MINIREVIEW

Growth Hormone Secretion: Molecular and Cellular Mechanisms and *In Vivo* Approaches

LLOYD L. ANDERSON,*†,[1] SRDIJA JEFTINIJA,† AND COLIN G. SCANES*†

Department of Animal Science and †Department of Biomedical Sciences, Iowa State University, Ames, Iowa 50011

Growth hormone (GH) release is under the direct control of hypothalamic releasing hormones, some being also produced peripherally. The role of these hypothalamic factors has been understood by *in vitro* studies together with such *in vivo* approaches as stalk sectioning. Secretion of GH is stimulated by GH-releasing hormone (GHRH) and ghrelin (acting via the GH secretagogue [GHS] receptor [GHSR]), and inhibited by somatostatin (SRIF). Other peptides/proteins influence GH secretion, at least in some species. The cellular mechanism by which the releasing hormones affect GH secretion from the somatotrope requires specific signal transduction systems (cAMP and/or calcium influx and/or mobilization of intracellular calcium) and/or tyrosine kinase(s) and/or nitric oxide (NO)/cGMP. At the subcellular level, GH release (at least in response to GHS) is accomplished by the following. The GH-containing secretory granules are moved close to the cell surface. There is then transient fusion of the secretory granules with the fusion pores in the multiple secretory pits in the somatotrope cell surface. Exp Biol Med 229:291–302, 2004

Key words: growth hormone; growth hormones secretagogues; cellular mechanisms; signal transduction systems

Introduction

A series of stimulatory and inhibitory releasing hormones of hypothalamic and also peripheral origins controls the release of growth hormone (GH) from somatotropes. Until recently, the consensus was of two hypothalamic releasing hormones for GH, respectively GH-releasing hormone (GHRH; stimulatory GH) and somatostatin (SRIF; inhibitory) with GH release and synthesis reflecting a balance between these. *In vivo* approaches such as stalk sectioning provide strong evidence for the physiological control of GH release (discussed in detail below). There is at least one other hypothalamic releasing hormone for GH with the identification by Kojima and colleagues of ghrelin as the natural ligand for the GH secretagogue (GHS) receptor (GHS-R; Ref. 1). Moreover, in at least some species, GH secretion is influenced by other peptides/proteins produced in the hypothalamus and in the periphery, including gonadotropin releasing hormone (GnRH), insulin-like growth factor 1 (IGF-1), leptin, pituitary adenylate cyclase activating polypeptide (PACAP), and thyrotropin releasing hormone (TRH).

There is increasing knowledge of the subcellular events leading to GH release. This includes signal transduction mechanisms and the physical nature of GH secretory granules fusing transiently with the cell membrane. Recently, our laboratory, in collaboration with Bhanu Jena, established that secretory granules in the somatotrope are moved close to the cell surface and then fused with a specific organelle, the fusion pores being located in the secretory pits in the cell surface (2). The present article reviews GH secretion under the following topics:

1. hypothalamic hormones that influence GH release and synthesis (with particular attention to other peptides as well as GHRH, SRIF, and ghrelin),
2. specific signal-transduction systems,
3. cell biology for controlled GH secretion involving movement of secretory granules and fusion pores secretory pits of somatotropes,

This work was supported by research grant USDA NRI 2003-35206-12817, the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, and by Hatch Act and State of Iowa funds.

[1] To whom requests for reprints should be addressed at Department of Animal Science, Iowa State University, 2356 Kildee Hall, Ames, IA 50011-3150. E-mail: llanders@iastate.edu

1535-3702/04/2294-0000$15.00

4. *in vivo* approaches to understanding the physiology of GH secretion.

Where possible, examples from studies using domestic animals will be employed.

1. The Hypothalamic Hormones Controlling GH Release and Synthesis

a. Growth Hormone Releasing Factor (GHRH). The major function of GHRH, released from neurosecretory terminals in the median eminence, is to stimulate the release and synthesis of GH. GHRH is expressed in the arcuate nucleus of the hypothalamus together with other tissues, for example, intestine, gonads, immune tissues, and the placenta (reviewed in Ref. 3). GHRH immunoreactive (IR) neurons have been identified in coronal and sagittal frozen sections of bovine and porcine hypothalami (4). Rounded bipolar GHRH IR perikarya are localized in ventrolateral regions of the arcuate nucleus (ARC) in both species. GHRH-immunoreactive fibers projected ventrally into the median eminence (ME) in both species.

Signal transduction involves G_s-protein, adenylate cyclase (isoform II and/or IV), cyclic 3′, 5′adenosine monophosphate (cAMP), and protein kinase A. There is a cAMP response element (CRE) upstream from the coding region of the GH gene. GHRH increases GH expression (e.g., cattle, Ref. 5; chickens, Refs. 6, 7). Intracellular Ca^{2+} concentrations are increased by GHRH. This involves both influx of Ca^{2+} (via L- and T-type voltage-sensitive Ca^{2+} channels) and by phospholipase C hydrolysis of phosphatidyl inositol, leading to mobilizing intracellular Ca^{2+} stores (reviewed in Ref. 3).

b. Somatostatin (SRIF). Somatostatin (SRIF) is a cyclic 14- or 28-amino-acid residue containing peptide. A major function of SRIF is suppressing the release, but not synthesis, of GH by the somatotrope (reviewed in Ref. 8). SRIF-IR neurons have been identified in bovine and porcine hypothalamic tissue (4). Bipolar SRIF-IR perikarya are located about the third ventricle in the periventricular nucleus. SRIF-IR fibers project ventrally into the ME. SRIF-IR fibers densely innervate the ventromedial and arcuate (ARC) nuclei in pigs, but are not as distinguishable as in cattle (4). Double immunostaining reveals a close apposition of SRIF-IR fibers with GHRH-IR perikarya in the ARC and ventromedial nuclei and apposition of SRIF- and GHRH-IR varicosities in the ME (4).

The SRIF receptor (sstr) is encoded by five different genes (*sstr*1–5; reviewed in Ref. 8). The dominant sstr influencing GH release from the somatotrope appears to be *sstr*-2 (rat; Ref. 9). Signal transduction involves G-protein coupled reduction in L- and T-type voltage-sensitive Ca^{2+} influx/channels and increased K^+ channels (reviewed in Ref. 8).

c. Ghrelin. Ghrelin is a natural ligand for the GH-secretagogue receptor (GHS-R; Ref. 10). It is produced by the stomach, small intestine, and central nervous system; for example, the hypothalamus, specifically including the ARC (1, 10, 11). Ghrelin acts by binding to the GHS receptor. The GHS-R is a seven transmembrane domain G-protein coupled receptor (12). Signal transduction involves activation of phospholipase C (via G protein), generation of inositol phosphate and diacyl glycerol, and increased intracellular Ca^{2+} (reviewed in Ref. 13). Ghrelin stimulates GH release both directly, acting at the level of the anterior pituitary gland, and by enhancing GHRH release (for details, see Section 4). The GHS-R is expressed in various hypothalamic and thalamic nuclei, the dentate gyrus, substantial nigra, ventral tegmentum, and facial nuclei of the brainstem, thus implicating a possible central role for ghrelin (14–17). For instance, ghrelin plays a critically important role in the control of appetite, stimulating food intake.

Ghrelin, when injected intracerebroventricular (icv) into rodents, stimulates feeding behavior and an increase in body weight (18, 19). Synthetic GHSs and the endogenous hormone, ghrelin, induce immediate gene c-*fos* only in the ARC, the site that also contains neuropeptide Y (NPY; Refs. 20–22). Additionally, the ARC and NPY neurons possess GHS-R mRNA, and these neurons also project to orexin- and melanin-concentrating hormone (MCH)—containing neurons in the lateral hypothalamus, neurons that affect feed intake (23, 24). The effects of ghrelin on feed intake appear to be mediated via NPY. Preadministration of the Y1 NPY receptor antagonist (BIB03304) blocks both the stimulatory effects of icv injection of ghrelin or GHRP-6 on food intake in rats, and the decreased body-core temperature (25, 26). Thus, NPY neurons of the ARC are likely a primary site for ghrelin increasing food intake.

It might be speculated that motilin may have analogous effects to ghrelin. Motilin is a highly conserved, 22-amino-acid polypeptide (27) secreted by enterochromaffin cells of the small intestine. This peptide stimulates gastrointestinal motor activity and seems to play a physiological role in the regulation of fasting motility patterns (28). Both IR motilin and mRNA expression are found in the gastrointestinal tract, brain, nerves, and other endocrine glands in several species, including monkey, man, pig, sheep, and rabbit (29). The motilin receptor, designated motilin-R1A (MTL-R1A), is a heterotrimeric, guanosine triphosphate-binding, protein (G protein)-coupled receptor. It was isolated from human stomach, and its amino acid sequence was found to be 52% identical to the human GHSR (30). The high amino-acid-sequence identity between MTL-R1A and the GHSR suggests a role for motilin in the control of GH secretion.

d. Other Hypothalamic Peptides Influencing GH Release Directly. There are multiple peptides produced by the hypothalamus and periphery that can influence GH release directly. This section will exclude the role of peptides within the hypothalamus and the role of insulin-like growth factors and their binding proteins. The effects of these hypothalamic peptides, some of which are

predominantly produced peripherally, may be biologically relevant or pharmacological. However, it is questioned whether the physiological control of GH secretion is much more complex than the two or three releasing hormone model previously envisioned.

i. Thyrotropin-Releasing Hormone (TRH). The modified tripeptide, TRH, can stimulate the release of GH in many species under some, but not under all, physiological circumstances. For instance, in domestic animals, TRH stimulates GH release in cattle (31, 32), fetal pigs (33), and young chickens (34). Moreover, TRH potentiates the effect of GHRH on GH in young chickens (35, 36) and also postnatal pigs (37). In the case of the latter, the magnitude of the potentiation diminished with age (37). In contrast, administration of TRH had little discernible effect in sheep (fetus or neonates, Ref. 38; lambs, Ref. 39) or postnatal pigs (37) or some fish (turbot, Ref. 40). In contrast, in adult horses, there seems to be an unusual situation with TRH inhibiting provoked GH release. Administration of TRH *per se* does not influence circulating concentrations of GH in horses (41). However, unexpectedly, TRH reduces the GH secretory response to GHRH (42) and blocks the effects of various physiological stimulators of GH release, including exercise and asparate administration (43). Just as TRH has direct stimulatory effects on GH release in the chicken and augments GHRH-induced GH release acting at the level of the somatotrope in the pituitary gland (see above), analogous inhibitory effects can be envisioned in the horse.

ii. Pituitary Adenylate Cyclase-Activating Peptide (PACAP). Pituitary adenylate cyclase-activating peptide (PACAP) is a potent GH secretagogue, and has even been proposed as the ancestral releasing factor for GH (reviewed in Ref. 44). In mammals, PACAP 27 and PACAP 38 stimulate GH release *in vitro* (GH_3 cells, Ref. 45; bovine and porcine somatotropes, Refs. 46, 47) and *in vivo* (cattle, Ref. 48). In addition, PACAP increases the synthesis of GH as indicated by elevated GH mRNA levels (pig, Ref. 47). In addition, PACAP 27 and 38 have also been demonstrated to stimulate GH release from pituitary cells of birds (chickens, Ref. 49) and fish (50–52).

