Minireview

Nanobiology and physiology of growth hormone secretion

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Abstract

Growth hormone (GH) secretion is controlled by hypothalamic releasing hormones from the median eminence together with hormones and neuropeptides produced by peripheral organs. Secretion of GH involves movement of secretory vesicles along microtubules, transient 'docking' with the porosome in the cell membrane and subsequent release of GH. Release of GH is stimulated by GH releasing hormone (GHRH) and inhibited by somatostatin (SRIF). Ghrelin may be functioning to stimulate GH release from somatotropes acting via the GH secretagogue (GHS) receptor (GHSR). However, recent physiological studies militate against this. In addition, ghrelin does influence GH release acting within the hypothalamus. Release of GH from the somatotropes involves the GH-containing secretory granules moving close to the cell surface followed by transitory fusion of the secretory granules with the porosomes located in multiple secretory pits in the cell membrane. Other peptides/proteins can influence GH secretion, particularly in species of non-mammalian vertebrates.

Keywords: growth hormone, growth hormone secretagogues, cellular mechanisms, signal transduction systems, porosomes, secretory pits

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Introduction

There is an exquisite control system directly influencing secretion of growth hormone (GH) from the somatotropes. We reviewed the then available information on GH secretion in 2004.[1] There are considerable numbers of more recent studies available providing a new insight into our understanding of the control of GH secretion. The consensus view is that there are three hypothalamic releasing hormones for GH, respectively, GH releasing hormone (GHRH; stimulatory), ghrelin (stimulatory) and somatostatin (SRIF; inhibitory) with GH release and synthesis reflecting a balance between these.[1] There are in addition a series of neuropeptides and hormones that have been demonstrated to be capable of influencing GH secretion from the somatotrope. The subcellular mechanism of release from secretory granules in somatotropes is increasingly understood. Secretory granules are moved to the vicinity close to the cell membrane and then fuse with porosomes in the secretory pits in the cell membrane.[2,3]

The present review considers recent advances in our understanding of the control of GH secretion and updates our review of the topic.[1] Where possible, the review will include examples from studies using domestic animals. The control of GH secretion is discussed under the following areas:

- Nanobiology of GH secretion;
- Physiological roles of GHRH, SRIF and ghrelin directly influencing the control of GH release from the somatotrope;
- Other neuropeptides, hormones and factors that modulate GH secretion;
- Evolution of the control of GH release;
- Somatotrope number and their effect on GH secretion;
- Neuropeptides acting on GH release at the level of the hypothalamus.

Nanobiology of GH secretion

Biological processes such as neurotransmission, and the secretion of enzymes and hormones, require fusion of membrane-bounded secretory vesicles at the cell plasma membrane and rapid expulsion of vesicular contents. It was commonly accepted that exocytosis requires incorporation of secretory vesicle membrane into the cell plasma membrane for expulsion of vesicular contents (e.g. see refs[4,5]). Studies in the last decade demonstrate that this is not the case.[2,3,6–12]

If membranes surrounding secretory vesicles fuse with the cell plasma membrane irreversibly to release their

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contents, there would be several corollaries: (1) the number of secretory vesicles would decline after secretion is stimulated; (2) secretory vesicles would be full; and (3) the surface area and hence volume of cells would increase. However, earlier transmission electron microscopy (TEM) studies on mast cells demonstrate that, after stimulation of secretion, intact as well as empty and partly empty secretory vesicles are present.[8] Quantitative TEM on stimulated and resting bovine chromaffin cells of the adrenal cortex showed no changes in the number of peripheral dense-core vesicles after stimulation of secretion.[2] Similarly, combined studies using atomic force microscopy (AFM) and TEM clearly demonstrate no change in the total number of secretory vesicles following secretion in pancreatic acinar cells.[13]

Is the number of secretory vesicles in somatotropes of the pituitary changed after secretion is stimulated?

No changes in the number of secretory vesicles were observed in porcine somatotropes after exposure to the GH secretagogue L-692,585 for 90 s (Table 1; Figure 1; data[14]). The number of filled vesicles is decreased 53% following exposure of somatotropes to the GH secretagogue L-692,585 (Table 1). Concomitantly, there are large increases in the number of partial filled or empty vesicles in somatotropes following exposure to the GH secretagogue L-692,585 (Table 1).[14] These changes are summarized in Figure 1. There is strong evidence that the loss of secretory vesicle contents in somatotropes is the GH that was secreted.[15] Immunocytochemical (GH antibodies and colloidal gold) labeling only occurred in filled (electron dense) GH vesicles in either control or stimulated cells (Figures 2a and b).[14] Moreover, there was an absence of immunogold-labeling in empty vesicles and, as expected, in other areas of the cytoplasm.[14] These results clearly demonstrate that following exposure of live somatotropes to the secretagogue, the filled GH-containing secretory vesicles empty very rapidly, releasing their contents, and then the empty or partly empty vesicles return to the cytoplasmic compartments of the cell. Transient fusion of secretory vesicles with the cell membrane, most likely by docking at the plasma membrane, is supported. Earlier studies on mast cells also demonstrated an increase in the number of spent and partly spent vesicles following stimulation of secretion, without any demonstrable increase in cell size.[2]

How are porosomes essential to secretion?

Porosomes are organelles with a 100–150-nm diameter opening in the cell membrane of secretory cells, for instance, somatotropes, pancreatic acinar cells and in synapses. These cone- or basket-shaped structures play a pivotal role in the 'transiently' docking of secretory vesicles (or neurotransmitters in synaptic vesicles) and their consequent emptying (e.g. refs[8–10,16–21]). Secretory vesicles fuse at depressions within the porosome, resulting in a 20–40% increase in the diameter of the depression.

Somatotropes, like pancreatic acinar cells, are slow secreting cells with only transient fusion of secretory vesicles after stimulation. In fast secreting cells such as nerve (neuronal porosome 12–15 nm diameter) or mast cells, the number of secretory vesicles fusing at the plasma membrane at one time is likely much greater than in slow secreting cells. Majó *et al.*[22] suggested similar secretory mechanisms for synaptic vesicles and secretory organelles in both neuronal and endocrine cells that have a highly regulated secretory pathway for intracellular communication.

Structure of the porosome

The molecular structure of the porosome has been elucidated and its composition is partially characterized (see Figures 3 and 4; see e.g. refs[20,21,23]). Furthermore, the native porosome has been isolated and functionally reconstituted in liposomes. There is a stepwise increase in the capacitance of the plasma membrane of somatotropes.[24] This may be the result of rapid transient fusion of secretory vesicles with the cell membrane. This is consistent with the AFM observations.[3] These demonstrated the presence of 'pits' which contain 'depressions' or porosomes at the plasma membrane of the somatotropes. These pores increase in diameter after GH secretagogue L-692,585 exposure.[3]

A set of eight protein units line the porosome. Fusion of secretory vesicles with the cell membrane involves calcium ions and an array of specialized proteins, SNAP receptors or *N*-ethylmaleimide-sensitive factor (NSF)-attachment protein receptor (SNARE) or, in the opposing membrane bilayers, respectively, the following:

Table 1 Effect of very short exposure to a GH secretagogue of somatotrophs on the number of GH secretory vesicles and to their filled, partially filled and empty status (data from ref.[14])

Treatment for 90 s	Total number of vesicles per μm^2 of cell area	Number of filled vesicles per μm^2 of cell area	Number of partially filled vesicles per μm^2 of cell area	Number of empty vesicles per μm^2 of cell area
Vehicle control (PBS)	6.6 ± 0.14	4.9 ± 0.21	1.1 ± 0.08	0.6 ± 0.13
GH secretagogue L-692,585	6.1 ± 0.36	$2.3 \pm 0.23^{***}$	$2.6 \pm 0.12^{***}$	$1.2 \pm 0.16^{*}$

Explants of neonatal anterior pituitary glands (1 mm^3) were exposed to vehicle. Explants of the contralateral sagittal section were exposed to the GH secretagogue, L-692,585, 20 μmol/L for 90 s. The tissue was then fixed (in 4% paraformaldehyde and 2.5% glutaraldehyde for 2 h followed by 1% osmium tetroxide for 1 h). After sectioning, GH was immunolocalized using antisera to porcine GH and colloidal gold. Numbers of GH secretory vesicles, filled vesicles, partially filled vesicles and empty vesicles were counted in somatotropes

