Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11165–11169, November 1995
Biochemistry

Growth hormone secretagogues: Characterization, efficacy, and minimal bioactive conformation

ROBERT S. MCDOWELL*†, KATHLEEN A. ELIAS‡, MARK S. STANLEY*, DANIEL J. BURDICK*, JOHN P. BURNIER*, KATHRYN S. CHAN*, WAYNE J. FAIRBROTHER§, R. GLENN HAMMONDS¶, GLADYS S. INGLE‡, NEIL E. JACOBSEN§, DEBORAH L. MORTENSEN‡, THOMAS E. RAWSON*, WESLEY B. WON‡, ROSS G. CLARK‡, AND TODD C. SOMERS*

Departments of *Bioorganic Chemistry, ‡Endocrine Research, §Protein Engineering, and ¶Protein Chemistry, Genentech, Inc., South San Francisco, CA 94080

Communicated by William H. Daughaday, University of California, Irvine, CA, August 14, 1995

ABSTRACT **Another class of growth hormone (GH) secretagogues has been discovered by altering the backbone structure of a flexible linear GH-releasing peptide (GHRP). *In vitro* and *in vivo* characterization confirms these GH secretagogues as the most potent and smallest (M_r < 500) reported. Anabolic efficacy is demonstrated in rodents with intermittent delivery. A convergent model of the bioactive conformation of GHRPs is developed and is supported by the NMR structure of a highly potent cyclic analog of GHRP-2. The model and functional data provide a logical framework for the further design of low-molecular weight secretagogues and illustrate the utility of an interdisciplinary approach to elucidating potential bound-state conformations of flexible peptide ligands.**

Growth hormone (GH) regulates optimal statural growth in children and body composition in adults. The pulsatile release of GH from the pituitary somatotrophs is controlled by a complex endocrine interaction involving hypothalamic neuronal hormones that induce (via GH-releasing hormone, GHRH) or suppress (via somatostatin) GH secretion. Discovered before GHRH, the GH-releasing peptides (GHRPs) were derived by systematically optimizing the GH-releasing activities of Met-enkephalin (1, 2). The GHRPs and GHRH directly stimulate GH secretion by the pituitary (3, 4) via different mechanisms (5, 6), and a synergistic response is observed when the two are co-administered (7, 8). More potent GH secretagogues have subsequently been developed by further modification of the GHRPs (9), and recently a nonpeptidyl lead series was identified by screening (10). Despite considerable interest in these molecules as a potential alternative to injectable recombinant human GH therapy, their clinical use is currently limited by low oral bioavailability and a poor understanding of the pharmacodynamic profile required for optimal efficacy. Neither an endogenous counterpart nor its receptors have been reported.

The intrinsic (*in vitro*) secretagogue activity of the prototype peptide GHRP-6 (His-DTrp-Ala-Trp-DPhe-Lys-NH_2) results primarily from the charged side chain of the N-terminal histidine and the central aromatic residues; the C-terminal lysine residue, although not absolutely required for *in vitro* activity, appears to contribute significantly to *in vivo* potency (11). Activity is enhanced when DTrp[2] is replaced by D-2-(2-naphthyl)alanine (D2Nal), and the N-terminal charge is delivered by the stereochemically analogous backbone nitrogen of D-alanine (GHRP-2, DAla-D2Nal-Ala-Trp-DPhe-Lys-NH_2) (9, 12). No potent cyclic analogs of GHRP have been reported, and little progress has been made in determining the topographical requirements for GHRP bioactivity, thus frustrating efforts to design secretagogues with improved pharmacological properties.

We describe here a strategy that has produced a highly active cyclic analog of GHRP-2 that is structured in water. We have also determined a minimal set of functional interactions that are actually required for GHRP activity, leading to the development of a highly potent secretagogue with a molecular mass <500 Da. Potential regimens for extended treatment with these molecules are examined.

MATERIALS AND METHODS

Peptide Synthesis. Synthesis of peptides was done by solid-phase peptide synthesis using either *t*-butoxycarbonyl (Boc) or 9-fluorenylmethoxycarbonyl (Fmoc) protocols (13) using commercially available reagents. C-terminal hydroxymethyl derivatives were prepared by cleaving the N-Boc amino acid from Wang resin with excess lithium borohydride in tetrahydrofuran followed by treatment with trifluoroacetic acid. The purity and identity of all compounds were established by analytical reverse-phase liquid chromatography (methanol/water/trifluoroacetic acid gradient), electrospray mass spectrometry (MS), high-resolution fast atom bombardment MS, and either amino acid analysis or ^1H and ^{13}C NMR, as appropriate.