Based on studies with rat somatotropes, the signal transduction mechanism, via binding to the PACAP type II receptor, for PACAP stimulation of adenyl cyclase/cAMP/protein kinase A, increases calcium entry via L-type, voltage-sensitive, calcium channels into the cells (44, 53, 54). Pharmacological blockers to phospholipase C-IP3-protein kinase C only partially depress effectiveness of PACAP (55). The signal-transduction mechanism appears to be conserved through vertebrate evolution as PACAP acts via PACAP receptors, cAMP and phospholipase C-IP3-protein kinase C, and voltage-sensitive calcium channels in goldfish (52). There is also evidence for the involvement of tetrodotoxin-sensitive sodium channels in the cellular response to PACAP (56).

iii. Gonadotropin-Releasing Hormone. In higher vertebrates, gonadotropin-releasing hormone (GnRH) does not stimulate GH release. However, in some species, at specific physiological phases, GnRH can evoke GH secretion. For instance, GnRH stimulates GH release from pituitary cells from neonatal rats (57). In fish, GnRH markedly stimulates GH secretion (e.g., goldfish, Refs. 58, 59; carp, Ref. 60). While GnRH acts via cAMP, there is also evidence that a nitric oxide mechanism may also be involved (61).

iv. Leptin. The effects of leptin on GH release appear to be predominantly stimulatory. The presence of leptin receptors in/on somatotropes is well established (e.g., Refs. 62–65). There are reports of leptin *per se* influencing GH release, either stimulating (66, 67) or increasing the responsiveness to GHRH (e.g., Refs. 67–70), or inhibiting (71) or reducing responsiveness to GHRH, for example, in human pituitary adenoma (72). The signal transduction mechanism for leptin appears to be predominantly via JAK II and STAT (reviewed in Ref. 72). In addition, not only does leptin increase nitric oxide (NO) production by pituitary cells, but also an NO donor-stimulated GH release. Inhibitors of nitric oxide synthesis from L-arginine (Ng-monomethyl-L-arginine) inhibited both basal and leptin-induced GH release (73). This would be consistent with leptin acting via NO and NO-sensitive soluble guanylase cyclase.

v. Others (FMRF Amide, Corticotropin-Releasing Hormone, Galanin, and NPY). Studies, particularly in lower vertebrate species, suggest that somatotropes are promiscuous in terms of the peptides that will stimulate GH release. In some species of lower vertebrates, corticotropin-releasing hormone (CRH) has been reported to stimulate GH secretion (e.g., reptiles, Ref. 74; fish, Ref. 75). Neuropeptide Y has a direct effect of GH release in some species of fish (76, 77). In contrast, galanin can inhibit GH release (78–80). Moreover, peptides related to the neuropeptide Phe-Met-Arg-Phe-NH_2 (FMRF amide), originally described in mollusks, stimulate GH release in amphibians. Similar peptides have been identified in brains of vertebrates (i.e., rat, chicken, frog, carp) with a similar RF amide motif at their C-termini (81–84). A novel hypothalamic RF amide peptide localized in the hypothalamus of the bullfrog has GH-releasing activity and was designated frog GH-releasing peptide (fGRP; Ref. 85).

2. Signal Transduction Mechanism Systems

a. Adenylate Cyclase/cAMP/Protein Kinase A and Phospholipase C-IP3-Protein Kinase C. Somatotropes, excitable cells that exhibit spontaneous action potential, when treated with GHRH, depolarize the cell membrane potential and stimulate Ca^{2+} influx via transmembrane Ca^{2+} channels, resulting in increases in intracellular free Ca^{2+} ($[Ca^{2+}]$; Ref. 86). This involves both an influx of Ca^{2+} (via L- and T-type voltage-sensitive Ca^{2+} channels) and phospholipase C hydrolysis of phosphatidyl inositol, leading to mobilizing intracellular Ca^{2+} stores (reviewed in Ref. 3). It is well established that the signal

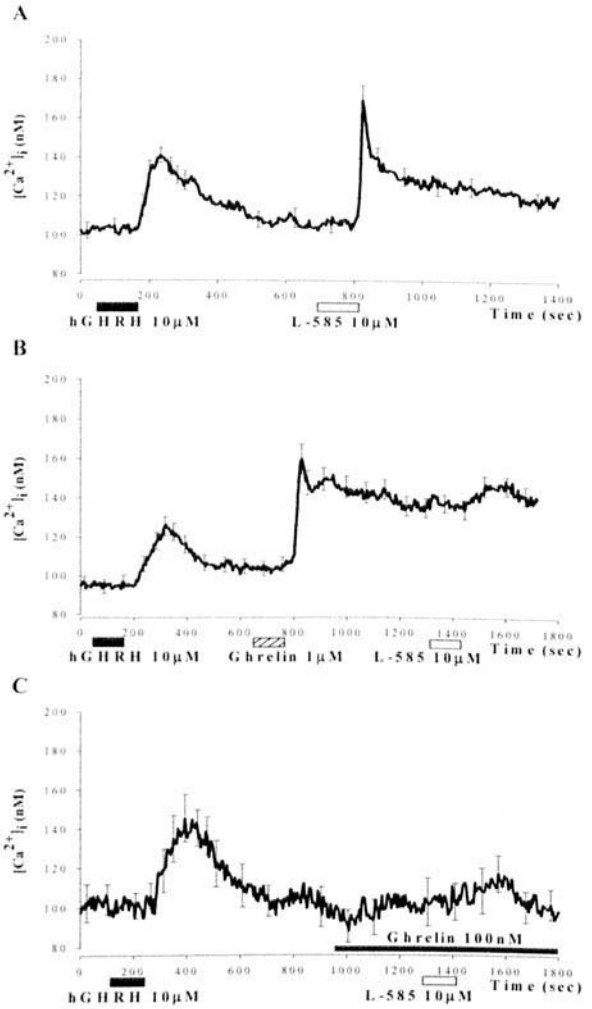


Figure 1. Inhibitory effects of ghrelin on calcium transients evoked by L-585 in isolated porcine somatotrophs. (a) Control studies on calcium transients in isolated porcine somatotropes evoked by a 2-min application of human growth hormone releasing-hormone (hGHRH) (10 μ*M*) and L-585 (10 μ*M*; *n* = 29). (b) Application of L-585 (10 μ*M*) 10 mins after administration of ghrelin (1 μ*M*) did not have an additive effect on $[Ca^{2+}]_i$ in isolated porcine somatotropes (*n* = 58). (c) Ghrelin (100 μ*M*) alone did not affect $[Ca^{2+}]_i$, but the changes in $[Ca^{2+}]_i$ evoked by 10 μ*M* L-585 were almost completely blocked (*n* = 7). (Reprinted from *Neuroendocrinology*, Vol. 77, Glavaski-Joksimovic A, Jeftinija K, Scanes CG, Anderson LL, Jeftinija S. Stimulatory effect of ghrelin on isolated porcine somatotropes, pp366–378. Copyright 2003, with permission from Karger, Basel.)

transduction for GHRH, and probably PACAP, involves the following:

1. binding to specific receptors in the plasma membrane of the somatotropes,
2. activation of adenylate cyclase (isoform II and/or IV) via the coupling protein, G_s-protein cyclic 3′, 5′adenosine monophosphate (cAMP),
3. activation of protein kinase A. In addition, there is activation of phospholipase C-IP3-protein kinase C and voltage-sensitive calcium channels.

Signal transduction for ghrelin and synthetic analogues or GHS (e.g., GHRP-6 and nonpeptidergic GHS such as L-692, 585, MK-677) involves activation of phospholipase C (via G protein), generation of inositol phosphate and diacyl glycerol, and increased intracellular Ca^{2+} (reviewed in Ref. 13). Modification of the somatotrope's electrophysiological properties leads to changes in sensitivity to ghrelin or its analogues. The GHRP-6 increases pituitary-specific transcription factor expression in somatotropes, but the mechanism by which ghrelin analogues exert these effects on the pituitary gland is not clear. K^+ conductance is the predominant membrane ion conductance at resting membrane potential, which regulates cell excitability (87). Long-term (48-hr) treatment with GHRP-2 increased excitability of ovine somatotropes and the levels of voltage-gated K^+ currents. The increase in the voltage-gated K^+ currents requires a functional protein kinase C (PKC) system and is achieved through an increase in K^+ channel protein synthesis (87). Down-regulation of PKC with phorbol 12-myristate 13-acetate at the time of adding GHRP-2 blocked the increase in K^+ currents. Calphostin C (PKC inhibitor), but not H_{89} (protein kinase A inhibitor), reduced the increase in K^+ currents by GHRP-2, whereas inclusion of actinomycin D (transcription inhibitor) or cycloheximide (protein synthesis inhibitor) abolished the increase in K^+ currents. Thus, GH secretagogues increase the excitability of somatotropes and the voltage-gated K^+ currents on the membrane. The increase in the synthesis of K^+ currents is achieved through an increase in synthesis of K^+ channels, and normal function of the PKC system is required for the increase in voltage-gated K^+ currents.

