Journal of
Medicinal
Chemistry

Volume 41, Number 17 August 13, 1998

Perspective

Peptidomimetic Growth Hormone Secretagogues. Design Considerations and Therapeutic Potential

Ravi P. Nargund,*,† Arthur A. Patchett,† Mark A. Bach,‖ M. Gail Murphy,§ and Roy G. Smith‡

Departments of Medicinal Chemistry, Clinical Pharmacology, Clinical Sciences, and Biochemistry and Physiology, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Received May 23, 1997

Introduction

Human growth hormone (GH) has been in clinical use since the late 1950s. Until the mid-1980s it was isolated from human cadaveric pituitaries and used for replacement therapy in GH-deficient children. In this patient population GH replacement therapy was efficacious and remarkably well-tolerated with minimal side effects. However, human GH was scarce, expensive, and sometimes contaminated with a virus that has been linked to Jakob-Creutzfeldt disease, a fatal degenerative neurological disorder. In the mid-1980s recombinant human GH (rhGH) became available for treatment of short stature in GH-deficient children and adolescents. Several pharmaceutical and biotechnology companies currently market native rhGH or methionyl-rhGH for treating GH deficiency in adults and children, short stature in association with renal insufficiency, AIDS-related wasting, and short stature associated with Turner's Syndrome.[1]

Availability of rhGH has spurred numerous investigational applications for GH replacement therapy. These include age-related muscle loss (sarcopenia), improvement in perisurgical or parenteral nutrition and other catabolic states, obesity, non-GH-deficient short stature, and a variety of catabolic states.[1] There has also been considerable interest in evaluating GH replacement therapy for use in treating cardiovascular disease and in enhancing the immune system. Interest in effects of GH on cardiovascular disease stems from clinical observations that GH is involved in regulating hepatic biosynthesis of lipoproteins and LDL receptors.[2] Furthermore, GH-deficient adults show a predisposition to cardiovascular disease.[3] In the immune system, GH has been shown using in vitro experiments to stimulate cell proliferation in primary lymphoid tissue including bone marrow and thymus. In humans, most evidence suggests that it increases the T-cell population.[4]

Normal human aging is associated with decreased GH secretion compared to young healthy adults.[5,6] Mean GH concentrations in the elderly are about half those in young adults, and many people over 60 are GH deficient by young adult standards. The resulting "hyposomatomedinemia" is accompanied by reductions in the levels of insulin-like growth factor (IGF-1) which is released from the liver in response to GH stimulation.[7,8] The reductions in GH and IGF-1 levels with aging may contibute to age-related decreases in muscle mass and strength.[9]

Rudman and co-workers were the first to explore potential applications of GH replacement therapy in the geriatric population.[10,11] In 1990 they reported that administration of rhGH to 21 healthy men from 61 to 81 years old led to marked improvements in muscle tone, skin thickness, lean body mass, and density of the lumbar vertebrae. These increases in lean body mass were coupled with a statistically significant loss of adipose tissue. On the basis of these studies, Rudman and co-workers concluded that "the effects of 6 months GH treatment on lean body mass and adipose tissue were equivalent in magnitude to the changes incurred

† Department of Medicinal Chemistry.
‖ Department of Clinical Sciences.
‡ Department of Biochemistry and Physiology.
§ Department of Clinical Pharmacology.

S0022-2623(97)00342-7 CCC: $15.00
Published on Web 07/17/1998

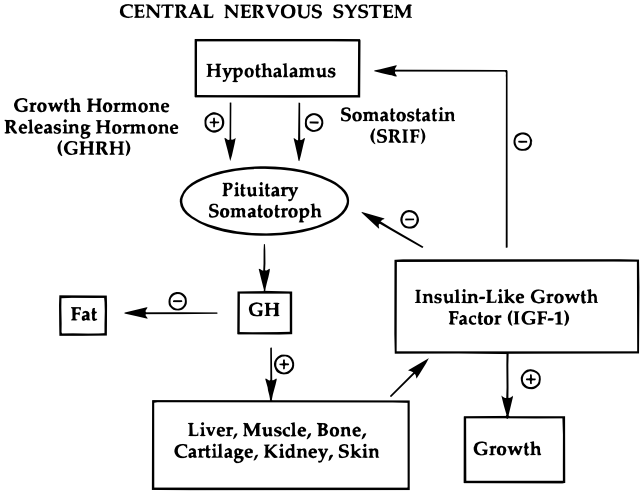


Figure 1. Regulation of growth hormone (GH) secretion by the hypothalamic-pituitary axis. The hypothalamic peptide, growth hormone-releasing hormone (GHRH), acts on pituitary somatrophs to release GH while the inhibitory peptide, somatostatin (SRIF), blocks GH release by interacting with its receptors that are expressed in the pituitary gland. GH pulsatility is regulated by the interplay of two hormones, GHRH and SRIF, which counterpoise one another. Following stimulation of GH release, concentrations of the anabolic hormone insulin-like growth factor (IGF-1) increase due to the actions of GH on the liver. GH stimulates growth by interacting with specific GH receptors that are widely distributed in body tissues and indirectly through the effects of IGF-1. IGF-1 is a feed-back inhibitor of GH synthesis and release by acting on the pituitary gland and hypothalamus.

during 10−20 years of aging". Other efforts to provide GH replacement therapy in elderly individuals have also resulted in improved body composition but, with the exception of one study,[11a] have not demonstrated increased strength or function.[12,13] In addition, GH has been poorly tolerated in elderly subjects, often requiring dose reduction or discontinuation from studies. The most common side effects include fluid retention in the lower extremities, diffuse arthraglia of both small and large joints, carpal tunnel syndrome, and insulin resistance leading to higher serum fasting glucose levels. It is clear that beneficial effects of GH replacement have been realized in pediatrics, but longer-term studies are essential to evaluate whether GH replacement therapy will be benefical in geriatrics.

Recombinant GH has also found widespread use in animal husbandry. Monsanto markets recombinant bovine GH (bovine somatotrophin; BST) as Posilac to increase milk production in dairy cows. Recombinant porcine GH (porcine somatotrophin; PST) has also been investigated extensively as a growth promotant in swine. Administration of PST by injection to finisher pigs has been reported to cause marked increases in feed efficiency and significant improvements in the carcass composition, i.e., fat/muscle ratio.[14]

Growth hormone (GH) is released in a pulsatile manner from somatotroph cells of the anterior pituitary in all mammalian species tested so far, including humans. As shown in Figure 1, GH pulsatility is regulated by the interplay of two hypothalamic hormones which counterpoise one another: growth hormone releasing hormone (GHRH) stimulates GH release whereas somatostatin (SRIF) inhibits GH release. Many neurotransmitters (e.g., noradrenaline and/or adrenaline, and acetylcholine) and neuropeptides (e.g., opioid peptides and galanin) are also involved in the control of GH secretion with both stimulatory and inhibitory effects.[1] These effects are exerted mainly via GHRH and/or somatostatin rather than directly at the pituitary level. Other stimuli including sleep,[15] exercise,[16] and hormonal changes during puberty[17] also have a profound effect on the GH secretory pattern. Therefore, it is clear that the control of pulsatile release of GH is a complex process involving multiple regulators.

Growth hormone is the primary hormone responsible for growth in mammals, and it accelerates metabolic processes such as lipolysis and protein synthesis. The anabolic effects of GH are mediated through two major pathways. GH exerts its action directly by interacting with specific GH receptors that are widely distributed in body tissues[18] and indirectly through the effects of insulin-like growth factor.[7] In addition to its well-documented anabolic effects, IGF-1 has been reported to be a feedback inhibitor of GH synthesis and release by acting primarily at the level of the hypothalamus and pituitary.[19]

Growth hormone (191 amino acids; MW = 215 000) is a relatively large peptide with poor oral bioavailability. Therefore, it must be administered parenterally. The current yearly expense for treating a GH-deficient child in the US exceeds $10 000. Furthermore, as discussed above, there are side effects associated with GH replacement therapy in the elderly which tend to limit widespread use. Injections of rhGH result in supraphysiologic concentrations of GH and override the physiologic feedback mechanisms that govern the pulsatile release of GH. Most GH deficiencies involve suboptimal release of GH due to insufficient hypothalamic input, rather than insufficient GH content in the pituitary. Therefore, drug discovery strategies have focused on identifying specific GH secretagogues that will provide a more physiologically relevant, pulsatile release of endogenous GH from the pituitary gland. It is anticipated that inducing or augmenting a normal episodic GH profile may provide the beneficial effects of GH with fewer side effects. Toward this end, the endogenous peptide, growth hormone releasing hormone (GHRH), and the growth hormone releasing peptides (GHRPs) have been investigated extensively during recent years as potential alternatives to GH replacement therapy.

Growth Hormone Releasing Hormone (GHRH)

Human GHRH is a C-amidated 44-amino acid peptide that was first isolated from pancreatic tumors of a patient suffering from acromegaly.[20] Subsequently, it was shown to be present in the hypothalamus and to cause GH release from the anterior pituitary by interacting with specific GHRH receptors.[21] GHRH (1−29)-NH_2 is the shortest fragment that possesses GH-releasing capability and binding properties similar to that of GHRH (1−44)NH_2 suggesting that the first 29 residues are essential for biological activity.[22] Mechanistic studies have shown that GHRH-mediated GH release from pituitary somatotrophs proceeds by a signaling pathway that utilizes cAMP.[23]

GHRH has been evaluated extensively in humans as an alternative to GH replacement therapy and in

animals for growth enhancement and to stimulate lactation. In the clinic, GHRH was shown to acutely stimulate GH release in normal and GH-deficient subjects.[24,25] Unlike GH replacement therapy, exogenous administration of GHRH (1−44)NH_2 twice daily by injection does not abolish GH pulsatility. Instead, continuous intravenous infusion of GHRH to young adult men for 14 days led to enhanced GH pulsatility and significant increases in IGF-1.[26] Long-term GHRH administration accelerated growth velocity in GH-deficient children, with no subsequent desensitization of acute GHRH-stimulated responses, suggesting that the somatotrophs remain responsive to subsequent stimulation.[27] In these studies GHRH was generally free of side effects.

GHRH is a relatively large peptide with poor oral bioavailability. Furthermore, in humans GHRH (1−44)NH_2 is rapidly metabolized with a terminal half-life of 6.7 min.[28] Therefore, considerable effort has been spent to identify more metabolically stable and longer-acting GHRH analogues, and several groups have synthesized super-potent analogues with somewhat improved pharmacological properties.[29−31] All these GHRH analogues are peptidyl in nature and are used subcutaneously. Thus far, GHRH and its analogues have not demonstrated significant improvements in efficacy or oral bioavailability over rGH. Therefore, use of GHRH and GHRH analogues in humans and in animals has been limited.

Growth Hormone Secretagogues: Growth Hormone Releasing Peptides (GHRPs)

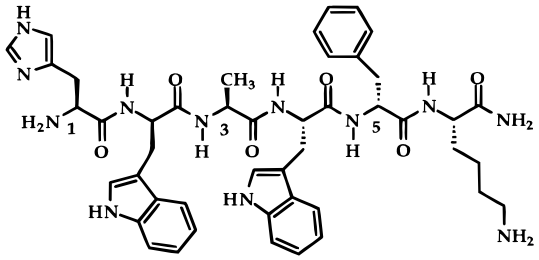
An alternative pathway for releasing endogenous GH from the pituitary was discovered by Bowers and co-workers before the isolation of GHRH in 1982.[32−37] They developed synthetic peptides with six and seven residues called the growth hormone releasing peptides (GHRPs) which are potent releasers of GH from the pituitary across all species tested so far, including humans. The GHRPs were discovered without knowledge of their molecular target or an endogenous counterpart. Following disclosure of the potent hexapeptide secretagogue **1** (GHRP-6; His^1-D-Trp-Ala^3-Trp-D-Phe^5-$LysNH_2$) by the Bowers group[36] in 1984, significant advances have been made in developing small-molecule mimetics of it. For the purposes of this paper, collectively, peptidomimetic and peptidyl analogues of the GHRPs are termed GH secretagogues (GHS). Pharmaceutical design of GHS is of interest since these ligands are agonists. Furthermore, peptidyl and peptidomimetic GH secretagogues are presently undergoing clinical evaluation as an alternative to GH replacement therapy. Considerable progress has been made in recent years in understanding their mechanism of action and their role in amplifying pulsatile GH secretion.

This Perspective highlights the discovery strategies that were employed in developing the GHRPs and the small-molecule GH secretagogues. We will discuss some results from clinical investigations and provide some perspective on possible clinical utilities of GH secretagogues. Finally, progress in understanding the mechanistic basis for GH secretagogue activity will be discussed.

Design and Discovery of GHRPs

Momany and Bowers designed the hexapeptide secretagogue **1** (GHRP-6) using an empirical approach and a theoretical approach that utilized conformational energy calculations, and disclosed their results in 1984.[36] The first series of GHRPs had been described by the Bowers group in 1977. They evolved from an assessment of C-amidated met- and leu-enkepkalins and their analogues for GH releasing activity from pituitary incubates. Replacement of Gly^2 of met-enkephalin-NH_2 (Tyr-Gly^2-Gly-Phe-MetNH_2) by D-Trp as found in **2** (Tyr-D-Trp^2-Gly-Phe-MetNH_2) or by D-Phe (**3**; Tyr-D-Phe-Gly-Phe-MetNH_2) resulted in compounds that in vitro released GH from the pituitary at relatively high concentrations (~10 μg/mL).[33] Later it was discovered that incorporation of D-Trp at position 3 (**4**; Tyr-Gly-D-Trp^3-Phe-MetNH_2) also released GH in vitro at somewhat lower concentrations.[34,35] Peptides **2**, **3**, and **4** were found to be specific for releasing GH and were devoid of opiate activity. Momany and co-workers designed several peptides starting from **2**, **3**, and **4** that enhanced the conformational properties thought to be responsible for activity. Conformational energy calculations on peptides **2** and **3** established that L and D stereochemistries at the 1 and 2 positions, respectively, resulted in an interaction of the aromatic rings of Tyr^1 and D-Trp^2.[35] Increased activity was later found with **5** (Tyr-Ala-D-Trp^3-Phe^4-Met-NH_2). Conformational analysis of **5** now found the aromatic side chains of D-Trp^3 and Phe^4 to be stacked with each other. Therefore, L−D or D−L stacking of the aromatic side chains was proposed by Momany et al. to be important for GH-releasing activity.[35] Furthermore, it was concluded that a calculated bend structure of these peptides was an important feature of the bioactive conformation of the GHRPs. To test this hypothesis they designed **6** (Tyr-D-Trp-D-Trp-Phe-NH_2) and found that it had enhanced GH-releasing activity. Particularly noteworthy is the negligible resemblance that the more potent secretagogue **6**, with its L−D−D−L arrangement, bears to the enkephalin peptides **2** and **3** from which it was derived. Following these breakthroughs, attempts were made to insert a spacer between the L−D and D−L ring sets of **6** while maintaining the conformational properties of the original peptide. From this work emerged a more active secretagogue **7** (Tyr^1-D-Trp-Ala^3-Trp-D-Phe^5-NH_2) that released GH at concentrations as low as 10−30 ng/mL.[34,35] Conformational analysis of **6** revealed interactions between the aromatic side chains that are initiated by a turn-like structure around the first four residues. SAR studies at the Tyr^1 and C-terminal positions then led to the discovery of the first potent peptidyl secretagogue **1** (GHRP-6; His^1-D-Trp-Ala^3-Trp-D-Phe^5-Lys-NH_2; EC_{50} = 10 nM).[37,38] Although the C-terminal lysine residue was not absolutely essential for in vitro activity, it appears to contribute significantly to in vivo efficacy.

Momany and Bowers have recently reported progress in understanding the solution conformation of **1** and its bioactive analogues with the working hypothesis that this conformation will resemble the bioactive conformation when bound to the receptor.[39] These studies were guided by molecular and dynamic computational methods, experimental results from solution NMR studies



1 (GHRP-6)

and conformational analysis of biologically active cyclic peptides Cys[1,9]-Ala-GHRP-6 (~30% GH-releasing activity of **1**) and Cys[1,9]-*N*-methyl-D-Phe[7]-GHRP-6 (~25% GH-releasing activity of **1**). Biologically active peptides bearing N-methylated residues and proline substitutions were identified which minimize the number of low-energy conformations of **1**. Both computational studies and solution NMR studies found that when His[1] is uncharged, **1** and its active analogues have stable conformations in water and the "core" GHRP-6 conformation is retained in its active analogues, including the two cyclic peptides. Analogues that perturbed this active "core" lost activity. In its low-energy conformation **1** adopts a folded conformation in which the polar N-terminal His and C-terminal Lys are close in space. The hydrophobic indole units from D-Trp[2] and Trp[4] are directed toward one another and the phenyl unit from D-Phe[5] appears to interact with the amino unit of Lys[6]. Therefore, a triaromatic "core" with a basic amine has been suggested to comprise the essential pharmacophore of **1**.

Biological Activities of GHRP-6

Hexapeptide **1** caused a concentration-dependent increase in GH from pituitary incubates of 21-day-old female rats at concentrations ranging from 1 to 30 ng/mL of medium.[36] In vitro, the GH response to 30 ng of GHRP-6 was completely inhibited by 100 ng of somatostatin-28 (SRIF-28).[36] Unlike the earlier compounds, **1** was potent in vivo in 21-day-old female rats in which it caused a dose-dependent increase in serum GH after sc administration at doses ranging from 3 to 30 μg per rat. The GH response was almost immediate, with the peak response occurring 10−15 min after dosing. The in vivo GH-releasing activity of **1** (3 μg; sc) was abolished if SRIF-28 (20 μg; sc) was given concomintantly, demonstrating that SRIF-28 is a powerful inhibitor of GHRP-6-mediated release of GH. Subsequently, Bowers and co-workers showed that **1** was active in a number of species including monkeys,[40] barrows,[41] steers[42] and under special circumstances chickens,[32] suggesting that the mechanism for GHRP-induced GH release was conserved across species. The in vitro GH release produced by **1** was specific since large doses elicited a marked release of GH but not of thyroid-stimulating hormone (TSH), luteinizing hormone (LH), or follicle-stimulating hormone (FSH).[36] Specificity for GH release with respect to prolactin (PRL) release was demonstrated in vitro but not in vivo. In dogs, **1** was potent for releasing GH after iv administration, and the rise in GH levels was followed by transient increases in cortisol levels.[43] Similar increases in cortisol levels were also seen in humans after iv administration of **1**.[44] The anabolic potential of **1** was evaluated by Bowers and co-workers using chronic in vivo studies in immature female rats.[36] A statistically significant increase in body weight gain was observed when **1** was given ip to 17-day-old rats once or twice daily for 9−25 days at doses ranging from 30 to 100 μg.[36,45] Chronic administration to cows enhanced milk production.[46]

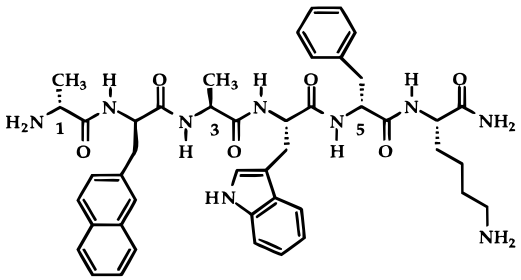
Identification of More Potent GHRPs

In 1993, Bowers and co-workers reported two more potent analogues of **1** (GHRP-6; His[1]-D-Trp-Ala[3]-Trp-D-Phe[5]-LysNH$_2$; EC_{50} = 10 nM).[47,48] Specifically, Bowers found that the in vitro activity of **1** was enhanced by incorporation of D-β-naphthylalanine for D-Trp[2] and, by use of the stereochemically inverted D-Ala for the N-terminal L-His, thus producing **8** (GHRP-2; D-Ala[1]-D-β-Nal[2]-Ala[3]-Trp-D-Phe[5]-LysNH$_2$; EC_{50} = 3 nM).[47] Furthermore, His[1] could be derivatized with an Ala to provide the potent heptapeptide GH secretagogue **9** (GHRP-1; Ala-His[2]-D-β-Nal-Ala[4]-Trp-D-Phe[6]-LysNH$_2$; EC_{50} = 6 nM).[48] In their design of hexarelin (**10**; His-D-2-methylTrp[2]-Ala-Trp-D-Phe-LysNH$_2$), Deghenghi and co-workers[49] utilized D-2-methylTrp in place of the D-Trp[2] of **1**. In rats, **8** and **9** are 2−3-fold more potent than **1** in releasing GH after iv administration. Hexarelin (**10**) was initially believed to have a longer duration of action after iv administration to rats, but subsequent experiments indicated an iv potency and duration of action which are comparable to **1**.[32]

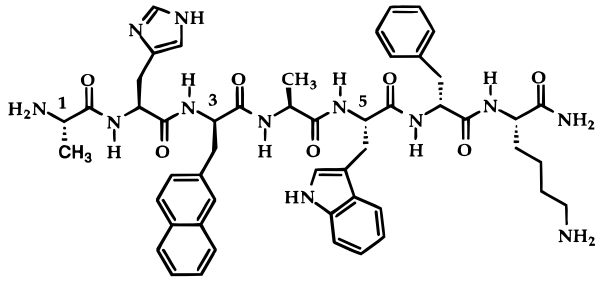
Early in vitro mechanism of action studies by Bowers found that **1** released considerably more GH from hypothalamic-pituitary incubates than from the pituitary alone, suggesting that hypothalamic input was important for GHRP-mediated GH release.[50] Later, it was shown that **1** synergized with GHRH and caused significantly larger amounts of GH to be released from cultured rat pituitary cells than the GH output after stimulation by GHRH or **1** alone.[51,52] Synergy between **1** and GHRH was eventually demonstrated in humans.[44] In cultured pituitary cells, complete desensitization to **1** occurs within a few minutes. Surprisingly, however, the in vivo effects are markedly different; continuous intravenous infusion of **1** to rats resulted in sustained elevated GH secretion and pulsatility.[53] There were also significant increases in serum IGF-1 levels. Mechanistically, **8**, **9**, and **10** are indistinguishable from **1**.

