MINI REVIEW

Neuroendocrine regulation of growth hormone

Jérôme Bertherat1*, Marie Thérèse Bluet-Pajot and Jacques Epelbaum

U159 INSERM, Centre Paul Broca, Paris, France; Peptide Biology Laboratory2, Salk Institute, La Jolla, CA, USA


This short review is focused on the neuroendocrine regulation of growth hormone (GH) pulsatile secretory pattern and GH gene expression. The neuronal network involved in the central control of GH includes extrahypothalamic neurons such as the noradrenergic and cholinergic systems, which regulate the two antagonistic neurohormonal systems: somatostatin (SRIH) and GH-releasing hormone (GHRH). Intrahypothalamic Proopiomelanocortin- and Galanin-containing interneurons also participate in the regulation of SRIH and GHRH neuronal activity, which also is dependent on sex steroids and GH and/or insulin-like growth factor I (IGF-I) feedback. cAMP (controlled mainly by GHRH and SRIH), thyroid and glucocorticoid hormones, IGF-I and activin are among the factors that regulate GH gene expression at the transcriptional level and may play a role in somatotroph differentiation and proliferation during ontogeny as well as physiological and pathological states such as acromegaly.

J Epelbaum, U159 INSERM, Centre Paul Broca, 2 ter rue d’Alixia, 75014 Paris, France

In all mammalian species tested so far, including man, growth hormone (GH) is secreted in a pulsatile manner from anterior pituitary somatotroph cells. Such a pulsatile pattern is important for the numerous metabolic actions of GH resulting in normal growth. Normal growth also is dependent on sequential crucial gene activation events occurring in somatotrophs during development and throughout adult life under the control of multiple neuroendocrine factors. Insight into the regulation of GH secretory pattern and gene expression has been approached successfully in recent years. In this review we shall focus on some recent studies on the extra- and intra-hypothalamic neuronal networks involved in the control of GH secretion, as well as on the transcriptional regulation of GH expression by hypothalamic and peripheral hormones. Interestingly, these last studies also led to a better knowledge of somatotroph differentiation and proliferation and the role of hypothalamic neurohormones in pituitary development.

Neuronal networks involved in GH regulation

Pulsatile GH secretion results from interacting neuroendocrine pathways. In addition to the two antagonistic neurohormones—GH-releasing hormone (GHRH) and somatostatin (SRIH)—many neurotransmitters and neuropeptides are involved in the neural control of GH secretion with both stimulatory and inhibitory effects on GH release. These effects are exerted mainly via GHRH and/or SRIH rather than directly at the pituitary level. Among these compounds, neurotransmitters such as noradrenaline (and/or adrenaline) and acetylcholine and neuropeptides such as opioid peptides and galanin seem to play an important role and have been the subject of many studies in these last few years.

Adrenergic systems

Among monoaminergic systems, adrenergic mechanisms are the most involved in the neural control of GH release. Their actions are complex, with stimulatory and inhibitory effects mediated by different receptors. Growth hormone secretion is enhanced by α2-agonists but modulated negatively by α1- and β-agonists. Clonidine, an α2-agonist, stimulates GH release and this effect is suppressed by an α2-antagonist (1). Indeed, clonidine has been used in clinical settings as a GH secretagogue. Methoxamine, an α1-agonist, reduces the amplitude of GH pulses in the rat as well as basal and stimulated GH secretion in the dog (2), and isoproterenol, a β-agonist, inhibits the release of GH in response to GHRH (3). The mechanism of the α2-adrenergic stimulation of GH secretion is unclear. In vitro data suggest that clonidine releases GHRH (4). Moreover, pretreatment with antiserum to GHRH...
abolishes the GH response to clonidine (5). However clonidine also acts by inhibiting SRIH release because it can potentiate the GH response to GHRH in the presence of increased somatostatinergic tone (6, 7). Finally, it cannot be excluded that the effects of clonidine are mediated by other factors. Recently, Aulakh et al. (8) proposed that α2-adrenoceptors, located on serotoninergic presynaptic nerve terminals, stimulate serotonin and subsequently GHRH release.

Stimulation of α1- and β-adrenoceptors probably increases SRIH release. In young rats, Cella et al. (5) have shown that, after administration of anti-SRIH serum, the GH lowering effect of methoxamine, an α1-agonist, is lacking completely. Isoproterenol, a β-agonist, suppresses the GH response to GHRH and this effect is blocked also by an anti-SRIH treatment (3). Reciprocally, β-adrenergic antagonists enhance the GH response to GHRH (9). On the other hand, isoproterenol is able to increase portal venous and arterial levels of SRIH (2).