We have recently demonstrated, using pharmacological blockers, that both ghrelin and a synthetic GHS can increase intracellular calcium concentrations in porcine somatotrope (88, 89). This two-phase phenomenon involves, first, mobilization of intracellular calcium, followed by calcium entry via L channels with somatotrope depolarization (see Fig. 1; Refs. 88, 89). The effects of GHS are blocked by inhibitors of adenylate cyclase and phospholipase C, supporting the involvement of both these, adenylate cyclase/cAMP/protein kinase A and phospholipase C; in the generation of inositol phosphate and diacyl glycerol and increased intracellular Ca^{2+}.

b. Nitric Oxide. There is evidence that nitric oxide (NO) and cGMP may mediate GH release induced by some secretagogues. *In vivo*, infusion of a precursor of NO, L-arginine, slightly elevated circulating concentrations of GH and increased the response to GHRH (90). Moreover, an inhibitor of NO synthesis, Nω-nitro-L-arginine methyl ester (NAME), suppressed GHRH-induced GH release (90). There is also evidence for guanylyl cyclase activity in somatotropes and increased cellular concentrations of cGMP following GHRH *in vitro* (91). Moreover, leptin increases NO production and GH release by pig pituitary glands (92). In fish, sodium nitroprusside (NO donor) increases GH secretion but not in the presence of an inhibitor of NO-sensitive soluble guanylase cyclase or NO scavengers (61).

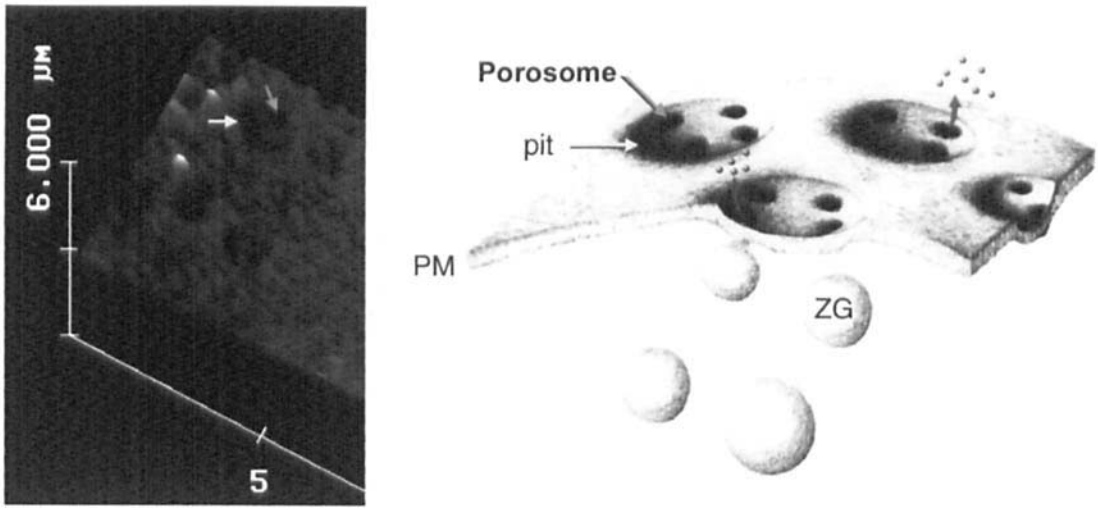


Figure 2. Porosome 1 demonstrates an atomic-force microscopy (AFM) micrograph of the plasma membrane at the apical end of live pancreatic acinar cells, demonstrating the presence of circular pits (yellow arrow) and the 100–150-nm diameter porosomes (blue arrow) within. The right panel is a schematic diagram depicting the porosomes within pits, at the base of which secretory vesicles (ZG) dock and fuse to release vesicular contents. (Courtesy of Bhanu P. Jena.)

3. Cellular Mechanism for GH Release

Physiological processes, such as neurotransmission, and the secretion of hormones or enzymes, require fusion of membrane-bounded secretory vesicles at the cell plasma membrane and the consequent expulsion of vesicular contents. This final step in the secretory process was little understood. It was believed that, following stimulation of secretion, membrane-bounded secretory vesicles dock and completely incorporate at the cell plasma membrane, to be retrieved by endocytosis later. This would allow the intravesicular contents to be released passively. This common model of exocytosis and endocytosis operating in cellular secretion appeared fine at first; however, severe logistic questions arose. Besides problems of rates and energy requirements for the process, the appearance of empty and partially empty secretory vesicles immediately following secretion were unexplained by the model. Finally, after almost half a century, the molecular mechanism of this vital cellular process has been resolved (2, 93–96).

The molecular mechanism of cellular secretion has recently been resolved by a series of exquisite studies from Bhanu Jena's laboratory (2, 93–96). These studies reveal,

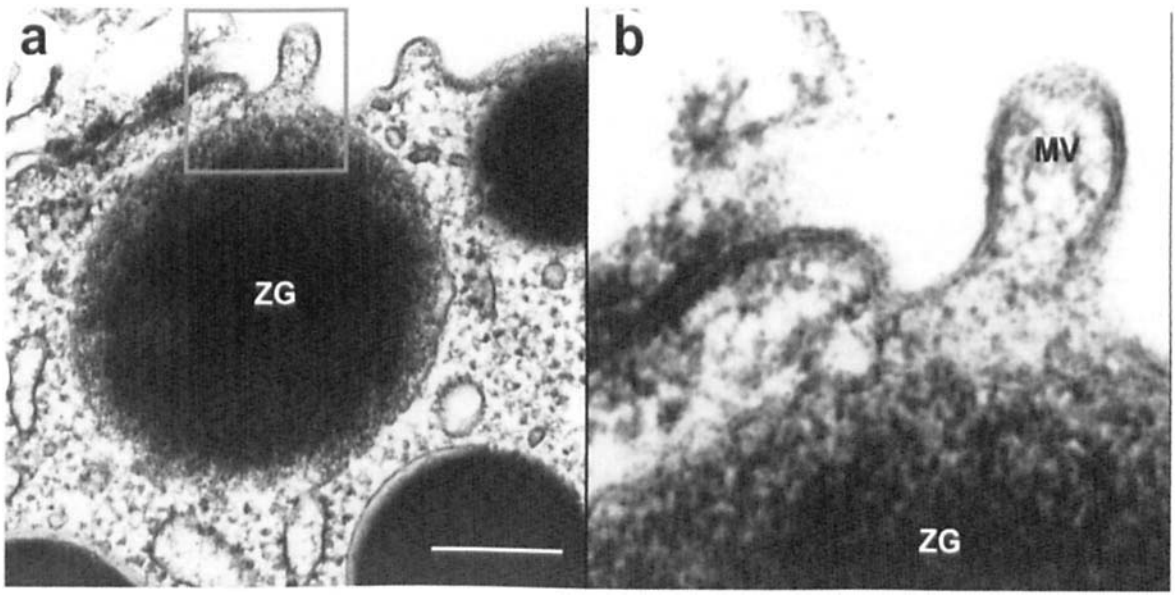


Figure 3. Transmission electron micrograph of a porosome associated with a docked secretory vesicle at the apical end of a pancreatic acinar cell. (a) Part of the apical end of a pancreatic acinar cell demonstrating, within the green bordered square, the presence of a fusion pore or porosome and an associated zymogen granule (ZG), the electron-dense secretory vesicle of the exocrine pancreas. (Bar = 400 nm.) The area within the green square in (a) has been enlarged to show the apical microvilli (MV) and a section through porosome and the ZG. Note the ZG membrane bilayer is attached directly to the base of the porosome cup. (Courtesy of Bhanu P. Jena.)