Clinical Findings: GHRPs and Hexarelin

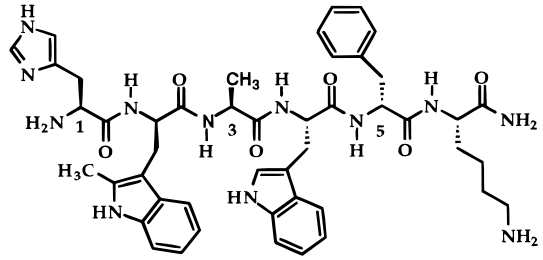
The GHRPs have been tested numerous times in healthy young and old human subjects as well as in various disease states.[54a] In healthy subjects, following a single intravenous, oral, or intranasal dose of a peptide GH secretagogue, a single broad GH peak is detected.[44,54a] Where measured, this GH peak is accompanied by smaller peaks of adrenocorticotropic hormone-mediated cortisol secretion and prolactin secretion.[44] In addition, the GHRPs effectively release GH in patients with diabetes,[54b] obesity,[54c,d] acromegaly,[54e] chronic renal failure,[54f] and polycystic ovary syndrome[54g] and in the presence of corticosteroid excess.[54d] However, GH secretagogues do not elicit a GH response in patients with hypothalamic-pituitary disconnection.[55,56] In a revealing study, Jaffe et al.[57] infused **1** (1 μg/kg/h) or saline



8 (GHRP-2)



9 (GHRP-1)



10 (Hexarelin)

into eight healthy young adult men over a 24 h period. Serum was sampled for GH levels every 10 min. A sustained, upregulated pulsatile pattern of GH secretion resulted. A similar observation was reported by Huhn et al.[58] Thus, the GH response to sustained serum concentrations of secretagogue was fundamentally different from that of a bolus dose.

Desensitization, resulting in lower peak GH responses after multiple doses of hexarelin, has been reported.[59] Other authors have not observed desensitization and have reported that the GH response to **10** is sustained after 15 days of dosing in the elderly.[60] Despite this, these investigators observed no significant increase in serum IGF-1 levels. In growth hormone deficient children, Mericq et al.[61] and Pihoker et al.[62] have reported similar observations using **8**. After 4−6 months of treatment, no significant increase in serum IGF-1 was observed, but both groups reported an increase in growth velocity. Laron et al.[63] treated non-GH-deficient short children with **10** (60 μg/kg; tid; intranasally) for up to eight months and reported a significant increase in serum IGF-1 as well as in growth velocity. The apparent variability in serum IGF-1 response to multiple doses of peptide secretagogues may be related to differences between secretagogues, differences in patient populations, or a large variability in individual responses, making detection of a small response difficult.

Development of Peptidomimetics of GHRP-6

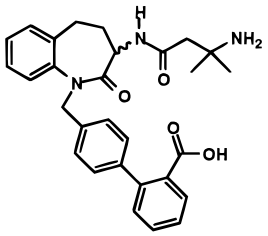
While their relevance to a putative natural mediator is unknown, the high potency of the GHRPs for releasing GH, their relatively good specificity and demonstrated anabolic effects in chronic administration studies have validated the GHRP mechanism for GH release as a viable alternative to GH replacement therapy. Unfortunately, the oral bioavailability of **1** in humans has been reported to be approximately 0.3%, and its $t_{1/2}$ in humans is only about about 20 min.[64,65] Therefore, considerable efforts have been made to identify peptidomimetics for GHRP-6 with improved pharmacokinetic properties.

Design of Benzolactam-Based GH Secretagogues. A group from Merck was the first to disclose nonpeptidyl mimetics of GHRP-6.[66a,b] When this research was initiated, nonpeptidyl agonists for peptide ligands were rare. The most notable examples were the opiates and tifluadom which are mimetics of the endogenous opiate peptides.[67a,b] From the SAR work of Momany and Bowers it was evident that a basic amine at position 1 was crucial for GHRP activity. Furthermore, aromatic amino acids were favored at positions 2, 4, and 5, and as discussed above, use of the unnatural D-Trp at position 2 converted the original opioid peptide to a GH secretagogue.[33] The Merck group utilized these attributes in their selection of nonpeptidyl compounds from the Merck sample collection for evaluation in their rat pituitary cell assay. From this directed screening, they identified a lead compound **11** (EC_{50} = 3 μM). Replacement of the carboxylic acid group in this compound by a tetrazole increased potency and independent synthesis of its *R* enantiomer then afforded the first potent nonpeptidyl GHRP mimetic **12** (L-692,429; EC_{50} = 60 nM).[66a,b] Benzolactam **12** caused GH release from cultured rat pituitary cells with a maximal 300% increase in 15 min.[68]

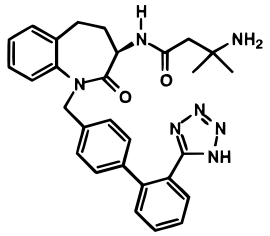
The discovery of **12** was a seminal breakthrough because it demonstrated that relatively small peptidomimetic agonists for **1** could be designed. Further SAR studies around **12** enhanced its potency even further by N-terminal derivatization with a (*R*)-2-hydroxypropyl unit to afford **13** (L-692,585; EC_{50} = 3 nM).[69,70] Hansen and co-workers have disclosed potent naphtholactam derivative **14** (EC_{50} = 4 nM) which is significantly more potent than **12** for releasing GH from cultured rat pituitary cells.[71] Unfortunately, the oral bioavailability of **14** in rats is less than 5%.[71]

Benzolactam Secretagogues: In Vivo Activities

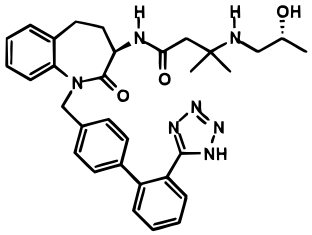
In vivo efficacy studies with **12** were conducted using dog models because, unlike rats and mice, dogs have a very steady basal GH concentrations. Acute iv administration of **12** to beagles resulted in an immediate increase in GH concentrations. Maximal serum concentrations of GH up to 90-fold greater than baseline values occurred within the first 30 min and returned to pretreatment levels by 120 min.[43] In an iv study with a balanced crossover design using four male and four female beagles, dose-dependent increases in GH levels occurred after iv administration of 0.1, 0.25, or 1.0 mg/kg of **12**. The specificity of **12** for GH release was evaluated in dogs after a single iv dose of 5 mg/kg. Significant increases in cortisol and adrenocorticotropic



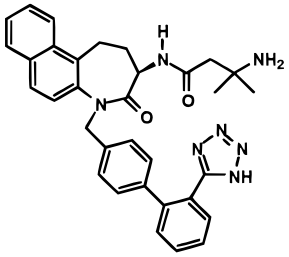
11



12



13



14

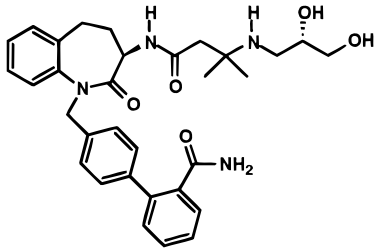
hormone (ACTH) were observed whereas the other measured hormones, including PRL, LH, insulin, thyroxine-3 (T_3), and thyroxine-4 (T_4), were in the normal range. As noted above, Hickey et al. had demonstrated similar increases in ACTH and cortisol levels in dogs after iv administration of **1**.[43] Therefore, despite marked structural differences, the specificity of **12** in dogs appears to be similar to **1**. The acute iv potency of **13** was considerably greater than **12** with a minimum effective dose of 0.005 mg/kg in beagles.[72] Since the minimum effective iv dose of **1** in dogs is 0.01 mg/kg,[72] benzolactam **13** appears to be 2-fold more potent intravenously than **1**. In a chronic study, after once-daily iv administration of **13** at 0, 0.01 and 0.10 mg/kg for 14 days, desensitization of the GH response did not occur and although transient increases in IGF-1 were evident 6 h after dosing, IGF-1 levels returned to baseline within 24 h. Prolactin, insulin, and thyroxine levels were unaffected.[72] A 12 h infusion of **13** into guinea pigs during which GH was monitored every 10 min resulted in a sustained amplification of episodic GH release, similar to that observed with **1**.[53] Intriguingly, GH pulsatility was initiated by **13** suggesting that this class of GH secretagogues has the capacity to reset the pulsatility of GH release.

Clinical Investigations with Benzolactam Secretagogue 12 (L-692,429)

Administration of benzolactam **12** to healthy young men (1 mg/kg, iv) provided a mean peak GH concentration of 82.5 μg/L, demonstrating that it was substantially more efficacious than GHRH at 1 μg/kg (highest dose tested).[73a] Like **1**, a single dose of **12** produced transient increases in cortisol and PRL in addition to GH. Continuous 24 h iv infusions of **12** stimulated pulsatile GH release and increased mean circulating GH concentrations in healthy older adults.[74] Furthermore, GH secretagogues may be able to reverse the catabolic effects of glucocorticoids since **12** elevated GH in patients undergoing short-term, high-dose treatment with prednisolone.[75]

Development of Other Benzolactam GH Secretagogues

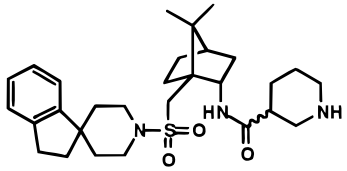
Unfortunately, the oral bioavailability of **12** in dogs is less than 5%.[76] The poor oral bioavailability of **12** could be due to poor absorption resulting from its zwitterionic nature. It is, therefore, of interest that DeVita and co-workers have shown the tetrazole moiety can be replaced with a carboxamide group to provide a potent secretagogue **15** (EC_{50} = 3 nM) that is orally active in dogs at 5 mg/kg.[77] Use of neutral and basic heterocyclic replacements for the 2′-tetrazole group in the benzolactam design has also provided potent compounds with in vitro GH-releasing activity comparable to **12**.[78] Another group found slightly higher rat pituitary cell activities when the tetrazole group was replaced by an oxadiazole or triazole.[78d] The effects of these changes on absorption and oral bioavailability of this class of GH secretagogues are still to be reported.



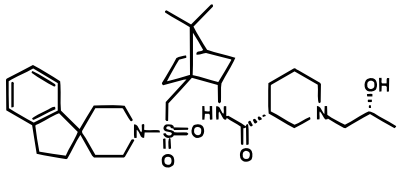
15

Quest For New Structural Leads: Privileged Structure Design

A weakly active camphor-based GH secretagogue **16** (EC_{50} = 300 nM) was discovered at Merck from directed screening of compounds in the rat pituitary GH release assay. Camphor sulfonamide **16** was also a potent oxytocin receptor antagonist (IC_{50} = 68 nM). Use of the (*R*)-nipecotic acid amino side chain and N-terminal derivatization with a 2(*R*)-hydroxypropyl unit provided a more potent GH secretagogue **17** (EC_{50} = 90 nM),[79] which, however, retained activity on the oxytocin receptor (IC_{50} = 130 nM).



16



17

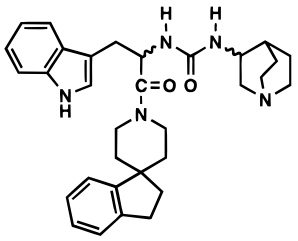
Although no potent and specific GH secretagogues were synthesized from the camphor sulfonamide, the spiroindanylpiperidine component of these compounds was selected for additional derivatization, acting on a suggestion made several years earlier by Evans and co-

workers[80] that a useful way of designing receptor agonists and antagonists was to derivatize frequently occurring structural units such as benzodiazepines. They called these recurring core units "privileged structures". The recognition of such core structures goes back to Ariens and co-workers[81] who noted the presence of hydrophobic double-ring systems which contributed importantly to the receptor affinity of many biogenic amine antagonists. They suggested this motif "must bind to *accessory binding sites* of a predominantly hydrophobic nature located next to the receptor for the corresponding agonist".[81] These receptor binding units may afford "multipotent competitive antagonists" which they illustrated, for example, with chlorpromazine whose pharmacology includes anticholinergic, antihistaminic, antiserotonergic and α-adrenergic blocking activities. Recurring structural units were also illustrated in CNS drugs by Andrews and Lloyd[82] and more recently in a review of peptidomimetics from natural products by Wiley and Rich.[83]

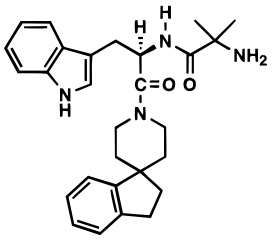
Following the suggestion of Evans and co-workers,[80] the derivatization of selected privileged structures was an approach taken at Merck to initiate new lead discoveries. At the time, the spiroindanylpiperidine group was hypothesized to be one of these privileged structures since the camphor sulfonamide GHS lead also had oxytocin antagonist activity and the spiroindanylpiperidine group was known to be present in σ receptor antagonists (subsequently published by Chambers and co-workers).[84] Later there were NK-1,[85,86] NK-2,[87] dual NK-1 and NK-2 antagonists[88] and C5a agonists[89] described which contain a spiropiperidine group further substantiating its categorization as a privileged structure. Presumably these receptors, for whatever reason, contain similar binding sites whose size and hydrophobic properties are well suited to accommodate a spiropiperidine group. If recurring structural units bind near receptor "active sites" as suggested by Ariens and coworkers, then Patchett and co-workers hypothesized that their derivatization with amino acids and small peptides might afford agonists or antagonists for peptide receptors.[90]

Design of Orally Active Spiroindoline Sulfonamide 20 (MK-0677)

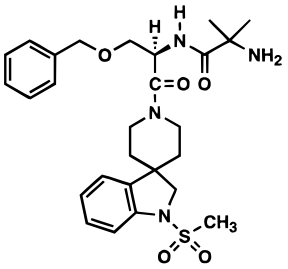
One of the first discoveries to come out of this privileged structure derivatization effort was **18**. Even as a mixture of four diastereomers it caused GH release in the rat pituitary cell culture assay with excellent potency (IC_{50} = 50 nM).[90] Spiropiperidine **18** did not elevate GH in dogs after an oral dose of 5 mg/kg. However, good oral activity was achieved in a D-tryptophan analogue **19** in which the ureidoquinuclidene part of structure **18** was replaced by an aminoisobutyric acid (AIB) group.[91] The latter is a preferred amino side chain in the benzolactam secretagogues and the D-Trp stereochemistry of **19** corresponds to *R* stereochemistry at the benzolactam 3-position of potent benzolactam secretagogues such as **12**. Additional potency and oral bioavailability improvements were achieved by introducing a methylsulfonylamido group and by replacing D-Trp by *O*-benzyl-D-serine. The resulting compound **20** released GH in the rat pituitary cell assay with an EC_{50} = 1.3 nM.[90] Furthermore, although it was considered to be a privileged structure derivative, the IC_{50} values of **20** in over 50 receptor assays were greater than 10 μM.[90,92] These included opiate, galanin, σ, benzodiazepine, substance P, and muscarinic receptors. Subsequently, it was shown that neither GHRH nor somatostatin would displace [^{35}S]-radiolabeled **20** from its receptor.[93]



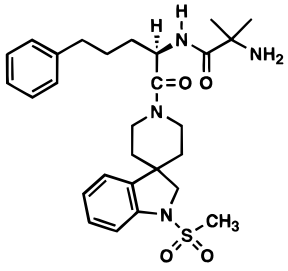
18



19



20 (MK-0677)



21 (L-163,255)

In Vivo Properties of 20 (MK-0677)

Secretagogue **20** has an oral bioavailability in dogs greater than 60%.[90] It elevated GH in beagles with intravenous doses down to 0.025 mg/kg and orally with doses as low as 0.125 mg/kg.[90] Following a single oral dose of 1 mg/kg in three dogs, mean GH peak levels increased more than 10-fold and GH levels remained elevated for 360 min. An iv injection of 0.25 mg/kg of **20** significantly increased peak GH concentrations 20-fold with a duration up to 180 min after treatment. IGF-1 levels were elevated 25% at 360 min and total cortisol was increased 2.3-fold over the saline control. Insulin and glucose were higher, LH and prolactin were unchanged, and thyroxine T4 was slightly lower but all of these changes were within normal limits for dogs.[9] The only significant difference from controls was in the cortisol data. As discussed above, cortisol elevation had been seen earlier with **1**[43,44] and with the benzolactam **12** in beagles.[43,72] In the clinic, increases in cortisol concentrations were seen with **12**,[73a] although, within normal ranges.

Results of a chronic study in which **20** was given orally, once daily to beagles for 14 days have been reported.[95] A significant down-regulation of the GH response was observed by day 2 which then stabilized with growth hormone levels remaining significantly elevated throughout the study at doses of 0.5 and 1.0 mg/kg. The GH elevation was characterized by an amplified pulsatile profile. IGF-1 concentrations were increased up to 126%, and daily pretreatment levels were higher than placebo values. However, when the animals were treated on alternate days with **20**, desensitization did not occur and basal IGF-1 concentrations had returned to control concentrations. The authors

suggest that IGF-1 elevation was involved in the GH down regulation which is consistent with a report that IGF-1 reduces GH concentrations in humans.[19] It is also interesting that repeated oral dosing of **20** attenuated the elevated cortisol concentrations and this decreased response was not observed on alternate day treatment. IGF-1 increase or desensitization of the growth hormone secretagogue (GHS) receptor may have caused this down regulation. To explore these possibilities, dogs were treated on day 1 with **20** and on days 2 and 3 the dogs were treated with porcine GH at a dose sufficient to cause a sustained increase in IGF-1. On day 4, after GH had returned to basal levels but IGF-1 levels were still elevated, the dogs were challenged with **20**. As observed with dogs treated for 4 days with **20**, the response to **20** on day 4 compared to day 1 was clearly attenuated. These results suggest that reduced responsiveness to repeated **20** treatment is largely caused by reduced responsiveness of the GH/GHRH axis associated with sustained increases in IGF-1.[95]

On the basis of its potency, selectivity, oral bioavailability, and sustained activity in dogs to elevate GH and IGF-1, **20** was selected for safety assessment studies and entered clinical studies as MK-0677.

In Vivo Tests with the MK-0677 Analogue 21 (L-163,255)

A structurally related compound **21** (L-163,255), with an intrinsic potency (EC_{50} = 1.5 nM) like that of **20**, has also been studied extensively in animals. Of interest is a chronic study that investigated its anabolic and functional effects in a dog hind limb immobilization protocol.[96] In this experiment the right hind limbs of beagles were kept immobile in a fixation device for 10 weeks, and then for 5 weeks the dogs were allowed to move about freely. Throughout both phases of the experiment the animals were given either **21** (5.0 mg/kg po, once daily) or the vehicle control. IGF-1 concentrations were elevated 60% in the treated group and were modestly decreased in the control group. At the end of the experiment the dogs treated with **21** had lost 0.21 ± 0.20 kg compared to a loss of 1.13 ± 0.19 kg in the controls. Muscle strength was measured in the immobilized limbs by isometric torque. An equal decline was noted in both groups during the first 10 weeks; however, at week 15 muscle strength had increased 43% in treated vs 16% in the control group when compared to their respective week 10 strength data. A correlation between the diameter of the vastus lacteralis muscle diameter and torque was also noted.[96] The results of this study suggest that GH secretagogues may be of value in rehabilitation therapy.

Secretagogue **21** has also been studied in swine. Given ad libitum in feed at 360 ppm over a 72 h period, it elevated mean plasma IGF-1 concentrations by 62% compared to 15% in the controls. Once daily iv administration of 1 mg/kg for 14 days resulted in an initial marked GH increase on day 1 followed by the maintenance of much reduced but significantly elevated GH concentrations out to day 14. Plasma cortisol concentrations also tended to be increased on day 1; however, no significant elevations were seen on days 7 and 14 when compared to controls. The IGF-1 increases measured on days 4, 7, 10, and 14 were approximately 60%.[97] Results are awaited from more extended studies with GH secretagogues in this species in respect to growth, feed efficiency, and carcass quality.

Clinical Experience with 20 (MK-0677)

In humans, single-dose administration of **20** stimulated GH secretion in a dose-dependent fashion with a threshold dose of 5 mg (G. Murphy, unpublished observations). Responses increased in magnitude with increasing dose up to 100 mg. The 100 mg dose of **20** resulted in a pronounced GH response (peak GH, 71.2 ± 10.4 μg/L; n = 6). The GH peak was seen 30−90 min after dosing. Like **1**[44] and **12**,[73a] single oral doses of **20** produced transient increases in cortisol and PRL in addition to GH. Peak GH release and GH area under the curve (AUC) that was determined from 0 to 8 h following morning and evening dosing with 25 mg of **20** were not significantly different. GH response to 25 mg of **20** administered following a high carbohydrate breakfast, although delayed, was not substantially different in magnitude than the response to 25 mg administered in the fasted state.

Dietary energy restriction induces a predictable catabolic response in normal subjects.[98,99] This loss of nitrogen is associated with a decrease in IGF-1 and an increase in GH, suggesting development of GH resistance.[100,101] However, the resistance is not absolute since administration of exogenous GH will effect an increase in IGF-1 and promote nitrogen retention.[98,99] The effect of 7 days of daily oral treatment with 25 mg of **20** on GH secretion and nitrogen balance has been evaluated in a model of short-term diet-induced nitrogen wasting in healthy young men.[102] Seven days of treatment with **20** in this model resulted in a sustained increase in the serum concentrations of GH, IGF-1, and IGFBP-3. Furthermore, nitrogen wasting was reversed with administration of **20** in this model, suggesting the drug may be useful in treating catabolic conditions.