NMR Structure Determination. Peptide (3.4 mg) was dissolved in 500 μl of 90% H_2O/10% ^2H_2O/50 mM sodium [$^2H_3$]acetate, pH 4.5. All NMR spectra were acquired at 7°C on a Bruker AMX500 spectrometer. Standard spectra were recorded including correlated (COSY), total correlated (TOCSY) [isotropic mixing sequence (14) applied for 70 ms], and nuclear Overhauser enhancement (NOESY) spectroscopies (mixing time of 300 ms). Spectra were processed and analyzed using FELIX (Biosym Technologies, San Diego). Nuclear Overhauser effect cross peaks were characterized as strong, medium, or weak, corresponding to upper bound distance restraints of 3.0, 4.0, and 5.0 Å, respectively; pseudoatom corrections were added when necessary (15). Dihedral angle restraints for ϕ and χ_1 were derived from coupling constant and nuclear Overhauser effect data. Distance geometry calculations were carried out using DGII (Biosym Technologies). One hundred structures were calculated, and the 25 with the lowest total restraint violations were further refined with restrained molecular dynamics and minimization using the DISCOVER program (Biosym Technologies). The all-atom AMBER force field (16, 17) was used with a 15.0-Å cutoff for nonbonded interactions and a distance-dependent dielectric constant ($\varepsilon = 4r$).

Computational Studies. Ensemble molecular dynamics and minimization (18) were used to explore consensus conformations of the aromatic side chains of the active cyclic analogs and the tetrapeptide series. One hundred dynamics trajectories were calculated, starting from different random conformations. During each 200-ps trajectory, tethering restraints were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GH, growth hormone; GHRH, growth hormone-releasing hormone; GHRP, growth hormone-releasing peptide; D2Nal, D-2-(2-naphthyl)alanine; SD, Sprague–Dawley.

†To whom reprint requests should be addressed.

applied to align the side-chain rings (r_0 = 1.0 Å; k = 10.0 kcal/Å^2) and backbone α carbons (r_0 = 1.5 Å) of the aromatic residues. No attempt was made to align the N or C termini. Calculations were performed with DISCOVER using the AMBER force field as described above. Systematic conformation searching was performed with the CSEARCH module of SYBYL (Tripos Associates, St. Louis) using 10°-angle increments. Apparent local minima from this search were minimized using MM2 (19), and redundant structures were removed.

Biological Assays. *In vitro* activity was assessed by measuring GH release from primary pituitary cell cultures. Anterior pituitaries from 200- to 220-g female Sprague–Dawley (SD) rats were enzymatically dispersed, and the cells were plated at 100,000 cells per ml in incubation/challenge medium. Cells were challenged with test compounds after 3 days at 37°C in 5% CO_2. Compounds were evaluated at six concentrations with six wells per concentration. Three to five EC_{50} values from independent experiments were used to derive the mean and SEM. *In vivo* potency was established in anesthetized female SD rats using the protocol described by Sartor *et al.* (20). Anabolic efficacy was assessed using 150-day-old female SD rats. Compounds (100 μg per rat per day) were administered either continuously by s.c. pump infusion or twice daily by s.c. injection for a total of 2 weeks. Weight gain was monitored daily. Animal procedures and protocols were approved by the Genentech Institutional Animal Care and Use Committee.

Hormone Assays. The concentrations of rat GH in medium and serum were determined by using a specific two-site rat GH ELISA. The intraassay and interassay variations were <6 and <10%, respectively. Luteinizing hormone, follicle-releasing hormone, thyroid-stimulating hormone, and prolactin levels were determined by using commercially available RIA kits (Amersham); adrenocorticotropin levels were determined using an RIA kit from ICN.