GH, growth hormone; PBS, phosphate-buffered saline

Difference between somatotropes following exposure to vehicle or the GH secretagogue L-692,585 $^{*}P < 0.05$; $^{***}P < 0.001$

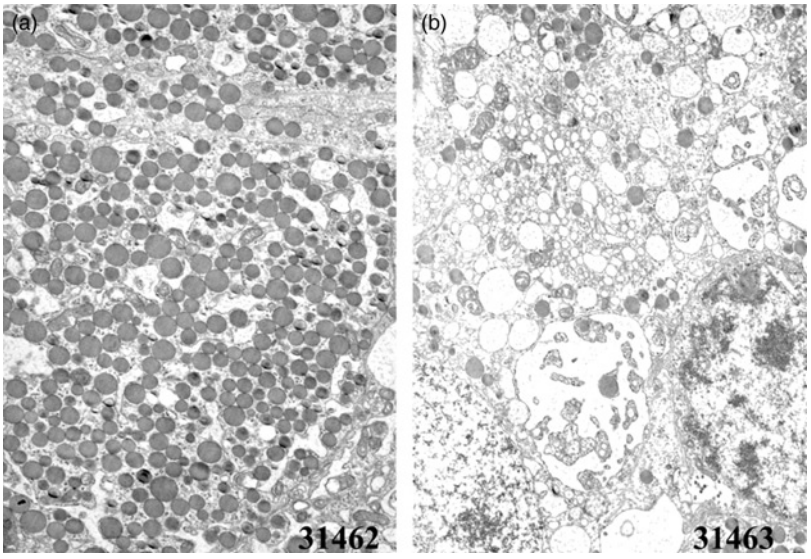

Figure 1 Transmission electron microscopy images of secretory vesicles in growth hormone cells of porcine pituitary. Note the high number of filled vesicles in control cells exposed to phosphate-buffered saline (a) and the high number of empty and partly empty vesicles in stimulated cells exposed to the secretagogue, L-692,585, for 90 s (b). Magnification, ×22,400

- Synaptosomal-associated protein 25 (SNAP-25) and syntaxin termed as target SNAREs (t-SNAREs) present at the base of the porosome in the cell membrane;
- Vesicle-associated protein or synaptobrevin or vesicular-SNARE (v-SNARE) in the vesicle membrane.

In the presence of calcium ions, the proteins assemble into a ring with a channel fusing the vesicle with the porosome in the cell membrane and establishing continuity between the two. The SNARE complex undergoes disassembly in the presence of the soluble ATPase NSF and ATP.

The lipids in the bilayers of the cell membrane and secretory vesicles influence secretion. For instance, the curvature of the cell membrane is influenced by cholesterol (negatively) and lysophosphatidylcholine (LPC) (positively). Membrane fusion is respectively increased and decreased by cholesterol and LPC.[25]

Swelling of the secretory vesicle

Concurrently with docking, there is swelling of the secretory vesicle. This is required to discharge the contents of the vesicle. This is accomplished by GTP-induced and G_o-mediated water entry and consequent vesicle swelling of vesicles. This involves GTPase activity of GTP-binding G(αo) protein-induced water channels, aquaporin-6 and the

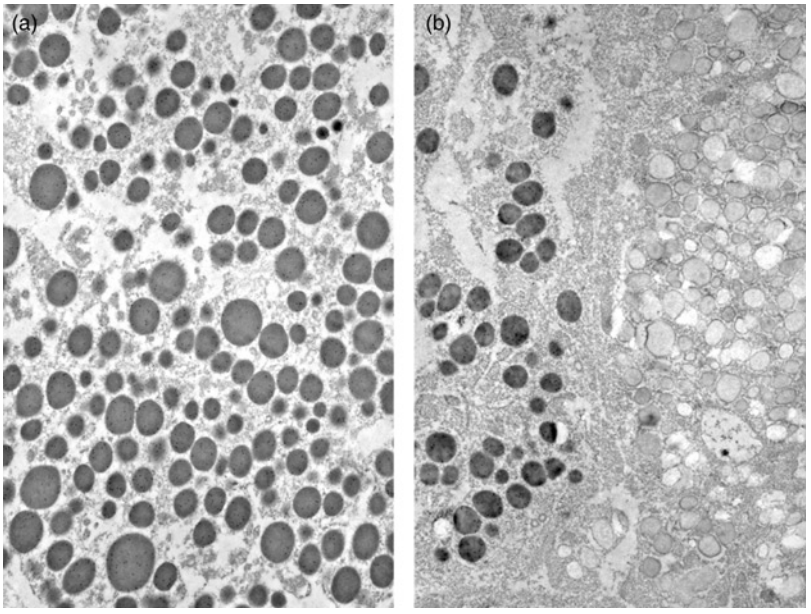

Figure 2 Transmission electron microscopy images of immunogold-labeled secretory vesicles in growth hormone (GH) cells of porcine pituitary. Note that localization of GH is only in electron-dense vesicles in both control (a) and stimulated (b) cells. After stimulation of GH secretion with the secretagogue, L-692,585, for 90 s, there was a complete absence of immunogold-labeled GH antibody in empty vesicles (b). Magnification, ×27,300

