A Tripeptidyl Ensemble Perspective of Interactive Control of Growth Hormone Secretion

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Key Words

Growth hormone · Growth hormone-releasing hormone · Growth hormone-releasing peptide · Ghrelin · Somatostatin · Insulin-like growth factor I · Threefold peptide control pathway · Sex-steroid hormones · Gender · Oestrogen · Oestradiol · Women

Abstract

Current investigational tools in molecular biology, biochemistry and integrative physiology have revealed an increasing array of signals that influence growth hormone (GH) secretion. The present perspective combines these factors under a simplified final-common pathway model of threefold joint control by GH-releasing hormone, GH-releasing peptide/ghrelin and somatostatin. This concept is highlighted from the viewpoint of sex steroid- and age-dependent modulation of the peptide trilogy listed above.

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Overview of Growth Hormone Neuroregulation

Principal anatomical components of the somatotrophic axis comprise the hypothalamic regulatory centres, the anterior pituitary gland and the peripheral target organs. Targets of growth hormone (GH) action include the liver, kidney, skeleton, brain, breast, ovary, testis, heart, skele-

tal muscle, gastrointestinal tract, spleen, thymus, bone marrow and skin. GH stimulates the cell-specific biosynthesis of insulin-like growth factor I (IGF-I), which in turn mediates many, but not all, growth effects. In certain cells, such as prechondrocytes and mature adipocytes, GH acts independently of local IGF-I production or reception. Organotropic hormones also drive in situ IGF-I gene expression and regulate IGF-receptor and -binding protein activities. Homeostasis proceeds by multivalent signalling from GH, IGF-I and an ensemble of regulatory peptides, and is discussed below. Adaptive control requires repeated gradual adjustments in feedforward and feedback drive. The sensitivity and efficacy of the feedback drive implies that the dose-response interactions are modulated by homologous and heterologous effectors, sex steroids, cortisol, thyroxine, systemic metabolites and intrapituitary factors [1–4].

The majority of somatotrophic signals converge on a final-common trilogy of regulatory peptides (fig. 1). In this model, GH-releasing hormone (GHRH) and somatostatin exert opposing (i.e. stimulatory and inhibitory) actions on somatotrope cells and achieve (paradoxical) synergy when presented in appropriately timed succession [5–8]. Ghrelin, a potent acylated peptide synthesized in the stomach and anterior pituitary gland, drives GH secretion via a novel cascade of hypothalamo-hypophysial mechanisms [9–12]. All three peptidyl effectors and cognate signalling pathways are regulated by a reversible autocrine feedback enforced by GH and IGF-I (fig. 1a) and spe-

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0301-0163/03/0607-0086\$19.50/0

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cific actions of oestradiol (fig. 1b) [1]. The physiological importance of oligopeptide control is illustrated by the ability of the combined infusion of *L*-arginine (a presumptive repressor of somatostatinergic outflow) and GHRH or GH-releasing peptide (GHRP) to elicit near-maximal GH secretion throughout the human lifetime [13–18]. In contrast, single administrations of GHRH, GHRP or *L*-arginine cause variable release of GH depending on age, sex, body composition and nutrient status.

GH-Releasing Hormone: A Prime Secretagogue

The core component of the dynamics of GH uses an essential feedforward stimulation by GHRH [1–3, 8, 13, 14, 19–25]. Direct monitoring of the pulsed release of GHRH into hypothalamo-pituitary portal blood has established strong (50–70%) temporal concordance of GHRH and GH peaks in four mammalian species [26–28]. The secretagogic role of GHRH has been observed in studies of GH secretion in rare genetic diseases and interventional experiments conducted both in vitro and in vivo. For example, the mean concentrations of GH and the GH pulse amplitude are reduced significantly by: (1) passive immunoneutralization of GHRH; (2) pharmacological blockade of the GHRH receptor; (3) chemical or electrolytic lesioning of GHRH neurons in the arcuate nucleus; and (4) loss-of-function mutations of the GHRH receptor in humans (initially described in the so-called ‘dwarfs of Sindh’) and mice (*lit/lit* dwarf) (fig. 2a) [1, 6, 14, 20, 29].