RESULTS

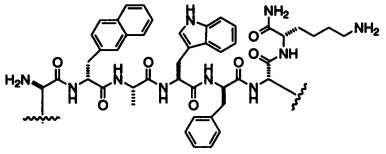
Cyclic Peptides. Starting from GHRP-2, the side chain of an N-terminal D-amino acid (replacing DAla[1]) was linked to the side chain of an amino acid inserted between DPhe[5] and Lys[6], thus enclosing the critical aromatic residues within a cyclic backbone. The activities of this cyclic series are summarized in Table 1. The most potent cyclic GHRP analog reported, **1a** has ≈10-fold greater activity than GHRP-6 *in vitro* and comparable acute efficacy *in vivo*. The activity of this series is highly sensitive to the composition of the linkage amide. Transposing the amide carbonyl to the N-terminal side of the -NH (**1b**) reduces activity 100-fold. An even greater reduction is seen when the amide bond of **1a** is shifted toward the N terminus (**1c**). Deleting a methylene group on either side of the amide bond (**1d**, **1e**) likewise produces decreased activity. These results suggest that **1a** supports a specific, productive conformation of the aromatic core that is not readily accessible to the other molecules.

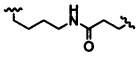
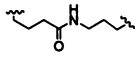
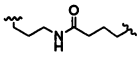
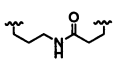
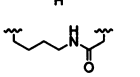
NMR studies indicate that **1a** is structured in water. The final set of refined structures contain no distance violations >0.1 Å and no dihedral violations. (NMR restraints and structures for **1a** are available on written request.) As shown in Fig. 1, the D2Nal-Ala-Trp-DPhe fragment adopts a compact conformation with nested hairpin turns initiated at DLys[1] and Ala[3]. The cyclic portion of this peptide is well defined (average pairwise root-mean-square deviation = 0.52 ± 0.21 Å), and the average pairwise deviation of the D2Nal-Ala-Trp-DPhe backbone is 0.27 ± 0.16 Å. The C-terminal lysine is disordered and is not shown. On either side of the DLys[1]-Glu[6] amide bond, the cyclization linkage makes a sharp 90° bend. Modeling studies suggest that the linkages of the less active analogs **1b–1e** do not readily adopt a similar conformation.

A Distinctive Tetrapeptide Series. In their initial discovery of GH secretagogues, Momany *et al.* (1) converted the weakly active tetrapeptide Tyr-DTrp-DTrp-Phe-NH_2 to a progenitor of GHRP-6 by inserting an alanine between the central DTrp residues and inverting the stereochemistry of the two C-terminal amino acids. The activity of the resulting peptide, Tyr-DTrp-Ala-Trp-DPhe-NH_2, was subsequently enhanced by replacing the N-terminal tyrosine with histidine and by appending a lysine to the C terminus to produce GHRP-6, the first of this series to show *in vivo* activity (11). The structural implications of these manipulations were intriguing: if the initial tetrapeptide and GHRP-6 act by a common mechanism (presumably by binding to a common site), the bioactive conformations of these molecules should share a similar three-dimensional presentation of the key aromatic residues.

We therefore conducted an analogous evolution of the shorter tetrapeptide series starting from Tyr-DTrp-DTrp-Phe-NH_2. Key intermediates in the sequential optimizations of both the longer (**2a–e**) and shorter (**3a–f**) series are shown in Table 2. Replacing Tyr[1] of **3a** by histidine (**3b**) did not significantly improve activity, although adding a C-terminal lysine (**3c**) produced a molecule that was only 4-fold less potent than GHRP-6 (**2c**). Substituting D2Nal for DTrp[2] (**3d**) and aminovaleric acid for His[1] (**3e**) further increased activity, similar to the trend observed in **2d** and **2e**. The activity of **3e** was further enhanced by replacing DTrp[3] by D2Nal to produce a compound

Table 1. Effect of ring size and linkage on *in vitro* activity of cyclic GHRP-2 analogs



No.	Sequence	Linker	*In vitro* EC_{50} ± SE, nM
1a	**k** b A W f **E** K-NH_2		0.43 ± 0.11
1b	(**adp**) b A W f **O** K-NH_2		44 ± 9
1c	**o** b A W f (**Adp**) K-NH_2		>100
1d	**o** b A W f **E** K-NH_2		28 ± 10
1e	**k** b A W f **D** K-NH_2		84 ± 10

Single-letter amino acid codes are used; lowercase letters denote D-stereochemistry. -NH_2, C-terminal carboxamide; b, D2Nal; adp and Adp, D- and L-2-aminoadipic acid, respectively. Boldface letters represent amino acids changed in each optimization step.