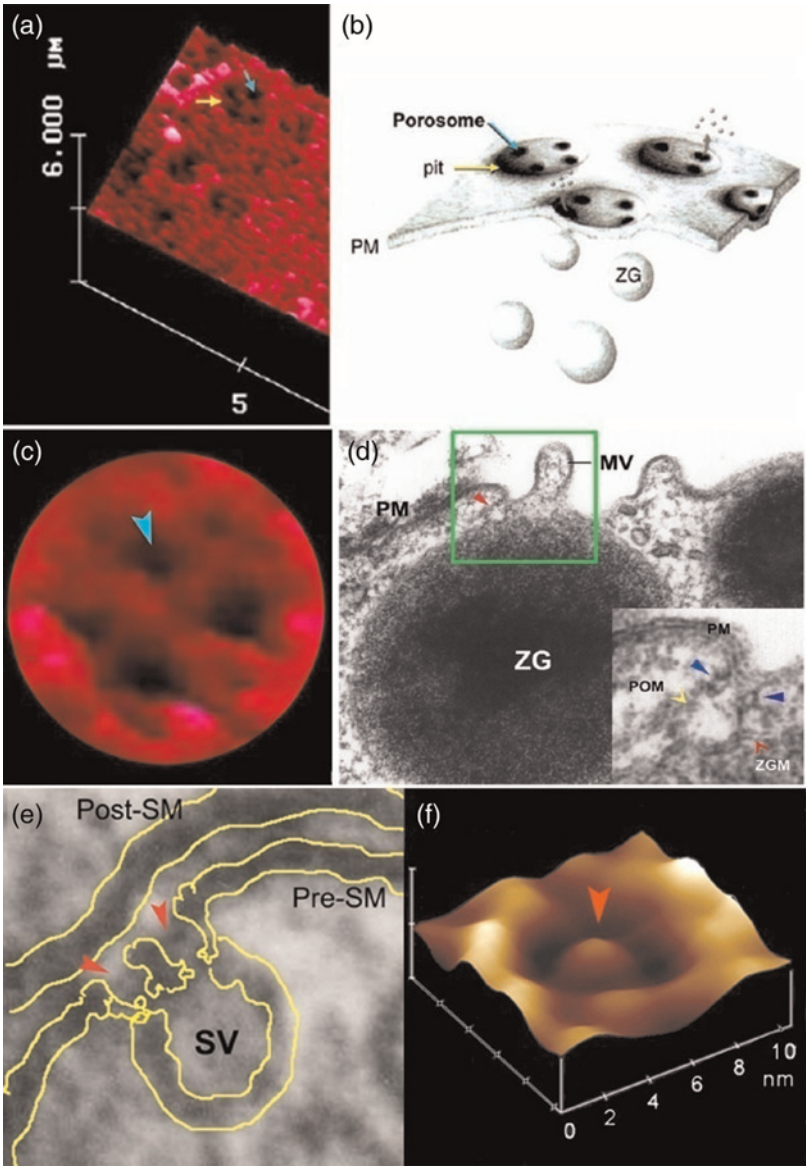

Figure 3 Porosomes or previously referred to as 'depressions' at the plasma membrane in pancreatic acinar cells and at the nerve terminal. (a) Atomic force microscopy (AFM) micrograph depicting 'pits' (yellow arrow) and 'porosomes' within (blue arrow), at the apical plasma membrane in a live pancreatic acinar cell.[8] (b) To the right is a schematic drawing depicting porosomes at the cell plasma membrane (PM), where membrane-bound secretory vesicles called zymogen granules (ZG) dock and fuse to release intravesicular contents.[8] (c) A high-resolution AFM micrograph shows a single pit with four 100–180 nm porosomes within.[8] (d) An electron micrograph depicting a porosome (red arrowhead) close to a microvilli (MV) at the apical plasma membrane (PM) of a pancreatic acinar cell.[12] Note the association of the porosome membrane (yellow arrowhead) and the zymogen granule membrane (ZGM) (red arrowhead) of a docked ZG (inset). Cross-section of a circular complex at the mouth of the porosome is seen (blue arrowhead). (e) The bottom left panel shows an electron micrograph of a porosome (red arrowhead) at the nerve terminal, in association with a synaptic vesicle (SV) at the presynaptic membrane (Pre-SM). Note a central plug at the neuronal porosome opening.[18] (f) The bottom right panel is an AFM micrograph of a neuronal porosome in physiological buffer, also showing the central plug (red arrowhead) at its opening.[18] It is believed that the central plug in neuronal porosomes may regulate its rapid close-open conformation during neurotransmitter release. The neuronal porosome is an order of magnitude smaller (10–15 nm) in comparison with porosome in the exocrine pancreas (courtesy of Prof Bhanu P Jena, Wayne State University School of Medicine; Detroit, MI, USA)

proton pump vH(+)-ATPase.[26–28] The swelling of the secretory vesicles results 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.

How are secretory vesicles moved to the porosomes and what is the energy requirement for this?

Secretory vesicles such as the GH-containing vesicles in somatotropes are transported to the plasma membrane along microtubules. This is driven by proteins of the kinesin superfamily molecular motors walking along the microtubule,[29] with each step requiring one ATP molecule.[30–32] For instance, F_1 is such a motor. There are three β subunits that undergo conformational changes that induce torque when they hydrolyze ATP. While this causes rotation, the structural basis for the linkage between torque and unidirectional rotation remains to be fully elucidated.[33,34] Kinesin proteins move along a microtubule at a velocity of about 3 μm s^{-1}.[30–32] As a single step is

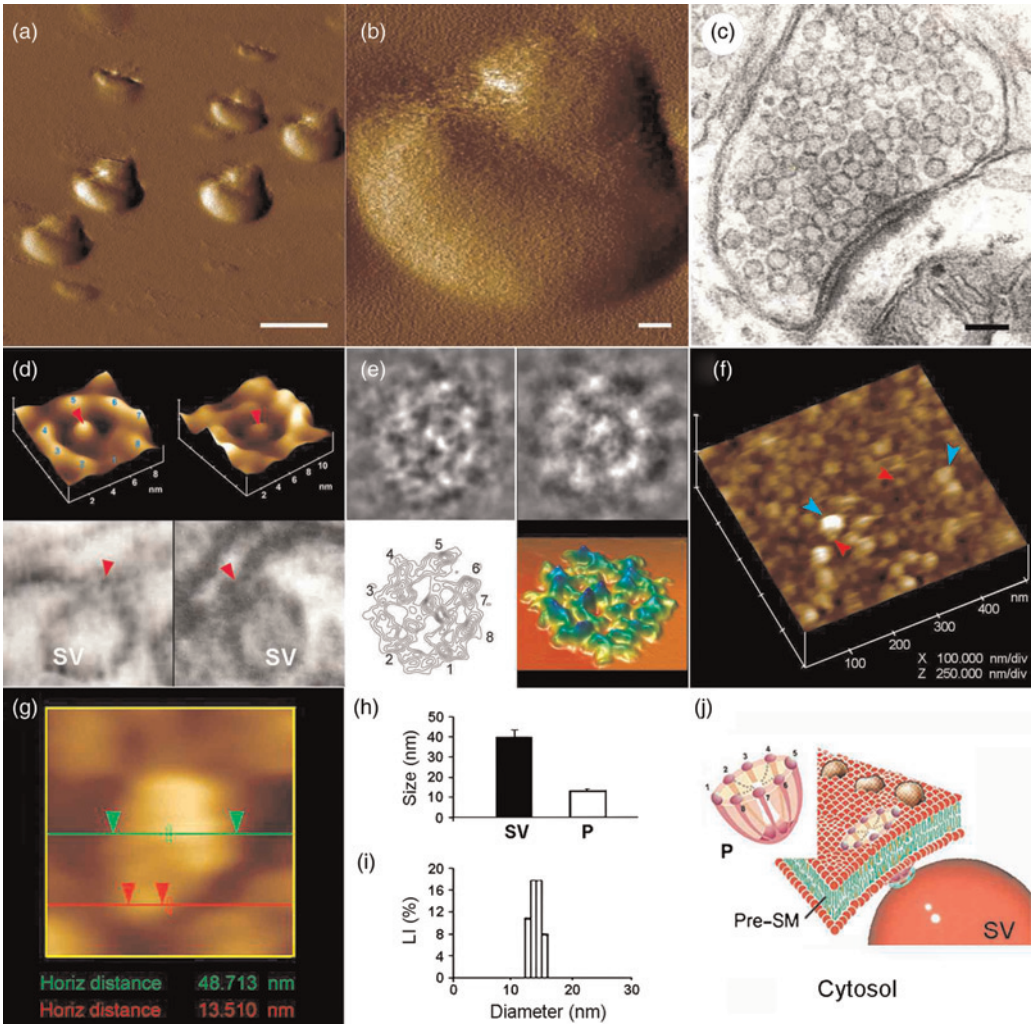

Figure 4 Structure and organization of the neuronal porosome complex at the nerve terminal. (a) Low-resolution atomic force microscopy (AFM) amplitude image bar = 1 μm (a) and high-resolution AFM amplitude image bar = 100 nm (b) of isolated rat brain synaptosomes in buffered solution. (c) Electron micrograph of a synaptosome,[18] bar = 100 nm. (d) Structure and arrangement of the neuronal porosome complex facing the outside (d, top left), and the arrangement of the reconstituted complex in the phosphatidylcholine:phosphatidylserine membrane (d, top right). Lower panels are two transmission electron micrographs demonstrating synaptic vesicles (SV) docked at the base of the cup-shaped porosome, having a central plug (red arrowhead).[17] (e) EM, electron density and 3D contour mapping (e) provides at the nanoscale, the structure and assembly of proteins within the complex.[17] (f) AFM micrograph of inside-out membrane preparations of isolated synaptosome. Note the porosomes (red arrowheads) to which synaptic vesicles are found docked (blue arrowhead).[18] (g) High-resolution AFM micrograph of a synaptic vesicle docked to a porosome at the cytoplasmic compartment of the presynaptic membrane.[18] (h) AFM measurements ($n = 15$) of porosomes (P, 13.05 $\pm$ 0.91) and synaptic vesicles (SV, 40.15 $\pm$ 3.14) at the cytoplasmic compartment of the presynaptic membrane.[17] (i) Photon correlation spectroscopy (PCS) of immunoisolated neuronal porosome complex demonstrating a size of 12–16 nm.[17] (j) Schematic illustration of a neuronal porosome at the presynaptic membrane, showing the eight ridges connected to the central plug[17] (courtesy of Prof Bhanu P Jena, Wayne State University School of Medicine, Detroit, MI, USA)

roughly 8 nm, the motor protein makes about 375 steps per second.[30–32] Assuming that 1000 secretory vesicles are being transported along microtubules of a stimulated somatotrope,[30,31] then the total rate of energy consumption as a result of vesicle transport is approximately 3.75×10^5 ATP molecules per second.[30–32] Since the total number of ATP molecules in a cell at any given moment is $\sim 10^9$, and since these molecules are used up and completely replaced in ~1–2 min, the total rate of consumption by the GH cells is approximately 10^7 ATP molecules per second.[30,32] Thus, the kinesin-on-microtubule motors use approximately 4% of the individual somatotropes' ATP on transporting 1000 vesicles. This is a modest estimation of the energy requirement as it takes no account of the energy required for transient docking and it is arguably a low estimate of the number of secretory vesicles in a GH cell that secrete on stimulation.