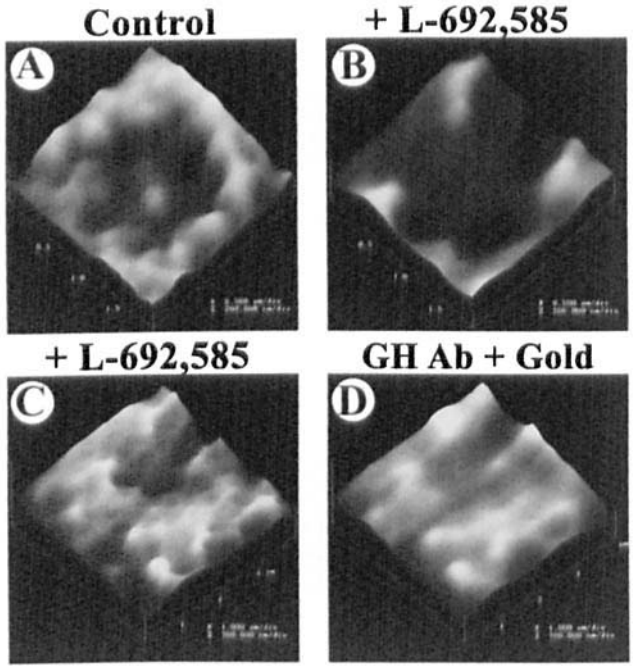
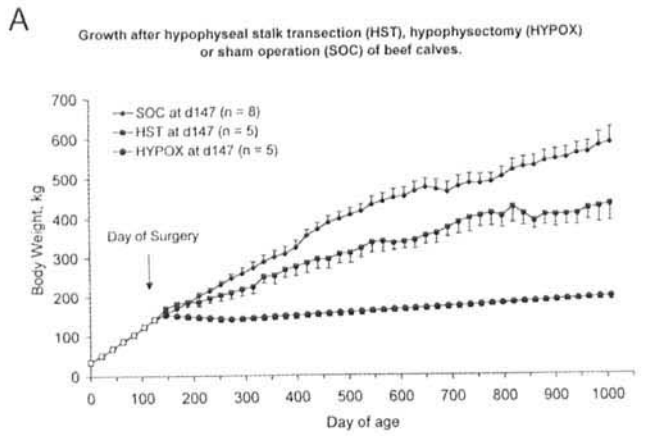
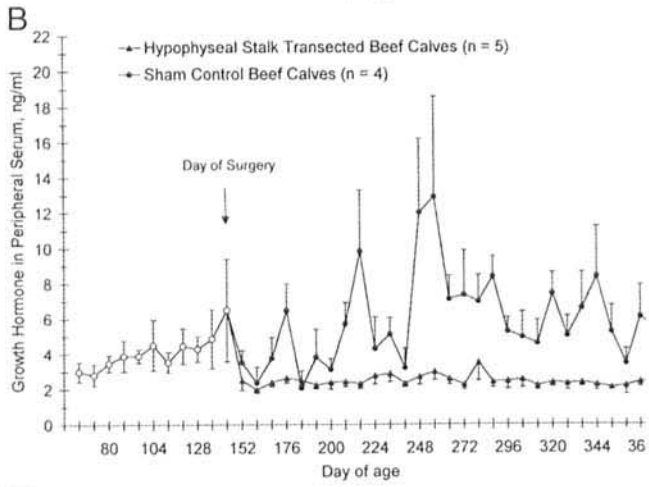
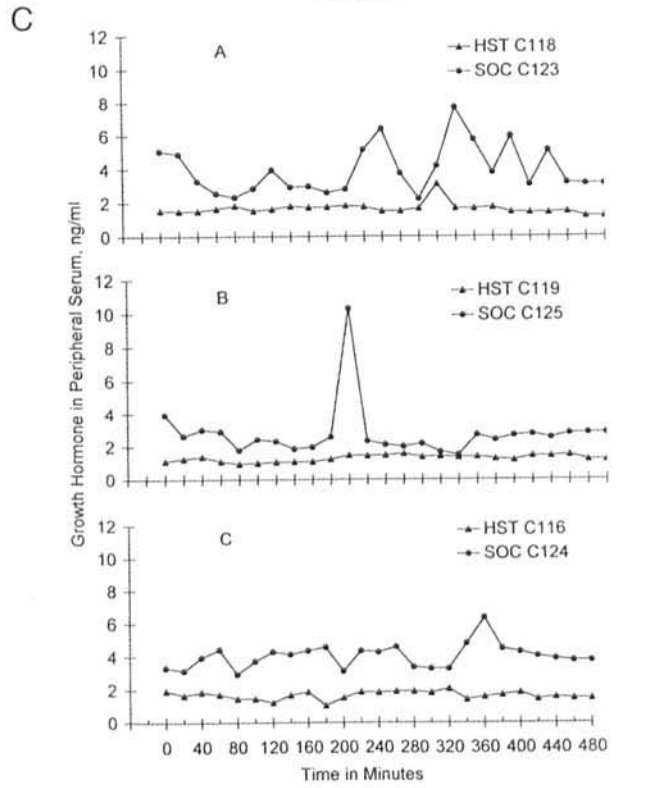


Figure 4. High-resolution atomic-force microscopy (AFM) performed on resting and stimulated growth hormone (GH)-secreting cells. AFM micrographs of a pit with depressions in resting cell (A), clearly demonstrating the enlargement of depressions or fusion pores following stimulation of secretion (B). Exposure of pits in a stimulated cell (C) to 30-nm gold-tagged GH antibody, results in binding of released growth hormone at the site to 30-nm gold-tagged GH antibody (D). Note the loss of fusion pores due to large amounts of gold-tagged antibody binding at these sites. (Reprinted from *Endocrinology*, Vol. 143, Cho SJ, Jeftinija K, Glavaski A, Jeftinija S, Jena BP, Anderson LL. Structure and dynamics of the fusion pores in live GH-secreting cells revealed using atomic force microscopy, pp1144–1148. Copyright 2002, with permission from The Endocrine Society, Chevy Chase, MD.)







→

Figure 5A. Growth in beef calves subjected to hypophysectomy (●), hypophyseal stalk transection (■), or sham operation (◆) at 146 ± 2 days of age. Birth weight averaged 35 ± 1 kg with subsequent body weights at 21-day intervals. Number of calves is indicated *in parentheses.* Values are mean ± SE. (Reprinted from *Endocrinology*, Vol. 140, Anderson LL, Hard DL, Trenkle AH, Cho SJ. Long-term growth after hypophyseal stalk transection and hypophysectomy of beef calves, pp2405–2414. Copyright 1999, with permission from The Endocrine Society, Chevy Chase, MD.)

Figure 5B. GH concentration in peripheral serum at 4-day intervals before surgery (○) and after hypophyseal stalk transection (HST) (▲) and sham-operated control (SOC) (●) of beef calves to day 372 of age. Number of calves is indicated *in parentheses.* Values are mean ± SE. (Reprinted from *Endocrinology*, Vol. 140, Anderson LL, Hard DL, Trenkle AH, Cho SJ. Long-term growth after hypophyseal stalk transection and hypophysectomy of beef calves, pp2405–2414. Copyright 1999, with permission from The Endocrine Society, Chevy Chase, MD.)

Figure 5C. Growth hormone (GH) concentration in peripheral serum from sequential blood samples at 20-min intervals during 8 hrs in hypophyseal stalk transection (▲) and sham-operated control (●) beef calves. The number denotes the individual calf ear tag identification. (Reprinted from *Endocrinology*, Vol. 140, Anderson LL, Hard DL, Trenkle AH, Cho SJ. Long-term growth after hypophyseal stalk transection and hypophysectomy of beef calves, pp2405–2414. Copyright 1999, with permission from The Endocrine Society, Chevy Chase, MD.)

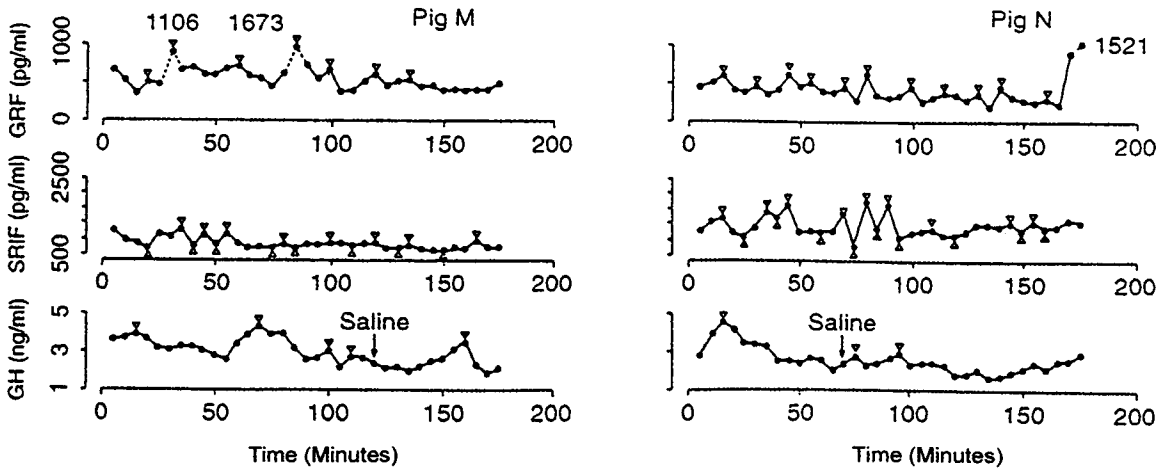


Figure 6. Growth hormone-releasing factor (GRF) and somatostatin (SRIF) concentration in hypophyseal portal blood and growth hormone (GH) concentration in jugular blood of 2 of 6 conscious, castrated pigs. Each sample represents a 5-min continuous collection. The sampling period was approximately 175 mins for each pig. Significant pulses and SRIF troughs, detected using ULTRA, are indicated by △ and □, respectively. Missing samples are shown as breaks in the splined data points. Values above the *y*-axis scale are connected by dotted lines. (Reprinted from *Proceedings of the Society for Experimental Biology and Medicine*, Vol. 217, Drisko JE, Faidley TD, Chang CH, Zhang D, Nicolich S, Hora DF Jr, McNamara L, Rickes E, Abribat T, Smith RG, Hickey GJ. Hypophyseal-portal concentrations of growth hormone-releasing factor and somatostatin in conscious pigs: relationship to production of spontaneous growth hormone pulses, pp188–196. Copyright 1998, with permission from the Society for Experimental Biology and Medicine, Maywood, NJ.)

for the first time, the existence of a new cellular structure at the cell plasma membrane, termed porosome. The porosome is a basket-like structure at the cell plasma membrane, having a 100–150 nm diameter opening to the cell exterior (see Fig. 2). Following stimulation of secretion, membrane-bound secretory vesicles dock and transiently fuse at the base of the porosome, expelling vesicular contents. In live cells, stimulation of secretion causes the porosome opening to dilate (20%–35%), returning to resting size following completion of the process (97). Exposure of cells to cytochalasin B, a fungal toxin that inhibits actin polymerization, resulted in a 15%–20% decrease in porosome size, and a consequent 50%–60% loss in stimulated secretion (97). Target SNAREs, or t-SNAREs, present at the base of the porosome interact with vesicle SNARE, or v-SNARE, in a circular array to form a conducting channel, thus establishing continuity between the two compartments (2). Concurrently, following stimulation of secretion, secretory vesicles swell (98, 99), resulting in a buildup of turgor pressure for the expulsion of vesicular contents through the t-/v-SNARE channel and the porosome to the cell exterior. Both the molecular mechanism of vesicle swelling (98, 99) and the molecular structure and partial composition of the porosome (95, 96), have been elucidated (see Figs. 2 and 3). Furthermore, the native porosome has been isolated and functionally reconstituted in liposomes (96).