We therefore conducted an analogous evolution of the shorter tetrapeptide series starting from Tyr-DTrp-DTrp-Phe-NH_2. Key intermediates in the sequential optimizations of both the longer (**2a–e**) and shorter (**3a–f**) series are shown in Table 2. Replacing Tyr[1] of **3a** by histidine (**3b**) did not significantly improve activity, although adding a C-terminal lysine (**3c**) produced a molecule that was only 4-fold less potent than GHRP-6 (**2c**). Substituting D2Nal for DTrp[2] (**3d**) and aminovaleric acid for His[1] (**3e**) further increased activity, similar to the trend observed in **2d** and **2e**. The activity of **3e** was further enhanced by replacing DTrp[3] by D2Nal to produce a compound

FIG. 1. Stereo plot of refined NMR structures of **1a**. The side chains of the aromatic residues are color-coded as follows: D2Nal[2] (green), Trp[4] (pink), and DPhe[5] (yellow). The C-terminal lysine, which is disordered, is eliminated for clarity.

Table 2. Optimization of an alternate GHRP backbone

No.	Sequence	*In vitro* EC_{50} ± SE, nM
2a	Y w A W f-NH_2	>1000
2b	**H** w A W f-NH_2	10 ± 3
2c	H w A W f **K**-NH_2	6.2 ± 1.5
2d	H **b** A W f K-NH_2	0.60 ± 0.3
2e	(**ava**) b A W f K-NH_2	0.20 ± 0.03
3a	Y w w F-NH_2	>1000
3b	**H** w w F-NH_2	>1000
3c	H w w F **K**-NH_2	26 ± 4
3d	H **b** w F K-NH_2	6.8 ± 1.2
3e	(**ava**) b w F K-NH_2	4.6 ± 1.5
3f	(ava) b **b** F K-NH_2	2.8 ± 0.4

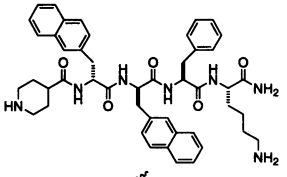
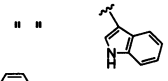
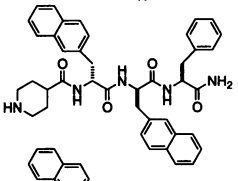
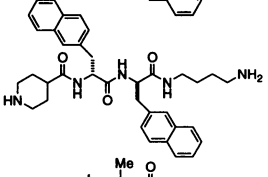
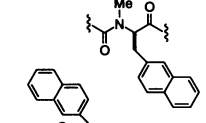
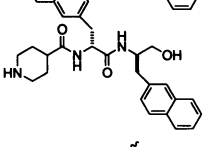
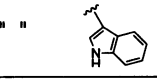
The *in vitro* structure activity relationships for the **2a–e** and **3a–f** series are summarized. Amino acids changed in each optimization step are shown in boldface type; lowercase letters denote D-stereochemistry; b, D2Nal, ava, 5-aminovaleric acid.

(**3f**) which demonstrated comparable *in vivo* potency to **2e** (ED_{50} = 2.6 vs. 1.5 μg, respectively) and was chosen as a lead for medicinal chemistry. Activity was dramatically improved by the incorporation of isonipecotic acid (inip) at the N terminus (**4a**, Table 3), resulting in a compound over 10-fold more potent *in vitro* than **3f** and 30-fold more potent than GHRP-6.

Mechanistic Characterization. This class of secretagogues, represented by **4a**, shares the mechanistic profile characteristic of GHRP-6 and related molecules (2, 5, 8). Repeated exposure of **4a** induces homologous desensitization of GH release from pituitary cells (Fig. 2*A*), although responsiveness to GHRH is maintained. While a combination of GHRP-6 and **4a** does not increase maximal GH release, GHRH in combination with either **4a** or GHRP-6 enhances GH secretion (Fig. 2*B*), indicating that **4a** and GHRH act via different sites. Somatostatin, which suppresses GHRP-stimulated GH release, has a similar effect on **4a** (Fig. 2*C*). Compound **4a** releases GH in a dose-dependent manner with no significant release of luteinizing hormone, follicle-stimulating hormone, adrenocorticotropic hormone, or thyrotropic hormone. A 1.5-fold increase in prolactin was seen, which is also seen with GHRP-6 incubation. Similar results were found for **1a** (K.A.E., unpublished data), further supporting the hypothesis that **1a**, **4a**, and GHRP-6 interact at a common site.