The GHRH receptor-effector pathway has the unique and multifold capability to: (1) rapidly stimulate the exocytotic release of GH-containing secretory granules; (2) subacutely induce transcriptionally dependent *de novo* GH biosynthesis; (3) promote chronic somatotrope cell hypertrophy, hyperplasia and tumour formation; (4) up-regulate (in the immature rat) and down-regulate (in the adult animal) the cognate pituitary receptor; (5) increase expression of pituitary ghrelin peptide and receptor; (6) synergize with GHRP-receptor agonists in vivo (fig. 2b); (7) promote somatostatin secretion in vivo and in vitro; (8) induce marked (rebound-like) GH secretion by somatostatin-primed somatotropes [5, 19, 20, 30–35].

No current network-like algebraic formalism embodies this unique collection of regulatory properties. Non-aromatizable androgens up-regulate the production and reception of GHRH in the rodent through indirect mechanisms, as the androgen receptor is not detectable in

Fig. 1. **a** Concept of ensemble control of GH secretion by GHRH, GHRP/ghrelin, somatostatin (SS), GH and IGF-I. **b** Sites and mechanisms of action of oestradiol on feedback by SS (top left) and feedforward by GHRH and GHRP-2 (top right and bottom left) discerned by infusion of a single effector in post-menopausal women.

Fig. 2. a Profound (>30-fold) attenuation of GH secretory burst mass in two young-adult siblings with a homozygous truncational mutation of the GHRH receptor. The generation of recurrent GH pulses ($p < 10^{-6}$) and unfolding of 24-hour rhythmicity ($p < 10^{-3}$), however, are clearly retained. Serum concentrations of GH were measured by high-sensitivity immunofluorometric assay of blood samples collected every 10 min for 24 h. In both patients, bolus injection of GHRP-2 stimulated release of GH by several fold. The normal daily GH pulse frequency shows that factors other than GHRH receptor-dependent signalling direct the timing properties of pulse renewal. Similarly, the nycthemeral rhythmicity of GH release is independent of GHRH signalling in timing, but not in absolute amplitude. Approximate entropy (ApEn) estimates are elevated by >5 standard deviations (SD) above age- and sex-matched control values. The latter anomaly indicates profound erosion of normal sub-pattern reproducibility otherwise maintained via repeated incremental feedforward adjustments that include GHRH. Adapted, with permission, from Roelfsema et al. [29]: © The Endocrine Society. **b** Marked (120-fold) feedforward drive by combined intravenous infusion of GHRH and GHRP-2 or saline (placebo) continuously over 24 h in a post-menopausal woman.

GHRH neurons. The oestrogen receptor (OR) subtype alpha is expressed in two-thirds of the arcuate-nucleus GHRH neurons in both men and women. Oestrogen agonists down-regulate hypothalamic synthesis and pituitary reception of the GHRH stimulus in the adult rat; and, conversely, transgenic silencing of OR- α expression de-

pletes IGF-I in the female mouse [1]. In contrast, human studies show that oestradiol up-regulates pituitary sensitivity to GHRH, when monitored under putative somatostatin withdrawal caused by pre-treatment with *L*-arginine. In theory, the last mechanism could contribute to elevated GH secretory-burst mass in puberty (fig. 3).

Fig. 3. a The impact of puberty in boys and girls on the mass of GH secreted per burst ($\mu\text{g/l}$). GH output was monitored by chemiluminescence assay of serum samples collected at 5-min intervals for 12 h. Data show the extreme range, interquartile boundaries and median values. The absolute range of burst mass was 40-fold among individuals. The mass of hormone secreted per burst is the time integral of the underlying (deconvolved) secretory pulse. Burst frequency and GH half-life do not change in puberty. Reproduced, with permission, from Veldhuis et al. [36]: ©The Endocrine Society. **b** Multifold mimicry of pubertal GH outflow induced by constant intravenous infusion of a potent, synthetic, hexapeptide analogue of ghrelin (GHRP-2 [$1 \mu\text{g/kg}$]) intravenously over 24 h. Subpanels give measured outcomes of GHRP-2 drive. Blood was collected every 10 min beginning at 08.00 h (time zero). Technical explanations follow in the text concerning changes in pattern irregularity (approximate entropy), the 24-hour rhythmic mean concentration of GH (mesor), plasma concentration of IGF-I and autofeedback on the GHRP-2 stimulus.

