the hGH isoforms have not been isolated, hence, their biological significances remain unknown.

Nevertheless, certain progress is being made, as in a recent study in which an extraordinarily stable dimeric 45-kDa hGH isoform was isolated and structurally characterized [18]. The mercaptoethanol-resistant (MER) 45-kDa hGH was shown to be an inter-chain disulfide-linked 22-kDa hGH homodimer which does not readily dissociate into monomers even when incubated in 10% 2-mercaptoethanol at 100 °C for extended periods of time. Development of a method for preparation of dimeric MER-45-kDa hGH suitable for biological testing [19] has led us to the current work assessing its capacity to interact with lactogenic and somatogenic receptors and its ability to stimulate proliferation of rat Nb2 lymphoma cells and T47-D human breast cancer cells. Our findings demonstrate that dimerization of hGH through unusually stable disulfide bonds attenuates hGH's receptor-binding and cell proliferative functions.

2. Materials And Methods

2.1. Chemicals

EGTA, bromocriptine (Lot 107H1244), sodium azide, sodium phosphate, sodium chloride, Fischer's medium, BSA, penicillin and streptomycin were purchased from Sigma (St. Louis, MO, USA). EDTA, ammonium bicarbonate and sodium bicarbonate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Tris, was purchased from EM Science (Gibbstown, NJ, USA). Gelding horse serum was purchased from Animal Technologies, Inc. (Tyler, TX, USA). Fetal calf serum, Dulbecco's modified Eagle's medium and Ham nutrient mixture F-12 medium 50:50 (DMEM/Ham's F12), and RPMI-1640 medium were purchased from Gibco (Gibco BRL Div. of Invitrogen, Gaithersburg, MD, USA). Trypsin-EDTA was purchased from HyClone (Logan, UT, USA).

2.2. Hormone preparations

Bovine GH (bGH; NHPP AFP10325C), monomeric hGH (monomeric NIH-hGH; NIDDK-NIH AFP 9755A), radioiodination grade hGH (radioiodination grade monomeric NIH-hGH, hGH AFP11019B), ovine PRL (oPRL; NIDDK-NIH AFP10692C), and human PRL (NHPP-hPRL; recombinant human prolactin, NHPP AFP 795) were obtained from Dr. A.F. Parlow of the National Hormone and Pituitary Program (Harbor-UCLA Medical Center, Torrance, CA, USA). MER-45-kDa hGH was previously isolated [19].

2.3. Protein determination

Quantification of protein [20] was carried out using the Microassay Procedure for microtiter plates (Bio-Rad, Hercules, CA, USA) and bovine serum albumin as a standard.

2.4. Radioiodination of hormones

Bovine GH, radioiodination grade monomeric NIH-hGH, and oPRL were each radiolabeled using an established procedure [21] with modifications [22]. The specific activities of the bGH, radioiodination grade monomeric NIH-hGH, and oPRL preparations were 62 μ Ci/μ g, 63 μ Ci/μ g, and 44 μ Ci/μ g, respectively.

2.5. Experimental animals

All animal experimentation was conducted in accordance with the accepted standards of humane animal care. All research animals were acquired and used in compliance with federal, state, and local laws and institutional regulations. Animals were maintained in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals.

2.6. Membrane preparations

Bovine liver membranes were prepared as described [23]. Lactating rabbit mammary gland membranes were prepared as described [22]. The lactating New Zealand White Rabbit, obtained from Harlan (Indianapolis, IN, USA), was subcutaneously injected at times 0, 12, and 24 hr with 1.0 mL/injection of a 1.0 mg bromocriptine/mL solution then sacrificed at 36 h. The mammary glands were subsequently dissected, frozen on dry ice then stored at 0 °C until used for preparation of microsomal membranes.

2.7. Non-linear curve fitting and statistical analyses

Simultaneous non-linear curve fitting of families of sigmoidal dose-response curves of radiolabelled hormone displacement data obtained in the RRAs and of rat Nb2 lymphoma cell number data obtained in the hormone-induced cell proliferation bioassay were carried out using the ALLFIT [24]. The ED_{50} values for curves within an assay were tested for

significant differences using One-Way ANOVA. If the ANOVA F-test indicated that ED_{50} values were significantly different from each other ($P < 0.05$), a *post hoc* Bonferroni Multiple Comparison Test was used to determine differences among the ED_{50}s ($P < 0.05$). An unpaired two-tailed Student's t-test was used when comparing ED_{50} values for significant differences ($P < 0.05$) in assays that had only two displacement curves.

2.8. Bovine liver GH radioreceptorassay

The bovine liver GH RRA was carried out as described [23] using [^{125}I]-bGH (20,000 cpm) with each dose point assayed in duplicate (n=2).