Consensus Structures. Each ensemble dynamics trajectory converged to one of the two consensus alignments illustrated in Fig. 3 *A* and *B*. The average pairwise backbone fluctuations of these structures are 1.35 ± 0.52 Å and 0.92 ± 0.32 Å, respectively. The alignments both feature a stacked arrangement of the Trp-DPhe (or DTrp-Phe) side chains and differ primarily in the orientation of D2Nal[2]. The backbone conformation of the D2Nal-Ala-Trp-DPhe residues shown in Fig. 3*B* is remarkably similar to the conformation observed in the NMR structure of **1a**: the average deviation in this region between

Table 3. Minimum pharmacophore for GH secretagogue activity

No.	Sequence	Structure	*In vitro* EC_{50} ± SE, nM	*In vivo* ED_{50}, μg
4a	(inip) b **b** F K-NH_2 M_r = 798		0.18 ± 0.04	0.20
4b	(inip) b **w** F K-NH_2 M_r = 786		1.1 ± 0.4	0.070
4c	(inip) b b F-NH_2 M_r = 670		0.28 ± 0.07	1.7
4d	(inip) b **b** (bda) M_r = 593		17 ± 3	3.3
4e	(inip) b (**nmb**) (bda) M_r = 607		0.38 ± 0.08	1.7
4f	(inip) b (**bol**) M_r = 508		1.8 ± 0.8	10
4g	(inip) b (**wol**) M_r = 496		10 ± 6	0.80

Sequential reduction and optimization of the pseudopentapeptide **4a** to the simple, low-molecular-weight compound **4g**, which maintains high *in vitro* and *in vivo* potency. inip, Isonipecotic acid; bda, butane diamine; nmb, *N*-methyl-D2Nal; bol, D-2-naphthylalanol; wol, D-tryptophanol. Lowercase letters denote D-stereochemistry.

the NMR and dynamics structures is 0.87 ± 0.23 Å. The principal discrepancy between the two, the χ_1 angle of D2Nal[2], is resolved if the side chain of this residue in the tetrapeptide adopts an alternate, low-energy rotamer that overlaps well with the NMR structure of **1a**.

Systematic Reduction of 4a. We next investigated the minimal functional pharmacophore of these compounds with the aim of reducing their molecular weight and complexity. Table 3 shows the sequential reduction of **4a** to the small peptidomimetic **4g**. In contrast to the GHRP-6 series (**2a–2e**), the C-terminal lysine of **4a** can be removed while retaining *in vivo* potency (**4c**). Replacing the third aromatic ring with an alkyl amine (**4d**) greatly simplifies the C terminus, but a drop in intrinsic activity is observed. Potency is restored by N-methylating D2Nal[2] (**4e**); this modification has little effect on other molecules within this series. The hydroxymethyl derivative **4f** retains high *in vitro* potency, although most other modifications that removed the amine from the C terminus of **4d** greatly reduced activity. Substituting an indole for the second naphthalene ring generates the lowest-M_r GH secretagogue reported with significant *in vivo* activity (**4g**).

A systematic search over all internal torsions of **4f** and **4g** confirms that these molecules share a minimum-energy backbone conformation that is identical to the consensus alignment of **4a** shown in Fig. 3*B*. We believe that this conformation, shown in Fig. 3*C*, represents a good model for the essential pharmacophore of GHRPs, although the biologically relevant rotamers of the aromatic side chains have yet to be determined.