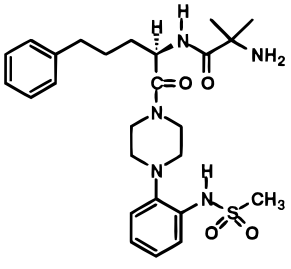
In a recently published study, healthy elderly men and women were treated with MK-0677 (10 or 25 mg) or placebo orally for 14 days.[103] In addition, GH was sampled every 20 min for 24 h before treatment and on day 14. On day 14, there was an increase in GH peak amplitude and AUC, but no detectable change in peak number. Two weeks of dosing with **20** resulted in an approximately 40% (10 mg) to 60% (25 mg) increase in serum IGF-1 concentrations as well ($p < 0.05$). Thus, repetitive dosing with this oral GH secretagogue resulted in an upregulation of pulsatile GH secretion rather than a single postdose peak of GH as was demonstrated with hexarelin.[60] In this study, treatment with **20** was generally well tolerated. There were no serious adverse experiences, although there were a few reports of increase in appetite and abdominal pain in MK-0677-treated subjects. As with the GHRPs, there were initial concerns about hormonal specificity. To address these concerns, serum cortisol and prolactin were measured every 20 min for 24 h at baseline and on day 14. There were no significant differences in cortisol AUC, or in its ultradian secretory pattern. The mean serum prolactin level increased 24% after the 25 mg dose of MK-0677 (7.0 ± 0.5 to 8.6 ± 0.7 mg/L). There were statistically significant dose-dependent increases in mean fasting serum glucose in this study. Whether

these changes persist over time, and their clinical relevance, will need to be determined in longer-term studies.

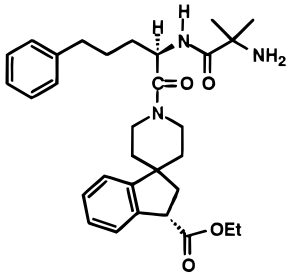
The GH response to classical stimuli of GH secretion is generally suppressed in obesity.[104] However, Cordido et al.[105] have shown that single dose administration of GHRP-6 is able to elicit a significant GH response in obese adults. Recently, the effect of 8 weeks of daily oral treatment with **20** (25 mg) on GH secretion and body composition has been evaluated in otherwise healthy obese males.[106] This regimen caused a sustained increase of the serum levels of GH, IGF-1, and IGFBP-3. Fat-free mass determined with DEXA scan increased significantly by approximately 3 kg in the MK-0677 group. Total and visceral fat were not significantly changed. Basal metabolic rate (BMR) was significantly increased after two but not eight weeks of treatment with **20**. Changes in body composition and energy expenditure were of an anabolic nature with a sustained increase in fat free mass and a transient increase in basal metabolic rate. In this study, it is difficult to explain the increase in body weight, fat-free mass and BMR in combination with unchanged body fat without an increased food intake. Recently, it has been reported that GHRP-6 injection activates neuropeptide Y cells in the rat arcuate nucleus,[107] which could indicate a positive effect on appetite.[108] Further studies would be needed to evaluate if a higher dose or a prolonged treatment period of **20** can result in a fat-reducing effect or whether increased food intake is limiting any possible benefit on fat mass.

Other Peptidomimetics of the GHRPs

The original camphor sulfonamide lead **16** had both GH secretagogue agonist and oxytocin antagonist activities. In analogy with oxytocin antagonists,[109] piperazine-based analogues of **20** were also synthesized. Not surprisingly, a high level of activity was observed in such compounds, exemplified by **22** (EC_{50} = 6.3 nM).[110]



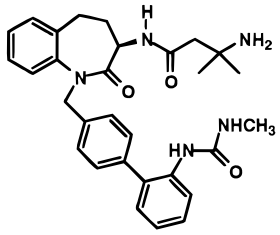
22



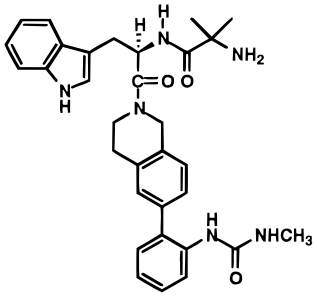
23

Another variation of the spiroindane group was described by Tata and co-workers[111] in which the 1,1-spiroindane 3-position was substituted by carboxylic acids and esters. The derived secretagogues were potent, orally active, and characterized by a much shorter duration of action than **20**. Representative of these compounds is ester **23** which exhibited an EC_{50} = 1.9 nM in the rat pituitary cell GH release assay and a $t_{1/2}$ in dogs of only 1.7 h, compared to 4.7 h for **20**. Following once-a-day administration to dogs for several days, the short-acting compound **23** produces less down regulation of GH response than does **20**, although IGF-1 elevations are also lower.

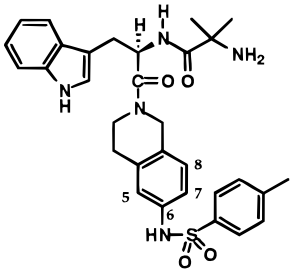
A series of tetrahydroisoquinoline/isoindoline-based GH secretagogues that have low nanomolar activities in the rat pituitary GH release assay were disclosed by Lefker and co-workers.[112] The design of this new of class of secretagogues originated from a project that sought to prepare hybrid structures between benzolactam **24** (EC_{50} = 50 nM)[77,112,113] and the spiropiperidine **19**. From this approach, a conformationally restricted urea derivative **25** of moderate GH-releasing activity was identified with EC_{50} = 100 nM. Further work in this series led to the discovery of a highly active *p*-tolylsulfonamide **26** (EC_{50} = 3 nM). This compound released GH with ED_{50} >10 mg/kg following iv administration to 4-week-old anesthetized Sprague−Dawley rats. The iv potency in rats was increased to 0.2 mg/kg by excising the terminal phenyl group, reversing the sulfonamide attachment and quite interestingly, moving this polar substituent from the 7 to the 6 position on the aromatic ring, as depicted in **27** (EC_{50} = 3.0 nM). The *N*,*N*-dimethylsulfonamide derivative **28** (EC_{50} = 30 nM) was 10-fold less active intravenously in rats (ED_{50} = 3 mg/kg). In dogs, **28** was orally active at 3 mg/kg with short a duration of action for GH release.



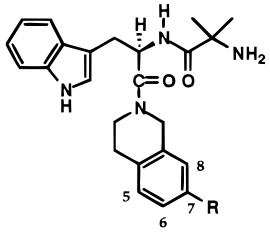
24



25



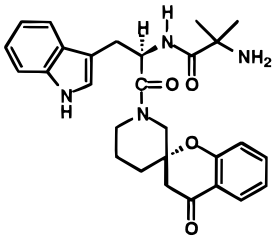
26



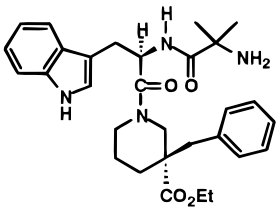
27 R = SO_2NH_2
28 R = $SO_2N(CH_3)_2$

More recently, highly active GH secretagogues such as **29** (EC_{50} = 1.8 nM) and **30** (EC_{50} = 1.6 nM) were disclosed in which a 3,3-substituted piperidine was utilized in place of the privileged structure component of **20**.[114,115] Compound **30** is a short-acting GH secretagogue in dogs. In the patent literature, GH secretagogues such as **31** in which the spiroindoline sulfonamide unit of **20** has been replaced by a new hydrophobic unit have been disclosed.[116] Descriptions of the biological properties of the new structural types are awaited.

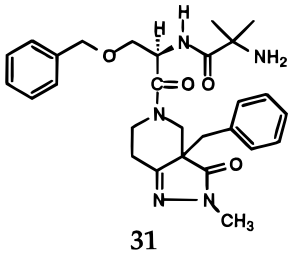
In addition, patent publications by Dodge and co-workers[117,118a] claim 2-acylaminopropanamines and 2-acylaminopropanamides, such as **32** and **33**, as GH



29

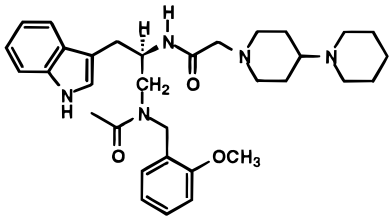


30

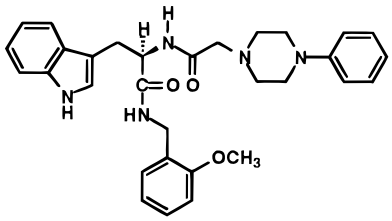


31

secretagogues. These compounds were described earlier as neurokinin-1 antagonists.[118b]



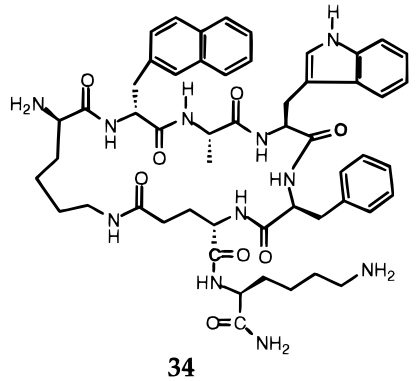
32



33

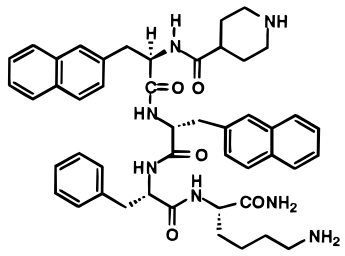
A multidisciplinary approach was utilized by McDowell and co-workers in their discovery of a new series of small molecule GH secretagogues.[119] To investigate the topographical requirements of the GHRPs, they developed **34** ($EC_{50} = 0.43 \pm 0.11$ nM), a cyclic analogue of the linear hexapeptide **8**. NMR studies showed that its D-2-Nal-Ala-Trp-D-Phe part structure adopts a compact conformation with nested hairpin turns initiated at D-Lys[1] and Ala[3]. Other cyclic analogues of **8** that did not readily adopt this conformation were considerably less active suggesting that a precise arrangement of the three aromatic side chains was crucial for GH releasing activity.

Extensive medicinal chemistry studies around **1** and **8** by McDowell and co-workers identified isonipecotic acid as a useful N-terminal group. An interesting finding from their studies culminating in the discovery of **35** ($EC_{50} = 0.18 \pm 0.04$ nM) was that the D-Trp-Ala-Trp-D-Phe triaromatic core of **1** could be presented as D-β-Nal-D-2-Nal-Phe. Further optimization of **35** by modifying its C-terminus gave the sub-nanomolar secretagogue **36** ($EC_{50} = 0.34 \pm 0.2$ nM). Remarkably, even

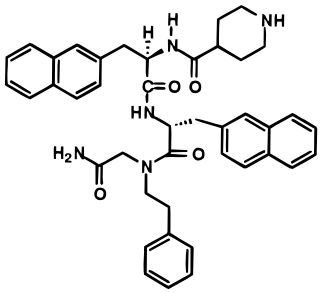


34

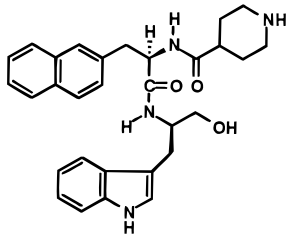
dipeptide derivatives such as **37** had good potency ($EC_{50} = 10.6 \pm 6.2$nM). At 100 nM concentrations, **35** and **37** were quite specific in their release of GH except for small increases in ACTH and PRL as expected of secretagogues acting via the GHRP pathway.[120]



35



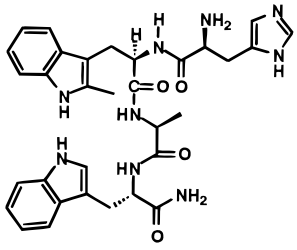
36



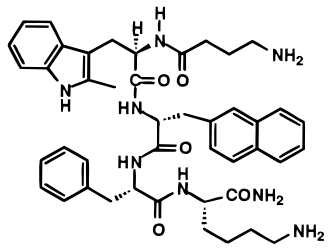
37

Interesting anabolic and diabetogenic data were reported for **35** ($EC_{50} = 0.18$ nM)[119,121] when 100 μg per day were administered to 150-day-old female Sprague–Dawley rats either by twice daily sc injection or by continuous infusion. Body weight gain at the end of 14 days was significantly greater with intermittent dosing. Continuous infusion apparantly induced tachyphlaxis.[119] The diabetogenic effects of **35** and rhGH were evaluated as single therapy and in combination with rhIGF-1 in 6-week-old obese male Zucker rats.[121] Serum fasting glucose levels over a 3-week period were elevated in the group that received 100 μg per rat per day of **35** by twice daily sc injection. This elevation was attenuated in the animals given **35** plus rhIGF-1. Administering rGH caused significant insulin resistance, as measured by serum glucose and insulin levels and by an insulin challenge. Secretagogue **35** produced body weight gain when given alone which was increased in combination with rhIGF-1.[121] These findings suggest that IGF-1 is an important factor in moderating the effects of rGH and GH secretagogues on serum glucose, and in maximizing the anabolic potential of these agents.

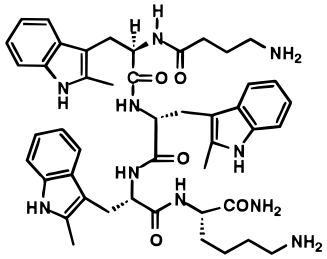
In their efforts to reduce secretagogue size, Deghenghi and co-workers[122,123] found that tetrapeptide **38** (His-D-2-methylTrp-Ala-TrpNH_2) had GH-releasing properties after iv administration to infant rats. Further work by this group has shown that GH releasing activity can be significantly improved by using a γ-aminobutyric acid (GAB) side chain for the His[1] of **10**.[123] The GH output with **39** (GAB-D-2-methylTrp-D-β-Nal-Phe-LysNH_2; plasma GH 198 ± 13.2 ng/mL) is nearly twice as much as the GH release with **10** (plasma GH 104 ± 1.9 ng/mL).[123] Further work in this series has yielded **40** (GAB-D-2-methylTrp-D-2-methylTrp-D-2-methylTrp-Lys-NH_2) and **41** (AIB-D-2-methylTrp-D-2-methylTrp-NH_2).[124,125] The former at 300 μg/kg sc potently releases GH and is a highly effective releaser of GH in the infant rat with elevations to 160−200 ng/mL. The tripeptide **41** is also reported to elevate GH levels into the 160−200 ng/mL range in the infant rat assay. Both **40** and **41** are orally active in dogs and in man where they were reported to elevate GH levels without increasing cortisol.[124,125] Other tripeptides have also been described.[126]



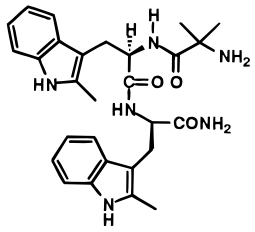
38



39

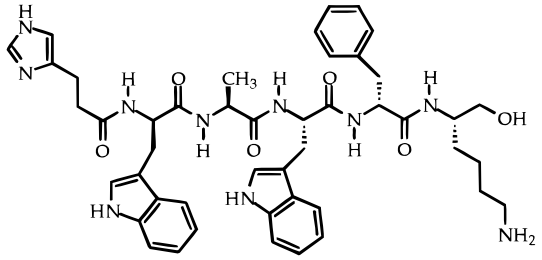


40

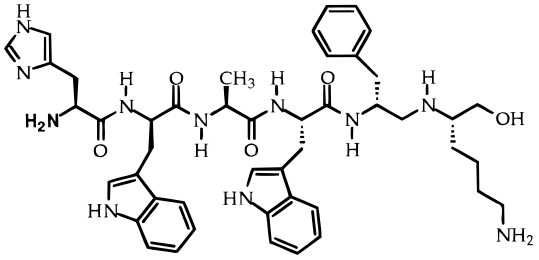


41

GHRP analogues with amide bond isosteres and lower molecular weight mimetics of **1** have been disclosed.[127−132] Hansen and Raun[127] have reported that imidazolyl propionic acid derivative **42** (EC_{50} = 5 nM) showed iv potency and efficacy (measured as maximal GH release) comparable to **1** and **8** in pentobarbitol anesthetized rats and conscious rats. However, **42** was nearly 20-fold less active than **1** and **8** after iv administration to conscious female pigs. These workers have suggested that species variations in the GHS receptors or in the regulation of GH release are possible explanations for these differences. Pharmacokinetic studies in rats by Johansen et al.[128] showed that **1**, **8**, and **42** have rather short terminal half-lives of 22 ± 1.4, 9 ± 0.3 and 11 ± 1 min, respectively, and high systemic clearance rates of 25 ± 3.5, 23 ± 1.2, and 39 ± 6.3 mL/min/kg, respectively. Biliary excretion was the major route of elimination of these peptides. Peptidyl secretagogue **42** was readily transported across the nasal epithelium with an intranasal bioavailability of 22−68%. An interesting finding by Johansen and co-workers is that analogue **43** (EC_{50} = 0.5 nM) with an aminomethylene substitution between the D-Phe[5] and Lys[6] positions displayed a 20-fold increase in potency in an anesthetized rat model when compared with **1**.[129]

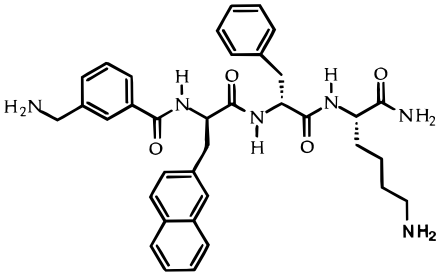


42

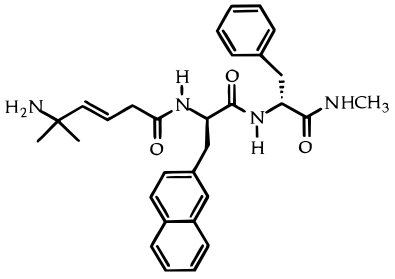


43

Peschke and co-workers used 3-aminomethylbenzoic acid as a dipeptide mimetic and incorporated it in peptides such as 44 (EC_{50} = 7 nM).[130] Related compounds bearing aminomethylthiophene-2-carboxylic acids have also been described.[130] The possible effects of these structural changes on oral potency or bioavailability have not yet been described. Di-, tri-, and tetrapeptides have been recently claimed in published patents as GH secretagogues.[131,132] Compound **45** is illustrative of one of the specifically claimed compounds.



44



45

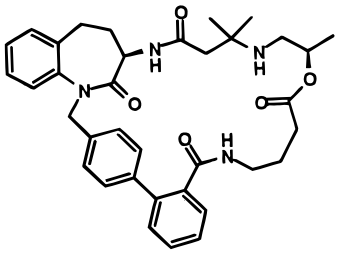
Pharmacophore Proposals

The preparation of [^{35}S]MK-0677[133] allowed identification of a saturable, high-affinity binding site for growth hormone secretagogues in porcine and rat anterior pituitary membranes.[93] Its relevance to GH secretion was supported by correlation of the rat receptor K_i values of **1**, **10**, **12**, and **20** with their relative potencies as releasers of GH in the rat pituitary cell assay. Importantly, competition binding experiments showed that **1** binds competitively with **20**.[93] These results provide some support for modeling comparisons of GHRP-6 with **20**, although receptor agonists as well as antagonists need not share identical binding sites on receptors.[134,135]

The subsequent cloning of the GH secretagogue receptor by Howard and co-workers[136] provided additional understanding on a structural level. A functionally important residue was shown to be Glu^{124} which is located in transmembrane helix 3 (TM3) in a position similar to that of Asp^{113} in the beta-adrenergic receptor[137] and to Asp^{122} in the somatostatin type 2 receptor.[138] The agonist ligands of these receptors contain an essential amino group which has been proposed to make an ionic interaction with these Asp residues near the top of TM3. To investigate the potential importance of Glu^{124} in the GH secretagogue receptor, this residue was replaced by Gln and the mutant receptor was expressed in HEK-293 cells which also contained the stably expressed calcium activated photoprotein aequorin. With this mutant receptor, [^{35}S]MK-0677 failed to give measurable binding up to 10 nM (higher concentrations were precluded by high background binding). As expected, 100 nM of **20** or of **1** increased Ca^{2+} concentrations in HEK-293 cells in which aequorin and wild-type receptor were expressed. However, at 100 nM they did not elevate Ca^{2+} significantly in cells transfected with the E124 → Q124 mutant. Assuming no conformational changes in the receptor, the data are consistent with but do not establish a common amine binding site near Glu^{124} for **1** and **20**.[139]

Schoen and co-workers[66b,140] used modeling techniques to investigate possible structural similarities of **1** and the benzolactam **12**. Overlays were generated of a folded conformer of **1** with low-energy conformers of **12**. Their preferred alignment placed the N-terminal amines close to one another, the benzolactam and D-Trp of **1** were similarly located and the tetrazole-bearing phenyl group was in the vicinity of the D-Phe^5 of **1**. DeVita and co-workers[141] have recently disclosed a potent macrocyclic benzolactam **46** (EC_{50} = 21 nM) in which the basic amine and the 2′-carboxamide group of the 2′-biphenyl unit are connected via a seven-atom ester linkage. The high activity of **46** supports a turn conformation in secretagogues when they are bound to the receptor.

Using this same model of **1**, an overlay with **20** was generated which aligned their N-terminal dipeptide fragments and placed the spiroindoline group of **20** in the vicinity of the Trp^4 of **1**. To test the implications of this alignment, Yang and co-workers[142] synthesized compounds which contained aromatic groups including indoles positioned to overlay with Trp^4. High activity was achieved when aromatic D-amino acids were substituted in place of the spiroindoline group in compounds



46

such as AIB-D-Trp-D-Trp-OEt and AIB-D-Trp-D-homoPhe-OEt. Their EC_{50} values of 6 and 3 nM, respectively, are lower than that of **1** (EC_{50} = 10 nM) in the rat pituitary cell assay. The success of this approach provides a striking, and to our knowledge a unique instance, in which a peptidomimetic was used to generate a simpler, more potent peptide. These peptides also illustrate a minimum GH secretagogue pharmacophore consisting of a basic amine and two aromatic residues.