2.9. Human IM-9 lymphocyte hGH radioreceptorassay

The human IM-9 lymphocyte hGH RRA was carried out as reported [25,26] with some modifications. Human IM-9 lymphoblastoid cells were grown in suspension culture in RPMI-1640 medium supplemented with 2 g/L sodium bicarbonate, 10% (v/v) fetal calf serum, 50 U/mL penicillin, 50 μ g/mL streptomycin at pH 7.5 in a humidified 5% CO_2 atmosphere at 37 °C. Before initiating the RRA, the RPMI-1640 medium was replaced with phosphate assay buffer [10 mM sodium phosphate, 150 mM sodium chloride, 10 mM EDTA, 10 mM EGTA, 0.1% (w/v) BSA, pH 7.6]. Cells were aliquoted into 12 × 75 mm polypropylene tubes (2×10^6 cells/tube) in phosphate assay buffer along with monomeric [^{125}I]-NIH-hGH (160,000 cpm/tube) in a total volume of 900 μl and incubated at 25 °C for 1 h. Subsequently, 100 μl aliquots containing various amounts (1 pg – 10 μg) of unlabelled monomeric NIH-hGH, NHPP-hPRL or dimeric MER-45-kDa hGH were added to the tubes, and the incubation was resumed for another 2 hr at 25 °C. Then 1 mL of assay buffer (5 °C) was added, the cells were centrifuged for 15 min at 10,000 × g at 5 °C and the supernatant was aspirated. The cells were washed with 4 mL assay buffer (5 °C) and the amount of [^{125}I]-NIH-hGH associated with the pellet determined. Each dose point was assayed in triplicate (n=3).

2.10. Lactating rabbit mammary gland PRL radioreceptorassay

The lactating rabbit mammary gland PRL RRA was carried out as described [22] using [^{125}I]-oPRL (20,000 cpm). Each dose point was assayed in duplicate (n=2).

2.11. Rat Nb2 lymphoma cell proliferation bioassay

The rat Nb2 lymphoma cell proliferation was performed as described [27] with some modifications. Rat Nb2-11 cells were cultured in assay media (Fischer's medium supplemented with 10% v/v fetal calf serum, 10% v/v horse gelding serum, 50 U/mL penicillin, 50 μg/mL streptomycin, 50 mM 2-mercaptoethanol, 1.2 mM sodium bicarbonate, at pH 7.5) in a humidified 5% CO_2 atmosphere at 37 °C. Twenty-four hours before initiating the assay, the cells were rinsed twice in cell-culture media without fetal calf serum and incubated in the same media for 24 hr. Cells were then harvested (0.05% Trypsin-EDTA), rinsed twice in cell-culture media without fetal calf serum, and aliquots of cells (50 μl containing 25,000 cells) were incubated for 72 hr in wells of a 96 well microtiter plate with varying amounts (0 – 25 ng) of NHPP-hPRL, monomeric NIH-hGH or dimeric MER-45-kDa hGH in a final assay volume of 100 μ l. The cell growth was measured by the MTS Assay [28] using the CellTiter 96® AQueous One Solution Cell Proliferation Assay Reagent (Promega, Madison, WI, USA). The numbers of rat Nb2-11 lymphoma cells were calculated using a standard curve having a linear correlation between a known cell number and absorbance at 490 nm. Each dose point was assayed in triplicate (n=3).

2.12. T47-D human breast cancer cell proliferation bioassay

T47-D human breast cancer cells were purchased from the American Type Culture Collection. The cell proliferation assay [29] was modified. Cells were cultured in DMEM/Ham's F12 supplemented with 2 mM glutamine, 100 units of penicillin/mL, 100 μg of streptomycin/mL and 10% (v/v) fetal bovine serum (FBS) in a humidified 5% CO_2 atmosphere at 37 °C. Prior to trypsinization [1 mL 0.25% (v/v) trypsin/25 cm^2 growth area of culture flask], the cultures were rinsed twice with sterile 120 mM phosphate buffered saline (PBS, pH 7.4) and serum-starved for 36 hr in DMEM/Ham's F-12 media containing no FBS. Cell density was determined [30] and cells were seeded at 8×10^3 cells/well then cultured for 70 hours in the presence of 9.1×10^{-9} M monomeric NIH-hGH standard, 9.1×10^{-9} M dimeric MER-45-kDa hGH or vehicle [0.01 M $NaHCO_3$, 0.1% (w/v) bovine serum albumin, 0.1% (v/v) glycerol] in a total volume of 100 μL. At the end of the incubation cell numbers were determined using the MTS Assay. Each dose point was assayed triplicate (n=3). A One-Way ANOVA was employed to test the equality of the means among the treatment groups. If the means among the groups were found to statistically differ, then a *post hoc* Student-Neuman-Keuls post hoc test was employed to reveal significances between the groups. Differences were considered to be significant at $p < 0.05$. Statistical analyses were conducted using the GraphPad Prism v4.0 statistical suite (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Attenuated binding of dimeric MER-45-kDa hGH to GH receptors in a bovine GH radioreceptorassay