Fig. 4. a Concept of deconvolution analysis to unravel the components of hormone concentration peaks into their underlying secretion (S) and kinetic contributions. **b** Example of stable kinetics of recombinant human (rh)GH infused across three major strata of pubertal development in boys. Knowledge of the GH distribution volume is important, as deconvolution secretory burst mass and amplitude and secretion rates are normalized to unit volume of distribution.

Somatostatin: Inhibitory and Facilitative Roles

The anterior pituitary gland expresses the five primary somatostatin receptor (SSTR) gene transcripts in the rat and human (yet not so evidently in the sheep) [21, 37, 38]. SSTR-1, SSTR-2 and SSTR-5 predominate. The arcuate nucleus manifests both SSTR-1 and SSTR-2 gene transcripts in approximately 15% of GHRH-secreting neurons in the adult male rat. Subtype-specific SSTR expression is sexually dimorphic, i.e. there is a large number of SSTR-1 in the arcuate nucleus and SSTR-2 predominates in the pituitary gland in males in comparison with females [39].

Intermittent exposure to somatostatin, although initially viewed as paradoxical, sensitizes somatotrope cells to stimulation by GHRH in vitro and in vivo [35, 40]. The outcome is an elevation in GH secretory burst mass, which may be measured directly in vitro through perfusion and indirectly in vivo by deconvolution analysis

(fig. 4). This positive interaction is important physiologically as repeated administration of a linear hexapeptidyl SSTR antagonist inhibits weight gain and linear growth in the immature male rat [25]. From a mechanistic viewpoint, somatostatin facilitates GH secretion through both the pituitary gland and hypothalamus. In somatotrope cells, somatostatin: (1) blocks the release but not the de novo biosynthesis of GH, thus favouring accumulation of releasable GH stores; (2) activates cellular phosphatases, which may mediate resensitization of the GHRH receptor; and (3) bidirectionally autoregulates cognate gene expression by rapid stimulation and delayed repression [1, 41]. In the arcuate nucleus, somatostatin: (1) up-regulates SSTR-2 (an autostimulatory pathway) and down-regulates SSTR-1 (an autoinhibitory pathway); and (2) represses GHRH outflow [42].

Both consecutive exposure to somatostatin and withdrawal of somatostatin cause a rebound-like pulse of GH

Fig. 5. Illustration of approximate entropy as a statistical measure of graded subpattern disruption despite preservation of coarse structure (seen here as a simple cosine rhythm). More disorderly neurohormone release signifies deterioration of feedback and/or feedforward control within the ensemble. Autonomous tumoural secretion, exogenous GHRH and/or GHRP-2 stimulation, defective GHRH-receptor signalling and IGF-I feedback withdrawal induce highly irregular secretion of GH, whereas infusions of somatostatin and rhIGF-I by way of enforced feedback endow more reproducible subpatterns of GH outflow. Reproduced, with permission, from Hartman et al. [43]. Permission conveyed through the Copyright Clearance Center.

secretion. A possible basis for this *in vivo* event would involve a sequential four-part mechanism, for example:

- (1) Pituitary accumulation of releasable GH stores by the blockade of exocytosis by somatostatin.
- (2) Recovery of somatotrope GHRH-receptor sensitivity by activation of phosphatases.
- (3) Restraint of hypothalamic somatostatin release during the rebound phase by removal of somatostatinergic autoneuronal stimulation via SSTR-2.
- (4) Simultaneous induction of arcuate-nucleus GHRH secretion by withdrawal of somatostatinergic inhibition.

Regarding this last point, a single injection of a somatostatin analogue (octreotide) in the conscious ram represses and then (on octreotide removal) stimulates hypothalamic GHRH release into hypophyseal-portal blood. In a functional context, passive immunoneutralization of GHRH activity in the adult male rat antagonizes rebound GH release [1].