As noted above, McDowell and co-workers[119] and Deghenghi and co-workers[125] also generated potent GH secretagogues which contain only three essential residues. McDowell et al. analyzed their potent cyclic peptide by NMR and molecular modeling.[119] Consensus alignment of minimum energy conformers of their peptide derivative **37** with the conformationally constrained peptide allowed them to propose a three-dimensional pharmacophore for GHRP activity consisting of a basic group and two aromatic rings presented on opposite faces of a central amide bond.

Design Considerations

Until recently the discovery of peptidomimetic agonists was a rarity; however, the synthesis of GH secretagogues and agonists for the C_{5a},[89] CCK-A,[143] bradykinin[144] receptors, and partial agonists of angiotensin receptor[145] highlights a change in this situation. It also appears that narrowly defined pharmacophores need not be required for agonist activity. For example, recent patent applications and publications describe secretagogues related to **20** which contain 4-heterocyclic piperidines,[146] 3,3-disubstituted piperidines,[116] 3,4-disubstituted piperidines,[147] 3,4-benzofused piperidines,[112] and 3,4-heterocyclic ring fused piperidines.[115] Also there is considerable latitude in the amine and aromatic components of active benzolactam and spiropiperidine analogues as noted above and as claimed in these patents.

The synthesis of **20** and the discovery of the benzolactam secretagogues illustrate the value of privileged structures in receptor ligand design. The recurring hydrophobic double-ring systems of Ariens and co-workers[81] were proposed to bind in "accessory binding sites". Thus compounds such as chlorpromazine could be considered to be biogenic amine antagonists since their tricyclic component bound in an accessory hydrophobic site and a complete biogenic amine pharmacophore is not expressed. Such an analysis may be applicable to spiropiperidine-containing antagonists. However, the spiroindanylpiperidine secretagogues are agonists. Quite possibly a peptidomimetic agonist need not present from its hydrophobic anchor the same pharmacophore as the peptide. However, **20** is a derivatized dipeptide

whose GH secretory activity was largely retained when an aromatic amino acid ester was substituted for the spiropiperidine group.[142] Given this interchangeability, the privileged structure may not be binding in an accessory site but, in fact, may contribute part of the tripeptide's minimal pharmacophore. This privileged structure approach was complementary to the structure-based approach of McDowell et al.,[119] and it is noteworthy that both methods arrived at similar tripeptide analogues.

Another aspect of the MK-0677 discovery, as noted by Patchett et al.,[90] was the presence in the lead of three modular units whose origins were in other secretagogues. Two of these units came from weak non-peptide leads and the third came from GHRP-6 itself. Presumably the high potency of the lead **18** resulted from affinities that were contributed by each modular unit enhanced by an energy term arising from their linkage. An elegant example of this design principle was described recently by Shuker and co-workers[148] in which compounds with affinities of 2 and 100 μM were linked together guided by NMR-derived distance constraints to afford a ligand for the FK506 binding protein with a $K_d = 19$ nM.

It is interesting that non-peptide **17** is simultaneously an agonist of the GHS receptor and an antagonist of the oxytocin receptor. Similarly, Hirschmann and co-workers[149] have synthesized β-D-glucose derivatives that are agonists of the somatostatin receptors and antagonists of the NK1 receptor. Potent peptidomimetic antagonists have not yet been reported of the GHS receptor. Perhaps some receptors are easier to inhibit than others. If so, it would be fascinating to understand such differences on a molecular level.

GH Secretagogues: Mechanism of Action of GHRP-6

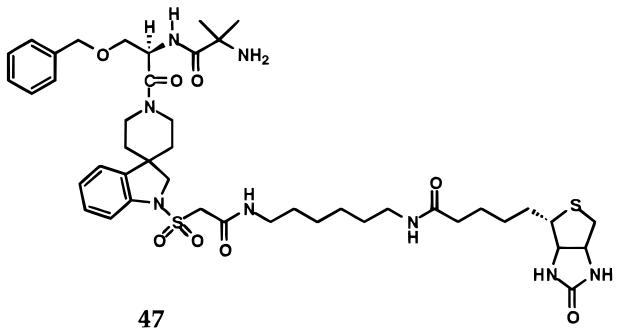
An understanding of the mechanism of action of **1** and how it might modify the actions of the two hypothalamic hormones, GHRH and somatostatin, that are involved in the control of GH secretion has been studied extensively. It was initially believed that the GHRPs were acting via the GHRH receptor since both **1** and GHRH released GH from the pituitary, their actions were blocked by SRIF-28 and both increased GH response following pretreatment with SRIF antiserum.[36] Although the endocrine factors involved in the GHRP-induced release of GH were not clearly understood, early mechanistic studies with the GHRPs and GHRH showed that the GHRPs release GH by a pathway independent of GHRH. GHRP-6 was shown to act directly on the somatotroph to cause GH release, to be a functional antagonist of somatostatin, and to potentiate GHRH through several mechanisms.[150] In contrast to GHRH, which increases cAMP in somatotrophs, **1** alone did not release intracellular cAMP, but when combined with GHRH, **1** potentiates cAMP production.[52] GHRP-6 was also shown to activate L-type Ca^{2+} channels and to depolarize somatotrophs by inhibiting K^+ channels.[150−153] GHRP-6 apparently transduces its signal through phospholipase C, resulting in production of IP_3 and diacylglycerol to cause redistribution of intracellular stores of Ca^{2+} and activation of protein kinase C.[154−158] These studies gave the first clear indication that **1** acted on a receptor distinct from that of the GHRH receptor and were consistent with the notion that the GHRP-6 receptor was G-protein coupled.

Characterization of a Receptor for the GH Secretagogues in the Pituitary Gland and Hypothalamus

Although GHRP-6 and its analogues were derived from met-enkephalin, the GH releasing activity of the GHRPs was not blocked by co-administration of naloxone.[159] Also, the peptidomimetics **12** and **20** lacked activity on different opiate receptor subtypes and in more than 50 different receptor assays.[66a,b, 90,92]

The receptor for MK-0677 was characterized with high specific activity [^{35}S]MK-0677 (800−1100 Ci/mmol). This radioligand was shown to bind with high affinity ($K_d = 140$ pM) and limited capacity ($B_{max} = 8$ fmol/mg of protein) to porcine pituitary membranes.[93,150] Such a low concentration of binding sites is remarkable and in rat pituitary membranes the concentration of binding sites is even lower (2 fmol/mg of protein). [^{35}S]MK-0677 binding was displaced by **12** and **13** and by the peptides **1** and **8**, but not by GHRH or somatostatin.[93] The K_i values in the binding assay correlate well with the EC_{50} values of GH release in the rat pituitary cell assay. Consistent with binding to a G-protein-coupled receptor [^{35}S]MK-0677 binding is dependent upon Mg^{2+} (5 mM), is displaced by GTP-γ-S (10 nM) but not by ATP-γ-S.[92,93] Compound L-692,429 (**12**) and GHRP-6 (**1**) are competitive inhibitors of MK-0677 binding[93] and GTP-γ-S is an allosteric inhibitor.[93] To explore further the specificity of [^{35}S]MK-0677 binding, met-enkephalin, gonadotrophin-releasing hormone (GnRH), TRH, galanin, gastrin-releasing peptide, substance P, melanocyte-stimulating hormone (MSH), isoproterenol, dopamine, bromocryptine, propanolol, and clonidine were evaluated at a concentration of 1 μM; none competed for [^{35}S]MK-0677 binding.[93]

To determine whether **20** was localized to somatotrophs, a biotinylated analogue of it, **47**, was prepared for immunofluorescence studies.[160] Biotinylated probe **47** was shown to be an excellent competitor for [^{35}S]MK-0677 binding ($IC_{50} = 0.2$ nM) and stimulated GH release with an EC_{50} of 2.5 nM. Rat pituitary cells were incubated with **47** for 3 min at 37 °C and treated with avidin-Texas Red. To detect GH, cells were then labeled with fluorescein-conjugated goat anti-rabbit IgG. This dual fluorophor labeling and confocal microscopy showed that binding of **47** was only observed on cells that contained GH.[161]



47

[^{35}S]MK-0677 also binds with high affinity ($K_d = 170$ pM) to membranes isolated from rat hypothalamus. The

concentration of binding sites (B_{max} = 8 fmol/mg of protein) is higher than that found in rat anterior pituitary membranes.[162] Binding was Mg^{2+} dependent and inhibited by nonhydrolyzable analogues of GTP such as GTP-γ-S and GMP−PNP.[162] Membranes isolated from liver, thalamus, cerebral cortex, medulla, pons, and posterior pituitary membranes were also assayed; however, high-affinity [^{35}S]MK-0677 binding was not detected, demonstrating the tissue specificity of MK-0677 binding to rat anterior pituitary and hypothalamus.[162] Binding to the hypothalamic membranes was highly selective for **1**, **8**, **12**, **13,** and **20**. Thus the hypothalamic receptor of MK-0677 has characteristics identical to those of the receptor identified in the anterior pituitary gland.

Signal Transduction Pathway Activated by Peptidomimetics

The peptidomimetics, in contrast to GHRH, do not increase intracellular cAMP levels in rat pituitary cells.[90,92] However, in combination with GHRH they were shown to amplify GHRH-induced increases in cAMP and potentiate GH release.[90] At doses of **20** that maximally stimulated GH release in vitro additional stimulation was not observed in the presence of maximally stimulating concentrations of **1** or **12**. Furthermore, a peptidyl antagonist of **1**, His-D-Trp-D-Lys-Ala-Trp-D-Phe-LysNH_2, blocked GH release by the secretagogues indicating, in agreement with [^{35}S]MK-0677 binding studies, that the effects of **1** and **12** are mediated through the same receptor. The synergistic effects of the peptidomimetics on the GHRH pathway were mimicked by phorbol myristic acetate (PMA), and incubation of rat pituitary cells for 24 h with PMA prior to treatment with **20** markedly attenuated the stimulation of GH release by **20**.[90] These results, and observations that **1**, **12**, and **20** cause increases in IP_3 turnover, increases in free intracellular Ca^{2+} and translocation of protein kinase C,[90,156−158,163] suggested that **12** and **20** were mimetics of **1** and that they interacted with a G-protein-coupled receptor that activates phospholipase C.[92,93] Elias and co-workers[120] have reported the mode of action of their small-molecule secretagogues **35** and **37** to be identical to **1** and distinct from that of GHRH. These results support the designation of **20**, **12**, **35**, and **37** and the other secretagogues as peptidomimetics in the GHRP pathway.

The role of Ca^{2+} signaling in the transduction pathway activated by **12** and **20** in somatotrophs was confirmed using fura-2 fluorescence ratio imaging.[66a,92] Nifedipine, omega-agatoxin IIIA, but not conotoxin prevented both increases in intracellular Ca^{2+} and GH release caused by the peptidomimetics suggesting activation of L-type Ca^{2+} channels.[164−166] Electrophysiology studies showed that **1** and the peptidomimetics blocked K^+ currents, resulting in depolarization of somatotrophs to enhance Ca^{2+} entry.[153,167,168] The depolarizing effects of **12** were confirmed using the membrane sensitive dye bisoxanol.[66a] Depolarizing agents such as the potassium channel blockers tetraethylammonium and 4-aminopyridine and the sodium channel agonist veratridine are weak GH secretagogues but were highly effective in amplifying GHRH-induced GH release. However, these agents did not affect either GHRP-6 or peptidomimetic-induced GH release. Thus, depolarization of pituitary cells partially explains the effects of **1**, **12**, and **20**. This property explains the observed functional antagonism of somatostatin since somatostatin causes hyperpolarization of somatotrophs by increasing potassium conductance.[169] Figure 2 summarizes current understanding of the signal transduction pathway mediated by the **1, 12**, and **20**. The receptor is G-protein-coupled, transducing its signal through the phospholipase C pathway, resulting in modulation of Ca^{2+} and K^+ channels. Although the GHRH receptor is also G-protein-coupled,[170] it activates a different signal transduction pathway to that of the MK-0677 receptor. Potentiation between the two transduction pathways is perhaps mediated by crosstalk between the $G_{\beta\gamma}$ subunits associated with the MK-0677 receptor and $G_{\alpha s}$ of the GHRH receptor complex.[171]

Cloning the GH Secretagogue Receptor (GHS-R)

An expression cloning approach for the MK-0677 receptor was utilized by Howard and co-workers[136] based on evidence that MK-0677 binds to a G-protein-coupled receptor signaling through phospholipase C. The nucleotide sequence of the pig MK-0677 receptor cDNA defined as GHS-R1a predicted a protein of 366 amino acids with seven transmembrane (7-TM) spanning domains, three intra- and extracellular loops, and a G-protein-coupled receptor (GPC-R) triplet signature sequence.[136] Subsequently the human cDNA was cloned. Genomic analysis by Southern blotting indicated that a single highly conserved gene was present in human, chimpanzee, bovine, rat, and mouse.[136] Amino acid sequence of the human Ia GHS-R with predicted membrane topology is shown in Figure 3. The closest identity of hGHS-R1a open reading frame with other hGPC-Rs was to the neurotensin (NT) and TRH receptors with ∼35% and 29% identity, respectively.[136] Interestingly, additional cDNAs clones were obtained from the pig and human libraries that encode a shorter form of the receptor of MK-0677 defined as GHS-R1b.[136] The receptor 1b cDNA predictably encodes a polypeptide of 289 amino acids that lacks transmembrane domains six and seven of the 1a receptor.[136] The truncated receptor is identical to the 1a receptor from the translation initiation codon to Leu-265 after which the cDNA is fused to a short contiguous reading frame of 24 amino acids followed by a translation stop codon. This short peptide sequence is highly conserved in the pig and human receptor genes.[136]

The pharmacological properties of MK-0677 receptors 1a and 1b were evaluated in functional assays using aequorin bioluminescence in *Xenopus* oocytes.[136] In addition, aequorin bioluminescent assays (in HEK293 cells) and [^{35}S]MK-0677 competition binding assays (with COS cells membranes) were performed. In *Xenopus* oocytes or COS-7 cells expressing 1a or 1b, aequorin bioluminescence was only induced by MK-0677 with the 1a clone. Expression of clone 1a but not 1b, in COS-7 cells resulted in high affinity binding of [^{35}S]MK-0677 to COS-7 membranes. Binding was inhibited by **1** and **8** but not by GHRH or somatostatin. *Thus the MK-0677 receptor should also be considered the receptor for the GHRP class of GH secretagogues (GHS-R).* No function has yet been assigned to receptor 1b. It may

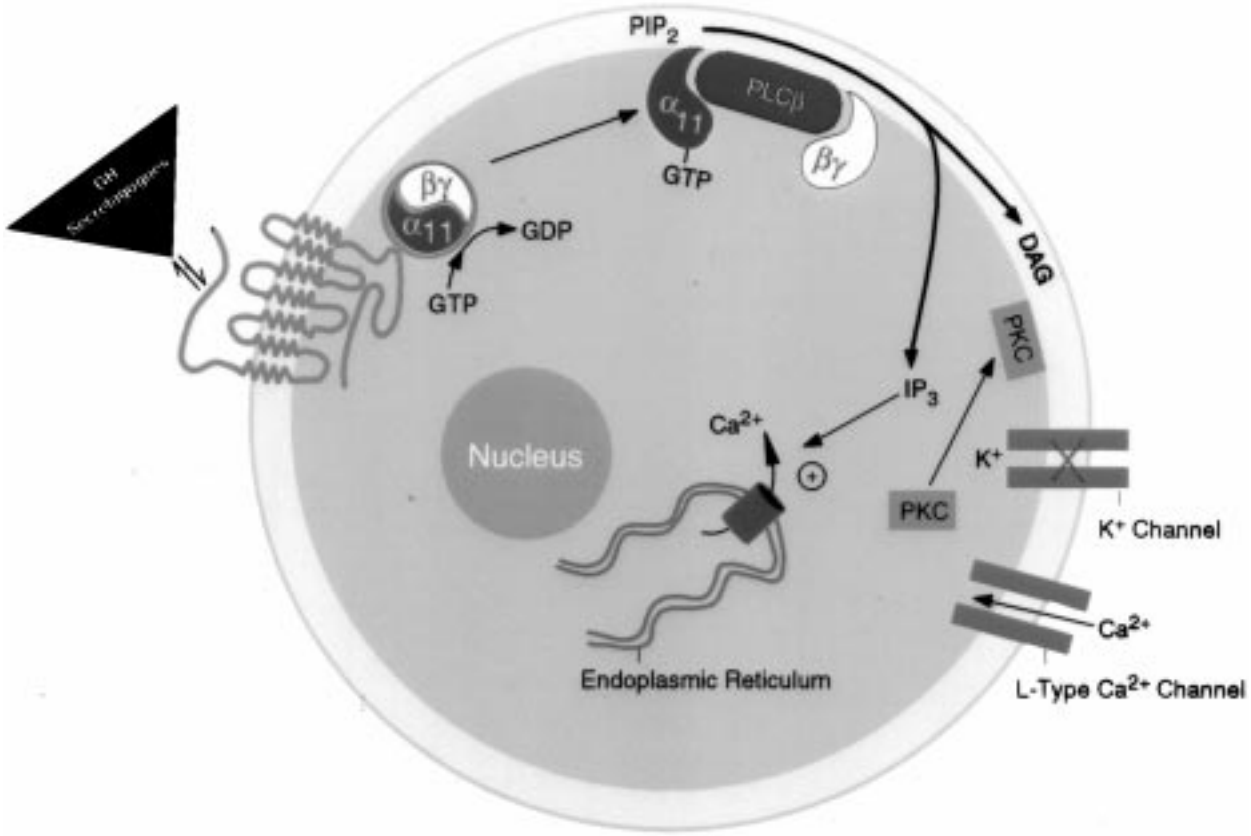


Figure 2. Signal transduction pathway activated by ligands that interact with the receptor defined with MK-0677. GH secretagogues, including MK-0677, apparently transduce their signal by interacting with a G-protein-coupled receptor that activates phospholipase C (PLC_β) resulting in production of inositol triphosphate (IP_3) and diacylglycerol (DAG). Increases in IP_3 cause redistribution of intracellular stores of Ca^{2+} from the endoplasmic reticulum and translocation of protein kinase C (PKC). Fluorescence ratio imaging studies show that peptidomimetics release GH from somatotrophs by activating L-type Ca^{2+} channels. Electrophysiology studies show that GH secretagogues block K^+ channels resulting in deoplarization of somatotrophs to enhance Ca^{2+} entry and facilitate GH secretion. Adapted from ref 150b and used with permission from the Endocrine Society.

play a regulatory role in modifying the function of a related G-protein-coupled receptor because it has been demonstrated that inactive truncated forms of a G-protein coupled receptor can be coexpressed to rescue function.[172]

A dendrogram of the GHS-R and other G-protein-coupled receptors suggests that it presents a new family of the NT-R, TRH-R branch of the phylogenetic tree (Figure 4).

The human GHS-R has been mapped by fluoresence in situ hybridization to band 3Q26.2.[173] Other genes whose deficiencies affect GH release do not map to this region. However, 3Q26.2 is close to a pre- and postnatal growth-deficiency map position reported for the Brachmann-de-Lange Syndrome.[174] Given the close proximity between the GHS-R gene and the presumed Brachmann-de-Lange location, it will be of great interest to determine whether these subjects respond to treatment with **20** and whether they have alterations in the gene encoding the GHS-R.

Peptidyl Secretagogue GHRP-6 and the Peptidomimetics in the CNS

Central effects of GHRP-6 (**1**) and the peptidomimetics are inferred by the demonstration that in guinea pigs the dose required to cause GH release is much (10−100-fold) lower when injected into the third ventricle than when administered peripherally.[175,176] The hypothalamic hormone GHRH is required for maximal effects of the GHS-R ligands. For example, their effects are reduced by prior infusion of GHRH antiserum[177] or by hypothalamic pituitary stalk transection.[178] In stalk transected animals a full response to the peptidomimetics is observed following replacement of GHRH.[178] It has been hypothesized that the GHS-R ligands stimulate the release of GHRH from neurons in the arcuate nucleus. Indeed, in sheep increases in GHRH levels have been measured in hypothalamic/pituitary portal vessels following GHRP-6 injection.[179,180]

Intravenous administration of GHRP-6 (**1**) and the peptidomimetics **12** and **13** into rats and mice increases Fos expression in the arcuate nucleus in a concentration-dependent manner consistent with their relative GH releasing activities.[175,181−183] In rats treated with **1**, approximately 25% of the neurons showing an increase in Fos immunoactivity contained GHRH and 51% expressed neuropeptide Y (NPY) mRNA.[107] The effect on NPY containing neurons might explain the increased feeding behavior observed in rats treated with **1**[184] as well as explain increased secretion of corticosteroids and ACTH.[185,186] It should be noted that other neurons in the brain may be activated that do not express c-*fos* and, of course, those expressing c-*fos* might be stimulated indirectly by activation or derepression.