Role(s) of GHRH, SRIF and ghrelin in the control of GH release from somatotrope

Growth hormone releasing factor

GHRH was first isolated from a human tumor[35,36] and later from hypothalamic tissue.[37] It was demonstrated to stimulate specifically the secretion of GH.[35–38] It is now clear that the principal role of GHRH is to stimulate release of GH. Pulsatile or ultradian secretion of GH is initiated by GHRH in adult humans and rats.[39–42] In the rat, the content of GHRH mRNA in the arcuate nucleus changes

inversely with the GH ultadian rhythm, peaking at the time of minimal GH secretion.[43] There is evidence of non-pituitary effects of GHRH. For instance, GHRH is expressed and can stimulate cell proliferation in peripheral tissues; the effect is mediated by a splice variant of GHRH receptor.[44,45]

GHRH is expressed in the arcuate nucleus of the hypothalamus and is released from neurosecretory terminals in the median eminence.[46] GHRH neurons are localized in ventrolateral regions of the bovine and porcine arcuate nucleus with fibers projecting ventrally to the median eminence.[47]

Expression of GH in the anterior pituitary gland is increased by GHRH (e.g. cattle,[48] chickens[49,50]). Expression of the GHRH-R gene is affected by multiple hormones and neuropeptides. GHRH can either down- or up-regulate GHRH expression. For instance, *in vitro*, GHRH acutely decreases GHRH expression with rat pituitary cells *in vitro* but with prolonged exposure to GHRH, expression is elevated. Moreover, *in vivo* studies where endogenous GHRH is masked by immunoneutralization, there is decreased expression of GHRH receptors.[51] GHRH decreased GHRH receptor expression in studies with ovine[52] and chicken pituitary cells[53] *in vitro*. Expression of GHRH receptor can be either up- or down-regulated by ghrelin in a concentration-dependent manner.[52] In chicken anterior pituitary cells, GHRH-receptor expression is depressed by SRIF and completely suppressed by glucocorticoids *in vitro*.[53]

The signal transduction mechanism for GHRH encompasses the following. There is first receptor binding and then the signal transduction mechanism 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 intracellular Ca^{2+} concentrations both by stimulating the influx of Ca^{2+} (via L- and T-type voltage-sensitive Ca^{2+} channels) and by mobilization of intracellular Ca^{2+} stores through phospholipase C hydrolysis of phosphatidyl inositol[46] (Figure 5).

Somatostatin (SRIF)

Somatostatin (SRIF), a cyclic 14- or 28-amino-acid residue peptide, acts to inhibit the release, but not synthesis, of GH from somatotropes.[1,54] Moreover, there is growing consensus that SRIF is physiologically the major inhibitor of GH release in mammals and probably through vertebrates. SRIF neurons have been identified in bovine and porcine hypothalamic tissue.[47] Bipolar SRIF perikarya are located in the anterior periventricular nucleus[47] with fibers projecting ventrally into the median eminence and innervate the ventromedial and arcuate nuclei (e.g. in pigs).[47] The content of SRIF mRNA in the anterior third periventricular nucleus varies with the ultradian GH rhythm, peaking at the time of maximal GH secretion.[43]

There are five different genes for the SRIF receptor (SSTR); namely *sstr*1–5.[54] These are G-protein-coupled receptors that couple reduction in L- and T-type voltage-sensitive Ca^{2+} influx/channels and increased K^+ channels.[54] The SSTR on the somatotropes is SSTR subtype 2 (rat[55]). However, there is evidence that SSTR type 5 polymorphisms are associated with agromegaly.[56] Use of specific

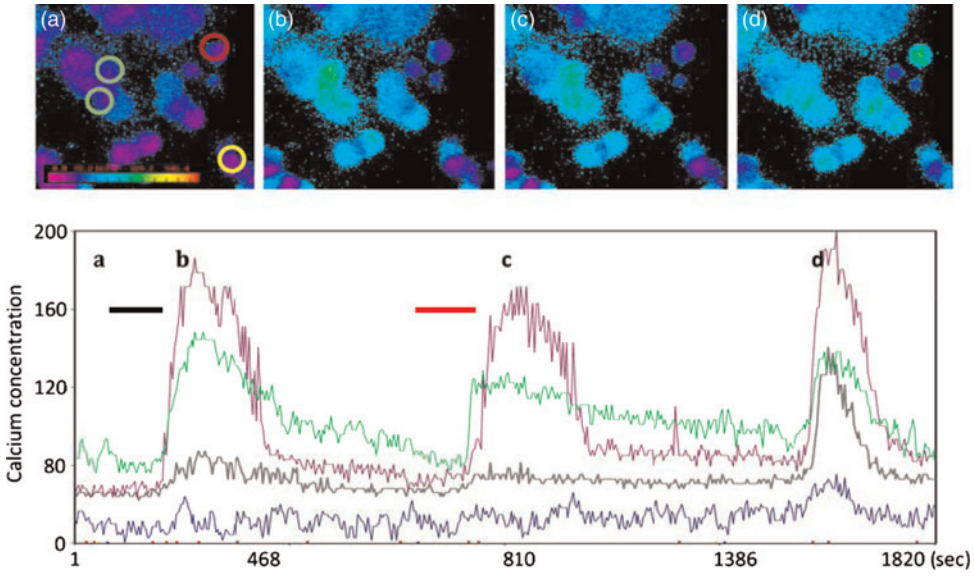

Figure 5 The sequence of color images (top panels) and kinetics of the $[Ca^{2+}]_i$ changes (bottom panel) illustrate response of isolated pituitary cells to application of 10 mmol/L hGHRH, 10 mmol/L L-585 and 50 mmol/L K^+. Pseudocolor images on the top panel are captured at the corresponding times marked on the bottom panel. Scale (in a) shows that the blue color corresponds to a baseline calcium concentration of 80–90 nmol/L, while warmer colors like green and yellow correspond to increased calcium concentrations. On the bottom panel are shown individual responses of four cells that are marked with O in color image '(a)'. Perfusion with hGHRH (10 mmol/L) for two minutes (black line) significantly increased $[Ca^{2+}]_i$ in two cells (green circles in a upper panel). Of the cells that responded to hGHRH, all (100%) also responded to L-585, which was applied 10 min after the application of hGHRH. Perfusion with 10 mmol/L L-585 for two minutes (red line) produced a prompt transient increase in $[Ca^{2+}]_i$ followed by the sustained decline to a plateau above the basal level. Potassium at a concentration of 50 mmol/L (dotted line), a non-selective stimulus, elevated $[Ca^{2+}]_i$ in all cells that responded to hGHRH and L-585 and additional neuroendocrine non-GH cells (read circle cell in panel a). In addition, there were cells that failed to respond to any stimuli (yellow circle in panel a)

agonists demonstrated that SRIF-induced suppression of Ca^{2+} currents in rat somatotropes is via SSTR2 while the increase in K^+ currents is via both SSTR2 and SSTR4.[57,58] In addition in the chicken, the effect of SRIF is via a SRIF receptor subtype (SSTR2). Evidence for this comes from the ability of the selective SSTR2 agonist to inhibit both basal and GHRH-stimulated GH release from chicken somatotropes *in vitro*. In contrast, specific SSTR1 and SSTR3 agonists did not influence GH release and at high concentrations, a selective SSTR4 agonist stimulated GH release. Moreover, there is expression of SSTR2 in the chicken anterior pituitary gland.[59] There does not appear to be an effect of SRIF on GH synthesis.[60]

Not only does SRIF act on somatotropes but also within the hypothalamus. The effects of SRIF are mediated in the hypothalamus by SSRT1 receptors on the GHRH neurons[61] on the arcuate nucleus with amounts of SRIF binding relating to the GH ultradian rhythm.[62]

Ghrelin

Ghrelin is an *n*-octanoylated peptide with 28-amino-acid residues which was originally isolated from the stomach.[63,64] This acylated peptide is highly conserved throughout the vertebrates that binds to the type 1a GH secretagogue receptor (GHS-R1a), a G-protein coupled receptor.[64] Ghrelin is produced by the regions of the gastrointestinal tract, for example, the stomach and the central nervous system including the hypothalamus.