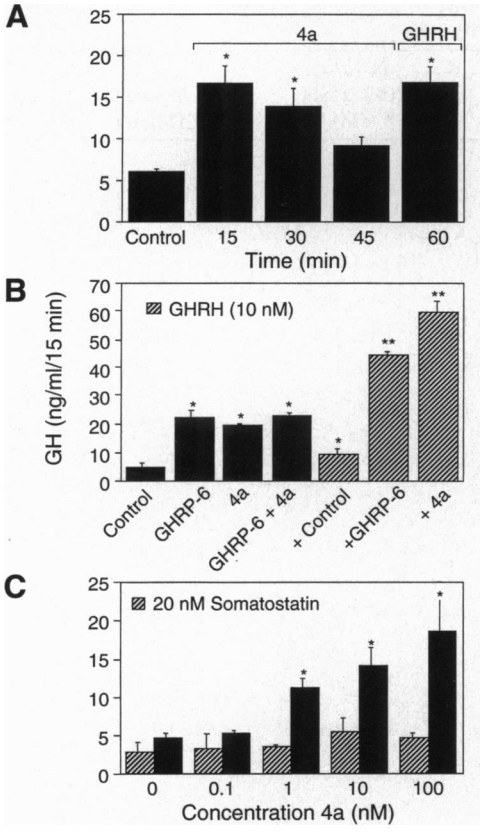


FIG. 2. *In vitro* characterization of **4a**. (*A*) Down-regulation of GH release from a single pituitary cell culture after three sequential 15-min incubations with **4a** (100 nM). After 45 min, **4a** induced no significant release of GH, although these cells still responded to GHRH (10 nM). (*B*) GH responses to GHRP-6 (100 nM) and **4a** (100 nM) were significantly greater than control, but when both were added in combination, GH release was not increased. GHRP-6 or **4a** in combination with GHRH (10 nM) elicited a synergistic GH response. Statistical significance ($P < 0.05$) vs. basal and GHRH alone are denoted by * and **, respectively. (*C*) GH release in response to increased concentrations of **4a** is suppressed with 20 nM somatostatin.

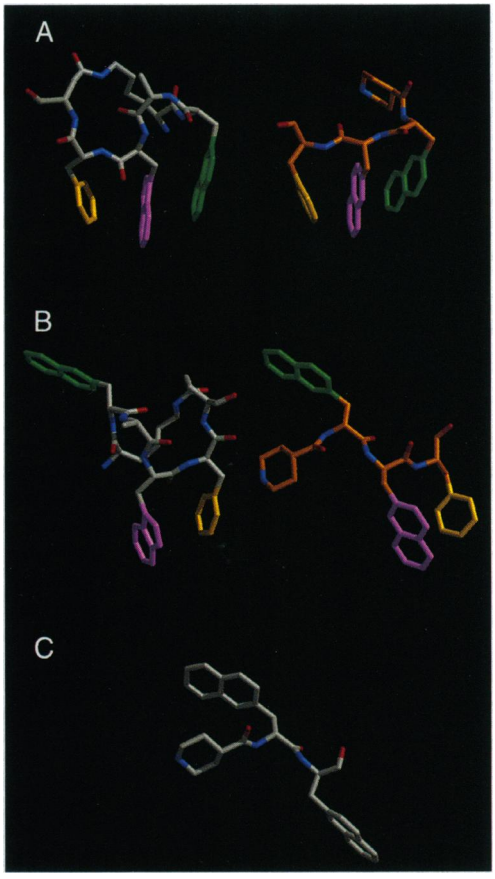


FIG. 3. Consensus alignments of topologically distinct peptide backbones. (*A*) Consensus model of **1a** (left) and **4a** (right) showing a collinear arrangement of the three aromatic rings. Side chains are color-coded as in Fig. 1; the C-terminal lysine is omitted for clarity. (*B*) Alternate consensus model of **1a** and **4a** in which D2Nal[2] is projected on the opposite face of the peptide. This conformation is consistent with the NMR structure of **1a**. (*C*) Minimum-energy structure of **4f** from systematic conformational searching. This simple structure, in which the two aromatic rings are presented on opposite faces of a central amide bond, represents a minimal three-dimensional pharmacophore for the GHRPs.

***In Vivo* Profiles.** Fig. 4 shows an *in vivo* comparison of **4b** and **4g** with the most potent reported hexapeptide (GHRP-2; EC_{50}

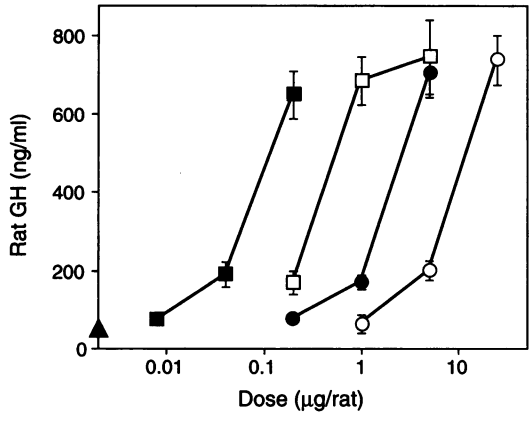


FIG. 4. *In vivo* comparison of structurally diverse GH secretagogues. Serum GH responses to excipient (▲), three doses of the benzolactam L-692,585 (○), the hexapeptide GHRP-2 (□), and compounds **4b** (■) and **4g** (●) (means ± SEMs; six rats per group).