The competitive displacement of [^{125}I]-bGH from bovine liver microsomal membrane GH-binding sites by unlabelled bGH, monomeric NIH-hGH, dimeric MER-45-kDa hGH, or NHPP-hPRL is shown in Figure 1. The displacement curve for dimeric MER-45-kDa hGH was horizontally shifted to the right of both monomeric hGH and monomeric bGH indicating a weaker ability to compete for binding to GH receptors. Table 1 lists the ED_{50} values and relative potencies of each of the hormones for binding to the GH receptors. The inability of NHPP-hPRL to displace [^{125}I]-bGH from bovine liver membranes demonstrated the specificity of the assay for GHs. In this heterologous GH RRA, dimeric MER-45-kDa hGH's relative GH-receptor binding potency was 38.8% that of bovine GH and 40.8% that of monomeric NIH-hGH.

3.2. Attenuated binding of dimeric MER-45-kDa hGH to GH receptors in a human IM-9 lymphocyte hGH radioreceptorassay

IM-9 lymphocyte hGH receptor occupancy by NIH-[^{125}I]-hGH and its competitive displacement by unlabelled monomeric NIH-hGH, dimeric MER-45-kDa hGH, or NHPP-hPRL is shown in Figure 2. The dose-response curve for dimeric MER-45-kDa hGH was horizontally positioned to the right of monomeric hGH indicating an impaired ability to compete for binding to hGH receptors. Table 2 lists the ED_{50} values and relative potencies of each of the hormones for binding to the hGH receptors. The inability of NHPP-hPRL to displace monomeric [^{125}I]-NIH-hGH from human IM-9 lymphocytes demonstrated the specificity of the assay for hGHs. In this hGH RRA, dimeric MER-45-kDa hGH's relative hGH-receptor binding potency was 26.2% that of monomeric NIH-hGH.

3.3. Attenuated binding of dimeric MER-45-kDa hGH to PRL receptors in a lactating rabbit mammary gland PRL radioreceptorassay

Results of the lactating rabbit mammary gland radioreceptor assay showing competitive displacement of [^{125}I]-oPRL from PRL-binding sites by unlabelled oPRL, monomeric NIH-

hGH, dimeric MER-45-kDa hGH, or bGH is shown in Figure 3. The displacement curve for dimeric MER-45-kDa hGH was horizontally located to the right of both oPRL and monomeric hGH indicating abated ability to compete for binding to PRL receptors. Table 3 lists the ED_{50} values and relative potencies of each of the hormones for binding to the PRL receptors. The inability of bGH to displace [^{125}I]-oPRL from lactating rabbit mammary gland membranes demonstrated the specificity of the assay for lactogens. In this PRL RRA, dimeric MER-45-kDa hGH's relative PRL-receptor binding potency was 14.8% that of oPRL and 16.8% that of monomeric NIH-hGH.

3.4. Weak potency of dimeric MER-45-kDa hGH in stimulating proliferation of rat Nb2 lymphoma cells

The stimulation of rat Nb2 lymphoma cell proliferation by NHPP-hPRL, monomeric NIH-hGH and dimeric MER-45-kDa hGH is shown in Figure 4. The stimulus-response curve for dimeric MER-45-kDa hGH was horizontally situated to the right of both hPRL and monomeric hGH indicating weaker ability to stimulate proliferation of rat Nb2 lymphoma cells. Table 4 lists the ED_{50} values and relative potencies of each of the hormones for stimulating proliferation of rat Nb2 lymphoma cells. In this in vitro bioassay for lactogenic hormones dimeric MER-45-kDa hGH's relative efficacy in stimulating proliferation of rat Nb2 lymphoma cells was 18.0% that of monomeric NIH-hGH.