Opposite effects resulting from the activation of various adrenergic receptors can be relayed by multiple adrenergic afferent systems with different target structures in the hypothalamus. The A1, A2 and A6 groups of neurons supply the noradrenaline (NA) innervation of the hypothalamus. Recently, we have shown that the negative effects of NA on GH release are mediated, at least in part, by noradrenergic neurons from the locus coeruleus (LC). These neurons interfere with GH pulsatility by modulating the amplitude (but not frequency) of GH secretory episodes, as documented by experiments of LC lesions (2). The effects of LC lesions can be mimicked by the α1-antagonist prazocin. Most NA projections of the LC reach the hypothalamus by the dorsal noradrenergic bundle, a major afferent to the paraventricular nucleus (PVN) (2). Involvement of the PVN in LC effects on GH secretion is substantiated by the effect of lesions of that structure, which also result in increased amplitude of GH rhythmic secretion (2). Moreover, the inhibiting effect of α1-agonists is no longer observed in PVN-lesioned animals, suggesting that α1-receptors mediating GH inhibition are located in this nucleus. The exact nature of the transmitter(s) involved in the processing by the PVN of inhibitory inputs originating in the LC and forwarded to neurosecretory SRIH-producing neurons of the periventricular nucleus is not yet elucidated.

Fibers involved in the positive regulation of GH by NA remains to be identified.

**Cholinergic systems**

Acetylcholine plays an important role in the neural control of GH release, both in animals (10) and in man (11). Muscarinic antagonists such as methscopolamine, atropine or pirenzepine have been shown to inhibit GH response elicited by sleep, exercise, arginine, clonidine and opioids in man. Conversely, cholinomimetic agents are able to increase basal GH levels.

The mechanisms whereby cholinergic systems affect GH response and the neuroanatomical level at which this action takes place are not yet defined. A direct stimulatory effect on the pituitary is unlikely (12). It seems that acetylcholine acts by inhibiting hypothalamic SRIH release rather than by stimulating GHRH release. Indeed, Massara et al. (13) reported that the muscarinic receptor antagonist pirenzepine completely abolished the rise in plasma GH elicited by GHRH and, conversely, the enhancement of the cholinergic tone by a cholinesterase inhibitor potentiated GH responsiveness to GHRH. In addition, acetylcholine and neostigmine inhibited SRIH release from rat hypothalamum in vitro, an effect reversed by atropine treatment (13). Finally, effects of cholinergic agonists and antagonists on GHRH-induced GH response are abolished in rats pretreated with cysteamine, a SRIH-depleting agent (14).

**Opioid peptides**

Although a role for endogenous opioid peptides in maintaining GH secretion has not been demonstrated as yet on basal plasma GH concentrations (15, 16), they were shown repetitively to increase plasma GH levels. No modification in GH secretion is detected from pituitary cells in culture, suggesting that they act in the brain. Activation of hypothalamic µ-opioid receptor on, or near, SRIH or GHRH neurons causes GH secretion (17). An action of opioid inputs via GHRH-secreting structures is supported by the fact that passive immunization of rats with an antiserum raised against rat GHRH completely inhibited the GH response to morphine (18) and that this response is decreased in rats rendered GHRH-deficient by neonatal exposure to monosodium glutamate (19). On the other hand, the enhancing effect of the met-enkephalin analog DAMM on GHRH-induced GH release in man suggests that the effect of opiates may be mediated also through inhibition of SRIH release (20).

Recent anatomical data (21) suggest that opioid neurons might be implicated in the reciprocal interactions between SRIH- and GHRH-containing neurons: in the arcuate nucleus, SRIH receptors are present on GHRH mRNAs and POMC mRNAs-containing cells and SRIH perikarya in the periventricular nucleus are innervated by POMC-derived peptides containing neurons.

**Galanin**

Growth hormone was the first pituitary hormone described to be affected by galanin, which is a 27 amino acid peptide distributed widely in the CNS, highly concentrated in the hypothalamus and partly colocalized with GHRH in median eminence nerve endings.
Table 1. Demonstrated afferents on hypothalamic somatostatin (SRIH) and growth hormone-releasing hormone (GHRH) neurons (see Refs. 51 and 21 for SRIH and Ref. 52 for GHRH).