= 1.0 ± 0.2 nM, ED_{50} = 0.33 μg) and nonpeptide (L-692,585; EC_{50} = 11 ± 4 nM, ED_{50} = 10 μg) secretagogues. The parallel i.v. dose–response curves for these structurally distinct compounds are consistent with a common, specific site of action. The pseudo-pentapeptide **4b** is the most potent GHRP-like compound ever reported *in vivo*. However, as evidenced by the *in vitro* and *in vivo* data for the pairs **4a**/**4b**, **4d**/**4e**, and **4f**/**4g** shown in Table 3, small changes in structure can have large and differential effects on the two measures of activity. Indeed, only a modest correlation is observed between the *in vitro* and *in vivo* assay results for a large sample of diverse GH secretagogues (Fig. 5).

The anabolic effects of continuous vs. intermittent administration of **4a** are contrasted in Fig. 6. At 150 days, the normal growth rate of female SD rats (5 g per day) has slowed below 2 g per week, and the anabolic effect of the secretagogues is significant. Weight gain was observed in these animals given twice daily injections of **4a**. This increase, evenly distributed over the organs and tissues, is consistent with the results seen with GH treatment in this model. By contrast, the same total daily dose given as a continuous infusion induced tachyphlaxis after an initial increase in growth velocity (**4a** is stable in a minipump under these conditions). This effect is reminiscent of the functional antagonism induced by super-agonists to other endocrine receptors and has not previously been reported for GH secretagogue-induced growth responses.

DISCUSSION

In exploring the structural requirements for GHRP activity, we have discovered a structured, cyclic analog with significant *in vivo* potency (**1a**), a peptide (**4a**) that demonstrates anabolic efficacy with intermittent exposure, and a low-molecular weight peptidomimetic (**4g**) that retains the *in vitro* and *in vivo* activity of GHRP-6. The discovery of **1a**, combined with a transfer of the aromatic residues onto a more compact peptide backbone, have enabled the derivation of a convergent structural model. The consensus structures from ensemble dynamics did not incorporate any restraints other than the intermolecular tethering potentials. Despite the considerable flexibility of the initial molecules, it is encouraging that only two possible alignments of the aromatic residues were ultimately identified, one of which was supported by the NMR structure of **1a**. These findings suggest that the conformational requirements for activity in a flexible linear molecule can be mapped by modifying the backbone chirality and the spacing between functionally important side chains. The ultimate precision of the resulting model is enhanced when structurally diverse cyclic analogs are included in the ensemble.

The high *in vivo* activity of **4g** indicates that only a subset of the aromatic core is required for full secretagogue function.

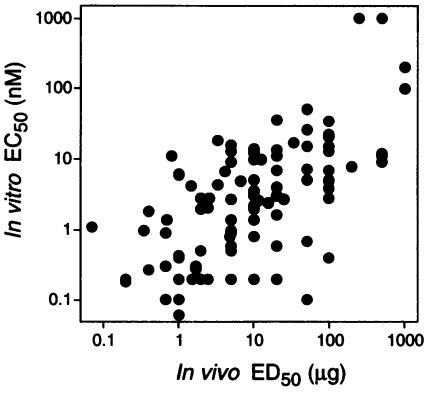


FIG. 5. Comparison of *in vitro* and *in vivo* activities of 107 GH secretagogues. GH release *in vitro* (EC_{50} in nM) vs. GH secretion *in vivo* (ED_{50} in μg per rat) for structurally diverse secretagogues.

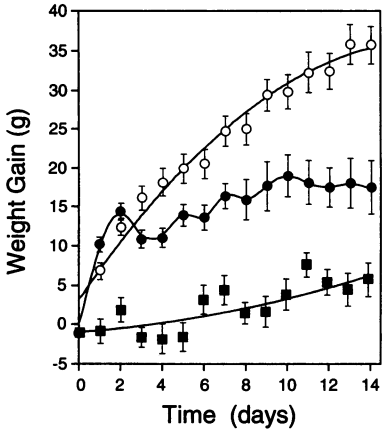


FIG. 6. Weight gain in rats treated continuously or intermittently with **4a**. Body weight gain in 150-day female SD rats treated with excipient (■) or **4a** (100 μg per rat per day) given by s.c. minipump infusion (●) or twice daily s.c. injection (○) for 14 days (means ± SEMs; eight rats per group).