3.5. Inability of dimeric MER-45-kDa hGH to stimulate proliferation of T47-D human breast cancer cells

The effects of dimeric Mer-45-kDa hGH and monomeric NIH-hGH standard on proliferation of T47-D human breast cancer cells are shown in Figure 5. Monomeric NIH-hGH standard (solid black bar) stimulated proliferation of T47-D cells ($4.54 \pm 0.30 \times 10^4$ cells) 2.2-fold above vehicle control (checkered bar; $2.05 \pm 0.20 \times 10^4$ cells) ($p < 0.001$). In contrast, dimeric Mer-45-kDa hGH (solid white bar) significantly reduced the growth of T47-D cells ($1.59 \pm 0.20 \times 10^4$ cells) to 77.6% below the vehicle control (checkered bar) ($p < 0.05$). These findings demonstrate that dimeric Mer-45-kDa hGH, unlike monomeric NIH-hGH standard, does not promote proliferation of T47-D human breast cancer cells but instead partially blocks their proliferation.

4. Discussion

A dimeric MER-45-kDa hGH isoform isolated from human pituitaries [18] was shown to be a homodimer of 22-kDa hGH subunits held together by unusually stable inter-chain disulfide bridges. Ample evidence indicates that the dimeric MER-45-kDa hGH is a naturally occurring hGH isoform. For instance, SDS-PAGE analysis showed that it was present in extracts of fresh pituitary glands [31]. The isoform was also observed in culture media of human pituitary adenomas [32]. In addition, dimeric MER-45-kDa hGH was detected in the circulation [8,17]. It is estimated that dimeric MER-45-kDa hGH comprises 2%% of the pituitary hGH [6,8,33] and approximately 1% of serum hGH [6]. A procedure for the isolation of MER-45-kDa hGH was recently developed that provided a preparation suitable for biological testing [19]. Therefore, in the present work, to ameliorate the gap in our knowledge about the functional significance of dimeric MER-45-kDa hGH, we have reported our pharmacological evaluation of its interactions with lactogenic and somatogenic receptors and its efficacies in promoting proliferation of either rat Nb2 lymphoma cells or T47-D human breast cancer cells.

GHs, PRLs, placental lactogens and related proteins are structurally and functionally similar [34–36]. However, individual hormones of this family are categorized as lactogenic or somatogenic based on their potencies in well-established bioassays and RRAs [37,38]. Thus,

in GH RRAs, a somatogen receptor will bind bGH more potently than oPRL and in PRL RRAs, a lactogen receptor will bind oPRL more potently than bGH. Most of the hormones in the GH family are strictly somatogenic or lactogenic. Human GH can bind to both types of receptors and, accordingly, has both types of biological activities. Hence, the bioactivities and receptor-binding activities of hGH isoforms were measured in both GH (somatogenic) assays and PRL (lactogenic) assays.

Our data demonstrated that dimeric MER-45-kDa hGH could bind to both lactogenic and somatogenic receptors, however, its receptor-binding potencies were attenuated compared to those of monomeric hGH. The dimeric MER-45-kDa hGH also had diminished growth-promoting activity in an in vitro Nb2 lymphoma cell proliferation bioassay compared to monomeric hGH. In addition, dimeric MER-45-kDa hGH lacked growth-promoting activity in an in vitro T47-D human breast cancer cell proliferation bioassay compared to monomeric hGH. Hence, association of hGH monomers to form the extraordinarily stable disulfide-linked dimeric MER-45-kDa hGH isoform brought about a quaternary structural change that markedly attenuated hGH's receptor-binding and biological activities.

The activities of various hGH dimers and bGH dimers are compared to monomeric GHs in Table 5. The relative activities of the GH dimers with respect to the GH monomers vary according to the type of GH dimer and assay. The differences amongst the receptor-binding and biological activities of the various types of dimeric GHs are likely due to differences in the geometric symmetries of the associated momoners when assembled in their quaternary configurations. The non-covalent dimers are loosely associated partly through contributions from hydrophobic interactions [39,40]. The chemically cross-linked dimers associate through the formation of random inter-chain amide bonds [41] while the recombinant DNA-derived synthetic hGH dimers are joined together through a short connecting polypeptide linker [42]. In contrast, the dimeric MER-45-kDa hGH dimers are connected through interchain disulfide linkages [18]. Although the topologies of the inter-chain disulfide linkages in dimeric MER-45-kDa hGH are currently unknown, it is informative to note that bacterial expression of a recombinant bGH [43] produced a disulfide-linked bGH dimer with antiparallel disulfide-links and another disulfide-linked bGH dimer with a concatenane in which the two monomers had interlocking large disulfide loops. Studies of the various naturally-occurring and synthetic dimeric hGHs indicate that there is productive binding to the GH and PRL receptors which result either in agonistic or antagonistic activities. A model inferring that there is substantial flexibility in the GH receptor to accommodate GH dimers with two high-affinity binding sites and allow the receptor to adopt an active signaling conformation has been proposed [44]. Further studies will need to be carried out in order to gain a mechanistic understanding of the structure-function relationships of the dimeric MER-45-kDa hGH and other dimeric hGHs.