The rebound burst of GH secretion is limited. The suppression of GH release when systemic concentrations of somatostatin fall could originate via a twofold mechanism involving interneuronal and central nervous system (CNS)-pituitary regulation. In particular, somatostatin withdrawal would predictably suppress SSTR-1-mediated autoneuronal inhibition, thus releasing hypothalamic somatostatin from SSTR-1 cells. Somatostatin is able to suppress GHRH outflow and block GH exocytosis, and, consequently, terminate the burst. GHRH (like GH), however, induces the release of hypothalamic somatostatin both *in vivo* and *in vitro*. The somatostatin restraint that would follow would initiate a new cycle of GH store accumulation, somatotrope resensitization to GHRH and

inhibition of GHRH secretion. This type of intrahypothalamic scenario may be crucial to GH-pulse regeneration within a multipeak volley. The proposed series of events differs notably from concentration-dependent autotfeedback by systemic GH. Blood-borne GH appears to induce periventricular-nuclear somatostatin secretion after a more prolonged time delay and with greater persistence than intrahypothalamic GHRH. Slower onset and offset of peripheral GH-mediated autoinhibition would reflect the need for CNS uptake, circulatory elimination and interneuronal tissue-fluid dissipation of the GH signal. This long-loop (systemic-CNS) autoinhibitory pathway may enforce the interval nadir in which GH secretion becomes undetectable in the adult male rat, but is measurable by immunofluorometry and immunochemiluminometry in the human [1]. In contrast, the adult female rodent exhibits substantial (but incomplete) escape from GH-driven autorepression. The implications of the previous regulatory interactions can be explored in simplified biomathematical constructs. Such formulations, which include key relevant time-delayed linkages, forecast gender-relevant automaticity (self-renewal) of hypothalamic GHRH and hypophyseal GH pulses [2, 3]. A broad statistical tool 'approximate entropy' provides a non-invasive measure of adaptations in the overall regulatory linkages within a neuroendocrine ensemble (fig. 5).

The main connections between GHRH, somatostatin and GH are stabilized by complex regulatory redundancies. For example, other analyses have revealed temporally biphasic autoregulation of pituitary SSTRs, induction of pituitary SSTR-1 and SSTR-2 by GHRH and GH [24], and GH-dependent down-regulation of pituitary SSTR-2

Fig. 6. a Oestradiol mutes submaximal dose-dependent inhibition of pulsatile GH secretion of somatostatin (SS) in gonadoprival women. **b** Impact of oestradiol on the median half-maximally inhibitory dose of infused SS viewed in relation to the mean 3-hour GH concentration ($p < 0.01$) and GH approximate entropy (ApEn) (not significant). Reproduced, with permission, from Bray et al. [45]. © The Endocrine Society.

Fig. 7. Oestradiol potentiates dose-dependent stimulation of GH secretion by GHRP-2 in post-menopausal women. Adapted, with permission, from Anderson et al. [59]: © The Endocrine Society.

and up-regulation of SSTR-5 gene transcripts. The precise implications and physiological regulation of such homologous and heterologous interactions across development are unclear.

Oestradiol stimulates transcriptional activity of the human SSTR-2 gene promoter in pituitary and breast cancer cells in vitro, and induces SSTR-2 and SSTR-3 gene expression (and somatostatin binding) in the rodent pituitary gland and arcuate nucleus in vivo [38, 44]. In contrast, oestrogens reduce the abundance of pituitary SSTR-1 and SSTR-5 mRNA [38]. The down-regulation of SSTR-5, if applicable to the human, could account for the ability of short-term replacement of oestradiol to antagonize inhibition by exogenous somatostatin in post-menopausal women (fig. 1b, fig. 6) [45].

GHRP/Ghrelin: Unique Secretagogic, Appetitive and Metabotropic Peptides

GH-releasing peptides were first synthesized more than 20 years ago in the laboratory of Cyril Y. Bowers. Early compounds were oligopeptide derivatives of met-enkephalin, which stimulated in vitro GH secretion by two- to three-fold at nanomolar concentrations. The GHRP receptor was cloned, expressed and mapped topographically in 1996 [1], and a gene encoding the endogenous ligand, ghrelin, was sequenced from the rat and human stomach 3 years later [10, 11, 16, 31, 46, 47].