It was commonly thought that exocytosis required the total incorporation of secretory vesicle membrane with the cell plasma membrane for expulsion of vesicular contents; however, transmission electron microscopy (TEM) reveals that this rarely occurs. On the contrary, TEM studies demonstrate that, following stimulation of secretion, there is a presence of intact as well as empty, or partly empty, secretory vesicles (100). Using atomic force microscopy (AFM), the existence of the fusion pore in somatotropes and other endocrine cells together with exocrine cells was demonstrated (see Fig. 4; Refs. 2, 93, 94, 96, 98, 100). Moreover, the structure and dynamics of the fusion pore have been examined at near nanometer resolution and in real time. The composition of the fusion pore or porosome has been characterized in recent studies using AFM and immuno-AFM studies on isolated plasma membrane preparations (95). Immunoblot analysis of the immuno-isolated fusion pore revealed the presence of several isoforms of the proteins identified earlier, in addition to the association of chloride channels (95, 96). The TEM and AFM micrographs of the immuno-isolated fusion pore complex were superimposable, allowing its detailed structure to be revealed (96). Fusion pores have been reconstituted into liposomes and examined by TEM. These exhibit the typical cup-shaped, basketlike morphology, and are functional, as is demonstrated by their ability to fuse with isolated secretory vesicles (96).

Using AFM and live pancreatic acinar cells, fusion pores have been shown to have a diameter of 100–180 nm and dilate only 25%–35% during exocytosis (93, 97). A parallel study using TEM showed no loss of secretory vesicle number after exocytosis (93). In a recent study using AFM, we, in collaboration with Bhanu Jena, have identified similar structures at the plasma membrane of live somatotropes and implicated their involvement in GH release (see Fig. 4; Ref. 100). Pits containing 100–200-diameter depressions or fusion pores were identified in resting GH-secreting cells. Following stimulation of GH secretion by a ghrelin agonist, the size of the fusion pore increased 40% and gold-tagged GH antibody was found to bind to the pit

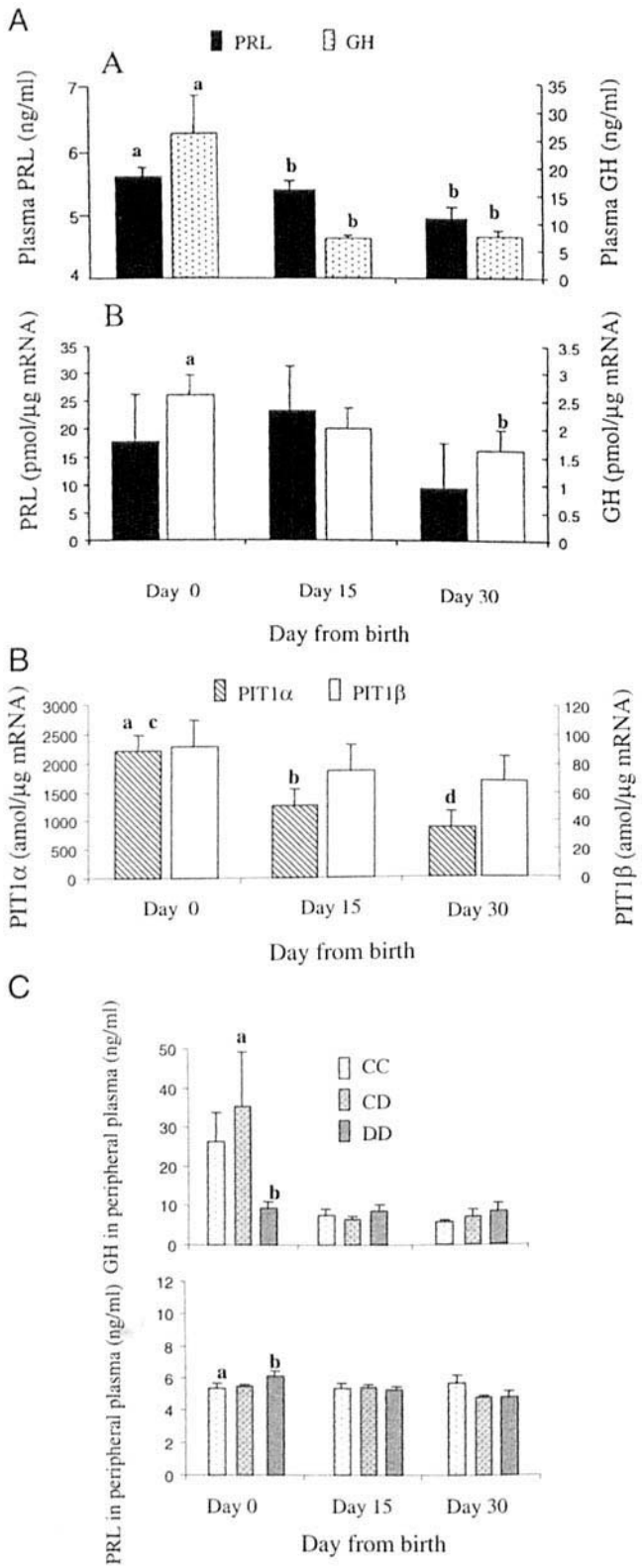


Figure 7A. Quantitative measurement of plasma prolactin (PRL) and growth hormone (GH) in Meishan pigs at Days 0, 15, and 30. Different letters within the same panel indicate significant differences ($P < 0.05$). (Reprinted from *Animal Reproduction Science*, Vol. 69, Sun HS, Anderson LL, Yu TP, Kim KS, Klindt J, Tuggle CK. Neonatal Meishan pigs show POU1F1 genotype effects on plasma GH and PRL concentration, pp223-237. Copyright 2002, with permission from Elsevier, Amsterdam.)

structures in these stimulated somatotropes. The fate of secretory vesicles has been examined in porcine somatotropes by TEM (101). Our study reveals that, after stimulation, membrane-bounded secretory vesicles transiently dock and fuse at the fusion pore to release vesicular contents and then are recycled. The number of secretory vesicles remained unchanged following GH release. It is clear that secretion of GH and presumably other protein hormones is due to transient fusion of secretory granules with the fusion pores or porosomes that are located in pits on the surface of the plasma membrane.

4. *In Vivo* Approaches to Understanding the Physiology of GH Secretion

Among the most powerful techniques used to examine the physiological controls of the anterior pituitary gland is hypophyseal stalk sectioning such that the influences of the hypothalamus are no longer present. There is direct evidence from such studies from pigs and cattle for a balance between stimulatory and inhibitory controls for GH release. This surgery reduces the GH content of the pituitary, but increases responsiveness to GHRH (see Fig. 5, A–C; Refs. 102–106). An interpretation of these observations is that the reduced pituitary GH content reflects depressed GH synthesis due to decreased GHRH stimulation. The increased responsiveness to GHRH results from lack of SRIF inhibition following stalk sectioning. In contrast, the nonpeptidal ghrelin agonist, L-692,585, was much less effective in eliciting GH release in pigs where the hypophyseal stalk had been surgically sectioned (and hence drastically reducing GHRH and SRIF bathing the pituitary; Ref. 107). This is consistent with ghrelin acting directly, either synergistically with GHRH on the somatotropes, or, more likely, exerting its major effect at the level of the hypothalamus by stimulating the release of GHRH and/or inhibiting the release of SRIF. Administration of a ghrelin agonist (the hexapeptide GH-releasing peptide) to sheep has been reported to enhance both GH release and the GHRH concentrations in the portal blood (108).

←

Figure 7B. Quantitative measurement of mRNA levels in Meishan pigs for POU1F1-α and POU1F1-β at Days 0, 15, and 30. Letters within the same panel indicate differences of $P < 0.05$ (a, b) or $P < 0.001$ (c, d). (Reprinted from *Animal Reproduction Science*, Vol. 69, Sun HS, Anderson LL, Yu TP, Kim KS, Klindt J, Tuggle CK. Neonatal Meishan pigs show POU1F1 genotype effects on plasma GH and PRL concentration, pp223-237. Copyright 2002, with permission from Elsevier, Amsterdam.)

Figure 7C. Statistical analysis identified a significant effect of POU1F1 genotype in Meishan pigs on Day 0 GH and PRL plasma hormone concentrations. Different letters within the same panel indicate significant differences ($P < 0.05$). (Reprinted from *Animal Reproduction Science*, Vol. 69, Sun HS, Anderson LL, Yu TP, Kim KS, Klindt J, Tuggle CK. Neonatal Meishan pigs show POU1F1 genotype effects on plasma GH and PRL concentration, pp223-237. Copyright 2002, with permission from Elsevier, Amsterdam.)

Another *in vivo* approach facilitates the determination of GHRH and SRIF concentrations in hypothalamo-hypophyseal portal blood vessels. Using a transorbital approach to the porcine pituitary (109), portal blood and jugular blood were collected sequentially for measurement of GHRH, SRIF, and GH concentrations (110). This is the first report of hypophyseal portal blood concentration of GHRH and SRIF in the conscious pig, and it revealed that there is a pulsatile pattern of release of GHRH and SRIF. Even though GH concentrations are similar across species, pigs have higher portal GHRH and SRIF concentrations compared to rats and sheep (111–114). Of 27 spontaneous GH pulses detected in 1050 mins among six pigs, GH peaks occurred nonrandomly within periods of portal SRIF descent (63%), whereas ascent of portal GHRH occurred in 48% in association with GH pulse maxima (Fig. 6; Ref. 110). The results suggest a close relationship with SRIF in GH pulse generation in the pig, but mediation of GH release cannot be explained simply by antagonism between GHRH and SRIF.