In summary, this is the first report to describe the receptor-binding and growth-promoting properties of dimeric MER-45-kDa hGH. Assessment of the biological significance of this previously uncharacterized dimeric hGH isoform gives us insight into the effects of a unique dimeric arrangement of hGH monomers on hGH's biological activity. The dimerization of hGH through unusually stable disulfide bonds forms a quaternary arrangement that attenuates hGH's receptor-binding and growth-promoting activities.

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IM-9 lymphocytes cells, as well as to Dr. A. Buckley (University of Cincinnati, Cincinnati, OH) for providing Nb2-11 cells. This work was supported by grants GM08194 and GM60655 of the National Institutes of Health.

Abbreviations

BSA	bovine serum albumin
DMS	dimethylsuberimidate
EDC	1-ethyl-3(3-dimethylaminopropyl) carbodiimide
EDTA	ethylenediaminetetraacetic acid
EGTA	ethylene glycol-bis(β-aminoethylether)-N, N, N′, N′-tetraacetic acid
GH	growth hormone
bGH	bovine growth hormone
hGH	human growth hormone
hPRL	human prolactin
MER-45-kDa hGH	mercaptoethanol-resistant 45-kDa human growth hormone
NHPP	National Hormone and Pituitary Program
NIDDK	National Institite of Diabetes, Digestive and Kidney Diseases
NIH	National Institutes of Health
oPRL	ovine prolactin
PRL	prolactin
RRA	radioreceptor assay
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
hGH-N	normal hGH gene

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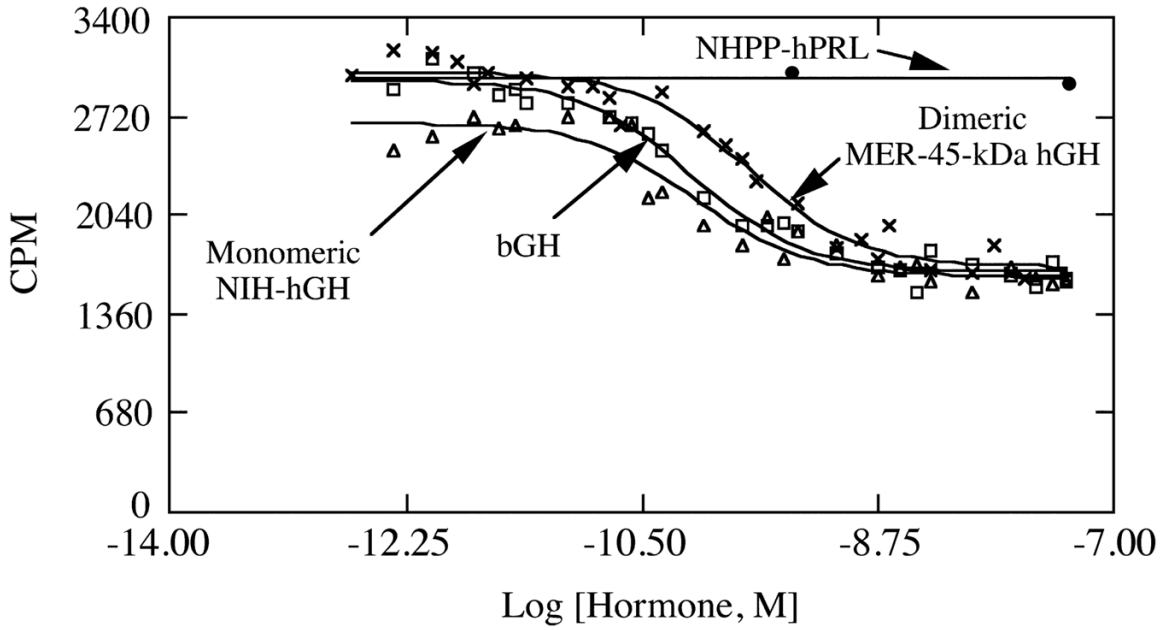

Figure 1. Bovine liver GH radioreceptorassay

In the GH RRA using bovine liver microsomal membranes, competitive displacement of [^{125}I]-bGH from GH receptors by unlabelled hormones was assessed. The abilities of unlabelled bGH (□–□), monomeric NIH-hGH (△–△), dimeric MER-45-kDa hGH (X–X) and NHPP-hPRL (●–●) to competitively displace [^{125}I]-bGH from bovine liver GH receptors are plotted as a function of hormone concentration.