Ghrelin is a 28-amino-acid peptide requiring fatty acylation of the N-terminal Ser³ residue (e.g. non-exclusively by octanoylic acid) for biological activity. A naturally occurring des-Gln¹⁴-ghrelin (27-amino-acid peptide) is also biologically active, but its physiological role is not defined.

Diverse GHRP-receptor agonists are able to:

- (1) synergize with GHRH in vivo but not in vitro (except in some perfusion systems)
- (2) oppose certain hypothalamic actions of somatostatin
- (3) stimulate release of arcuate-nucleus GHRH
- (4) partially overcome GH autonegative feedback
- (5) increase pituitary GH gene expression after prolonged administration in the immature (but not adult) animal
- (6) stimulate somatotrope expression of the *pit-1* gene (a pituitary-specific cytodifferentiation-enhancing transcription factor)
- (7) trigger secretion of prolactin and adrenocorticotropin transiently
- (8) exert motilin-like gastroprokinetic effects directly and stimulate peptic acid secretion indirectly via CNS pathways
- (9) serve as potent central appetitive signals (orexigens)
- (10) act as cardiotropic agents
- (11) antagonize (acutely) or facilitate (chronically) peripheral glucose disposal
- (12) block β -cell insulin secretion in vitro and augment insulin release in vivo
- (13) inhibit fat oxidation directly, thus promoting adipocyte hypertrophy
- (14) deplete intra-abdominal fat indirectly by stimulating pulsatile GH secretion [10, 11, 13, 18, 44–53].

Ghrelin

Immunoreactive (but not necessarily commensurately bioactive) ghrelin circulates at concentrations of 200–650 pmol/l in the rat and human. Higher values are approximately the same magnitude as the in vitro ligand-receptor equilibrium dissociation constant [11, 20]. The concentrations of ghrelin fall post-prandially and in obesity, and rise during fasting, after weight loss or gastrectomy and in anorexia nervosa [54, 55]. Caloric restriction unmasks the diurnal rhythmicity of the concentrations of ghrelin. In young adults, intravenous infusion of ghrelin (5.0 pmol/kg/min) for 20 min increases the concentrations of ghrelin by two- to three-fold, stimulates GH secretion by 50% of maximal output and enhances appetite compared with saline [56]. Clinical data are consistent with inferences in a transgenic model of molecular silenc-

Fig. 8. Impact of GHRP-2, GHRH and placebo on pulsatile GH secretion calculated by deconvolution analysis. Individual GH-concentration profiles were obtained by sampling blood every 10 min for 24 h. Data are from a post-menopausal volunteer studied on six occasions in randomly assigned order before and after oestradiol replacement [30, 58]. Each peptide was infused continuously at a rate of 1 µg/kg/h intravenously for 24 h.

ing of the hypothalamic GHRP receptor. Affected mice of either sex eat sparingly, gain weight at a poor rate and acquire little intra-abdominal fat. Only the female transgenic animal exhibits a significant reduction in pulsatile GH secretion and serum concentrations of IGF-I [57]. The latter gender contrast is not well explained, despite several examples of sex differences in GHRP receptor expression. In the human, the oestradiol-replete state acutely potentiates the stimulation of GH secretion by GHRP (fig. 7, 8) [56].

GHRP

GHRP and GHRH act synergistically in the presence of a functional hypothalamo-pituitary nexus. Peptide synergy reflects the complementary and convergent effects of these two secretagogues, which are:

- (1) GHRP evokes the release of GHRH from the arcuate nucleus in the sheep and opposes hypothalamic inhibitory effects of somatostatin in the rat.
- (2) GHRP down-regulates, whereas GHRH up-regulates, ghrelin and the cognate receptor in the pituitary gland.
- (3) GHRP induces GH gene expression after extended administration in the immature animal only (i.e. not adult) and stimulates maximal GH secretion in puberty, whereas GHRH drives GH gene transcription promptly at all ages and elicits maximal GH secretion earlier in life.
- (4) Feedforward by GHRP compared with GHRH is resistant to GH autofeedback.
- (5) GHRH and GHRP activate distinct cellular receptor-effector signalling pathways, which intersect more subtly at several distal loci [18, 60–62].