In vivo models coupled with molecular approaches have provided insights into the role of a member of the POU-domain family of genes, POU1F1 (also previously known as PIT-1 or GHF1) in the control of GH. For instance, Snell dwarf (*dw*) and Jackson dwarf (*dw-J*) mice have independent mutations in the POU1F1 gene that cause a lack of somatotropes, lactotropes, and thyrotropes (115). A role of POU1F1 in the control of GH expression is indicated from polymorphisms at POU1F1 that are associated with growth and other performance traits (i.e., fatness, carcass composition) in the pig (e.g., 116). In Chinese Meishan pigs, segregating a *MspI* POU1F1 polymorphism, there were effects on GH mRNA and on the circulating concentrations of GH at birth, but not thereafter (see Fig. 7, A–C; Ref. 117).

Conclusions

While there have been tremendous advances in our understanding of the control of GH secretion, many questions remain. These include the following:

1. Why are there multiple releasing hormones for GH?
2. Do all somatotropes respond to all secretagogues?
3. Is there a localized signal transduction mechanism(s)?
4. Are the receptors for releasing hormones arranged in close proximity to fusion pores?

1. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing peptide from stomach. Nature 402:656–660, 1999.
2. Cho SJ, Wakade A, Pappas GD, Jena BP. New structure involved in transient membrane fusion and exocytosis. Ann N Y Acad Sci 971: 254–256, 2002.
3. Frohman LA, Kineman RD. Growth hormone-releasing hormone: discovery, regulation, and actions. In: Kostyo JL, Goodman HM, Eds. Handbook of Physiology, Section 7, The Endocrine System, Vol V: Hormonal Control of Growth. American Physiological Society, New York: Oxford University Press. pp187–219, 1999.
4. Leshin LS, Barb CR, Kiser TE, Rampacek GB, Kraeling RR. Growth hormone-releasing hormone and somatostatin neurons within the porcine and bovine hypothalamus. Neuroendocrinology 59:251–264, 1994.
5. Silverman BL, Kaplan SL, Grumbach MM, Miller WL. Hormonal regulation of growth hormone secretion and messenger ribonucleic acid accumulation in cultured bovine pituitary cells. Endocrinology 122:1236–1241, 1988.
6. Vasilatos-Younken R, Tsao PH, Foster DN, Smiley DL, Bryant H, Heiman ML. Restoration of juvenile baseline growth hormone secretion with preservation of the ultradian growth hormone rhythm by continuous delivery of growth hormone-releasing factor. J Endocrinol 135:371–382, 1992.
7. Radecki SV, Deaver DR, Scanes CG. Triiodothyronine reduces growth hormone secretion and pituitary growth hormone mRNA in the chicken, *in vivo* and *in vitro*. Proc Soc Exp Biol Med 205:340–346, 1994.
8. Tannenbaum GS, Epelbaum J. Somatostatin. In: Kostyo JL, Goodman HM, Eds. Handbook of Physiology, Section 7, The Endocrine System, Vol V: Hormonal Control of Growth. American Physiological Society, New York: Oxford University Press, pp221–265, 1999.
9. Reed DK, Korytko AI, Hipkin RW, Wehrenberg WB, Schonbrunn A, Cuttler L. Pituitary somatostatin receptor (*sst*)1–5 expression during rat development: age-dependent expression of *sst*2. Endocrinology 140:4739–4744, 1999.
10. Lee HM, Wang G, Englander EW, Kojima M, Greeley GH Jr. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. Endocrinology 143:185–190, 2002.
11. Masuda Y, Tanaka T, Inomata N, Ohnuma N, Tanaka S, Itoh Z, Hosoda H, Kojima M, Kangawa K. Ghrelin stimulates gastric acid secretion and motility in rats. Biochem Biophys Res Commun 276: 905–908, 2000.
12. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffion PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LHT. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977, 1996.
13. Bowers CY. Growth hormone-releasing peptides. In: Kostyo JL, Goodman HM, Eds. Handbook of Physiology, Section 7, The Endocrine System, Vol V: Hormonal Control of Growth. American Physiological Society, New York: Oxford University Press, pp267–297, 1999.
14. Bennet PA, Thomas GB, Howard AD, Feighner SD, Van Der Ploeg LH, Smith RG, Robinson IC. Hypothalamic growth hormone secretagogue-receptor (GHS-R) expression is regulated by growth hormone in the rat. Endocrinology 138:4552–4557, 1997.
15. Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van Der Ploeg LH, Howard AD. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Brain Res Mol Brain Res 48:23–29, 1997.
16. Yokote R, Sato M, Matsubara S, Ohye H, Niimi M, Murao K, Takahara J. Molecular cloning and gene expression of growth hormone-releasing peptide receptor in rat tissues. Peptides 19:15–20, 1998.
17. Mitchell V, Bouret S, Beauvillian JC, Schilling A, Perret M, Kordan C, Epelbaum J. Comparative distribution of mRNA encoding the growth hormone secretagogue-receptor (GHS-R) in *Microcebus murinus* (primate, lemurian) and rat forebrain and pituitary. J Comp Neurol 429:469–489, 2001.

18. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. Nature 409:194–198, 2001.
19. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 407:903–913, 2000.
20. Dickson SL, Leng G, Robinson IC. Systemic administration of growth hormone-releasing peptide activates hypothalamic arcuate neurons. Neuroscience 53:303–306, 1993.
21. Hewson AK, Dickson SL. Systemic administration of ghrelin induces Fos and Egr-1 proteins in the hypothalamic arcuate nucleus of fasted and fed rats. J Neuroendocrinol 12:1047–1049, 2000.
22. Dickson SL, Luckman SM. Induction of c-*fos* messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6. Endocrinology 138:771–777, 1997.
23. Broberger C, DeLecea L, Sutcliffe JC, Hokfelt T. Hypocretin/orexin- and melanin-concentrating hormone-expressing cells from distinct populations in the rodent lateral hypothalamus relationship to the neuropeptide Y and agouti gene-related protein systems. J Comp Neurol 402:460–474, 1998.
24. Horvath TL, Diano S, van Den Pol AN. Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. J Neurosci 19:1072–1087, 1999.
25. Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inove G, Hosoda K, Kojima M, Kanagawa K, Nakao K. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50:227–232, 2001.
26. Lawrence CB, Snape AC, Baudoin FMH, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. Endocrinology 143:155–162, 2002.
27. Brown JC, Cook MA, Dryburgh T. Motilin, a gastric motor activity stimulating polypeptide: the complete amino acid sequence. Can J Biochem 51:533–537, 1973.
28. Yanaihara N, Yanaihara C, Mochizuki T, Iguchi K, Hoshino M. Chemical synthesis, radioimmunoassay, and distribution of immunoreactivity of motilin. In: Motilin. Z. Itoh, Ed. Tokyo: Academic Press, pp31–46, 1990.
29. Huang Z, De Clercq P, Depoortere I, Peeters TL. Isolation and sequence of cDNA encoding the motilin precursor from monkey intestine. Demonstration of the motilin precursor in the monkey brain. FEBS Lett 435:149–152, 1998.
30. Feighner SD, Tan CP, McKee KK, Palyha OC, Hreniuk DL, Pong SS, Austin CP, Figueroa D, MacNeil D, Cascieri MA, Nargund R, Bakshi R, Abramovitz M, Stocco R, Kargman S, O'Neill G, Van Der Ploeg LHT, Evans J, Patchett AA, Smith RG, Howard AD. Receptor for motilin identified in the human gastrointestinal system. Science 284: 2184–2188, 1999.
31. Convey EM, Tucker HA, SmithVG, Zolman J. Bovine prolactin, growth hormone, thyroxine and corticoid response to thyrotropin-releasing hormone. Endocrinology 92:471–476, 1973.
32. Johke T. Effects of TRH on circulating growth hormone, prolactin and triiodothyronine levels in the bovine. Endocrinol Japon 25:19–26, 1978.
33. Spencer GSG, Garssen GJ, Colenbrander B, Meijer JC. Effect of somatostatin and thyrotropin-releasing hormone on the levels of growth hormone in the circulation of the chronically catheterized pig fetus *in utero*. J Endocrinol 106:121–124, 1985.
34. Harvey S, Scanes CG, Chadwick A, Bolton NJ. The effect of thyrotropin releasing hormone (TRH) and somatostatin (GHRIH) on growth hormone and prolactin secretion *in vitro* and *in vivo* in the domestic fowl (*Gallus domesticus*). Neuroendocrinology 26:249–260, 1978.
35. Harvey S, Scanes CG. Comparative stimulation of growth hormone secretion in anaesthetized chickens by human pancreatic growth hormone-releasing factor (hpGRF) and thyrotrophin-releasing hormone (TRH). Neuroendocrinology 39:314–320, 1984.
36. Scanes CG, Harvey S. Stimulation of growth hormone secretion by human pancreatic growth-hormone-releasing factor and thyrotrophin-releasing hormone in anaesthetized chicken. Gen Comp Endocrinol 56:198–203, 1984.
37. Dubreuil P, Couture Y, Pelletier G, Petitclerc D, Delorme L, Lapierre H, Gaudreau P, Morisse, J, Brazeau P. Effect of long-term administration of porcine growth hormone-releasing factor and(or) thyrotropin releasing factor on growth hormone, prolactin and thyroxine in growing pigs. J Anim Sci 68:95–107, 1990.
38. Thomsett MJ, Marti-Henneberg C, Gluckman PD, Kaplin SL, Rudolph AM, Grumback MM. Hormone ontogeny in the ovine fetus. VII. The effect of thyrotropin-releasing factor on prolactin and growth hormone release in the fetus and neonate. Endocrinology 106:1074–1078, 1980.
39. Wrutniak C, Cabello G, Charrier J, Dulor J-P, Blanchard M, Barenton B. Effects of TRH and GRF administration on GH, TSH, T_4 and T_3 secretion in the lamb. Reprod Nutr Dév 27:501–510, 1987.
40. Rousseau K, La Belle N, Pichavant K, Marchelidon J, Chow BK, Boeuf G, Dufour S. Pituitary growth hormone secretion in the turbot, a phylogenetic recent teleost, is regulated by a specific pattern of neuropeptides. Neuroendocrinology 74:375–385, 2001.
41. Thompson DL Jr, Rahmanian MS, DePew CL, Burleigh DW, DeSouza CJ, Colborn DR. Growth hormone in mares and stallions: pulsatile secretion, response to growth hormone-releasing hormone, and effects of exercise, sexual stimulation, and pharmacological agents. J Anim Sci 70:1201–1207, 1992.
42. Kennedy SR, Thompson DL Jr, Pruett HE, Burns PJ, Deghenghi R. Growth hormone response to a novel GH releasing tripeptide in horses: interaction with GnRH, TRH, and sulpiride. J Anim Sci 80: 744–750, 2002.
43. Pruett HE, Thompson DL Jr, Cartmill JA, Williams CC, Gentry LR. Thyrotropin releasing hormone interactions with growth hormone secretion in horses. J Anim Sci 81:2343–2351, 2003.
44. Sherwood NM, Krueckl SL, McRory JE. The origin and function of the pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon superfamily. Endocr Rev 21:619–670, 2000.
45. Murakami Y, Koshimura K, Yamauchi K, Nishiki M, Tanaka J, Furuya H, Miyake T, Kato Y. Pituitary adenylate cyclase activating polypeptide (PACAP) stimulates growth hormone release from GH_3 cells through type II PACAP receptor. Regul Pept 56:35–40, 1995.
46. Hashizume T, Soliman EB, Kanematsu S. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP), prostaglandin E_2 (PGE_2) and growth hormone releasing factor (GRF) on the release of growth hormone from cultured bovine anterior pituitary cells in vitro. Domest Anim Endocrinol 11:331–337, 1994.
47. Martinez-Fuentes AJ, Malagon MM, Castano JP, Garrido-Gracia JC, Gracia-Navarro F. Pituitary adenylate cyclase-activating polypeptide (PACAP) 38 and PACAP27 differentially stimulate growth hormone release and mRNA accumulation in porcine somatotropes. Life Sci 62: 2379–2390, 1998.
48. Radcliff RP, Lookingland KJ, Chapin LT, Tucker HA. Pituitary adenylate cyclase-activating polypeptide induces secretion of growth hormone in cattle. Domest Anim Endocrinol 21:187–196, 2001.
49. Peeters K, Langouche L, Vandesande F, Darras VM, Berghman LR. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on cAMP formation and growth hormone release from chicken anterior pituitary cells. Ann N Y Acad Sci 865:471–474, 1998.
50. Xiao D, Chu MM, Lee EK, Lin HR, Wong AO. Regulation of growth hormone release in common carp pituitary cells by pituitary adenylate