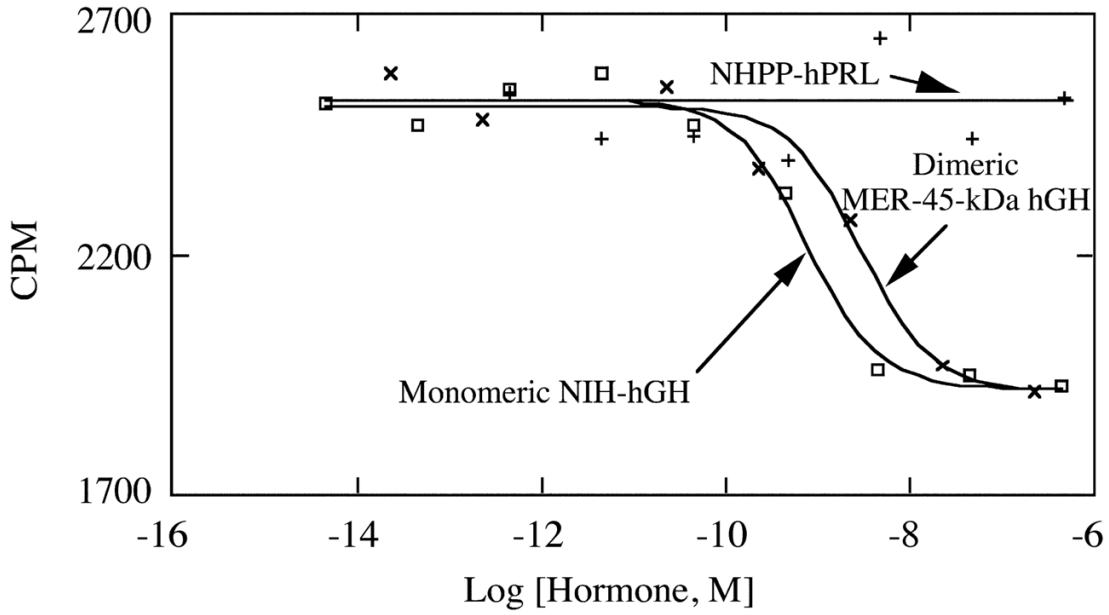

Figure 2. Human IM-9 lymphocyte hGH radioreceptorassay

In the hGH RRA using IM-9 lymphocytes, competitive dose-response displacement of radioiodination grade monomeric [^{125}I]-NIH-hGH from hGH receptors by unlabelled hormones was assessed. The abilities of unlabelled monomeric NIH-hGH (□– □), dimeric MER-45-kDa hGH (X–X) and NHPP-hPRL (+ – +) to competitively displace radioiodination grade monomeric [^{125}I]-NIH-hGH from IM-9 lymphoblastoid cell hGH receptors are plotted as a function of hormone concentration.