In rat brain GHS-R mRNA is also expressed in the anterior hypothalamus, suprachiasmatic nucleus, su-

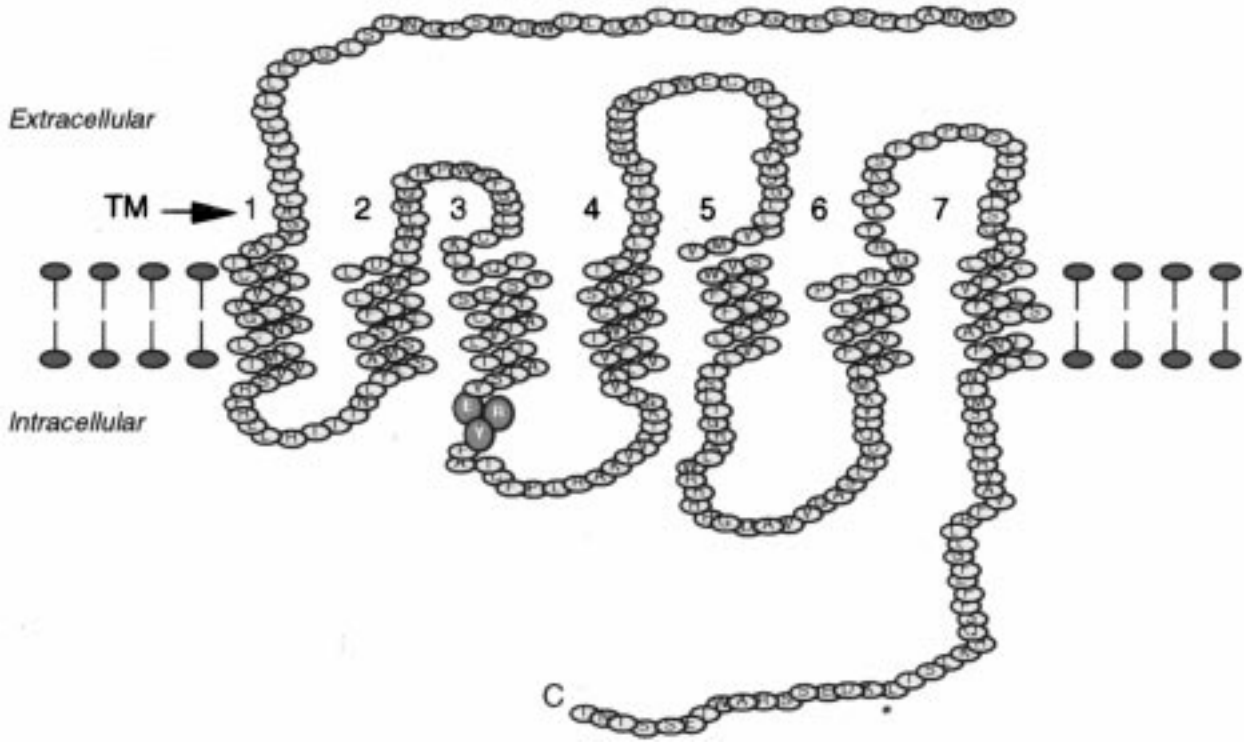


Figure 3. Amino acid sequence (1−366) of the human Ia GHSR with predicted membrane topology. TM denotes transmembrane spanning domains. The G-protein-coupled receptor signature sequence (ERY 142) is highlighted. N-Linked glycosylation sites and potential phosphorylation sites are omitted for clarity. Adapted from ref 150b and used with permission from the Endocrine Society.

praoptic nucleus, ventromedial hypothalamus, arcuate nucleus, dentate gyrus, tuberomamillary nucleus, pars compacta of substantia nigra, the ventral tegmental area, dorsal raphe nuclei, and median raphe nuclei.[187] The presence of GHS-R mRNA in regions of the brain outside the arcuate nucleus is intriguing. For example, the hippocampus is enriched with numerous neurotransmitter systems and has been implicated in learning and memory;[188,189] the substantia nigra and ventral tegmental areas are the main centers for dopaminergic cell bodies that are involved in many biological functions such as motor control and reinforcement behavior.[190] Dorsal and median raphe nuclei are centers for serotonergic neurons that project to different parts of the central nervous system implicated in a variety of functions including feeding, nociception and affective behaviors.[191] Clearly, the significance of the GHS-R in these brain regions, that are not obviously involved in GH release, will be the focus of future studies.

Regulation of Pulsatile GH Release

As discussed above GH secretion is pulsatile in all species studied.[1,193−195] Episodic secretion of GH appears to have biological importance because in deficient animals GH replacement is more effective when given in a pulsatile manner.[196] GH pulses are dependent on the release of GHRH[197] and somatostatin; secretion of the latter is increased during GH troughs.[198−200] When GHRH, **1**, or the peptidomimetics are constantly infused into humans, pulsatile GH release is amplified and sustained.[26,57,58,74] By contrast, administration of somatostatin or GH suppresses endogenous GH pulsatility.[201−203] The evidence for GH pulsatility being controlled at the hypothalamic level is compelling. Hypothalamic/pituitary stalk disconnection results in loss of normal GH pulsatility in sheep.[204]

A model for GH pulsatility that has been advanced by Smith and co-workers[92,150b] hypothesizes that GH sustains its own pulsatility through the coupling of three biological oscillators, somatostatin, GHRH, and the presumed natural ligand for the GHS-R. When ligands for the GHS-R are administered exogenously they reset the coupled oscillators by interrupting endogenous somatostatin tone, thus relieving repression on GHRH neurons. GHRH synergizes with the GHS-R ligand to cause GH release from the pituitary gland and then GH feeds back negatively on the hypothalamus to entrain a new cycle by increasing somatostatin tone on GHRH containing neurons, and perhaps by inhibiting neurons that release the natural ligand for the GHS receptor. This hypothesis is consistent with electrophysiology recordings from cells in a hypothalamic slice preparation showing that increases in activity of arcuate neurons caused by administration of **1** can be blocked by exogenous application of somatostatin.[205] Moreover, recent studies have shown that activation of arcuate neurons by **20** can be prevented by prior treatment with GH. The inhibitory effect of GH is apparently mediated by release of somatostatin from the periventricular nucleus and its activity on somatostatin subtype-2 receptors on arcuate neurons.[150b,206]

Future Directions

The GHRPs and their peptidomimetics present a novel approach for supplementing a key anabolic hormone, GH, whose levels decline with aging. Unlike GH replacement therapy which overrides normal physiol-

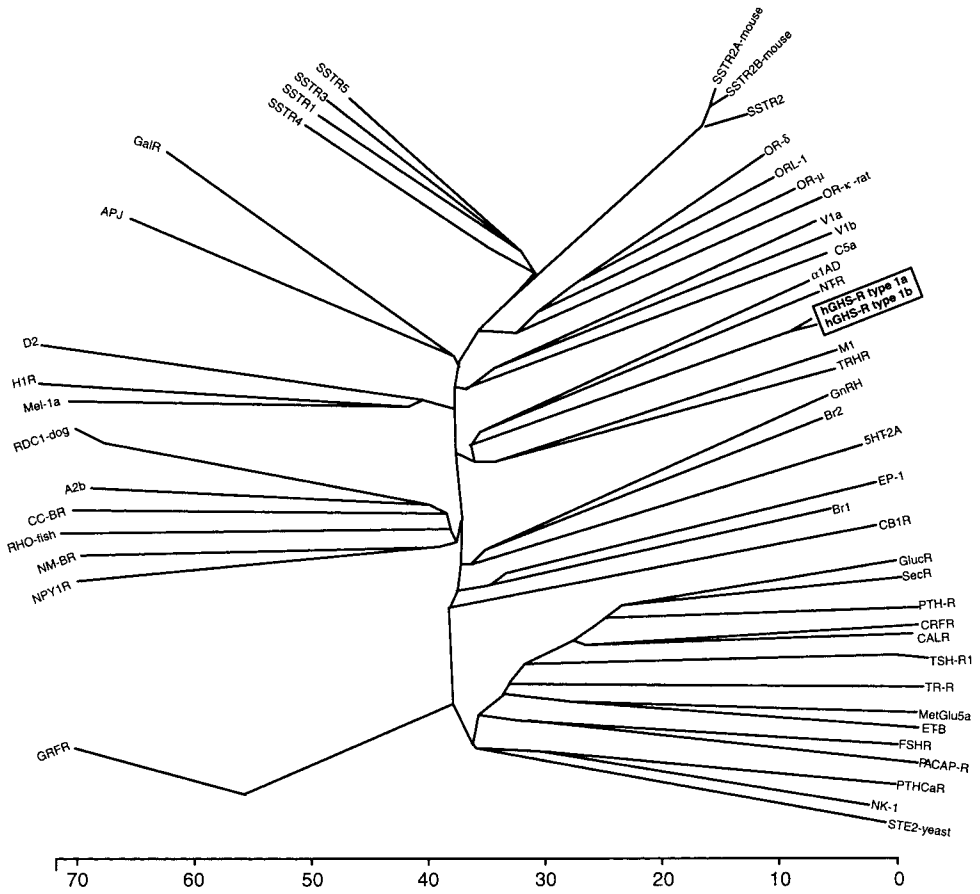


Figure 4. Dendrogram of the GH secretagogue receptor and other G-protein-coupled receptors. Database searches (Genbank 92, EMBL 43, Swiss-Prot 31, PIR 45, dEST (Gbest 92), Prosite 12), sequence alignments, and analysis of the GHS-R nucleotide and protein sequences were carried out using the GCG Sequence Analysis Software Package (Madison, WI; pileup, peptide structure and motif programs), FASTA and BLAST search programs, the PC/Gene software suite from Intelligenetics (San Francisco, CA; protein analysis programs), and Lasergene software (DNA Star, Madison, WI). The amino acid sequence of representative members (51 sequences) for all known classes (families I−IV and pheromone) of GPC-Rs were used to construct the dendrogram using the clustal method (PAM-250, gap, and length penalty = 10). The length of each pair of branches represents the distance between sequence pairs. The scale *below* the tree measures the distance between sequences. Units indicate the percent of substitution events. All receptor sequences aligned to human GHS-R type Ia and type 1b cDNAs were human unless otherwise noted, and assession numbers are for the SwissProt database, GenBank database (designated with "G"), or PIR database ("P"): 5HT-2A, serotonin, P28223; RDC-1, orphan receptor, A39714; somatostatin, A47457; V1a, vasopressin, A53046; V1b, vasopressin, A55089; A2b, adenosine, P29275; M1, muscarinic, P11229; APJ, orphan, P11229; C5a, chemotactic, P21730; CASR, extracellular calcium-sensing, P41180; CB1, cannabinoid, P21554; CRF, corticotropin-releasing factor, P34998; FSHR, FSH, P23945; CC-BR, gastrin/cholecystokinin type B, P32239; GRFR, GH-releasing factor, Q02643; GnRH, gonadotropin-releasing hormone, P30968; CALR, calcitonin, X69920 (G); GlucR, glucagon, U03469 (G); OR-d, opioid, U10504 (G); Mel-1a, melatonin, U14108 (G); SecR, secretin, U20178 (G); PTHCaR, parathyroid cell calcium-sensing, U20759 (G); Br1, bradykinin, U22346 (G); a1AD, α-1 adrenergic, L31772 (G); GalR, galanin, L34339 (G); H1R, histamine, D14436 (G); TRHR, TRH, D16845 (G); MetGlu5a, metabotropic glutamate, JC2132 (P); a1AD, α-1 adrenergic, JC2331 (P); Br2, bradykinin, JH0712 (P); NK-1, substance P, P25103; NM-BR, neuromedin-B, P28336; NT-R, neurotensin, P30989; NPY1R, neuropeptide Y, P25929; OR-m, opioid, P35372; RHO-fish, rhodopsin, P35356; EP-1, prostaglandin E1, P34995; PTH-R, parathyroid hormone, Q03431; OR-k, opioid-rat, S39015 (P); ORL-1, opioid-orphanin FQ, S43087 (P); ET-B, endothelin, S44866 (G); D2, dopamine, S62137 (G); STE2-yeast, pheromone a factor, P06842; SSTR2, SSTR3, SSTR5, SSTR2A-mouse, SSTR2B, SSTR1, somatostatin, P30874, P32745, P35346, P30875, P30934, P30872, respectively; PACAP-R, pituitary adenylate cyclase-activating peptide, D17516 (G); TR-R, thrombin, P25116; TSH-R1, TSH, S49816 (G). Adapted from ref 150b and used with permission from the Endocrine Society.

ogy, iv infusion of the GHRPs and the short-acting benzolactam **12** or chronic oral administration of long-acting **20** amplifies the pulsatile GH secretory pathway. Considerable progress has been made within the past several years in understanding the regulation of GH secretions and the GH secretagogues present an additional tool in these studies. The pharmacological effects of these secretagogues have been well-characterized across many species, including humans. Specific receptors for GH secretagogues are present in the anterior pituitary gland and the hypothalamus. That this pathway of GH regulation is highly conserved across species suggests that GH secretagogues mimic an unidentified hormone for this receptor that regulates pulsatile GH secretion in concert with GHRH and somatostatin. Nonetheless, there may be species differences and receptor subtypes involved in the GHRP action. In this context it is relevant that Chen and coworkers[207] have reported that **8** elevates cAMP in ovine pituitary cells although GHRP-6 does not. However, peptidyl secretagogue **8** does not cause cAMP accumulation in rat pituitary cells. These data suggest species

differences in the response of pituitary somatotrophs to the GHRPs and may be indicative of GH secretagogue receptor subtypes.

Perhaps normal aging is associated with reduced secretion of the putative endogenous ligand of the GH secretagogue receptor. If this speculation is true, animals lacking the GH secretagogue receptor or its natural ligand would have growth-retarded phenotypes because a factor which normally synergizes with GHRH would be lacking. Since somatostatin is also a key mediator in inducing an episodic profile of GH secretion, it also follows that mice lacking a specific subtype(s) of somatostatin receptor may not release GH in a pulsatile manner. To test these predictions it will be interesting to produce GHS-R −/− and somatostatin receptor −/− mice. Furthermore, the expression of the receptor in specific regions of the brain in addition to the pituitary and hypothalamus glands implies the possibility of CNS actions which are still to be characterized.

In the clinic the GHRPs and peptidomimetics present a number of therapeutic opportunities. The GH secretagogues may offer an alternative to injectable rGH with the potential advantages of oral dosing and a more physiologic GH profile. In some individuals, the physiologic GH profile may contribute to better tolerability than that achieved by injectable recombinant GH. However, in contrast to GH, secretagogues are subject to physiologic feedback mechanisms. Thus, the sustained supraphysiologic GH and IGF-1 levels that can be attained with GH are not possible with secretagogues. For some conditions associated with relative GH resistance, the efficacy of secretagogues may therefore be limited. Another, nonendocrine medicinal chemistry analogy are the SSRI's such as Prozac, which in effect are indirect 5-HT agonists with an upper limit on serotonergic drive that can be elicited.

It is also unclear at this time what type of pharmacodynamic profile will result in optimal efficacy. It should be noted that chronic administration of short-acting peptidyl secretagogues such as **8** and **10** has been shown to stimulate GH release without any apparent desensitization of the GH response and without causing a significant increase in serum IGF-1 levels. Furthermore, improved linear growth of GH-deficient children has been reported without IGF-1 elevation. In contrast, once-daily administration of the long-acting MK-0677 causes marked down-regulation of GH levels and a significant increase in serum IGF-1.

To date, little long-term clinical safety and efficacy data are available for GH secretagogues. As the GH secretagogues demonstrate some lack of specificity in their stimulation of GH secretion, chronic use of these compounds will, no doubt, include monitoring their effects on cortisol, prolactin and glucose. It is also interesting that **10** has been shown to stimulate food intake in rats.[208] The feeding behavior does not appear to be mechanism-based since Locatelli and co-workers[208] were able to identify related compounds that induced feeding but did not release GH. The molecular basis for the feeding behavior is not known at this time. Finally, GH secretagogues also have the potential to produce some of the side effects which have been reported with rhGH.

Some cases of GH deficiency in children are thought to result from hypothalamic or neurosecretory defects. The GH secretagogues may be useful in helping to identify the etiology of GH deficiency, as the response to GHRH, GHRPs, or the combination may vary with the site of the secretory defect.

Growth hormone deficient children are candidates for treatment with GH secretagogues. However, published reports suggest that only a subset of these children will respond; these are likely children with an intact hypothalamic-pituitary connection and a functioning pituitary. To date, growth response to **8** in GH-deficient children has been modest; this may be a function of the specific individuals selected for the trials, a suboptimal dosing regimen, or inherently limited efficacy compared to that of GH. It is possible that different pharmacodynamic responses with longer-acting compounds (e.g., MK-0677) which elevate IGF-1 may result in a different efficacy profile.

Recombinant human GH is approved for treatment of growth failure in girls with Turner's Syndrome and in children with renal failure. Adults with chronic renal failure secrete GH in response to **10**. Since girls with Turner's Syndrome have an intact hypothalamic-pituitary axis, it is likely that the secretagogues will stimulate GH secretion in both patient populations. However, it is unknown whether sufficiently high levels of GH can be stimulated in order to demonstrate clinical efficacy. Pilot studies would be needed.

The use of GH has been explored in several adult patient populations. Classical GH deficiency in adults is now an approved indication for GH in several countries. However, as adults with this condition often do not have an intact hypothalamic-pituitary axis, some of these patients may not respond to secretagogues. Preliminary studies would be required to determine the number of potential "responders".

A number of other conditions which potentially could be treated with GH secretagogues include a variety of catabolic conditions, including postoperative recovery, malnutrition from a variety of causes, muscle wasting due to chronic exposure to corticosteroids, as a result of Cushing's syndrome or through iatrogenic causes, osteoporosis and frailty associated with age-related hyposomatomedinemia. In the treatment of frail elderly, if clinical efficacy can be demonstrated, GH secretagogues have the potential to cause a dextral shift in "age-function curve". In an aging population, the social impact of such changes could be significant. While scientific and medical challenges to the development of these interesting compounds remain, their therapeutic potential is tantalizing and significant.

Summary

Since the description of GHRP-6 by Bowers and coworkers in 1984, considerable progress has been made in developing small molecules that mimic the actions of **1** and in understanding the role of secretagogues in augmenting pulsatile GH secretion. GH secretagogues present a number of opportunities for therapeutic intervention. The design of these peptidomimetics is interesting since they are receptor agonists. Furthermore, their discovery was achieved without knowledge of the molecular target and without structural informa-

tion concerning the endogenous hormone that they presumably mimic. Only recently with the aid of peptidomimetic ligands has the receptor for the secretagogues been identified, characterized, and cloned and it should be useful in the search for the putative natural ligand which has still not been identified. Therefore, the discovery of small molecule GH secretagogues is an example of "retro drug discovery" or "reverse pharmacology".

Acknowledgment. We thank Dr. Matthew J. Wyvratt for helpful suggestions and Ms. Janice Shamus for preparation of the manuscript.

Biographies

Mark A. Bach completed his Ph.D. degree at the University of Chicago and received his M.D. degree from Baylor College of Medicine. Following training in Pediatrics at Baylor, and in Pediatric Endocrinology at the National Institutes of Health, he joined Merck Research Laboratories in Rahway, NJ, in 1993 where he is currently Director, Clinical Research, Endocrinology and Metabolism.

M. Gail Murphy received her M.D. degree from Jefferson Medical College. She is presently Senior Director, Clinical Pharmacology, at Merck Research Laboratories.

Ravi P. Nargund obtained his doctorate in chemistry from Indiana University under the guidance of Professor Paul A. Grieco. Following postdoctoral research in the laboratories of Professor Gilbert Stork at Columbia University, he joined Merck Research Laboratories in Rahway, NJ, in 1990 where he is currently Senior Research Fellow, Medicinal Chemistry.

Arthur A. Patchett completed his Ph.D. degree in chemistry at Harvard with Professor R. B. Woodward. Following postdoctoral research at the National Institutes of Health with Dr. Bernhard Witkop, he joined the Merck Research Laboratories in 1957 where he is currently Vice President, Medicinal Chemistry.

Roy G. Smith received his Ph.D. degree in Chemistry at London University, U.K. in 1970 with Professor Ray Bonnett. Following postdoctoral work at Vanderbilt University he joined the faculty of the Cell Biology Department at Baylor College of Medicine in Houston where he worked on the molecular mechanisms of steroid hormone action. After spending 15 years at Baylor, he joined Merck Research Laboratories in 1987 as a Senior Director. He is currently Vice President of Biochemistry and Physiology.

References

(1) For a comprehensive review on the actions of GH and its regulation see: (a) Rosen, T.; Johannsson, G.; Johansson, J.-O.; Bengtsson, B.-A. Consequences of growth hormone deficiency in adults and the benefits and risks of recombinant human growth hormone treatment. *Horm. Res.* **1995**, *43*, 93−99. (b) Strobl, J. S.; Thomas, M. J. Human growth hormone. *Pharmacol. Rev.* **1993**, *46*, 1−34.

(2) Rudling, M.; Norstedt, G.; Olivecrona, H.; Reihner, E.; Gustafsson, J. A.; Angelin, B. Importance of growth hormone for the induction of hepatic low-density lipoprotein receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 6983−6987.

(3) Rosen, T.; Bengtsson, B.-A. Premature death due to mortality due to cardiovascular disease in hypopituitarism. *Lancet* **1990**, *336*, 285−288.

(4) Gelato, M. C. Growth hormone-insulin like growth factor I and immune function. *Trends Endocrinol. Metab.* **1993**, *4*, 106−110 and references therein.

(5) Ho, K. Y.; Evans, W. S.; Blizzard, R. M.; Veldhius, J. D.; Merriam, G. R.; Samojlik, E.; Furlanetto, R.; Rogol, A. D.; Kaiser, D. L.; Thorner, M. O. Effects of sex and age on the 24-hour profile of growth hormone secretion in man: Importance of endogenous estradiol concentrations. *J. Clin. Endocrinol. Metab.* **1987**, *64*, 51−58.

(6) Zadik, Z.; Chalew, S. A.; McCarter, R. J. Jr.; Meistas, M.; Kowarski, A. A. The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. *J. Clin. Endocrinol. Metab.* **1985**, *60*, 513−516.

(7) Juul, A.; Bang, P.; Hertel, N. T.; Main, K.; Dalgaard, P.; Jorgensen, K.; Muller, J.; Hall, K.; Skakkebaek, N. E. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J. Clin. Endocrinol. Metab.* **1994**, *78*, 744−752.

(8) Schwander, J. C.; Hauri, C.; Zapf, J.; Froesch, E. F. Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. *Endocrinology* **1983**, *113*, 297.

(9) Thompson, L. V. Effects of age and training on skeletal-muscle physiology and performance. *Phys. Ther.* **1994**, *74*, 71−81.

(10) (a) Rudman, D.; Feller, A. G.; Hoskote, N. S.; Gergans, G. A.; Lalitha, P. Y.; Goldberg, A. F.; Schlenker, R. A.; Cohn, L.; Rudman, I. W.; Mattson, D. E. Effects of human growth hormone in men over 60 years old. *N. Engl. J. Med.* **1990**, *323*, 1−8. (b) Rudman, D.; Feller, A. G.; Cohn, L.; Shetty, K. R.; Rudman, I. W.; Draper, M. W. Effects of human growth hormone on body composition in elderly men. *Horm. Res.* **1991**, *36*, Suppl. 1, 73−81.