Ghrelin directly provokes secretion of GH from somatotropes across the vertebrates including rodents,[63] baboon,[65] chickens[66] and goldfish.[67] The signal transduction mechanism for ghrelin includes a nitric oxide/cGMP signaling pathway[68] as with gonadotropin-releasing hormone (GnRH) in goldfish.[68] While virtually all porcine somatotrophs respond to ghrelin with increased intracellular calcium concentrations,[69] there is elevated intracellular calcium in only about one-fifth of chicken somatotrophs in response to ghrelin.[70] Not only does ghrelin increase the release of GH but it also increases GH mRNA, for instance, in chicken anterior pituitary cells *in vitro*.[66] Moreover, ghrelin enhances the number of somatotropes (see Section on Control of somatotrope number). Expression of GHS receptor in somatotropes appears to be down-regulated by ghrelin. This is supported by *in vitro* studies where a GHS receptor agonist decreased GHS receptor expression in ovine pituitary cells.[53]

While ghrelin can stimulate GH secretion, it may be questioned whether ghrelin does act as a hypothalamo-hypophysiotropic regulator of GH release acutely. Alternatively, does either hypothalamic or gastrointestinal ghrelin play a physiological role in the control of GH release? In an elegant series of studies, Grouselle *et al*.[71] examined the relationship between peripheral plasma and cerebrospinal fluid (CSF) concentrations of ghrelin and those of GH together with the presumed linkage temporal between pattern of pulsatile release of ghrelin and that of GH in sheep. Analysis of the pulsatile patterns of either plasma ghrelin or CSF concentrations of ghrelin and the circulating concentrations of GH failed to demonstrate tight linkage with either.[71] Moreover, the concentrations of ghrelin were reported to be much higher in the peripheral plasma than in the CSF,[71] suggesting that even if ghrelin were to be involved in the physiological control of GH release, it would be of gastrointestinal and not hypothalamic origin. In rats, pulsatile GH secretion appears to be acutely under the control of two not three hypothalamic neuropeptides, specifically GHRH and SRIF.[72] While ghrelin is capable of stimulating GH release, it probably is not involved physiologically in the immediate control of GH release at the level of the somatotrope.[72] Centrally, ghrelin evokes a GH secretory pulse by increasing the release of the GHRH level[72] while also suppressing the inhibitory effect of SRIF at the level of the pituitary[72] (also discussed below in 'Neuropeptides acting on GH release at the level of the hypothalamus'). The effect of ghrelin in the periphery is via the vagus nerve, hence to the brain and hypothalamus, thereby increasing the release of GHRH and neuropeptide Y (NPY).

In conclusion and at least in rats, pulsatile GH secretion appears to be under the control of two hypothalamic neuropeptides, GHRH and somatostatin (SRIH), with ghrelin capable of stimulating GH release by stimulating GHRH release. Similar studies in sheep examining the relationship between peripheral plasma and CSF concentrations of ghrelin and of GH suggest that hypothalamic ghrelin has little direct role in the control of GH release and that this is most probably also the case with peripheral ghrelin.[71]

Neuropeptides, hormones and other factors modulating GH secretion

There is a considerable series of neuropeptides, neurotransmitters and hormones that can either increase or decrease GH release. These include hypothalamo-hypophysiotropic factors (or releasing factors) such as GnRH, leptin, pituitary adenylate cyclase activating polypeptide (PACAP) and thyrotropin-releasing hormone (TRH) together with peripherial peptide hormones such as leptin and insulin-like growth factor 1 (IGF-I). The ability of neuropeptides to influence GH secretion has been examined by both *in vitro* and *in vivo* studies where the release of GH is demonstrated to be stimulated (or inhibited) by challenges alone or in the presence of the known secretagogue, GHRH. However, because a neuropeptide can affect GH release, that does not *per se* demonstrate that physiologically the neuropeptide is directly controlling GH secretion. To preclude the neuropeptide acting at the level of the hypothalamus rather than directly on the somatotrope, the physiological role of the hypothalamic releasing factors can be examined by their ability to influence GH release following stalk sectioning. While a neuropeptide may be capable of influencing GH release directly, this does not imply that it does. This can be studied by determining the ability of antisera to the neuropeptide reducing endogenous GH secretion or correlating episodic release of the neuropeptide with that of GH. The GH response to the secretagogue depends on the number of somatotropes and the number of GHRH

receptors per somatotrope. Neuropeptides, hormones and other factors modulating GH secretion are discussed under the following topics:

(a) Stimulatory neuropeptides;
(b) Inhibitory neuropeptides, proteins and hormones;
(c) Direct effects of neurotransmitters on GH secretion.

It should be noted that there is considerable species variation in the control of GH release and while that is partially addressed in this section, it is also covered in the section on the evolution of the control of GH release. While there are many GH secretagogues, many hormones and neuropeptides do not affect GH release. For instance, VGF appears to have no effect on secretion or expression of GH.[73]

Stimulatory neuropeptides

These will be discussed in alphabetical order to reduce the risk of drawing unintended conclusions of their relative importance.

(i) *Adiponectin*: Based on *in vitro* studies, adiponectin can influence GH release. Short-term exposure of pituitary tissue to adiponectin for four hours is accompanied by reduced release of GH in the presence or absence of GHRH. In contrast, with 24-h exposure to adiponectin, there was increased expression of GHRH receptor and elevated GH release at high doses.[74]

(ii) *Bombesin*: see gastrin-releasing peptide.

(iii) *Corticotropin-releasing hormone (CRH)*: One of the many factors that stimulate GH release in fish is CRH. For instance, CRH stimulates GH release in the eel.[75]

(iv) *Galanin*: Galanin can directly stimulate GH release. For instance, galanin when peripherally administered, stimulates GH secretion.[76] There is increased secretion of GH in transgenic mice over-expressing galanin.[77] Galanin has been reported to stimulate GH secretion *in vitro* with pig pituitary cells[78] but the effect is not consistently observed.[79] It is likely that galanin is a surrogate for galanin-related peptide (GALP). This (GALP) peptide was originally isolated and cloned from pig hypothalamic.[80] Not only does GALP stimulate GH release,[81,82] but it is expressed in close proximity to the anterior pituitary, in both the posterior pituitary gland and hypothalamic arcute nucleus.[83]

(v) *Gastrin-releasing peptide*: Gastrin-releasing peptide can stimulate GH release based on studies with bombesin, mimicking endogenous gastrin-releasing peptide. Bombesin has been reported to stimulate GH release *in vivo* but not *in vitro* in rats.[84] However, a modest increase in GH release was observed from rat pituitary cells *in vitro* in the presence of bombesin.[85]

(vi) *GnRH*: In fish, increased GH release can be evoked by GnRH, with GnRH increasing GH release by a cGMP mechanism *in vitro* (e.g. goldfish[68]). Similarly, in young chickens, there are increased intracellular concentrations of calcium following exposure to GnRH.[68]

(vii) *Kisspeptin*: Kisspeptin is produced in the hypothalamus. There is some evidence, albeit scant, that kisspeptin can stimulate GH release. The *Kiss1* gene is translated to produce a 145-amino-acid protein. This is enzymatically clipped to form a 54-amino-acid residue containing peptide, kisspeptin-54, and further cleaved to form kisspeptins with, respectively, 14, 13 or 10 amino acid residues. Kisspeptin is the ligand for the former orphan receptor – G protein-coupled receptor-54 (GPR54).[86,87] Kisspeptin 10 *in vitro* has been reported to increase the secretion of both LH and GH with elevated intracellular concentrations of calcium in not only gonadotrophs but also in somatotrophs.[88] Similarly, kisspeptin 10 has been reported to increase plasma concentrations of GH in cows treated with estradiol and/or progesterone but not in cows not receiving steroids.[89]

(viii) *Leptin*: The satiety hormone, leptin, is produced by adipose tissue together with other tissues including the anterior pituitary gland. Leptin stimulates GH release in mammals (pigs,[90] rats,[91] cattle[92]). Moreover, leptin increases intracellular calcium in both chicken and porcine somatotropes.[70,93] It may be questioned whether peripheral leptin physiologically influences secretion of GH or whether the leptin affecting GH release is locally produced and exerts an autocrine/paracrine effect. Synthesis of leptin in the anterior pituitary gland has been established.[94,95]

(ix) *Motilin*: Motilin and ghrelin are members of the same peptide family.[64] It has been reported to stimulate GH release *in vitro* while immunoneutralization using an antiserum to motilin depresses circulating concentrations of GH.[96] It is remotely possible that the antiserum to motilin cross-reacted with ghrelin. Recently, we have reported that motilin increases intracellular calcium in isolated pig somatotropes (Figure 6).