Administration of GHRP-receptor agonists in near-maximal and intermittent doses produces rapid response desensitization *in vitro* and *in vivo*. On the other hand, delivery of a submaximal effective (or near-stimulus threshold) dose frequently or continuously sustains pulsatile GH secretion and elevates serum concentrations of IGF-I for at least 3–6 months; slows down fasting-associated nitrogen wasting; augments lean-tissue mass; reduces visceral adiposity; acutely antagonizes and chronically facilitates insulin action (the latter emerges as intra-abdominal fat mass decreases under GH stimulation); opposes oxidation of fat in adipocytes by GH receptor-independent mechanisms; and promotes bone remodeling [12, 18, 58, 63–70]. The impact of continuous intravenous infusion of GHRH or GHRP-2 separately for 24 h is illustrated in figure 8.

Interventional trials are being used to evaluate the growth-promoting efficacy of GHRP-receptor agonists in clinically pre-pubertal children with idiopathic short stature and non-destructive GH-deficient states. In available investigations, hexapeptides (hexarelin or GHRP-2) or the non-peptidyl GHRP mimetic, MK-0677 (ipamorelin), were administered intranasally, orally or subcutaneously 1–3 times daily for 6 months or more. These agents increase serum concentrations of IGF-I, GH and IGF-binding protein 3 (IGFBP-3) into the normal range. Responses tend to be biphasic over time, with an initial two- to threefold elevation, followed by a 30–40% decline to values that continue to exceed pre-study baseline determinations significantly. Collective data from 30 children in five studies produced cohort-mean annualized height velocities at 6 months of 6.0–8.3 cm/year (aggregate $p < 0.001$ vs. basal growth records) [64, 65, 67, 68].

In a prospective clinical study involving healthy, older (physiologically hyposomatotropic) adults, GHRP-2 was delivered by continuous subcutaneous infusion (1 µg/kg/h) for 1–3 months [69]. Detailed analyses of 24-hour GH release profiles showed tripartite stimulation of pulsatile, entropic (feedback-sensitive irregularity) and 24-hour rhythmic GH secretion. Concentrations of IGF-I increased significantly and remained at this level for 90 days. Daily pulsatile GH production rates (calculated by deconvolution analysis) increased initially by 2–3 times baseline values (at 1 week) and then decreased gradually to 1.5–2 times baseline values (for 3 months). Of note, peripheral concentrations of IGF-I did not fall as GH output reset to an intermediate value; the basis for this dissociation is not established, but one mechanism may be more effectual GH drive to the liver and other organs resulting from a GH secretory pattern similar to that

seen in puberty. The 30–35% decline in mean GH concentrations may reflect negative feedback by GH and IGF-I on the hypothalamo-pituitary unit [69]. An important insight in this study is that unvarying low-dose GHRP-receptor agonism (unlike continuous GnRH receptor stimulation) augments and maintains a physiological pattern of higher amplitude, more entropic and nycthemerally rhythmic GH secretion for at least 90 days. Continual intravenous infusion of GHRP-2 and/or GHRH for 24 h also elevates concentrations of IGF-I and enforces a pubertal-like pattern of GH secretion (fig. 1b, fig. 8) [58, 63].

The neuroendocrine phenotype induced by constant low-dose GHRP-2 infusion mirrors that of puberty. Specific similarities include: (1) increased GH secretory-burst mass and thereby greater incremental and maximal GH peaks; (2) a fixed GH pulse frequency and half-life; (3) a more irregular pattern of GH secretion (measured by elevated approximate entropy), which denotes strong feedback adaptations; (4) heightened diurnal GH rhythmicity; and (5) systemically elevated concentrations of IGF-I [36, 71].

The significant (1.5- to 3-fold) rise in mean serum concentrations of GH and IGF-I resulting from near-constant stimulation of GHRP would be predicted to repress GH secretion by autoinhibition. Thus, it would be of interest to investigate the basis for partial feedback escape in both settings. Possible adaptive mechanisms in this unique clinical context include: (1) a reduction in negative feedback caused by a mid-physiological (rather than pharmacological) elevation of GH and IGF-I; and/or (2) limited down-regulation of the GHRP-response pathways by continuous low-dose agonist exposure. Regarding the last point, repeated intragastric administration of a small yet effective dose of a non-peptidyl GHRP mimetic in pigs increases pulsatile GH secretion, the approximate entropy of GH release and plasma concentrations of IGF-I over a period of 5 days. In contrast, a threefold higher (near-maximally effective) dose of the same agonist represses GH secretion on the second day [72].