(11) Welle, S.; Thornton, C.; Statt, M.; McHenry, B. Growth hormone increases muscle mass and strength but does not rejuvenate myofibrillar protein synthesis in healthy subjects over 60 years old. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 3239- 3243.

(12) Papadakis, M. A.; Grady, D.; Black, D.; Tierney, M. J.; Gooding, G. A.; Schambelan, M.; Grunfeld, C. Growth hormone replacement in healthy older men improves body composition but not functional ability. *Ann. Intern. Med.* **1996**, *124*, 708−716.

(13) Yarasheski, K. E.; Zachwieja, J. J.; Campbell, J. A.; Bier, D. M. Effect of growth hormone and resistance exercise on muscle growth and strength in older men. *Am. J. Physiol.* **1995**, *268*, E268−76.

(14) (a) Chung, C. S.; Etherton, T. D.; Wiggins, J. P. Stimulation of swine growth by porcine growth hormone. *J. Anim. Sci.* **1985**, *60* (Suppl. 1), 118−130. (b) Moseley, W. M.; Paulissen, J. B.; Taylor, V. N.; Munyakazi, L.; Goodwin, M. C.; Alaniz, G. R.; Clafin, G. H. Recombinant bovine somatotrophin (rbST) improves growth performance in finishing beef steers. *J. Anim. Sci.* **1991**, *69* (Suppl. 1), 313.

(15) Takahashi, Y.; Kipnis, M.; Daughaday, W. H. Growth hormone secretion during sleep. *J. Clin. Invest.* **1968**, *47*, 2079−2090.

(16) Bunt, J. C.; Boileau, R. A.; Bahr, J. M.; Nelson, R. A. Sex and training differences in human growth hormone levels during prolonged exercise. *J. Appl. Physiol.* **1986**, *61*, 1796−1801.

(17) Zachmann, M. Interrelations between growth hormone and sex hormones: physiology and therapeutic consequences. *Horm. Res.* **1992**, *38* (Suppl. 1), 1−8.

(18) (a) Leung, D. W.; Spencer, S. A.; Cachianes, G.; Hammonds, R. G.; Collins, C.; Henzel, W. J.; Barnard, R.; Waters, M. J.; Wood, W. I. Growth hormone receptor and serum binding protein purification, cloning and expression. *Nature* **1987**, *330*, 537−543. (b) Wallis, M. Growth hormone receptor cloned. *Nature* **1987**, *330*, 521−522.

(19) Bermann, M.; Jaffe, C. A.; Tsai, W.; DeMott-Friberg, R.; Barkan, A. L.; Negative feedback regulation of pulsatile growth hormone secretion by insulin-like growth factor-1. *J. Clin. Invest.* **1994**, *94*, 138−145.

(20) (a) Guillemin, R.; Brazeau, P.; Bohlen, P.; Esch, F.; Ling, N.; Wehrenberg, W. B. Growth hormone releasing factor from a human pancreatic tumor that caused acromegaly. *Science* **1982**, *218*, 585−589. (b) Rivier, J.; Speiss, J.; Thorner, M.; Vale, W. Characterization of a growth hormone-releasing factor from a pancreatic islet tumor. *Nature* **1982**, *300*, 276−278.

(21) (a) Mayo, K. E. Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Mol. Endocrinol.* **1992**, *6*, 1734−1744. (b) Gaylinn, B. D.; Harrison, J. K.; Zysk, J. R.; Lyons, C. E.; Lynch, K. R.; Thorner, M. O. Molecular cloning and expression of a human anterior pituitary receptor for growth hormone-releasing hormone. *Mol. Endocrinol.* **1993**, *7*, 77−84.

(22) (a) Ling, N.; Baird, A.; Wehrenberg, W. B.; Ueno, N.; Munegumi, T.; Brazeau P. Synthesis and activity of C-terminal deleted analogs of human growth hormone-releasing factor. *Biochem. Biophys. Res. Commun.* **1984**, *123*, 854−861. (b) Campbell, R. M.; Lee, Y.; Rivier, J.; Heimer, E. P.; Felix, A. M.; Mowles, T. F. GRF analogs and fragments: correlation between receptor binding, activity and structure. *Peptides* **1991**, *12*, 569−574.

(23) Labrie, F.; Gagne, B.; Lefrevre, G. Growth hormone-releasing factor stimulates adenylate cyclase activity in the anterior pituitary gland. *Life Sci.* **1983**, *33*, 2229.

(24) (a) Low, L. C. K. Growth hormone-releasing hormone: clinical studies and therapeutic aspects. *Neuroendocrinology* **1991**, *53* (Suppl. 1), 37. (b) Thorner. M. O. On the discovery of growth hormone-releasing hormone. *Acta Pediatr.* **1993**, *388* (Suppl.), 2.

(25) Kubiak, T. M.; Friedman, A. R.; Moseley, W. M. Synthetic analogues of growth hormone releasing factor (GHRF) with improved pharmaceutical properties. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 31−52.

(26) Vance, M. L.; Kaiser, D. L.; Evans, W. S.; Furlanetto, R.; Vale, W.; Rivier, J.; Thorner, M. O. Pulsatile growth hormone secretion in normal men during a 24-hour continuous infusion of growth hormone-releasing factor (1-40): evidence for intermittent somatostatin secretion. *J. Clin. Invest.* **1985**, *75*, 1584−1590.

(27) Brain, C. E.; Hindmarsh, P. C.; Brook, C. G. D. Continuous subcutaneous GHRH (1-29)NH_2 promotes growth over one year in short, slow growing children. *Clin. Endocrinol.* **1990**, *32*, 153−163.

(28) Frohman, L. A.; Downs, T. R.; Williams, T. C.; Heimer, E. P.; Pan, Y. CE.; Felix, A. M. Rapid enzymatic degradation of growth hormone-releasing hormone by plasma in vitro and in vivo to a biologically inactive product cleaved at the NH_2 terminus. *J. Clin. Invest.* **1986**, *78*, 906−913.

(29) Smith, D. P.; Heiman, L. M.; Wagner, J. F.; Jackson, R. L.; Bimm, R. A.; Hsiung, H. M. Production and biological activity of hybrid growth hormone-releasing hormone propeptides. *Biotechnology* **1992**, *10*, 315−319.

(30) Campbell, R. M.; Bongers, J.; Felix, A. M. Rational design, synthesis, and biological evaluation of novel growth hormone-releasing factor (GRF) analogs. *Biopolymers* **1995**, *37*, 67−88.

(31) Kubiak, T. M.; Friedman, A. R.; Martin, R. A.; Ichhpurani, A. K.; Alaniz, G. R.; Clafin, W. H.; et al. Position 2 and position2/Ala15-substituted analogs of bovine growth hormone-releasing factor (bGRF) with enhanced metabolic stability and improved in vivo bioactivity. *J. Med. Chem.* **1993**, *36*, 888−897.

(32) For a comprehensive review on the discovery of the GHRPs, see: Bowers, C. Y. Xenobiotic growth hormone secretagogues: Growth hormone releasing peptides. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 9−28.

(33) Bowers, C. Y.; Chang, J.; Momany, F.; Folkers, K. Effect of the enkephalins and enkephalin analogs on release of pituitary hormones in vitro. In *Molecular Endocrinology*; MacInytre, I., Ed.; Elsevier/North-Holland: Amsterdam, pp 287−292.

(34) Bowers, C. Y.; Momany, F.; Reynolds, G. A.; Chang, D.; Hong, A.; Chang, K. Structure-activity relationships of a synthetic pentapeptide that specifically releases growth hormone in vitro. *Endocrinology* **1981**, *106*, 663−667.

(35) Momany, F. A.; Bowers, C. Y.; Reynolds, G. A.; Chang, D.; Hong, A.; Newlander, K. Design, synthesis, and biological activity of peptides which release growth hormone in vitro. *Endocrinology* **1981**, *108*, 31−39.

(36) Bowers, C. Y.; Momany, F. A.; Reynolds, G. A.; Hong, A. On the in vitro and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* **1984**, *114*, 1537−1545.

(37) Momany, F. A.; Bowers, C. Y.; Reynolds, G. A.; Hong, A.; Newlander, K. Conformational energy studies and in vitro and in vivo activity data on growth-hormone releasing peptides. *Endocrinology* **1984**, *114*, 1531−1536.

(38) The reported EC_{50} values correspond to the intrinsic GH secretory potency in the rat primary pituitary cell culture assay. EC_{50} is the concentration of compound required for half-maximal release of GH. For a detailed description of the assay, see: (a) Sartor, O.; Bowers, C. Y.; Chang, D. Parallel studies of His-D-Trp-Ala-Trp-DPhe-Lys-NH_2 and human pancreatic growth hormone-relasing factor-44-NH_2 in rat primary pituitary cell monolayer culture. *Endocrinology* **1984**, *116*, 952−957. (b) Badger, T. M.; Millard, W. J.; McCormick, G. F.; Bowers, C. Y.; Martin, J. B. The effects of growth hormone (GH) releasing peptides on GH secretion in perifused pituitary cells of adult male rats. *Endocrinology* **1984**, *115*, 1432−1438.

(39) Momany, F. A.; Bowers, C. Y. Computer-assisted modeling of xenobiotic growth hormone secretagogues. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 73−84.

(40) Walker, R. F.; Codd, E. E.; Barone, F. C.; Nelson, A. H.; Goodwin, T.; Campbell, S. A. Oral activity of the growth hormone releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 in rats, dogs and monkeys. *Life Sci.* **1990**, *47*, 29−36.

(41) Doscher, M. E.; Baker, P. K.; Kraft, L. A.; Ricks, C. A. Effect of a synthetic growth hormone releasing peptide (BI 679) and growth hormone-releasing factor (GRF) on serum growth hormone levels in barrows. *J. Anim. Sci.* **1984**, *59* (Suppl.), 109.

(42) Kraft, L. A.; Baker, P. K.; Doscher, M. E.; Ricks, C. A. Effects of a synthetic growth hormone releasing peptide on serum growth hormone levels in steers. *J. Anim. Sci.* **1984**, *59* (Suppl.), 218.

(43) Hickey, G.; Jacks, T.; Judith, F.; Taylor, J.; Schoen, W. R.; Krupa, D.; Cunningham, P.; Clark, J.; Smith, R. G. Efficacy and specificity of L-692,429, a novel nonpeptidyl growth hormone secretagogue, in beagles. *Endocrinology* **1994**, *134*, 695−701.

(44) Bowers, C. Y.; Reynolds, G. A.; Durham, D.; Barrera, C. M.; Pezzoli, S. S.; Thorner, M. O. Growth hormone (GH) releasing peptide stimulates GH secretion in normal men and acts synergistically with GH releasing hormone. *Endocrinology* **1990**, *70*, 975−982.

(45) Baker, P. K.; Conner, S. D.; Doscher, M. E.; Kraft, L. A.; Ricks, C. A. Use of a synthetic growth hormone releasing peptide to increase rate of body weight gain in rats. *J. Anim. Sci.* **1984**, *59* (Suppl.), 220.

(46) Croom, Jr J. W.; Leonard, E. S.; Baker, P. K.; Kraft, L. A.; Ricks, C. A. The effects of synthetic growth hormone releasing peptide BI 679 on serum growth hormone levels and milk production in lactating dairy cattle. *J. Anim. Sci.* **1984**, *59* (Suppl.), 109.

(47) Bowers, C. Y. In *Molecular And Clinical Advances In Pituitary Disorders*; Melmed, S., Ed.; Endocrine Research and Education: Los Angeles, CA, 1993; pp 153−157.

(48) (a) Bowers, C. Y. GH releasing peptides − structure and kinetics. *J. Pediatr. Endocrinol.* **1993**, *6*, 21−31. (b) Akman, A. S.; Girard, M.; O'Brien, L. F.; Ho, A. K.; Chik, C. L. Mechanisms of actions of a 2nd generation growth hormone-releasing peptide (Ala-His-D-beta-Nal-Ala-Trp-D-Phe-Lys-NH_2) in rat anterior-pituitary cells. *Endocrinology* **1993**, *132*, 1286−1291.

(49) Deghenghi. R.; Cananzi, M. M.; Torsello, A.; Battisti, C.; Muller, E. E.; Locatelli, V. GH-Releasing activity of hexarelin, a new growth hormone releasing peptide, in infant and adult rats. *Life Sci.* **1994**, *54*, 1321−1328.

(50) Reynolds, G. A.; Bowers, C. Y. *In vitro* studies with GH releasing peptides. 69th Annual Meeting of The Endocrine Society, Indianapolis, IN, 1987; p 49 (Abstract).

(51) Bowers, C. Y.; Sartor, A. O.; Reynolds, G. A.; Badger, T. M. On the actions of growth hormone releasing peptide, GHRP. *Endocrinology* **1991**, *128*, 2027−2035.

(52) Cheng, K.; Chan, W. W.-S.; Barreto, A.; Convey, E. M.; Smith, R. G. The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 on GHRF-stimulated GH release and intracellular adenosine 3′5′-monophosphate accumulation in rat primary pituitary cell culture. *Endocrinology* **1989**, *124*, 2791−2798.

(53) Fairhall, K. M.; Mynett, A.; Smith, R. G.; Robinson, I. C. A. F. Consistent GH responses to repeated injections of GH-releasing hexapeptide (GHRP-6) and the nonpeptide GH secretagogue, L-692,585. *J. Endocrinol.* **1995**, *145*, 417−426.

(54) (a) For a recent review on the clinical actions of GHRPs, see: Ghigo, E.; Arvat, E.; Muccioli, G.; Camanni, F. Growth hormone releasing peptides. *Eur. J. Endocrinol.* **1997**, *136*, 445−460 and references therein. (b) Micic, D.; Kendereski, A.; Popovic, V.; Sumarac, M.; Macut, D.; Zoric, S.; Colic, M.; Zivkovic, D.; Dieguez, C.; Casanueva, F. Growth-hormone response to GHRH, GHRP-6 and GHRH+GHRP-6 in non-insulin-dependent diabetes-mellitus. *Diabetologia* **1996**, *39*, 697. (c) Cordido, F.; Penalva, A.; Dieguez, C.; Casanueva, F. F. Massive growth-hormone (GH) discharge in obese subjects after the combined administration of GH-releasing hormone and GHRP-6−evidence for a marked somatotroph secretory capability in obesity. *J. Clin. Endocrinol. Metab.* **1993**, *76*, 819−823. (d) Dieguez, C.; Cordido, E.; Leal, A.; Pombo, M.; Pumar, A.; Penalva, A.; Casanueva F. F. GHRP-6 induced GH secretion in three states of pathological hyposomatotropism: Cushing's syndrome, obesity and short stature children. 10th International Congress of Endocrinology, San Francisco, CA, 1996; 228 (Abstract). (e) Giustina, A.; Bresciani, E.; Bugari, G.; Bussi, A. R.; Deghenghi, R.; Imbimbo, B.; Giustina, G. Hexarelin, a novel GHRP-6 analog, counteracts the inhibitory effect of hydrocortisone on growth hormone secretion in acromegaly. *Endocr. Res.* **1995**, *21*, 569−582. (f) Kyrgialanis, A.; Kakavas, I.; Voudiclari, S.; Deghenghi, R.; Moschogianni, H.; Tolis, G. Pituitary somatotrope responsiveness to two growth hormone secretagogues: GRF (1-29) and the GHRP-hexarelin in hemodialysed patients with chronic renal failure (CRF). *Nephrol. Dialysis Transplantation* **1995**, *10*, 985 (Abstract). (g) Micic, D.; Kendereski, A.; Sumarac, M.; Zoric, S.; Colic, M.; Macut, D.; Dieguez. C.; Casanueva, F. Growth hormone secretion in patients with polycystic ovary disease after administration of GHRH, GHRP-6 and GHRH+GHRP-6. 10th International Congress of Endocrinology, San Francisco, CA, 1996; 3−52 (Abstract).

(55) Pombo, M.; Barreiro, J.; Penalva, A.; Peino, R.; Dieguez, C.; Casanueva, F. F. Absence of growth-hormone (GH) secretion after the administration of either GH-releasing hormone (GHRH), GH-releasing peptide (GHRP-6), or GHRH plus GHRP-6 in children with neonatal pituitary-stalk transection. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 3180−3184.

(56) Popovic, V.; Damjanovic, S.; Micic, D.; Djurovic, M.; Doknic, M.; Dieguez, C.; Casanueva, F. Growth hormone secretion after GHRH and GHRP-6 in patients with hypothalamo-pituitary disconnection. *J. Endocrinol.* **1995**, *144*, P96 (Abstract).

(57) Jaffe, C. A.; Ho, P. J.; Demottfriberg, R.; Bowers, C. Y.; Barkan, A. L. Effects of a prolonged growth-hormone (GH)-releasing peptide infusion on pulsatile GH secretion in normal men. *J. Clin. Endocrinol. Metab.* **1993**, *77*, 1641−1647.