cyclase-activating polypeptide: signal transduction involves cAMP- and calcium-dependent mechanisms. Neuroendocrinology 76:325–338, 2002.

51. Montero M, Yon L, Rousseau K, Arimura A, Fournier A, Dufour S, Vaudry H. Distribution, characterization, and growth hormone-releasing activity of pituitary adenylate cyclase-activating polypeptide in the European eel, *Anguilla anguilla*. Endocrinology 139:4300–4310, 1998.
52. Wong AO, Li WS, Lee EK, Leung MY, Tse LY, Chow BK, Lin HR, Chang JP. Pituitary adenylate cyclase-activating peptide as a novel hypophysiotropic factor in fish. Biochem Cell Biol 87:329–343, 2000.
53. Alarcon P, Garcia-Sancho J. Differential calcium responses to the pituitary adenylate cyclase-activating peptide (PACAP) in the five main types of rat anterior pituitary. Pflugers Arch 440:685–691, 2000.
54. Murakami Y, Koshimura K, Yamauchi K, Nishiki M, Tanaka J, Kato Y. Roles and mechanisms of action of pituitary adenylate cyclase-activating polypeptide (PACAP) in growth hormone and prolactin secretion. Endocrinol J 48:123–132, 2001.
55. Martinez-Fuentes AJ, Castano JP, Gracia-Navarro F, Malagon MM. Pituitary adenylate cyclase-activating polypeptide (PACAP) 38 and PACAP27 activate common and distinct intracellular signaling pathways to stimulate growth hormone secretion from porcine somatotropes. Endocrinology 139:5116–5124, 1998.
56. Murakami Y, Tanaka J, Koshimura K, Kato Y. Involvement of tetrodoxin-sensitive sodium channels in rat growth hormone secretion induced by pituitary adenylate cyclase-activating polypeptide (PACAP). Regul Pept 73:119–121, 1998.
57. Andries M, Denef C. Gonadotropin-releasing hormone influences the release of prolactin and growth hormone from intact rat pituitary *in vitro* during a limited period in neonatal life. Peptides 1:527–532, 1995.
58. Murthy CK, Turner RJ, Wong AO, Rao PD, Rivier JE, Peter RE. Differential actions of a mammalian gonadotropin-releasing hormone antagonist on gonadotropin-II and growth hormone release in goldfish, *Carassius auratus*. Neuroendocrinology 59:561–571, 1994.
59. Murthy CK, Zheng W, Trudeau VL, Nahorniak CS, Rivier JE, Peter RE. In vivo actions of a gonadotropin-releasing hormone (GnRH) antagonist on gonadotropin-II and growth hormone secretion in goldfish, *Carassius auratus*. Gen Comp Endocrinol 96:427–437, 1994.
60. Li WS, Lin HR, Wong AO. Effects of gonadotropin-releasing hormone on growth hormone secretion and gene expression in common carp pituitary. Comp Biochem Physiol B Biochem Mol Biol 132:335–341, 2002.
61. Uetsky AD, Weiss BL, Yunker WK, Chang JP. Nitric oxide produced by a novel nitric oxide synthase isoform is necessary for gonadotropin-releasing hormone-induced growth hormone secretion via a cGMP-dependent mechanism. J Neuroendocrinol 15:667–676, 2003.
62. Shimon I, Yan X, Magoffin DA, Friedman TC, Melmed S. Intact leptin receptor is selectively expressed in human fetal pituitary and pituitary adenomas and signals human fetal pituitary growth hormone secretion. J Clin Endocrinol Metab 83:4059–4064, 1998.
63. Iqbal J, Pompolo S, Considine RV, Clarke IJ. Localization of leptin receptor-like immunoreactivity in the corticotropes, somatotropes, and gonadotropes in the ovine anterior pituitary. Endocrinology 141:1515–1520, 2000.
64. Lin J, Barb CR, Matteri RL, Kraeling RR, Chen X, Meinersmann RJ, Rampacek GB. Long form leptin receptor mRNA expression in the brain, pituitary, and other tissues in the pig. Domest Anim Endocrinol 19:53–61, 2000.
65. Lin J, Barb CR, Kraeling RR, Rampacek GB. Growth hormone releasing factor decreases long form leptin receptor expression in porcine anterior pituitary cells. Domest Anim Endocrinol 24:95–101, 2003.
66. Barb CR, Yan X, Azain MJ, Kraeling RR, Rampacek GB, Ramsay TG. Recombinant porcine leptin reduces feed intake and stimulates growth hormone secretion in swine. Domest Anim Endocrinol 15:77–86, 1998.
67. Mizuno I, Okimura Y, Takahashi Y, Kaji H, Abe H, Chihara K. Leptin stimulates basal and GHRH-induced GH release from cultured rat anterior pituitary cells in vitro. Kobe J Med Sci 45:221–227, 1999.
68. Carro E, Seoane LM, Senaris R, Considine RV, Casanueva FF, Dieguez C. Interaction between leptin and neuropeptide Y on in vivo growth hormone secretion. Neuroendocrinology 68:187–191, 1998.
69. Carro E, Senaris RM, Seoane LM, Frohman LA, Arimura A, Casanueva FF, Dieguez C. Role of growth hormone (GH)-releasing hormone and somatostatin on leptin-induced GH secretion. Neuroendocrinology 69:3–10, 1999.
70. Tannenbaum GS, Gurd W, Lapointe M. Leptin is a potent stimulator of spontaneous pulsatile growth hormone (GH) secretion and the GH response to GH-releasing hormone. Endocrinology 139:3871–3875, 1998.
71. Giusti M, Bocca L, Florio T, Corsaro A, Spaziante R, Schettini G, Minuto F. In vitro effect of human recombinant leptin and expression of leptin receptors on growth hormone-secreting human pituitary adenomas. Clin Endocrinol (Oxf) 57:449–455, 2002.
72. Lloyd RV, Jin L, Tsumanuma I, Vidal S, Kovacs K, Horvath E, Scheithauer BW, Couce ME, Burguera B. Leptin and leptin receptor in anterior pituitary function. Pituitary 4:33–47, 2001.
73. Baratta M, Saleri R, Mainardi GL, Valle D, Giustina A, Tamanini C. Leptin regulates GH gene expression and secretion and nitric oxide production in pig pituitary cells. Endocrinology 143:551–557, 2002.
74. Denver RJ, Licht P. Modulation of neuropeptide-stimulated pituitary hormone secretion in hatchling turtles. Gen Comp Endocrinol 77:107–115, 1990.
75. Rousseau K, Le Belle N, Marchelidon J, Dufour S. Evidence that corticotropin-releasing hormone acts as a growth hormone-releasing factor in a primitive teleost, the European eel (*Anguilla anguilla*). J Neuroendocrinol 11:385–392, 1999.
76. Peng C, Humphries S, Peter RE, Rivier JE, Blomqvist AG, Larhammar D. Actions of goldfish neuropeptide Y on the secretion of growth hormone and gonadotropin-II in female goldfish. Gen Comp Endocrinol 90:306–317, 1993.
77. Peng C, Chang JP, Yu KL, Wong AO, Van Goor F, Peter RE, Rivier JE. Neuropeptide-Y stimulates growth hormone and gonadotropin-II secretion in the goldfish pituitary: involvement of both presynaptic and pituitary cell actions. Endocrinology 132:1820–1829, 1993.
78. Arvat E, Gianotti L, Ramunni J, Grottoli S, Brossa PC, Bertagna A, Camanni F, Ghigo E. Effect of galanin on basal and stimulated secretion of prolactin, gonadotropins, thyrotropin, adrenocorticotropin and cortisol in humans. Eur J Endocrinol 133:300–304, 1995.
79. Giustina A, Bonfanti C, Licini M, Stefana B, Ragni G, Turano A. Effect of galanin on growth hormone (GH) response to thyrotropin releasing hormone of rat pituitary GH-secreting adenomatous cells (GH1) in culture. Life Sci 58:83–90, 1996.
80. Giustina A, Ragni G, Bollati A, Cozzi R, Licini M, Poiesi C, Turazzi S, Bonfanti C. Inhibitory effects of galanin on growth hormone (GH) release in cultured GH-secreting adenoma cells: comparative study with octreotide, GH-releasing hormone, and thyrotropin-releasing hormone. Metabolism 46:425–430, 1997.
81. Dockray GJ, Reeve JR Jr, Shively J, Gayton RJ, Barnard CS. A novel active pentapeptide from chicken brain identified by antibodies to FMRF-amide. Nature 305:328–330, 1983.
82. Chartrel N, Dujardin C, Leprince J, Desrues L, Tonon MC, Cellier E, Cosette P, Jouenne T, Simonnet G, Vaudry H. Isolation, characterization, and distribution of a novel neuropeptide, *Rana* RFamide (R-RFa), in the brain of the European green frog *Rana esculenta*. J Comp Neurol 448:111–127, 2002.