(x) *Neuropeptide Y*: There is evidence that NPY can stimulate GH release directly from somatotropes. There is, for instance, increased secretion of GH in the presence of NPY basal from porcine pituitary tissue *in vitro*.[97] However, NPY depresses the response to a GHRH challenge by pig pituitary cells *in vitro*.[98]

(xi) *Orexins*: There are direct effects of both orexin A and orexin B on GH release. Orexin A has been observed to increase further GH release in the presence of GHRH but not affecting basal GH release with ovine somatotropes, the effect mediated via the L-type voltage-gated Ca^{2+} current.[99] Not surprisingly, somatotrophs have been demonstrated to express orexin-1 receptor expression.[100] The neuropeptide has been reported to influence GH release from ovine anterior pituitary explants from sheep with the direction of the effect varying with whether the sheep had previously been on long day lengths (increased GH release) or short lengths (decreased GH release).[100] The physiological significance of a role for orexin A in pituitary function is more likely based on the observation that there is greater expression of orexin A in the hypothalamus of sheep on short day length than on long day length.[100]

(xii) *PACAP*: There is increased GH secretion in the presence of PACAP.[101] In the presence of PACAP, there is increased GH release from pituitary tissue (cattle,[102] chicken,[103] carp,[104] goldfish[105]). Chicken somatotropes respond to PACAP with increased intracellular calcium[70] and increased GH mRNA.[53] While PACAP knock-outs have been produced, there is no evidence of disturbance of GH release.[106,107] This would suggest that PACAP is not either involved in or essential for the control of GH

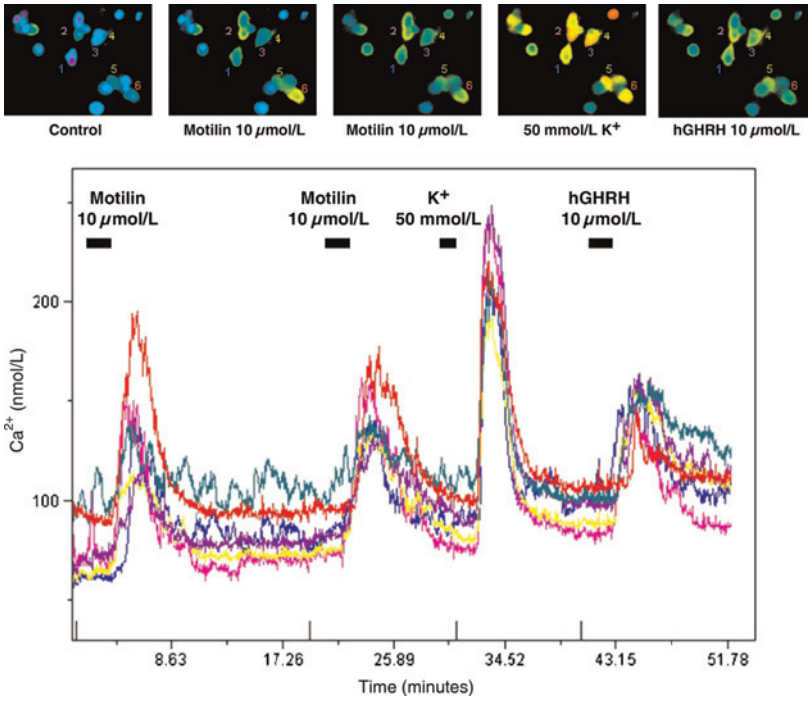

Figure 6 The sequence of pseudocolor images (a) and the kinetics of the $[Ca^{2+}]i$ changes (b) illustrate response of isolated porcine pituitary cells to applications of 10 μmol/L motilin, 50 mmol/L K^+ and 10 μmol/L hGHRH. The pseudocolor images correspond to peak calcium increases. Somatotropes were functionally identified by two-minute application of 10 μmol/L hGHRH. The repeated two-minute administration of motilin evoked similar response in six somatotropes labeled with color numbers and shown in the time course histogram with identical colors (b)

release or there is sufficient redundancy in the GH control system to overcome the absence of PACAP.

(xiii) *Resistin*: Another hormone produced by adipose tissue is resistin.[108] Resistin stimulates GH release from rat somatotropes.[108] The mechanism is Gs protein-dependent, involving an adenylate cyclase/cAMP/protein kinase A, phosphatidylinositol 3-kinase and protein kinase C with extracellular Ca^{2+} entry mediated via L-type voltage-sensitive Ca^{2+} channels.[109]

(xiv) *TRH*: TRH, pyro Glu-His-Pro-amide, was the first hypothalamic hypophysiotropic peptide to be characterized chemically. It is unique with its structure completely conserved through vertebrates. TRH binds to two G-protein-coupled receptors for TRH (namely TRH-R1 and TRH-R2).[110] Galas *et al*.[111] concluded that TRH has different roles in different vertebrate classes. In mammals, TRH stimulates the secretion of TSH, prolactin and GH.[111] In birds, TRH stimulates the secretion of TSH and GH[112] with increased intracellular calcium in somatotropes.[70] Moreover, TRH increases anterior pituitary GH mRNA in chicken anterior pituitary cells *in vitro*.[53] In amphibians, TRH stimulates the secretion of GH, prolactin and alpha-melanocyte-stimulating hormone (MSH).[111] In addition, TRH has a slight effect on TSH release in the adult.[80] In fish, TRH stimulates the secretion of GH, prolactin and MSH but does not affect TSH.[111] However, the effects of TRH on the release of GH effects vary with species within the class, development age and physiological/pathological state.[111] For instance, circulating concentrations of GH are increased following TRH administration in hypothyroid but not euthyroid dogs.[113]

Inhibitory neuropeptides, proteins and hormones

(i) *Cortistatin (CST)*: CST, like SRIF, is a cyclic peptide and has functional similarities to SRIF; binding to the SRIF receptor subtypes (SSTR1–5) with similar affinities. CST can bind other receptors (e.g. GHS-R and the MrgX2 receptor).[114] As might be expected based on its ability to bind to the SRIF receptor, CST can inhibit GH release. *In vivo*, both CST and somatostatin have been demonstrated to depress circulating concentrations of GH.[115–117] CST has structural similarities to somatostatin with there being homologies between the CST and the somatostatin genes.[118–121] CST, like somatostatin, binds to all somatostatin receptors. In contrast, CST but not somatostatin binds to the receptor (GHS-R1) for ghrelin.[114]

(ii) *IGF-I*: IGF-I inhibits GH release. For instance, GHRH-stimulated GH release *in vitro* is inhibited by IGF-I (e.g. chicken[122]). Moreover, rat somatotrophs express the IGF-I receptor (IGF-1R).[123] IGF-I not only decreases GH secretagogue receptor expression but also GH secretion in response to a GH secretagogue agonist in rat pituitary cell cultures.[124]

It may be questioned as to whether, physiologically, it is IGF-I of peripheral origin that is depressing GH release. There is evidence that would support an autocrine and/or paracrine effect of IGF-I on GH release. IGF-I is expressed in the human anterior pituitary gland, particularly in corticotrophs and also in some somatotrophes with IGF-I immunoreactivity confined to secretory granules in coexistence with either adrenocorticotropic hormone (ACTH) immunoreactivity or GH immunoreactivity suggesting constitutive

release of IGF-I when ACTH is released.[123] Similarly, both somatotrophs and many corticotrophs in the human express IGF-I.[124,125] Similarly, IGF-I is expressed in fish pituitary glands.[126]

Direct effects of neurotransmitters on GH secretion

In at least some species of fish, neurotransmitters influence GH release. For instance, in goldfish, dopamine stimulates GH release *in vitro* while norepinephrine, acting via alpha-2 adrenergic receptors, depresses basal and inhibits secretagogue (dopamine, GnRH)-stimulated GH secretion.[127,128] Similarly, serotonin inhibits GnRH-stimulated GH release in goldfish.[129]

Evolution of the control of GH release

GH and cytokines are members of the class I helical cytokines, with their receptors forming a family of receptors. While there are genes for both GH and prolactin in the gnathostomes (jawed vertebrates), there is only a single GH gene in the jawless vertebrate, lamprey.[130] This supports prolactin resulting from duplication of the GH gene prior to the separation of the lampreys from the gnathostomes.[131] There is scant evidence for even the existence of GH in invertebrate species with the exception of the early report of the detection of vertebrate GH-like immunoreactivity in the primitive chordates, the tunicate, Ciona.[132]

There is extensive information on control of release of GH through vertebrates (summarized in Table 2). In fish, GH secretion is stimulated by GHRH, adenylate cyclase-activating peptide (PACAP), ghrelin, GnRH, dopamine, CRH (see Table 2)[75] and TRH. Gahete *et al.*[133] concluded that there is an evolutionary trend to simplification with fewer stimulatory factors controlling GH release.