(58) Huhn, W. C.; Hartman, M. L.; Pezzoli, S. S.; Thorner, M. O. Twenty-four-hour growth hormone (GH)-releasing peptide (GHRP) infusion enhances pulsatile GH secretion and specifically attenuates the response to a subsequent GHRP bolus. *J. Clin. Endocrinol. Metab.* **1993**, *76*, 1202−1208.
(59) Klinger, B.; Silbergeld, A.; Deghenghi, R.; Frenkel, J.; Laron, Z. Desensitization from long-term intranasal treatment with hexarelin does not interfere with the biological effects of this growth hormone- releasing peptide in short children. *Eur. J. Endocrinol.* **1996**, *134*, 716−719.
(60) Ghigo, E.; Arvat, E.; Gianotti, L.; Grottoli, S.; Rizzi, G.; Ceda, G. P.; Boghen, M. F.; Deghenghi, R.; Camanni, F. Short-term administration of intranasal or oral hexarelin, a synthetic hexapeptide, does not desensitize the growth hormone responsiveness in human aging. *Eur. J. Endocrinol.* **1996**, *135*, 407−412.
(61) Mericq, V.; Cassorla, F.; Salazar, T.; Avila, A.; Iniguez, G.; Bowers, C. Y.; Merriam, G. R. Treatment with GHRP-2 accelerates the growth of GH deficient children. *J. Invest. Med.* **1996**, *44*, A 103.
(62) Pihoker, C.; Kearns, G. L.; Bowers, C. Pharmacokinetics (PK) and pharmacodynamics (PD) of growth-hormone releasing peptide-2 (GHRP-2)—a phase-I study in children. *J. Invest. Med.* **1996**, *44*, A 60.
(63) Laron, Z.; Frenkel, J.; Deghenghi, R.; Anin, S.; Klinger, B.; Silbergeld, A. Intranasal administration of the GHRP hexarelin accelerates growth in short children. *Clin. Endocrinol.* **1995**, *43*, 631−635.
(64) Bowers, C. Y.; Alster, D. K.; Reynolds, G. A.; Frentz, J. M. GH release following oral administration of synthetic hexapeptide (GHRP) in normal men and GH deficient children. 72nd Annual Meeting of the Endocrine Society, Atlanta, GA, 1990; p297 (Abstract).
(65) Bowers, C. Y.; Alster, D. K.; Frentz, J. M. The growth hormone-releasing activity of a synthetic hexapeptide in normal men and short statured children after oral administration. *J. Clin. Endocrinol. Metab.* **1992**, *74*, 292−298.
(66) (a) Smith, R. G.; Cheng, K.; Pong, S. S.; Hickey, H.; Jacks, T.; Butler, B.; Chan, W.-S.; Chaung, L. Y. P.; Judith, F.; Taylor, J. A.; Wyvratt, M. J.; Fisher, M. H. A Novel Non-Peptidyl Growth Hormone Secretagogue. *Science* **1993**, 260, 1640−1643. (b) Schoen, W. R.; Pisano, J. M.; Prendergast, K.; Wyvratt, M. J., Jr.; Fisher, M. H.; Cheng, K.; Chan, W. W. S.; Butler, B.; Smith, R. G.; Ball, R. G. A novel 3-substituted benzazepinone growth hormone secretagogue (L-692,429). *J. Med. Chem.* **1994**, *37*, 897−906.
(67) (a) Horwell, D. C. Kappa opioid analgesics. *Drugs Future* **1988**, *13*, 1061−1071. (b) Rees, D. C.; Hunter, J. C. Opioid receptors. In *Comprehensive Medicinal Chemistry*; Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: Oxford, Vol. 3, pp 805−846.
(68) Cheng, K.; Chan, W. W.-S, Butler, B.; Wei, L.; Schoen, W. R.; Wyvratt, M. J.; Fisher, M. H.; Smith, R. G. Stimulation of growth hormone release from rat primary cells by L-692,429, a novel non-peptidyl GH secretagogue. *Endocrinology* **1993**, *132*, 2729−2731.
(69) Schoen, W. R.; Ok, D.; DeVita, R. J.; Pisano, J. M.; Hodges, P.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Smith, R. G.; Wyvratt, M. J., Jr.; Fisher, M. H. Structure-activity relationships in the amino acid sidechain of L-692,429. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1117−1122.
(70) Ok, D.; Schoen, W. R.; Hodges, P.; DeVita, R. J.; Brown, J. E.; Chang, K.; Chan, W. W.-S.; Butler, B. S.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J., Jr. Structure activity relationships of non-peptidyl growth hormone secretagogue L-692,429. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2709−2714.
(71) Hansen, T. K.; Thogersen, H.; Hansen, S. S. Naphtho-fused azepines as potent growth hormone secretagogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2951−2954.
(72) Jacks, T. M.; Hickey, G. J.; Judith, F.; Taylor, J.; Chen, H. Y.; Krupa, D. A.; Feeney, W. P.; Schoen, W. R.; Ok, D.; Fisher, M. H.; Wyvratt, M. J., Jr.; Smith, R. G. Effects of acute and repeated intravenous administration of L-692,585, a novel non-peptidyl growth hormone secretagogue, on plasma growth hormone, IGF-1, ACTH, cortisol, prolactin, insulin, and thyroxine levels in beagles. *J. Endocrinol.* **1994**, *143*, 399−406.
(73) (a) Gertz, B. J.; Barrett, J. S.; Eisenhandler, R.; Krupa, D. A.; Wittreich, J. A.; Seibold, J. R.; Schneider, S. H. Growth hormone response in man to L-692,429, a novel nonpeptide mimic of growth hormone-releasing peptide-6. *J. Clin. Endocrinol. Metab.* **1993**, *77*, 1393−1397. (b) Aloi, J. A.; Gertz, B. J.; Hartman, M. L.; Huhn, W. C.; Pezzoli, S. S.; Wittreich, J. M.; Krupa, D. A.; Thorner, M. A. Neuroendocrine responses to a novel growth hormone secretagogue, L-692,429 in healthy older subjects. *J. Clin. Endocrinol. Metab.* **1994**, *79*, 943−949.
(74) Chapman, I. M.; Hartman, M. L.; Pezzoli, S. S.; Thorner, M. O. Enhancement of pulsatile growth hormone secretion by continuous infusion of a growth hormone releasing peptide (GHRP)-mimetic, L-692,429, in older adults. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 2874−2880.
(75) Gertz, B. J.; Sciberras, D. G.; Yogendran, L.; Christie, K.; Bador, K.; Krupa, D.; Wittreich, J. M.; James, I. L-692,429, a nonpeptide growth hormone (GH) secretagogue, reverses glucocorticoid suppression of GH secretion. *J. Clin. Endocrin. Metab.* **1994**, *79*, 745−749.
(76) For pharmacokinetic properties of L-692,429, see: Leung, K. H.; Cohn, D. A.; Miller, R. R.; Doss, G. A.; Stearns, R. A.; Simpson, R. E.; Feeney, W. P.; Chui, S.-H. L. Pharmacokinetics and disposition of L-692,429, a novel nonpeptidyl growth hormone secretagogue in preclinical species. *Drug Metab. Dispos.* **1996**, *24*, 753−760.
(77) DeVita, R. J.; Schoen, W. R.; Fisher, M. H.; Frontier, A. J.; Pisano, J. M.; Wyvratt, M. J., Jr.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Hickey, G. J.; Jacks, T. M.; Smith, R. G. Benzolactam growth hormone secretagogues: carboxamides as replacements for the 2′-tetrazole moiety of L-692,429. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2249−2254.
(78) (a) Patchett, A. A.; Smith, R. G.; Wyvratt, M. J. Orally Active Growth Hormone Secretagogues. In *Integration of Pharmaceutical Discovery: Case Histories*; Borchardt, R. T., Sawyer, T., Freidinger, R., Smith, P., Eds.; 1997, in press. (b) DeVita, R. J.; Schoen, W. R.; Ok, D.; Barash, L.; Brown, J. E.; Fisher, M. H.; Hodges, P.; Wyvratt, M. J., Jr.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Smith, R. G. Benzolactam growth hormone secretagogues: replacements for the 2′-tetrazole moiety of L-692,429. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1807−1812. (c) DeVita, R. J.; Wyvratt, M. J., Jr. Benzolactam growth hormone secretagogues. *Drugs Future* **1996**, *21*, 273−281. (d) Ankersen, M.; Peschke, B.; Hansen, B. S.; Hansen, T. K. Investigation of bioisosteres of the growth hormone secretagogue L-692,429. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1293−1298.
(79) Nargund, R. P.; Barakat, K. J.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Smith, R. G.; Patchett, A. A. Synthesis and biological activities of camphor-based non-peptide growth hormone secretagogues. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1265−1270.
(80) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. Methods for drug discovery: Development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.* **1988**, *31*, 2235−2246.
(81) Ariens, E. J.; Beld, A. J.; Rodrigues de Miranda, J. F.; Simonis, A. M. The pharmacon-receptor-effector concept, A basis for understanding the transmission of information in biological systems. In *The Receptors, A comprehensive treatise*, O'Brien, R. D., Ed.; Plenum Press: New York and London, 1979; pp 33−91.
(82) Andrews, P. R.; Lloyd, E. J. Molecular conformation and biological activity of central nervous system active drugs. *Med. Res. Rev.* **1982**, *2*, 355−393.
(83) Wiley: R. A.; Rich, D. H. Peptidomimetics derived from natural products. *Med. Res. Rev.* **1983**, *13*, 327−384.
(84) Chambers, M. S.; Baker, R.; Billington, D. C.; Knight, A. K.; Middlemiss, D. N.; Wong, E. H. F. Spiropiperidines as high-affinity, selective s ligands. *J. Med. Chem.* **1992**, *35*, 2033−2039.
(85) Elliott, J. M.; Cascieri, M. A.; Davies, S.; Huscroft, I. T.; Kelleher, F. J.; Lewis, R. T.; MacLeod, A. M.; Merchant, K. J.; Sadowski, S.; Stevenson, G. I. Serine derived NK1 antagonists. 211th American Chemical Society National Meeting, New Orleans, LA, 1996; MEDI 075 (Abstract).
(86) Lowe, J. A.; McLean, S. Tachykinin antagonists. *Curr. Pharm. Des.* **1995**, *1*, 269−278.
(87) Smith, P. W.; Cooper, A. W. J.; Bell, R.; Beresford, I. J. M.; Gore, P. M.; McElroy, A. B.; Pritchard, J. M.; Saez, V.; Taylor, N. R.; Sheldrick, R. L. G.; Ward, P. New spiropiperidines as potent and selective non-peptide tachykinin NK2 receptor antagonists. *J. Med. Chem.* **1995**, *38*, 3772−3779.
(88) Shah, S. K.; Hale, J. J.; Qi, H.; Miller, D. J.; Dorn, C. P.; Mills, S. G.; Sadowski, S. J.; Cascieri, M. A.; Metzger, J. M.; Eiermann, G. J.; Forrest, M. J.; MacIntyre, D. E.; MacCoss, M. Discovery of substituted spiroindolinepiperidines as orally active dual antagonists of NK1 and NK2 receptors. 212th American Chemical Society National Meeting, Orlando, FL 1996; MEDI 136 (Abstract).
(89) deLaszlo, S. E.; Allen, E. E.; Li, B.; Ondeyka, D.; Rivero, R.; Malkowitz, L.; Molineaux, C.; Siciliano, S. J.; Springer, M. S.; Greenlee, W. J.; Mantlo, N. A nonpeptide agonist ligand of the human C5a receptor: synthesis, binding affinity optimization and functional characterization. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 213-218.

(90) Patchett, A. A.; Nargund, R. P.; Tata, J. R.; Chen, M.-H.; Barkat, K. J.; Johnston, D. B. R.; Cheng, K.; Chan, W. W.-S.; Butler, B.; Hickey, G.; Jacks, T.; Schleim, K.; Pong, S.-S.; Chaung, L. Y.-P.; Chen, H. Y.; Frazier, E.; Leung, K. H.; Chiu, S.-H. L.; Smith, R. G. Design and biological activities of L-163,191 (MK-0677): A potent orally active growth hormone secretagogue. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7001−7005.

(91) Chen, M.-H.; Steiner, M. G.; Patchett, A. A.; Cheng, K.; Wei, L.; Chan, W. W.-S.; Butler, B.; Jacks, T. M.; Smith, R. G. Analogs of the orally active growth hormone secretagogue L-162,752. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2163−2168.

(92) Smith, R. G.; Pong, S.-S.; Hickey, G.; Jacks, T.; Cheng, K.; Leonard, R.; Cohen, C. J.; Arena, J. P.; Chang, C. H.; Drisko, J.; Wyvratt, M.; Fisher, M.; Nargund, R.; Patchett, A. Modulation of pulsatile GH release through a novel receptor in hypothalamus and pituitary gland. In *Recent Progress In Hormone Research*; Conn, P. M., Ed.; The Endocrine Society: Bethesda, 1996; Vol. 51, pp 261−286.

(93) Pong, S.-S.; Chaung, L.-Y. P.; Dean, D. C.; Nargund, R. P.; Patchett, A. A.; Smith, R. G. Identification of a new G-protein linked receptor for growth hormone secretagogues. *Mol. Endocrinol.* **1996**, 57−61.

(94) Jacks, T.; Smith, R.; Judith, F.; Schleim, K.; Frazier, E.; Chen, H.; Krupa, D.; Hora, D., Jr.; Nargund, R.; Patchett, A.; Hickey, G. MK-0677, a potent, novel, orally active growth hormone (GH) secretagogue: GH, insulin-like growth factor I, and other hormonal responses in beagles. *Endocrinology* **1996**, *137*, 5284−5289.

(95) Hickey, G.; Jacks, T.; Schleim, K.; Frazier, E.; Chen, H.; Krupa, D.; Feeney, W.; Nargund, R.; Patchett, A.; Smith, R. G. Repeat administration of the growth hormone secretagogue MK-0677 increases and maintains elevated IGF-1 levels in beagles. *J. Endocrinol.* **1997**,*152*, 183−192.

(96) Jacks, T.; Lieber, R.; Schleim, K.-D.; Mohler, R.; Haven, M.; Feeney, W.; Hora, D.; Hickey, G. L-163,255, a GH secretagogue, increased muscle torque in the remobilization phase of a canine hind limb model. 10th International Congress of Endocrinology, San Francisco, CA, 1996; P1-602 (Abstract).

(97) Chang, C. H.; Rickes, E. L.; McGuire, L.; Frazier, E.; Chen, H.; Barakat, K.; Nargund, R.; Patchett, A.; Smith, R. G.; Hickey, G. J. Growth hormone (GH) and insulin-like growth factor I responses after treatments with an orally active GH secretagogue L-163,255 in swine. *Endocrinology* **1996**, *137*, 4851−4856.

(98) Snyder, D. K.; Clemmons, D. R.; Underwood, L. E. Treatment of obese, diet restricted subjects with growth hormone for 11 weeks: Effects of anabolism, lipolysis and body composition. *J. Clin. Endocrinol. Metab.* **1988**, *67*, 54−61.

(99) Manson, J. McK.; Wilmore, D. W. Positive nitrogen balance with human growth hormone and hypocaloric intravenous feeding. *Surgery* **1986**, *100*, 188−97.

(100) Isley, W. L.; Underwood, L. E.; Clemmons, D. R. Dietary components that regulate serum somatomedin-C in humans. *J. Clin. Invest.* **1983**, *71*, 175−182.

(101) Ho, K. Y.; Veldhuis, J. B.; Johnson, M. L.; Furlanetto, R.; Evans, W. S.; Alberti, K. G. M.; Thorner, M. O. Fasting enhances growth hormone secretion and amplifies the complex synthesis of growth hormone secretion in man. *J. Clin. Invest.* **1988**, *81*, 968−975.

(102) Clemmons, D. R.; Plunkett, L. J.; Polvino, W. M. Administration of the GH secretagogue L-163,191 stimulates an anabolic response in calorically restricted normal volunteers. *Endocrinol. Metab.* **1997**, *4* (Suppl. A), 35 (Abstract).

(103) Chapman, I. M.; Bach, M. A.; Van Cauter, E.; Farmer, M.; Krupa, D.; Taylor, A. M.; Schilling, L. M.; Cole, K. Y.; Skiles, E. H.; Pezzoli, S. S.; Hartman, M. L.; Veldhuis, J. D.; Gormley, G. J.; Thorner, M. O. Stimulation of the growth hormone (GH)/IGF-I axis by daily oral administration of a GH secretagogue (MK-0677) in healthy elderly subjects. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 4249−4257.

(104) Veldhuis, J.; Iranmesh, A.; Waters, M.; et al. Dual defects in pulsatile GH secretion and clearance subserve the hyposomatotrophism in man. *J. Clin. Endocrin. Metab.* **1991**, *72*, 51−59.

(105) Cordido, F.; Penalva, A.; Dieguez, C.; Casanueva, F. F. Massive growth-hormone (GH) discharge in obese subjects after the combined administration of GH-releasing hormone and GHRP-6—evidence for a marked somatotroph secretory capability in obesity. *J. Clin. Endocrinol. Metab.* **1993**, *76*, 819−823.

(106) Svensson, J.; Lonn, L.; Jansson J.-O.; Murphy, G.; Wyss, D.; Krupa, D.; Cerchio, K.; Gertz, B.; Bosaeous, I.; Sjostrom, L.; Bengtsson, B.-A. Two months treatment of obese subjects with GH secretagogue MK-677 increases fat-free mass and insulin-like growth factor1 (IGF-I). *Endocrinol. Metab.* **1997**, *4* (Suppl. A), 35 (Abstract).

(107) Dickson, S.; Luckman, S. Induction of c-fos mRNA in NPY and GRF neurones in the rat arcuate nucleus following systemic injection of the GH secretagogue, GHRP-6. *Endocrinology* **1997**, *138*, 771−777.

(108) Clark, J.; Kalra, P.; Kairs S. Neuropeptide Y and human pancreatic polypeptide stimulate feeding in rats. *Endocrinology* **1984**, *115*, 427−429.

(109) Williams, P. D.; Anderson, P. S.; Ball, R. G.; Bock, M. G.; Carroll, L. A.; Chiu, S.-H. L.; Clineschmidt, B. V.; Culberson, J. C.; Erb. J. M.; Evans, B. E.; Fitzpatrick, S. L.; Freidinger, R. M.; Kaufman, M. J.; Lundell, G. F.; Murphy, J. S.; Pawluczyk, J. M.; Perlow, D. S.; Pettibone, D. J.; Pitzenberger, S. M.; Thompson, K. L.; Veber, D. F. 1-(((7,7-Dimethyl-2(S)-(2(S)-amino-4-(methylsulfonyl)butryramido)bicyclo[2.2.1]-heptan-1(S)-yl)methyl)-sulfonyl)-4-(2-methylphenyl)piperazine (L-368,899): an orally bioavailable, non-peptide oxytocin antagonist with potential utility for managing preterm labor. *J. Med. Chem.* **1994**, *37*, 565−571.

(110) Barakat, K. J.; Prendergast, K. J.; Cheng, K.; Jacks, T. M.; Schleim, K.; Chan, W. W.-S.; Butler, B.; Frazier, E.; Hickey, G. J.; Smith, R. G.; Patchett, A. A.; Nargund, R. P. Design and biological activities of aryl piperazine-based growth hormone secretagogues. *Bioorg. Med. Chem. Lett.*, in press.

(111) Tata, J. R.; Lu, Z.; Cheng, K.; Wei, L.; Chan, W. W.-S.; Butler, B.; Schleim, K. D.; Jacks, T. M.; Leung, K.; Chiu, S.-H. L.; Hickey, G.; Smith, R. G.; Patchett, A. A. The design and synthesis of orally active short duration spiroindane growth hormone secretagogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2319−2324..

(112) Lefker, B. A.; Carpino, P. A.; Pan, L. C.; Chidsey-Frink, K. L.; DiSilva-Jardine, P.; DiCapua, F. M.; Griffith, D. A.; Hada, W. A.; Inthavongsay, J. K.; Lewis, S. K.; Mangano, F. M.; Murray, M. C.; Ng, O.; Owens, J. G.; Pirie, C. M.; Rose, C. R.; Ryan, N. I.; Schafer, J. R.; Tucker, J. L.; Wright, A. S.; Zawistoski, M. P.; Thompson, D. D. The design, synthesis and biological activities of a series of tetrahydroquinoline/isoindole-based growth hormone secretagogues. In *Growth Hormone Secretagogues in Clinical Practice*; Bercu, B. B., Walker, R. F., Eds.; Marcel Dekker: New York, 1998; pp 107−120.

(113) Bochis, R. J.; Wyvratt, M. J.; Schoen, W. R. U.S. Patent No. 5,-283,241.

(114) Yang, L.; Morriello, G.; Prendergast, K.; Cheng, K.; Jacks, T.; Chan, W. W.-S.; Schleim, K. D.; Smith, R. G.; Patchett, A. A. Potent 3-spiropiperidine growth hormone secretagogues. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 107−112.

(115) Yang, L.; Morriello, G. J.; Patchett, A. A.; Leung, K.; Jacks, T.; Cheng, K.; Schleim, K.; Chan, W.; Chiu, S.-H. L.; Smith, R. G. L-163,540, a potent and orally bioavailable short duration growth hormone secretagogue. 215th American Chemical Society National Meeting, Dallas, TX, 1998; MEDI 020 (Abstract).

(116) Carpino, P. A.; Jardine, D. P. A.; Lefker, B. A.; Ragan, J. A. PCT Patent Publication WO97/24369.

(117) Dodge, J. A.; Hipskind, P. A. PCT Patent Publication WO97/07117.

(118) (a) Dodge, J. A.; Hipskind, P. A. PCT Patent Publication WO97/06803. (b) Hipskind, P. A.; Howbert, J. J.; Bruns, R. F.; Cho, S. S. Y.; Crowell, T. A.; Foreman, M. M.; Gehlert, D. R.; Iyengar, S.; Johnson, K. W.; Krushinski, J. H.; Li, D. L.; Lobb, K. L.; Mason, N. R.; Muehl, B. S.; Nixon, J. A.; Phebus, L. A.; Regoli, D.; Simmons, R. M.; Threlkeld, P. G.; Waters, D. C.; Gitter, B. D. 3-Aryl-1,2-diacetamidopropane derivatives as novel and potent NK-1 receptor antagonists. *J. Med. Chem.* **1996**, *39*, 736−748.

(119) McDowell, R. S.; Elias, K. A.; Stanley, M. S.; Burdick, D. J.; Burner, J. P.; Chan, K. S.; Fairbrother, W. J.; Hammond, R. G.; Ingle, G. S.; Jacobsen, N. E.; Mortensen, D. L.; Rawson, T. E.; Won, W. B.; Clark, R. G.; Somers, T. C. Growth hormone secretagogues: characterization, efficacy, and minimal bioactive conformation. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11165−11169.

(120) Elias, K. A.; Ingle, G. S.; Burnier, J. P.; Hammonds, R. G.; McDowell, R. S.; Rawson, T. E.; Somers, T. C.; Stanley, M. S.; Cronin, M. J. *In vitro* characterization of four novel classes of growth hormone-releasing peptides. *Endocrinology* **1995**, *136*, 5694−5699.

(121) Somers, T. K.; Elias, K. A.; Clark, R. G.; McDowell, R. S.; Stanley, M. S.; Burnier, J. P.; Rawson, T. E. PCT Patent Publication WO 96/15148.

(122) Deghenghi, R. Growth hormone releasing peptides. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 85−102.

(123) Deghenghi, R.; Boutignon, F.; Luoni, M.; Grilli, R.; Guidi, M.; Locatelli, V. Small peptides as potent releasers of growth hormone. *J. Pediat. Endocrinol. Metab.* **1995**, *8*, 311−313.

(124) Deghenghi, R.; Boutignon, F.; Luoni, M.; Grilli, R.; Guidi, M.; Locatelli, V. Characterization of the activity of new growth hormone secretagogues in the infant rat. 10th International Congress of Endocrinology, San Francisco, CA, 1996; P1-581 (Abstract).