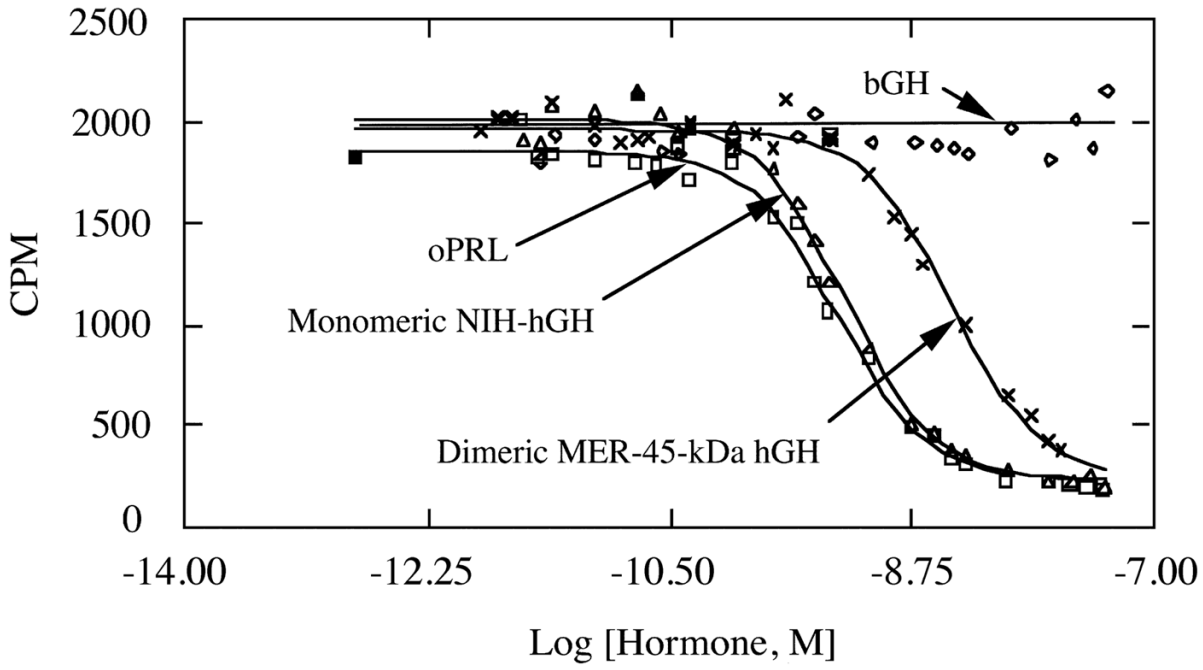

Figure 3. Lactating rabbit mammary gland PRL radioreceptorassay

In the PRL RRA using lactating rabbit mammary gland microsomal membranes, competitive dose-response displacement of [^{125}I]-oPRL from PRL receptors by unlabelled hormones was assessed. The abilities of unlabelled oPRL (□– □), monomeric NIH-hGH (△–△), dimeric MER-45-kDa hGH (X–X) and bGH (◇–◇) to competitively displace [^{125}I]-oPRL from lactating rabbit mammary gland PRL receptors are plotted as a function of hormone concentration.

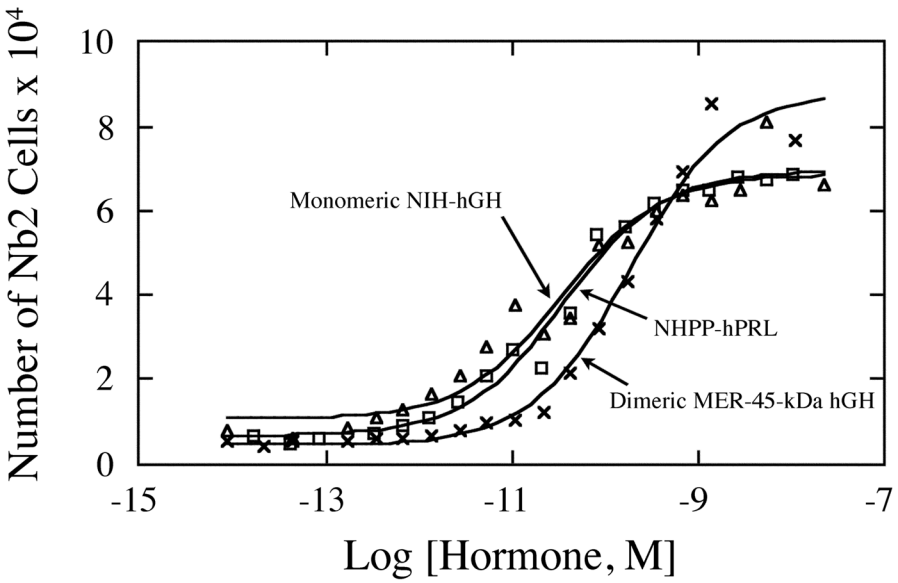

Figure 4. Rat Nb2 lymphoma cell proliferation bioassay

The proliferative response of rat Nb2 lymphoma cells to lactogenic hormones was assessed. Cells were incubated for 72 hr in the presence of varying amounts of NHPP-hPRL (□–□), monomeric NIH-hGH (△–△), and dimeric MER-45-kDa hGH (X–X) to stimulate the proliferation of Nb2 lymphoma cells. The extent of cell proliferation was expressed as the number of Nb2 lymphoma cells at the end of the incubation period as a function of hormone concentration.