GHRH is a member of the GHRH/PACAP/glucagon superfamily of peptides (for early reviews see refs.[134,135]). The prevailing view is that there have been multiple exon and gene duplications. There are two major branches of this superfamily:

Branch 1: GHRH, PACAP-related peptide (PRP), PACAP, vasoactive intestinal peptide (VIP), peptide HI (PHI) and secretin with the following arrangements of genes with an exon(s) encoding specific peptides along transcripts:

- GHRH (vertebrates);[136,137]
- PRP (mammals)/GHRH like peptide (non-mammalian chordates) and PACAP;[136,137]
- PHI/PHM and VIP;
- Secretin.

Branch 2: glucagon, glucagon-like peptide (GLP)-1 and GLP-2 together with gastric intestinal peptide, probably also a member of the glucagon branch. There are three exons for, respectively, glucagon, GLP-1 and GLP-2.

Multiple members of at least branch 1 can influence GH release. Not only is GH release increased by GHRH and PACAP (see above) but also by VIP. For instance, GHRH and VIP, but not PHI, can increase GH release from bovine pituitary cells.[138] The development of the GHRH/PACAP family involves initial exon duplication and multiple gene duplications. In the protochordate tunicate, Ciona (phylum Chordata), there are at least two genes each with exons that can be translated to homologs of GHRH/PRP and PACAP.[139] While there is not a robust GH secretory response to GHRH-like peptides,[140] GHRH stimulates GH release in non-mammalian vertebrates[136] as it does in mammals.

The deduced sequences of PACAP show tremendous homologies across the phylum Chordata with, for instance, the PACAP from a protochordate tunicate being 96% identical to human PACAP.[139] This is despite the 600 million years separation of the ancestors of the protochordate tunicate and that of humans. In contrast, the putative tunicate GHRH/GHRH-like peptide/PRP is 59% identical to human GHRH.[139] The PACAP gene is ancient in animal evolution, appearing before the divergence of protostomes (e.g. phylum Arthropods, Mollusca and Annelida) and deuterostomes (e.g. Chordata, Echinoderma, Hemichordata) about one billion years ago. There is strong evidence for the presence of PACAP-like peptide in mollusks,[141] annelids[142] and arthropods/insects. In Drosophila, the amnesiac gene encodes a peptide that is homologous with vertebrate PACAP. The peptide is expressed in neurons and acts via a cAMP signaling pathway in sleep regulation.[143]

It is possible to envision the control of GH secretion shifting from autonomous to being influenced by multiple hormones and neurotransmitters with varying degrees of specificity to a highly specific mechanism with a few major control points as is the case in mammals.

Table 2 Peptides, hormones and neurotransmitters that directly physiologically influence GH secretion in different vertebrate classes

Class	Stimulator	Probable stimulator	Possible stimulator	Inhibitor	Possible inhibitor
Mammals	GHRH	Ghrelin, PACAP	Leptin, adiponectin, kisspeptin, neuropeptide Y, orexin A and B	SRIF, IGF-I	Cortistatin, T_3
Birds	GHRH, TRH	Ghrelin, PACAP	Leptin, GnRH	SRIF, IGF-I, T_3	
Reptiles/ amphibians	GHRH, TRH	Ghrelin, PACAP		SRIF, IGF-I	
Fish	GHRH, TRH	CRH, ghrelin, PACAP, GnRH, dopamine		SRIF, IGF-I, serotonin, norepinephrine	

GHRH, growth hormone-releasing hormone; PACAP, pituitary adenylate cyclase activating polypeptide; SRIF, somatostatin; IGF-I, insulin-like growth factor; TRH, thyrotropin-releasing hormone; GnRH, gonadotropin-releasing hormone; CRH, corticotropin-releasing hormone

Control of somatotrope number

GH secretion is markedly affected by the number of somatotropes in the pituitary gland. In turn, the number is influenced by hormones and neuropeptides, including glucocorticoids, ghrelin and GHRH. Differentiation of somatotropes is induced by glucocorticoids, for instance, in prolonged culture of chick embryo pituitary cells with large increases in somatotropes and responsiveness to GHRH.[144,145] A critical role for ghrelin in the development of somatotropes is supported by the studies in transgenic animals. In GHS-R1a null mice and rats, there are considerably fewer somatotropes and much lower expression of Pit-1 and GH.[146] In addition, ghrelin has been demonstrated to stimulate cell proliferation of the rat pituitary cell line GH3.[147] Moreover, the development of somatotropes is depressed in zebra fish where ghrelin functioning was prevented by antisense oligonucleotides. This led to GH expression being abolished.[148] The effect was overcome in rescue experiments with ghrelin.[148]

There is evidence that GHRH can either increase or decrease somatotrope numbers. Evidence for the former comes from the following series of studies. *In vivo*, GHRH increased the number of somatotropes in pigs.[149] Chronic high concentrations of GHRH were achieved in progeny of dams that had received administration of a plasmid encoding a protease-resistant GHRH. Conversely, and somewhat surprisingly, prolonged treatment of chick embryo anterior pituitary cells with GHRH depressed the number of somatotropes.[144]

It is frequently assumed that somatotropes can transform into lactotropes and *vice versa*. However, there is evidence that does not support this, at least in the case of developing chick embryo. For instance, lactotropes differentiate prior to somatotropes[144] and in a separate lobe of the pituitary gland.[150] Moreover, somatotropes are not transformed into lactotropes *in vitro*.[150] Somatotropes themselves appear to influence somatotrope number. Recent studies have examined the effects of somatotrope ablation on their functioning using transgenic mice with somatotrope-specific expression of a modified influenza virus ion channel. The severity of the loss of somatotropes depended on transgene copy number with a disruption of the GH cell network organization and effects on cell–cell communication.[151]

Intrahypothalamic factors ultimately influencing GH secretion

There is evidence for multiple peptides indirectly influencing GH by effects with the hypothalamus. These peptides are either directly or indirectly influencing the release of hypophysiotropic factors from terminals in the median eminence. The neuropeptides will be considered in alphabetical order:

Bombesin: See gastrin-releasing peptide.