(125) Deghenghi, R.; Boutignon, F.; Luoni, M.; Rigamonti, A.; Cella, S. G.; Broglio, F.; Arvat, E.; Ghigo, E.; Locatelli, V. Small impervious peptides as GH secretagogues. 2nd International Symposium on Growth Hormone Secretagogues, Tampa, FL, February 13−16, 1997; P9 (Abstract).
(126) Deghenghi, R. PCT Patent Publication W097/2260.
(127) Hansen, B. S.; Raun, K. GH secretagogues − potency and efficacy are "model" dependent. 10th International Congress of Endocrinology, San Francisco, CA, 1996; P1 583 (Abstract).
(128) Johansen, P. B.; Hansen, K. T.; Andersen, J. V.; Jensen, M.; Jensen, M. Pharmacokinetics of growth hormone releasing peptides in the rat. 10th International Congress of Endocrinology, San Francisco, CA, 1996; P1 585 (Abstract).
(129) Johansen, N. L.; Hansen, B. S.; Kligaard, H.; Ankersen, M. Structure activity relationships of GHRP analogs. In 10th International Congress of Endocrinology, San Francisco, CA, 1996; P1 586 (Abstract).
(130) Peschke, B.; Madsen, K.; Hansen, B. S.; Johansen, N. L. Aminomethylthiophene-2-carboxylic acids as dipeptide mimetic in new growth hormone secretagogues. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1969−1972.
(131) Johansen, N. L.; Lau, J.; Madsen, K.; Thoegersen, H.; Lundt, B. F.; Peschke, B.; Hansen, T. K.; Hansen, B. S. PCT Patent Publication WO97/00894.
(132) Hansen, T. K.; Peschke, B.; Lau, J.; Lundt, B. F.; Ankersen, M.; Watson, B.; Madsen, K. PCT Patent Publication WO97/23508.
(133) Dean, D. C.; Nargund, R. P.; Pong, S.-S.; Chaung, L.-Y. P.; Griffin, P.; Melillo, D. G.; Ellsworth, R. L.; Van Der Ploeg, L. H. T.; Patchett, A. A.; Smith, R. G. Development of a high specific activity sulfur-35-labeled sulfonamide radioligand that allowed the identification of a new growth hormone secretagogue receptor. *J. Med. Chem.* **1996**, *39*, 1767−1770.
(134) Schwartz, T. W.; Rosenkilde, M. M. Is there a 'lock' for all agonist 'keys' in TTM receptors? *Trends Pharmacol. Sci.* **1996**, *17*, 213-216.
(135) Blake, A. D.; Bot, G.; Reisine, T. Structure-function analysis of the cloned opiate receptors: peptide and small molecule interactions. *Chem. Biol.* **1996**, *3*, 967−972.
(136) Howard, A. D.; Feighner, S. D.; Cully, D. F.; Arena, J. P.; Liberator, P. A.; Rosenblum, C. I.; Hamelin, M.; Hreniuk, D. L.; Palyha, O. C.; Anderson, J.; Paress, P. S.; Diaz, C.; Chou, M.; Liu, K. K.; McKee, K. K.; Pong, S.-S.; Chaung, L.-Y. P.; Elbrecht, A.; Dashkevicz, M.; Heavens, R.; Rigby, M.; Sirinathsinghji, D. J. S.; Dean, D. C.; Melillo, D. G.; Patchett, A. A.; Nargund, R. P.; Griffin, P. R.; DeMartino, J. A.; Gupta, S. K.; Schaeffer, J. M.; Smith, R. G.; Van der Ploeg, L. H. T. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* **1996**, *273*, 974−977.
(137) Strader, C. D.; Sigal, I. S.; Register, R. B.; Candelore, M. R.; Rands, E.; Dixon, R. A. Identification of residues required for ligand binding to the beta-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4384−4388.
(138) Strnad, J.; Hadcock, J. R. Identification of a critical aspartate residue in transmembrane domain three necessary for the binding of somatostatin to the somatostatin receptor SSTR2. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 913−921.
(139) Feighner, S. D.; Howard, A. D.; Prendergast, K.; Palyha, O. C.; Hreniuk, D. L.; Nargund, R.; Underwood, D.; Tata, J. R.; Dean, D. C.; Tan, C.; McKee, K. K.; Woods, J. W.; Patchett, A. A.; Smith, R. G.; Van der Ploeg, L. H. T. Structural requirements for the activation of the human growth hormone secretagogue receptor. *Mol. Endocrinol.* **1998**, *12*, 137−145.
(140) Wyvratt, M. J. Nonpeptidyl growth hormone secretagouges. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 103−117.
(141) DeVita, R. J.; Frontier, A. J.; Schoen, W. R.; Wyvratt, M. J.; Fisher, M. H.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Smith, R. G. Design and synthesis of potent macrocyclic benzolactam growth hormone secretagogues. *Helv. Chim. Acta* **1997**, *80*, 1244−1259.
(142) (a) Yang, L.; Nargund, R.; Morriello, G.; Barakat, K.; Pan, Y.; Prendergast, K.; Cheng, K.; Chan, W. W.-S.; Smith, R. G.; Patchett, A. A. Tripeptide growth hormone secretagogues. 210th American Chemical Society National Meeting, Chicago, IL, 1995; MEDI 011 (Abstract). (b) Yang, L.; Morriello, G.; Pan, Y.; Nargund, R.; Barakat, K.; Prendergast, K.; Cheng, K.; Chan, W. W.-S.; Smith, R. G.; Patchett, A. A. Tripeptide growth hormone secretagogues. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 759−764.
(143) Henke, B. R.; Aquino, C. J.; Birkemo, L. S.; Croom, D. K.; Dougherty, R. W., Jr.; Ervin, G. N.; Grizzle, M. K.; Hirst, G. C.; James, M. K.; Johnson, M. F.; Queen, K. L.; Sherrill, R. G.; Sugg, E. E.; Suh, E. M.; Szewczyk, J. W.; Unwalla, R. J.; Yingling, J.; Willson, T. M. Optimization of 3-(1H-indazol-3-ylmethyl)-1,5-benzodiazepines as potent, orally active CCK-A agonists. *J. Med. Chem.* **1997**, *40*, 2706−2725 and references therein.
(144) Aramori, I.; Zenkoh, J.; Morikawa, N.; Asano, M.; Hatori, C.; Sawai, H.; Kayakiri, H.; Satoh, S.; Inoue, T.; Abe, Y.; Sawada, Y.; Mizutani, T.; Inamura, N.; Nakahara, K.; Kojo, H.; Oku, T.; Notsu, Y. Nonpeptide mimic of bradykinin with long-acting properties at the bradykinin B_2 receptor. *Mol. Pharmacol.* **1997**, *52*, 16−20.
(145) Perlman, S.; Schambye, H. T.; Rivero, R. A.; Greenlee, W. J.; Hjorth, S. A.; Schwartz, T. W. Non-peptide angiotensin agonist. Functional and molecular interaction with the AT_1 receptor. *J. Biol. Chem.* **1995**, *270*, 1493−1496.
(146) Nargund, R.; Patchett, A. A. PCT Patent Publication WO 96/13265.
(147) Morriello, G.; Patchett, A. A.; Yang, L.; Chen, M.-H.; Nargund, R. PCT Patent Publication WO 95/13069.
(148) Skuker, S. B.; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. Discovering high-affinity ligands for proteins: SAR by NMR. *Science* **1996**, *274*, 1531−1534.
(149) Hirschmann, R.; Nicolau, K. C.; Pietranico, S.; Salvino, J.; Leahy, E. M.; Sprengler, P. A.; Furst, G.; Smith, A. B. III; Strader, C. D.; Casieri, M. A.; Candelore, M. R.; Donaldson, C.; Vale, W.; Maechler, L. Nonpeptidal peptidomimetics with a b-D-glucose scaffolding. A partial somatostatin agonist bearing a close structural relationship to a potent selective substance P antagonist. *J. Am. Chem. Soc.* **1992**, *114*, 9217−9218.
(150) (a) Smith, R. G.; Cheng, K.; Pong, S.-S.; Leonard, R. J.; Cohen, C. J.; Arena, J. P.; Hickey, G. J.; Chang, C. H.; Jacks, T. M.; Drisko, J. E.; Robinson, I. C. A. F.; Dickson, S. L.; Leng, G. Mechanism of Action of GHRP-6 and Nonpeptidyl Growth Hormone Secretagogues. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 147−163. 205. (b) Smith, R. G.; Van der Ploeg, L. H. T.; Howard, A. D.; Feighner, S. D.; Cheng, K.; Hickey, G. J.; Wyvratt, M. J., Jr.; Fisher, M. H.; Nargund, R. P.; Patchett, A. A. Peptidomimetic regulation of growth hormone secretion. *Endocr. Rev.* **1997**, *18*, 621−645.
(151) Pong, S.-S.; Chaung, L.-Y. P.; Smith, R. G. GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) stimulates growth hormone secretion by depolarization in rat pituitary cell cultures. 73rd Annual Meeting of The Endocrine Society, Washington, DC, 1991; p88 (Abstract).
(152) Pong, S.-S.; Chaung, L.-Y. P.; Smith, R. G.; Ertel, E. A.; Smith, M. M.; Cohen, C. J. Role of calcium channels in growth hormone secretion induced by GHRP-s (His-D-Trp-Ala-Trp-D-Phe-Lys-NH2) and other secretagogues in rat somatotrophs. 74th Annual Meeting of The Endocrine Society, San Antonio, TX, 1992; p146 (Abstract).
(153) Leonard, R. J.; Chaung, L.-Y. P.; Pong, S.-S. Ionic conductances of identified rat somatotroph cells studies by perforated patch recording are modulated by growth hormone secretagogues. *Biophys. J.* **1991**, *59*, 254a (Abstract).
(154) Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Barreto, A.; Smith, R. G. Evidence for a role of protein kinase-C in His-DTrp-Ala-Trp-DPhe-Lys-NH_2- induced growth hormone release from rat primary pituitary cells. *Endocrinology* **1991**, *129*, 3337−3342.
(155) Mau, S. E.; Witt, M. R.; Bjerrum, O. J.; Saermark, T.; Vilhardt, H. Growth hormone releasing hexapeptide (GHRP-6) activates the inositol (1,4,5)-trisphosphate/diacylglycerol pathway in rat anterior pituitary cells. *J. Recept. Signal Transduct. Res.* **1995**, *15*, 311−323.
(156) Herrington, J.; Hille, B. Growth hormone-releasing hexapeptide elevates intracellular calcium in rat somatotrophs by two mechanisms. *Endocrinology* **1994**, *135*, 1100−1108.
(157) Bresson-Bepoldin, L.; Dufy-Barbe, L. GHRP-6 induces a biphasic calcium response in rat pituitary somatotrophs. *Cell Calcium* **1994**, *15*, 247−258.
(158) Blake, A. D.; Smith, R. G. Desensitization studies using perifused rat pituitary cells show that growth hormone releasing hormone and His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 stimulates growth hormone release through distinct receptor sites. *J. Endocrinol.* **1991**, *129*, 11−19.
(159) Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Wei, L.; Smith, R. G. A novel nonpeptidyl growth hormone secretagogue. *Horm. Res.* **1993**, *40*, 109−115.
(160) Nargund, R. P.; Feighner, S.; Hreniuk, D.; Dashkevicz, M.; Cheng, K.; Chan, W.-W. S.; Van Der Ploeg, L. H. T.; Smith, R. G.; Patchett, A. A. Novel probes derived from MK-0677 for characterization of the growth-hormone secretagogue receptor. 212th American Chemical Society National Meeting, Orlando, FL 1996; MEDI 009 (Abstract).
(161) Hreniuk, D. L.; Howard, A. D.; Rosenblum, C. I.; Palyha, O. C.; Diaz, C.; Cully, D. F.; Paress, P. S.; McKee, K. K.; Dashkevicz, M.; Arena, J. P.; Liu, K. K.; Schaeffer, J. M.; Nargund, R. P.; Smith, R. G.; Van der Ploeg, L. H. T.; Feighner, S. D. Cytochemical identification and cloning of a G-protein coupled growth hormone secretagogue receptor. *Soc. Neurosci.* **1996**, *22*, 1300 (Abstract).

(162) Chaung, L.-Y. P.; Pong, S.-S.; Dean, D.; Schaeffer, J. M.; Smith, R. G. Characterization of a G-protein-linked receptor for peptidyl and nonpeptidyl growth hormone secretagogue in rat and porcine hypothalamic and pituitary membranes. 10th International Congress of Endocrinology, San Francisco, CA, 1996; P1-613 (Abstract).
(163) Adams, E. F.; Petersen, B.; Lei, T.; Buchfelder, M.; Fahlbusch, R. The growth hormone secretagogue, L-692, 429, induces phosphatidylinositol hydrolysis and hormone secretion by human pituitary tumors. *Biochem. Biophys. Res. Commun.* **1995**, *208*, 555−561.
(164) Adams, M. E.; Olivera, B. M. Neurotoxins: overview of an emerging research technology. *Trends Neurosci.* **1994**, *17*, 151−155.
(165) Olivera, B. M.; Miljanich, G. P.; Ramachandran, J.; Adams, M. E. Calcium channel diversity and neurotransmitter release: the omega-conotoxins and the omega-agatoxins. *Annu. Rev. Biochemistry* **1994**, *33*, 823−867.
(166) Ertel, E. A.; Warren, V. A.; Adams, M. E.; Griffin, P.; Cohen, C. J.; Smith, M. M. Type III omega-agatoxins: a family of probes for similar binding sites on L- and N-type calcium channels. *Biochemistry* **1994**, *33*, 5098−5108.
(167) McGurk, J. F.; Pong, S.-S.; Chaung, L.-Y. P.; Gall, M.; Butler, B. S.; Arena, J. P. Growth hormone secretagogues modulate potassium currents in rat somatotrophs. *Soc. Neurosci.* **1993**, *19*, 1559 (Abstract).
(168) Pong, S.-S.; Chaung, L.-Y. P.; Leonard, R. J. The involvement of ions in the activity of a novel growth hormone secretagogue L-692,429 in rat pituitary cell culture. 75th Annual Meeting of The Endocrine Society, Las Vegas, NV, 1993; p 172 (Abstract).
(169) Koch, B. D.; Blalock, J. B.; Schonbrunn, A. Characterization of the cyclic AMP-independent actions of somatostatin in GH cells. I. An increase in potassium conductance is responsible for both the hyperpolarization and the decrease in intracellular free calcium produced by somatostatin. *J. Biol. Chem.* **1988**, *263*, 216−225.
(170) Mayo, K. E.; Godfrey, P. A.; DeAlmeida, V.; Miller, T. Structure, function, and regulation of the pituitary receptor for growth hormone releasing hormone. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 53−71.
(171) Tang, W.-J.; Gilman, A. G. Type specific regulation of adenylyl cyclase by G protein bg subunits. *Science* **1991**, *254*, 1500−1503.
(172) Maggio, R.; Vogel, Z.; Wess, J. Reconstitution of functional muscarinic receptors by co-expression of amino-carboxyl-terminal receptor fragments. *FEBS* **1993**, *319*, 195−200.
(173) Aqua, M. S.; Rizzu, P.; Lindsay, E. A.; Shaffer, L. G.; Zackai, E. H.; Overhauser, J.; Baldini, A. Duplication of 3q syndrome: molecular delineation of the critical region. *Am. J. Med. Genet.* **1995**, *55*, 33−37.
(174) (a) Brachmann, W.E Fall von symmetrischer Monodaktylie durch ulnadefekt mit symmetrischer Flughautbildung in den Ellenbeugen, sowie, anderen Abnormitaten (Zwerghaftigheit, Halsrippen, Behaarung). *Jahrb Kinderkeilk.* **1916**, *84*, 224−235. (b) de Lange, C. Sur un type nouveau de degeneration (Typus Amstelodamensis). *Arch. Med. Enf.* **1933**, *36*, 713−718.
(175) Dickson, S. L. Evidence for a central site and mechanism of action of growth hormone releasing peptide (GHRP-6). In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 237−251.
(176) Fairhall, K. M.; Mynett, A.; Thomas, G. B.; Robinson, I. C. A. F. Central and peripheral effects of peptide and nonpeptide GH secretagogues in vivo. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 219−236.
(177) Wehrenberg, W. B.; Brazeau, P.; Luhen, R.; Bohlen, P.; Guillemin, R. Inhibition of the pulsatile secretion by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). *Endocrinology* **1982**, *111*, 2147−2148.
(178) Hickey, G. J.; Drisko, J. E.; Faidley, T. D.; Chang, C. H.; Anderson, L. L.; Nicolich, S.; McGuire, L.; Rickes, E. L.; Krupa, D. A.; Feeney, W. P.; Friscino, B. F.; Cunningham, P.; Frazier, E.; Chen, H. Y.; Laroque, P.; Smith, R. G. Mediation by the central nervous system is critical to the *in vivo* activity of the GH secretagogue L-692,585. *J. Endocrinol.* **1996**, *148*, 371−380.
(179) Guillaume, V.; Magnan, E.; Cataldi, M.; Dutour, A.; Sauze, N.; Renard, M.; Razafindraibe, H.; Conte-Devolx, B.; Deghenghi, R.; Lenaerts, V.; Oliver, C. Growth hormone (GH)-releasing hormone secretion is stimulated by a new GH-releasing hexapeptide in sheep. *Endocrinology* **1994**, *135*, 1073−1076.
(180) Fletcher, T. P.; Thomas, G. B.; Clarke, I. J. Growth hormone-releasing hormone and somatostatin concentrations in the hypophysial portal blood of conscious sheep during the infusion of growth hormone-releasing peptide-6. *Domes. Anim. Endocrinol.* **1996**, *13*, 251−258.
(181) Dickson, S. L.; Leng, G.; Dyball, R. E. J.; Smith, R. G. Central actions of peptide and nonpeptide growth hormone secretagogues in the rat. *Neuroendocrinology* **1995**, *61*, 36−43.
(182) Sirinathsinghji, D. J. S.; Chen, H. Y.; Hopkins, R.; Trumbauer, M.; Heavens, R.; Rigby, M.; Smith, R. G.; Van der Ploeg, L. H. T. Induction of c-fos mRNA in the arcuate nucleus of normal and mutant growth hormone-deficient mice by a synthetic non-peptidyl hormone secretagogue. *Neuro. Report* **1996**, *6*, 1989−1992.
(183) Bailey, A.; Smith, R. G.; Leng, G. Activation of hypothalamic arcuate neurons in the urethane-anesthetized rat following systemic injection of growth-hormone secretagogues. *J. Physiol. London* **1996**, *495*, 115−116.
(184) Locke, W.; Kirgis, H. D.; Bowers, C. Y.; Abdoh, A. A. Intracerebroventricular growth hormone-releasing peptide-6 stimulates eating without affecting plasma growth hormone responses in rats. *Life Sci.* **1995**, *56*, 1347−1352.
(185) Wahlestedt, C.; Skagerberg, G.; Ekman, R.; Heilig, M.; Sundler, F.; Hakanson, R. Neuropeptide (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. *Brain Res.* **1987**, *417*, 33−38.
(186) Alvers, H. E.; Ottenweller, J. E.; Liou, S. Y.; Lumpkin, M. D.; Anderson, E. R. Neuropeptide Y in the hypothalamus: effect of corticosteroid and single-unit activity. *Am. J. Physiol.* **1990**, *258*, R376−R382.
(187) Guan, X.-M.; Yu, H.; Palyha, O. C.; McKee, K. K.; Feighner, S. D.; Sirinathsinghji, D. J. S.; Smith, R. G.; Van der Ploeg, L. H. T.; Howard, A. D. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Mol. Brain Res.* **1997**, *48*, 23−29.
(188) Eichenbaum, H.; Otto, T.; Cohen, N. J. The hippocampus—what does it do? *Behav. Neural. Biol.* **1992**, *57*, 2−36.
(189) Pohorecki, R.; Domino, E. F. Synaptic pharmacology of the hippocampus. *Rev. Clin. Basic Pharm.* **1987**, *6*, 189−211.
(190) Fallon, J. G.; Loughlin, S. E. Substantia nigra. In *The Rat Nervous System*; Paxinos, G., Ed.; Academic Press: San Diego, 1995; pp 215−237.
(191) Halliday, G.; Harding, A.; Paxinos, G. Serotonin and tachykinin systems. In *The Rat Nervous System*; Paxinos, G., Ed.; Academic Press: San Diego, 1995; pp 929−974.
(192) Jansson, J. O.; Eden, S.; Isaksson, O. Sexual dimorphism in the control of growth hormone secretion. *Endocr. Rev.* **1985**, *6*, 128−150.
(193) Davis, S. L.; Ohlson, D. L.; Klindt, J.; Anfinson, M. S. Episodic growth hormone secretory patterns in sheep: relationship to gonadal steroid hormones. *Am. J. Physiol.* **1977**, *233*, E519.
(194) Miller, J. D.; Tannenbaum, G. S.; Colle, E.; Guyda, H. J. Daytime pulsatile growth hormone secretion during childhood and adolescence. *J. Clin. Endocrinol. Metab.* **1982**, *55*, 989−994.
(195) Chihara, K.; Minamitani, N.; Kaji, H.; Kodama, H.; Kita, T.; Fujita, T. Human pancreatic growth hormone-releasing factor stimulates release of growth hormone in conscious unrestrained male rabbits. *Endocrinology* **1983**, *113*, 2081.
(196) Gevers, E. F.; Wit, J. M.; Robinson, I. C. A. F. Growth, growth hormone (GH)-binding protein, and GH receptors are differentially regulated by peak and trough components of the GH secretory pattern in the rat. *Endocrinology* **1996**, *137*, 1013−1018.
(197) Lumpkin, M. D.; McDonald, J. K. Blockade of growth hormone-releasing factor (GRF) activity in the pituitary and hypothalamus of the conscious rat with a peptidic GRF antagonist. *Endocrinology* **1989**, *124*, 1522−1531.
(198) Tannenbaum, G. S.; Ling, N. The interrelationship of growth hormone (GH)-releasing factor and somatostatin in the generation of the ultradian rhythm of GH secretion. *Endocrinology* **1984**, *115*, 1952−1957.
(199) Plotsky, P. M.; Vale, W. Patterns of growth hormone-releasing factor and somatostatin into the hypophysial postal circulation of the rat. *Science* **1985**, *230*, 461−463.
(200) Chihara, K.; Minamitani, N.; Kaji, H.; Arimura, A.; Fujita, T. Intraventricularly injected growth hormone stimulates somatostatin release into rat hypophysial portal blood. *Endocrinology* **1981**, *109*, 2279−2281.
(201) Carlsson, L. M. S.; Jansson, J. O. Endogenous growth hormone (GH) secretion in male rats is synchronized to pulsatile GH infusions given at 3-hour intervals. *Endocrinology* **1990**, *126*, 6−10.
(202) Abe, H.; Molitch, M. E.; van Wyk, J. J.; Underwood, L. E. Human growth hormone and somatomedin C suppress the spontaneous release of growth hormone in unanesthetized rats. *Endocrinology* **1983**, *113*, 1319−1324.
(203) Clark, R. G.; Carlsson, L. M. S.; Robinson, I. C. A. F. Growth hormone (GH) secretion in the conscious rat: negative feedback of GH on its own release. *Endocrinology* **1988**, *119*, 201−209.
(204) Fletcher, T. P.; Thomas, G. B.; Willoughby, J. O.; Clarke, I. J. Constitutive growth hormone secretion in sheep after hypothalamopituitary disconnection and the direct *in vivo* pituitary effect

of growth hormone releasing peptide 6. *Neuroendocrinology* **1994**, *60*, 76−86.
(205) Dickson, S. L.; Doutrelant-Viltart, O.; McKenzie, D. N.; Dyball, R. E. J. Somatostatin inhibits arcuate neurons excited by GH-releasing peptide (GHRP-6) in rat hypothalamic slices. *J. Physiol. London* **1996**, *495*, 109−110.
(206) Wu, D.; Chen, C.; Zhang, J.; Bowers, C. Y.; Clarke, I. J. The effects of GH-releasing peptide-6 (GHRP-6) and GHRP-2 on intracellular adenosine 3′,5′-monophosphate (cAMP) levels and GH secretion in ovine and rat somatotrophs. *J. Endocrinol.* **1996**, *148*, 197−205.
(207) Locatelli, V.; Torsello, A.; Grilli, R.; Guidi, M.; Luoni, M.; Deghenghi, R.; Muller, E. E. Growth hormone-releasing peptides stimulate feeding idependently of their GH releasing activity. 1996 Growth Hormone Research Society Conference, London, U, November 13−16, 1996; O-063 (Abstract).

JM970342O