83. Fujimoto M, Takeshita K, Wang X, Takabatake I, Fujisawa Y, Teranishi H, Ohtani M, Muneoka Y, Ohta S. Isolation and characterization of a novel bioactive peptide, *Carassius* RFamide (C-RFa), from the brain of the Japanese crucian carp. Biochem Biophys Res Commun 242:436–440, 1998.
84. Ukena K, Iwakoshi E, Minakata H, Tsutsui K. A novel rat hypothalamic RFamide-related peptide identified by immunoaffinity chromatography and mass spectrometry. FEBS Lett 512:255–258, 2002.
85. Koda A, Ukena K, Teranishi H, Ohta S, Yamamoto K, Kikuyama S, Tsutsui K. A novel amphibian hypothalamic neuropeptide: isolation, localization, and biological activity. Endocrinology 143:411–419, 2002.
86. Chen C, Clarke IJ. Modulation of Ca^{2+} influx in the ovine somatotroph by growth hormone-releasing factor. Am J Physiol 268:E204–E212, 1995.
87. Chen C. The effect of two-day treatment of primary cultured ovine somatotropes with GHRP-2 on membrane voltage-gated K^+ currents. Endocrinology 143:2659–2663, 2002.
88. Glavaski-Joksimovic A, Jeftinija K, Jeremic A, Anderson LL, Jeftinija S. Mechanism of action of growth hormone secretagogue, L-692,585, on cultured porcine somatotropes. J Endocrinol 175:625–636, 2002.
89. Glavaski-Joksimovic A, Jeftinija K, Scanes CG, Anderson LL, Jeftinija S. Stimulatory effect of ghrelin on isolated porcine somatotropes. Neuroendocrinology 77:366–378, 2003.
90. Valverde I, Penalva A, Ghigo E, Casanueva FF, Diequez C. Involvement in nitric oxide in the regulation of growth hormone secretion in dogs. Neuroendocrinology 74:213–219, 2001.
91. Kostic TS, Andric SA, Stojilkovic SS. Spontaneous and receptor-controlled soluble guanylyl cyclase activity in anterior pituitary cells. Mol Endocrinol 155:1010–1022, 2001.
92. Baratta M, Saleri R, Mainardi GL, Valle D, Giustina A, Tamanini C. Leptin regulates GH gene expression and secretion and nitric oxide production in pig pituitary cells. Endocrinology 143:551–557, 2002.
93. Cho SJ, Kelly M, Rognlien KT, Cho J, Hörber JK, Jena BP. SNAREs in opposing bilayers interact in a circular array to form conducting pores. Biophys J 83:2522–2527, 2002.
94. Cho SJ, Quinn AS, Stromer MH, Dash S, Cho J, Taatjes DJ, Jena BP. Structure and dynamics of the fusion pore in live cells. Cell Biol Int 26:35–42, 2002.
95. Jena BP, Cho SJ, Jeremic A, Stromer MH, Abu-Hamdah R. Structure and composition of the fusion pore. Biophys J 84:1337–1343, 2003.
96. Jeremic A, Kelly M, Cho SJ, Stromer MH, Jena BP. Reconstituted fusion pore. Biophys J 85:2035–2043, 2003.
97. Schneider SW, Sritharan KC, Geibel JP, Oberleithner H, Jena BP. Surface dynamics in living acinar cells imaged by atomic force microscopy: identification of plasma membrane structures involved in exocytosis. Proc Natl Acad Sci U S A 94:316–321, 1997.
98. Cho SJ, Abdus Sattar AKM, Jeong EH, Satchi M, Cho J, Dash S, Mayes MS, Stromer MH, Jena BP. Aquaporin 1 regulates GTP-induced rapid gating of water in secretory vesicles. Proc Natl Acad Sci U S A 99:4720–4724, 2002.
99. Jena BP, Schneider SW, Geibel JP, Webster P, Oberleithner H, Sritharan KC. G_i regulation of secretory vesicle swelling examined by atomic force microscopy. Proc Natl Acad Sci U S A 94:13317–13322, 1997.
100. Cho SJ, Jeftinija K, Glavaski A, Jeftinija S, Jena BP, Anderson LL. Structure and dynamics of the fusion pores in live GH-secreting cells revealed using atomic force microscopy. Endocrinology 143:1144–1148, 2002.
101. Lee JS, Mayes MS, Stromer MH, Jena BP, Anderson LL. Number of secretory vesicles in GH cells of the pituitary remain unchanged after secretion (abstract). 42nd Annual Meeting of the American Society for Cell Biology, December 14–18, 2002, Moscone Convention Center. Proceedings of American Society for Cell Biology 13(Suppl. 1):86a.
102. Klindt J, Ford JJ, Berardinelli JG, Anderson LL. Growth hormone secretion after hypophysial stalk transection in pigs. Proc Soc Exp Biol Med 172:508–513, 1983.
103. Plouzek CA, Molina JR, Hard DL, Vale WW, Rivier J, Trenkle A, Anderson LL. Effects of growth hormone-releasing factor and somatostatin on growth hormone secretion in hypophysial stalk-transected beef calves. Proc Soc Exp Biol Med 189:158–167, 1988.
104. Anderson LL, Ford JJ, Klindt J, Molina JR, Vale WW, Rivier J. Growth hormone and prolactin secretion in hypophysial stalk-transected pigs as affected by growth hormone and prolactin releasing and inhibiting factors. Proc Soc Exp Biol Med 196:194–202, 1991.
105. Anderson LL, Hard DL, Carpenter LS, Awotwi EK, Diekman MA, Trenkle AH, Cho SJ. Pregnancy, parturition, and lactation in hypophyseal stalk-transected beef heifers. J Endocrinol 163:463–475, 1999.
106. Anderson LL, Hard DL, Trenkle AH, Cho SJ. Long-term growth after hypophyseal stalk transection and hypophysectomy of beef calves. Endocrinology 140:2405–2414, 1999.
107. Hickey GJ, Drisko J, Faidley T, Chang C, Anderson LL, Nicolich S, McGuire L, Rickes E, Krupa D, Feeney W, Friscino B, Cunningham P, Frazier E, Chen H, Laroque P, Smith RG. Mediation by the central nervous system is critical to the *in vivo* activity of the GH secretagogue L-692,585. J Endocrinol 148:371–380, 1996.
108. 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 135: 1073–1076, 1994.
109. Drisko JE, Faidley TD, Hora DH Jr, Niebauer GW, Feeney W, Friscino BF, Hickey GJ. Transorbital approach to the porcine pituitary. J Invest Surg 9:305–311, 1996.
110. Drisko JE, Faidley TD, Chang CH, Zhang D, Nicolich S, Hora DF Jr, McNamara L, Rickes E, Abribat T, Smith RG, Hickey GJ. Hypophyseal-portal concentrations of growth hormone-releasing factor and somatostatin in conscious pigs: relationship to production of spontaneous growth hormone pulses. Proc Soc Exp Biol Med 217: 188–196, 1998.
111. Plotsky PM, Vale W. Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophyseal portal circulation of the rat. Science 230:461–463, 1985.
112. Tannenbaum GS, Ling N. The interrelationship of growth hormone releasing factor and somatostatin in generation of the ultradian rhythm of growth hormone secretion. Endocrinology 115:1952–1957, 1984.
113. Cataldi M, Magnan E, Guillaume V, Dutour A, Conte-Devolx B, Lombardi G, Oliver C. Relationship between hypophyseal portal GRF and somatostatin and peripheral GH levels in the conscious sheep. J Endocrinol Invest 17:717–722, 1994.
114. Frohman LA, Downs TR, Clarke IJ, Thomas GB. Measurement of growth hormone-releasing hormone and somatostatin in hypothalamic-portal plasma of unanesthetized sheep: spontaneous secretion and response to insulin-induced hypoglycemia. J Clin Invest 86:17–24, 1990.
115. Li S, Crenshaw EB III, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG. Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. Nature 347:528–533, 1990.
116. Yu TP, Sun HS, Wahls SI, Sanchez-Serrano, Rothschild MF, Tuggle CK. Cloning of the full-length pig PIT1 (POU1F1) cDNA and a novel alternative PIT1 transcript, and functional studies of their encoded proteins. Anim Biotech 12:1–19, 2001.
117. Sun HS, Anderson LL, Yu TP, Kim KS, Klindt J, Tuggle CK. Neonatal Meishan pigs show POU1F1 genotype effects on plasma GH and PRL concentration. Anim Reprod Sci 69:223–237, 2002.