Calcitonin: In rats, spontaneous GH secretory pulses are suppressed by the intracerebroventricular administration of calcitonin.[152]

Cholecystokinin: Cholecystokinin influences GH secretion at the hypothalamic level. For instance, in goldfish, CCK, when centrally administered, decreases preprosomatostatin mRNA.[153]

Galanin: Galanin and/or galanin like peptide (GALP) act within the hypothalamus indirectly to increase GH release. Intraventricular infusion of GALP increases plasma concentrations of GH in rats[81] and rhesus monkeys.[154] Similarly, in rats and pigs, circulating concentrations of GH are increased by intracerebroventricular administration of galanin.[76,155]

Gastrin-releasing peptide: Exogenous bombesin, mimicking endogenous gastrin-releasing peptide, stimulates GH release in goldfish by suppressing expression of preprosomatostatin I and II in the forebrain.[156]

Ghrelin: Ghrelin stimulates GH secretion, for instance, in baboons.[65] There is considerable evidence that ghrelin exerts a major, if not its major, effect on GH release by decreasing the release of SRIF from neurosecretory terminals in the hypothalamus and/or hypothalamic synthesis of SRIF. In pigs, circulating concentrations of GH are increased by the intracerebroventricular administration of an agonist for the GHSR.[155] Interestingly, the effect of the GHSR agonist is attenuated by concomitant administration of SRIF or galanin or NPY.[155] In goldfish, peripheral administration of ghrelin increases circulating concentrations of GH while increasing expression of preprosomatostatin I and decreasing preprosomatostatin II in the forebrain.[157]

NPY: NPY is another hypothalamic neuropeptide affecting GH release by an intrahypothalamic effect.[155]

Obestatin: Obestatin is a peptide encoded by the ghrelin gene.[158] Obestatin binds to the orphan G protein-coupled receptor GRP39 and motilin. Obestatin suppresses food intake and gastrointestinal functions, and thus is implicated in meal initiation and body-weight regulation. Ghrelin administration increases food intake and decreases energy expenditure and weight gain. That obestatin is a cognate ligand for GPR39 suggests that GPR39 may evolve from a common ancestor but with divergent functions in body-weight regulation.[158]

Apelin: Apelin consists of 77 amino acids that are processed to produce several smaller peptide fragments: apelin-36, -17, -13 and -12. Apelin mRNA and peptide expression have been described in various tissues, including the gastrointestinal tract, adipose tissue, brain, lung, kidney, liver and the cardiovascular system.[159] Apelin may play a role in fluid homeostasis and food intake, as both apelin and the receptor (APJ) have been demonstrated to be particularly active in the supraoptic nuclei (SON) and paraventricular nuclei (PVN) of the hypothalamus.[160–162] These areas of the brain are known to be important in the regulation of food and water intake, and water deprivation has been shown to increase the mRNA expression of the apelin receptor in the SON and PVN.[163,164] Food intake causes a decrease in plasma GH concentrations in ruminants, which was reversed in the preruminant.[165] In goats, AVP stimulated ACTH secretion more than CRH did, and GH secretion was stimulated as well.[166]

Peptide YY (PYY): PYY, a 36-amino-acid peptide that shares a common tertiary structure with NPY and pancreatic polypeptide (PP),[167] was initially isolated from porcine intestine.[168] This peptide exists in two major circulating

forms, PYY_{1-36} or PYY_{3-36}.[169] PYY_{3-36} is a 34-amino-acid peptide, which is derived from PYY_{1-36} via cleavage from the N-terminal by dipeptidyl peptidase IV. PYY_{3-36} is able to cross the blood–brain barrier[170] and peripheral administration of the peptide has been demonstrated to reduce feed intake.[171] Peripheral administration of PYY_{3-36} in pigs can reduce feed intake.[172] Moreover, plasma PYY levels fluctuated with the changes in energy balance leading to the suggestion that plasma PYY_{3-36} levels influence satiety and contribute to the termination of feed intake in pigs. Furthermore, the effect of PYY_{3-36} was rapid and short-lived, reflecting the clearance (short half-life) of intravenous administered PYY_{3-36}. Moreover, intravenous injection of PYY_{3-36} did not affect plasma acyl-ghrelin levels in the fasted condition indicating that, at least in pigs, PYY_{3-36} is not a major regulator of ghrelin secretion.[172] However, since the administration of PYY_{3-36} increased plasma GH levels, it is suggested that PYY_{3-36}, apart from its influence on feed intake, may also have other metabolic functions that are crucial in the regulation of energy homeostasis in pigs.

GH plays a major role in the regulation of lipid metabolism and alterations in GH axis elicit major changes in fat distribution and mobilization.[173] Central chronic ghrelin administration regulates adipose lipid metabolism, mainly in a GH-independent fashion, as a result of increased mRNA, protein expression and activity levels of fatty acid metabolism enzymes. Central ghrelin regulates hepatic lipogenesis *de novo* in a GH-independent fashion but lipid mobilization in a GH-dependent fashion because carnitine palmitoyltransferase 1 was decreased in wild-type Lewis rats. These findings suggest the existence of a new central nervous system-based neuroendocrine circuit, regulating metabolic homeostasis of adipose tissue.

In thyrotoxicosis patients, GH responses to stimuli are diminished and the hypothalamic–pituitary–adrenal axis is hyperactive.[174] This blunted GH responsiveness to ghrelin, GHRP-6 and GHRH did not normalize after six months of normalization of thyroid function, although GH responses to all peptides are enhanced in approximately half of the patients. These results suggest that the different pathways of GH release stimulated by these peptides are similarly affected by thyroid hormone excess. Cortisol responsiveness to ghrelin and GHRP-6 was normal while ACTH responses to these peptides decrease during treatment. These results suggest that hypothalamic mechanisms of ACTH release modulated by ghrelins/GHS may be activated by thyroid hormone excess, but adrenal reserve is prevented.[174] Several protein-tyrosine phosphatases (PTPs) have been implicated in the control of GHR signaling, but none have been shown to affect growth *in vivo*. Pilecka *et al*. applied a battery of molecular and cellular approaches to test a family-wide panel of PTPs for interference with GHR signaling.[175] Among the subset of PTPs that showed activity in multiple readouts, PTP-H1/PTPN3 was selected for further *in vivo* studies and found that mice lacking the PTP-H1 catalytic domain show significantly enhanced growth over their wild-type littermates. PTP-H1 mutant animals also had enhanced plasma and liver mRNA expression of IGF-I, as well as increased bone density and mineral content. These observations point to a controlling role of PTP-H1 in modulating GHR signaling and systemic growth through IGF-I secretion.[175] Korbonits *et al*.[176] analyzed the effect of common genetic variations in the *GHSR* gene in the regulation of height, a highly heritable polygenic trait, in the human at the population level. They could not show any association of the *GHSR* gene and variation of stature in our population. They speculated that common genetic variants, not genotyped in their study, may exist and may modulate, at an early stage, the GH–IGF-I axis and hypothalamic programming. Other genetic and environmental factors may influence this axis later in life. However, if such factors exist, any such effects would be modest.[176]

GHSR1 agonists increase GH pulse amplitude by activating GHSR1a on hypothalamic GHRH neurons, augmenting GHRH-induced release through GHSR1a on somatotrophs, and antagonizing the suppressive effect of somatostatin (SST) on GHRH-induced GH secretion from somatotrophs.[177] GHSR1a on GHRH action is important because ghsr−/− mice exhibit reduced expression of pit1 associated with modest reductions in somatotrophs and lactotrophs in the anterior pituitary gland. Compared with wild-type mice, ghsr1a−/− mice exhibit 20% lower circulating IGF-I levels and are slightly smaller. Ghrelin and GHRH are intimately involved in the normal regulation of GH release and the absence of either signaling pathway results in impaired GH secretion. GH release from somatotrophs is inhibited by SST action mediated through somatostatin receptor subtype 5 (SST5) and subtype 2 (SST2). FRET studies revealed that SST5 signal transduction requires SST-induced formation of SST5:SST5 homomers. In the presence of GHSR1a, BRET analysis shows that SST5 constitutively forms GHSR1a:SST5 heteromers, thereby inhibiting SST action. Formation of heteromers is stabilized by ghrelin and destabilized by high concentrations of SST. High GH causes SST to increase providing negative feedback, and when GH is low, SST is low.[177]

Cruz and Smith[178] provide an extensive review of GHSR1a distribution and functions in different organ systems. Most GHSR1a receptors are concentrated in pituitary cells, and GHSR mRNA levels were greatly expressed in various nuclei of the hypothalamus, consistent with its neuroendocrine roles in GH secretion and energy balance. GH levels were significantly higher after ghrelin administration compared with levels obtained after treatment with either GHRH or GHSR agonist, hexarelin. Ghrelin also induced prolactin, ACTH, cortisol and aldosterone release in humans. Despite its potent induction of GH release, GHSR-null mice showed no phenotypic differences with wild-type mice, suggesting that ghrelin and its receptor do not exert dominant effects in this neuroendocrine process.[178]

Conclusion

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) Why do only some somatotropes respond to specific secretagogues?
(3) Is there a localized signal transduction mechanism(s)?
(4) Are the receptors for releasing hormones arranged in close proximity to porosomes?

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