These results are consistent with the activity of the benzolactam secretagogue L-692,429, which can be similarly construed as presenting a subset of the GHRP-6 functionality. Further constraint of **4g** should distinguish among the possible side-chain conformations, providing further refinement of this model for the design of other minimal GH secretagogues.

We thank M. Struble and J. Tom for peptide purification; D. Narindray and C. Quan for synthesis; Y. Ma for animal experiments; M. Cronin, R. Vandlen, K. Attie, and D. Artis for advice; the Research Immunochemistry group for GH ELISAs; and K. Andow, A. Bruce, and D. Wood for assistance with graphics.

1. Momany, F. A., Bowers, C. Y., Reynolds, G. A., Chang, D., Hong, A. & Newlander, K. (1981) *Endocrinology* **108**, 31–39.
2. Bowers, C. Y., Momany, F. A., Reynolds, G. A. & Hong, A. (1984) *Endocrinology* **114**, 1537–1545.
3. Bowers, C. Y., Sartor, A. O., Reynolds, G. A. & Badger, T. M. (1991) *Endocrinology* **128**, 2027–2035.
4. Akman, M. S., Girard, M., O'Brien, L. F., Ho, A. K. & Chik, C. L. (1993) *Endocrinology* **132**, 1286–1291.
5. Blake, A. D. & Smith, R. G. (1991) *J. Endocrinol.* **129**, 11–19.
6. Goth, M. I., Lyons, C. E., Canny, B. J. & Thorner, M. O. (1992) *Endocrinology* **130**, 939–944.
7. Bowers, C. Y., Reynolds, G. A., Durham, D., Barrera, C. M., Pezzoli, S. S. & Thorner, M. O. (1990) *J. Clin. Endocrinol. Metab.* **70**, 975–982.
8. Cheng, K., Chan, W. W.-S., Barreto, A., Jr., Convey, E. M. & Smith, R. G. (1989) *Endocrinology* **124**, 2791–2798.
9. Bowers, C. Y. (1993) in *Molecular and Clinical Advances in Pituitary Disorders*, ed. Melmed, S. (Endocrine Research and Education, Los Angeles), pp. 153–157.
10. Smith, R. G., Cheng, K., Schoen, W. R., Pong, S.-S., Hickey, G., Jacks, T., Butler, B., Chan, W. W.-S., Chaung, L.-Y. P., Judith, F., Tayler, J., Wyvratt, M. J. & Fisher, M. H. (1993) *Science* **260**, 1640–1643.
11. Momany, F. A., Bowers, C. Y., Reynolds, G. A., Hong, A. & Newlander, K. (1984) *Endocrinology* **114**, 1531–1536.
12. Bowers, C. Y. (1993) *J. Pediatr. Endocrinol.* **6**, 21–31.
13. Barany, G. & Merrifield, R. B. (1980) in *The Peptides*, eds. Gross, E. & Meienhofer, J. (Academic, New York), Vol. 2, pp. 1–284.
14. Cavanagh, J. & Rance, M. (1992) *J. Magn. Res.* **96**, 670–678.
15. Wüthrich, K., Billeter, M. & Braun, W. (1983) *J. Mol. Biol.* **169**, 949–961.
16. Weiner, S. J., Kollman, P. A., Nguyen, D. T. & Case, D. A. (1986) *J. Comput. Chem.* **7**, 230–252.
17. Weiner, S. J., Kollman, P. A., Case, D. A., Singh, U. C., Ghio, C., Alagona, G., Profeta, S., Jr., & Weiner, P. (1984) *J. Am. Chem. Soc.* **106**, 765–784.
18. McDowell, R. S., Blackburn, B. K., Gadek, T. R., McGee, L. R., Rawson, T., Reynolds, M. E., Robarge, K. D., Somers, T. C., Thorsett, E. D., Tischler, M., Webb, R. R., III, & Venuti, M. C. (1994) *J. Am. Chem. Soc.* **116**, 5077–5083.
19. Allinger, N. L. & Yuh, Y. H. (1981) *QCPE* **13**, 395.
20. Sartor, O., Bowers, C. Y., Reynolds, G. A. & Momany, F. A. (1985) *Endocrinology* **117**, 1441–1447.