Developmental Control of GH Secretion

The secretion and activity of GH, IGF-I, GHRH, GHRP and somatostatin differ in several respects *in utero*, and during infancy, childhood, puberty and adulthood. First, pulsatile GH secretion increases markedly, but transiently, in the newborn in association with initially reduced systemic concentrations of IGF-I [73, 74]. Sec-

ond, GHRH up-regulates and down-regulates the cognate pituitary receptor in the immature and adult rat, respectively [20, 32]. Third, post-aromatization oestrogen induces periventricular somatostatin gene expression in the first 5 days of post-natal life, but not consistently in the pre-pubertal, pubertal or adult animal. Finally, oestradiol stimulates GHRH gene expression in the arcuate nucleus of the infantile rat, but represses the same endpoint in the adult animal [14, 75]. Oestrogenic effects may be exerted by the OR- α gene, which is expressed in the majority (>65%) of single (adult) GHRH neurons. The genetic programs that control developmental expression of OR- α in this area are currently undefined.

GH Autonegative Feedback: Mechanisms of Autoinhibition and Escape

Time-delayed feedforward and feedback interactions among GH, GHRH and somatostatin offer self-renewable GH pulsatility in simplified biomathematical formulations [2, 3]. All three components – feedforward, feedback and time delay – are critical elements in driving pulse automaticity. In this framework, ghrelin serves as an amplifier from pituitary and systemic sources. The ghrelin gene is definitively expressed in the anterior pituitary gland. At present, however, immunoreactive ghrelin, but not the ghrelin transcript, is identifiable in disparate hypothalamic neurons. Accordingly, neurons might in principle accumulate (and not synthesize) the peptides.

GH autofeedback in the adult male rodent is not required in the overlap of the mechanistic pathway. Two of the requirements for this pathway are:

- (1) a functional GH receptor in the brain coupled positively to somatostatin secretion, as observed in antisense receptor-silencing experiments in the rat [76];
- (2) the SSTR-2 within the hypothalamo-pituitary unit, as established in the transgenic mouse [21, 77].

SSTR-2 could participate in this pathway by mediating somatostatinergic autoneuronal facilitation in the hypothalamus (thereby heightening somatostatin outflow during GH autofeedback) and in blocking granule exocytosis by somatotrope cells. Concomitantly, GH represses the synthesis and secretion of GHRH, thereby withdrawing the feedforward drive [2, 3, 78].

The female rat maintains minimal, but detectable, GH autoinhibition. This sex difference is caused mainly by the reduction of GH-inducible somatostatin outflow [1] and may reflect, in part, the ability of oestradiol to down-regulate the GH receptor gene in the arcuate nucleus. Tes-

Fig. 9. Oestradiol suppresses autonegative feedback by a single bolus of rhGH on GHRP-2 (but not basal or GHRH)-stimulated GH secretion in post-menopausal individuals. Panels illustrate feedback repression imposed by a pulse of rhGH on basal, GHRH- and GHRP-2-stimulated GH release following short-term supplementation of post-menopausal women with **a** oral placebo or **b** oestradiol repletion. Reproduced, with permission, from Anderson et al. [79]: © The Endocrine Society.

tosterone, however, induces SSTR-2 in the pituitary gland more effectively than oestradiol *in vivo*, thus accentuating the interpulse suppression of GH release under somatostatin outflow in the male rodent. In humans, oestrogen selectively suppresses GH-induced autonegative feedback on the GHRP-2 (but not basal, exercise or GHRH) stimulus (fig. 9) [79]. Such selectivity may occur through up-regulation of the GHRP receptor gene and can be observed in transcription assays *in vitro*.