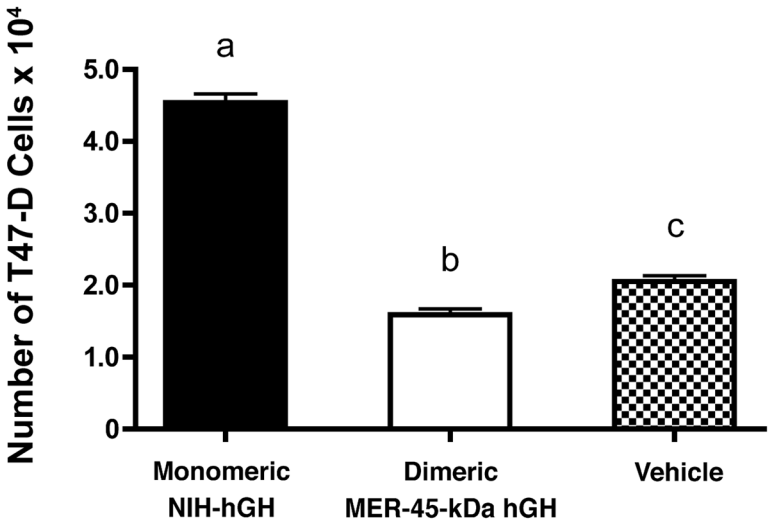

Figure 5. T47-D human breast cancer cell proliferation bioassay

The proliferative response of T47-D human breast cancer cells to hGHs was assessed. Cells were incubated for 72 hr in the presence of either 9.1×10^{-9} M monomeric NIH-hGH (solid black bar) or 9.1×10^{-9} M dimeric MER-45-kDa hGH (solid white bar) or in the presence of vehicle alone (checkered bar) to stimulate the proliferation of T47-D human breast cancer cells. The extent of cell proliferation was expressed as the number of T47-D human breast cancer cells at the end of the incubation period. A One-Way ANOVA testing the equality of the means among the treatment groups indicated statistical differences ($P < 0.05$) among values for the proliferation responses to hormonal stimulation. A *post hoc* Student-Neuman-Keuls *post hoc* test revealed differences between values for the proliferation responses to hormonal stimulation among the treatments ($P < 0.05$) that indicated grouping of data into statistically equavalent responses (a, b, or c) to hormonal stimulation.

TABLE 1

Bovine liver GH radioreceptorassay potency estimates and ED_{50} values.

Hormone	Statistical Grouping	ED_{50} (pM ± S.E.)	Points	Relative Potency
bGH	a	76.7 ± 13.7	26	100
Monomeric NIH-hGH	a	80.6 ± 18.0	25	95.1
Dimeric MER-45-kDa hGH	b	197.5 ± 33.3	26	38.8
NHPP-hPRL	c	∞	26	0

TABLE 2

Human IM-9 lymphocyte hGH radioreceptorassay potency estimates and ED_{50} values.

Hormone	Statistical Grouping	ED_{50} (nM ± S.E.)	Points	Relative Potency
Monomeric NIH-hGH	a	0.77 ± 0.25	9	100
Dimeric MER-45-kDa hGH	b	2.96 ± 0.99	7	26.2
NHPP-hPRL	c	∞	7	0

TABLE 3

Lactating rabbit mammary gland PRL radioreceptorassay potency estimates and ED_{50} values.

Hormone	Statistical Grouping	ED_{50} (nM ± S.E.)	Points	Relative Potency
oPRL	a	0.53 ± 0.04	24	100
Monomeric NIH-hGH	a	0.60 ± 0.04	24	88.0
Dimeric MER-45-kDa hGH	b	3.56 ± 0.24	24	14.8
bGH	c	∞	24	0

TABLE 4

Rat Nb2 lymphoma cell proliferation bioassay potency estimates and ED_{50} values.

Hormone	Statistical Grouping	ED_{50} (pM ± S.E.)	Points	Relative Potency
Monomeric NIH-hGH	a	34.46 ± 8.82	19	100
NHPP-hPRL	a	37.12 ± 8.81	19	92.8
Dimeric MER-45-kDa hGH	b	191.33 ± 42.74	18	18.0

Table 5

Percent potencies of dimeric GHs relative to monomeric GHs in various assays.

	Bovine liver GH RRA	**Human IM-9 lymphocyte GH RRA**	**Lactating rabbit mammary gland RRA**	**Rat Nb2 lymphoma cell proliferation**	**Human T47-D cell proliferation or signaling**	**Rat body weight gain**	**Rat tibial width**	**Pigeon crop sac**	**Rabbit liver GH RRA**	**Mouse costal cartilage in vivo $^{35}SO_4^{2-}$ incorporation**	**Human C14 fibrosarcoma JAK-2 activation**	**Mouse 3T3-F442A fibroblast JAK-2 activation**
MER-45-kDa hGH	40.8	26.2	16.8	18.0	22.4% inhibition vs monomer 2.2-fold stimulation							
Biosynthetic non-covalent hGH	20 [45]					21 [45]	29 [45]					
Pituitary non-covalent hGH				100 [46]								
Dissociable disulfide-linked hGH						10 [33]		100 [33]				
Dissociable disulfide-linked hGH	20 [46]			100 [46]								
Dissociable heterodimeric 22-kDa hGH and cleaved 22-kDa hGH	20 [46]			100 [47]								
DMS-crosslinked bGH						6 [48]			26 [48]			
EDC-crosslinked Recombinant bGH										Enhanced [41]		
EDC Crosslinked Pituitary hGH										Enhanced [41]		
Synthetic Tandem hGH G120R				< 1 [42]	Lower [42]						30 [44]	Similar [44]
Synthetic Tandem hGH				< 1 [42]	Similar [42]						30 [44]	Similar [44]