In adolescence, GH secretion is not repressed by high-amplitude GH pulses and elevated systemic concentrations of IGF-I [71]. One recent analysis disclosed two possible mechanisms to explain this paradox in mid-pubertal boys: (1) the marked suppression of GH pulsatility by a single intravenous pulse of recombinant human (rh) GH

Fig. 10. Time course of escape from autofeedback inhibition of GH release in three developmental strata of boys. A single pulse of rhGH was injected at time zero (before the illustrated record begins). Adapted, with permission, from Richmond et al. [80]; ©The Endocrine Society.

infused over a 6-min period 2 h earlier; and (2) a more rapid recovery of GH secretion following the induced nadir in mid-pubertal boys than in pre-pubertal boys or young men (fig. 10). These distinctions operate in the face of equivalent feedback inhibition of an exogenous GHRH stimulus [80]. Prominent autoinhibition of pulsatile GH secretion (defined in absolute [μg/l] and fractional [%] terms) in mid-adolescence could indicate a unique

Fig. 11. Reciprocal effects of a selective GH-receptor antagonist (pegvisomant) to deplete concentrations of IGF-I and release GH secretion in healthy young adults. Adapted, with permission, from Veldhuis et al. [81]; ©The Endocrine Society.

inhibitory susceptibility of arcuate-nucleus GHRH release to GH-imposed negative feedback. Accelerated escape of GH output after maximal suppression could indicate heightened GHRH and GHRP drive and/or an abbreviated duration of GH-induced somatostatin outflow [80]. Equivalent feedback inhibition of exogenous GHRH stimulus would indicate similar GH-induced somatostatin restraint before, during and after puberty.

Infusion of rhIGF-I inhibits pulsatile GH secretion in the rat and human. A novel strategy has been applied recently to reveal feedback suppression by systemic IGF-I. In this clinical study, IGF-I depletion was achieved through a single subcutaneous injection of pegvisomant 1 mg/kg (a selective GH receptor antagonist peptide) and compared with saline [81, 82]. Pegvisomant stimulated a 1.8-fold rise in overnight pulsatile and basal GH secretion while inducing a 32% reduction in serum concentrations of IGF-I within 72 h (fig. 11). The degree of unconstrained feedback was compared by sex in the cohort of nine men and seven women studied. Kinetic analyses of the data showed that GH receptor blockade does not alter the half-life of secreted GH or infused rhGH despite the attainment of a mean serum concentration of pegvisomant that was 3,000-fold higher than that of GH. Concentrations of GH also increase in successfully treated (IGF-I depleted) patients with acromegaly. GH kinetics are normal so the elevation in GH would indicate the preservation of feedback reactivity by a subpopulation of somatotropes.

Fig. 12. Left column Composite impact of oestradiol on exogenous drive by GHRH, somatostatin (SS) and GHRP-2 in post-menopausal individuals (top left box). Oestrogen supplementation augments GH secretory-burst mass and the irregularity of release patterns without altering deconvolution-estimated true (non-pulsatile) basal GH secretion (left middle box). Oestradiol repletion also depletes IGF-I, elevates IGFBP-1 and does not alter concentrations of IGFBP-3. The

first two adaptations predictably limit free IGF-I availability, which would mute negative feedback [79]. **Middle column** Schema of primary effector actions within GH-IGF-I axis. **Right column** Lack of equivalent single-effector studies of testosterone. Distinct mechanistic contrasts between testosterone and oestradiol, i.e. only testosterone stimulates computed basal (non-pulsatile) GH secretion and elevates serum concentrations of IGF-I [30, 58, 59, 79, 81, 83].

Summary

The regulation of GH secretion proceeds via a minimally tripeptidyl ensemble, comprising a combined input of GHRH, GHRP and somatostatin. Sex-steroid hormones, as well as other factors, modulate the synthesis, secretion and reception of the primary signals listed above (fig. 12). Collaborative experiments in molecular biology, cellular biochemistry and integrative physiology are beginning to unravel the mechanisms that coordinate the activity of this putative ensemble over the human lifetime.

Acknowledgements

The author would like thank Jean Plote for her excellent editorial assistance. This work was supported in part by the National Center for Research Resources via General Clinical Research Center grant M01 RR00585 to the Mayo Clinic and National Institutes of Health grants ROI AG 14799 and AG 19695.

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