<table>
<thead>
<tr>
<th>SRIH hypophysiotropic neurons* (Anterior periventricular area)</th>
<th>SRIH intrinsic neurons (Suprachiasmatic nucleus)</th>
<th>GHRH hypophysiotropic neurons (ventrolateral arcuate nucleus)</th>
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<td>SRH</td>
<td>Vasopressin</td>
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<td>GHRH</td>
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<td>CRH</td>
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<td>POMC-derived peptides</td>
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* CRH: corticotrophin-releasing hormone; PNMT: Phenylethanolamine-N. methyl transferase; POMC: proopiomelanocortin.

Galanin stimulates GH when injected intravenously in humans or intracerebroventricularly (icv) in rats (22). Furthermore, in adult male rats, icv injection of galanin antiserum leads to a dramatic alteration of pulsatile GH secretion with a reduction of GH pulse amplitude and an increase in pulse frequency, suggesting that galanin could be an endogenous modulator of GH secretion.

Pituitary effects of galanin on GH release reported by several authors are inconsistent and dependent on the culture condition and the age of animals (23, 24). At any rate, the pituitary effects of galanin are weak and cannot account for the action of the peptide on GH secretion. Thus, galanin may act predominantly through stimulation of GHRH secretion. The co-existence of galanin and GHRH in the same arcuate nucleus neurons (22) may provide the possibility of interaction between the two neuropeptides. Evidence for a stimulation of GHRH by galanin has been provided in vitro by Kitajuma et al. (cited in Ref. 22) using incubated hypothalamic slices. On the other hand, an action of galanin through an inhibition of SRIH release has been suggested by the fact that, in humans, galanin significantly potentiates GH response to GHRH infusion (25). Recently, Tanoh et al. (26) demonstrated such a hypothesis in conscious rats pretreated with neostigmine, cysteamine or specific anti-SRIH serum (in order to decrease the endogenous somatostatinergic tone). In such animals, galanin-induced GH secretion indeed was reduced. Taken together, these results suggest that the effects of galanin are mediated at different levels. Galanin given icv probably acts within the blood–brain barrier inside the hypothalamus but, given iv, it affects brain structures located outside the blood–brain barrier, such as the median eminence in which galanin-binding sites are highly concentrated (22).

**Somatostatin/GHRH interactions**

The organization of hypothalamic SRIH-containing neurons is essentially similar in all mammalian species studied so far, including man. Most hypothalamic areas contain an extensive network of SRIH-containing fibers and different populations of immunoreactive perikarya are present in the anterior periventricular area proper and the parvo cellular portion of the PVN and in the suprachiasmatic nucleus (SCN), arcuate nucleus and ventromedial nucleus (VMN) (27). By retrograde tracing coupled to immunohistochemistry, up to 78% of periventricular somatostatinergic neurons have been demonstrated to project to the median eminence (28), while other hypothalamic SRIH neurons do not innervate this neurohemal organ. Using various knife cuts and transsections (29), we had shown previously that the SRIH-containing hypophysiotropic neurons send their fibers in a lateral direction to the lateral hypothalamus, travel for a short distance in the medial forebrain bundle and re-enter the mediobasal hypothalamus at the level of the retrochiasmatic area to reach the external layer of the median eminence from which the peptide is released in the portal blood. All other hypothalamic SRIH neurons are intrinsic neurons involved in local circuitry; this has been outlined most elegantly for suprachiasmatic neurons, which display a circadian pacemaker activity (30). Semi-quantitative analysis of SRIH mRNA and immunocytochemical staining revealed that the hypophysiotropic neurons in the periventricular area display the highest levels of activity of the entire CNS, while SCN and arcuate–ventromedial neurons are four to nine time less active (31). Table 1 summarizes the association between neurochemically defined terminals and hypothalamic SRIH neurons. Glutamate probably could be added to this list, according to recent ultrastructural and physiological data suggesting a major excitatory role for this amino acid neurotransmitter on neuroendocrine neurons (32). While SRIH co-localizes extensively with GABA and neuropeptide Y in other brain regions, such co-localization patterns have not been demonstrated unequivocally in the hypothalamus. However, a moderate extent of co-localization with tyrosine hydroxylase was reported recently for the periventricular cell group and the cells located at the boundary between VMN and the arcuate nucleus (33).

In contrast to the wide distribution of SRIH neurons...
in hypothalamic and extrahypothalamic brain regions, the arcuate nucleus is the major source of GHRH neurons in primate and rodent brains (34). From these ventrolateral arcuate perikarya only, GHRH fibers innervate the external layer of the median eminence. The second GHRH-containing cell group encapsulates the caudal part of the VMN in its dorsal extent. However, this second cell group does not project to the median eminence but innervates other hypothalamic and perihypothalamic nuclei. Finally, it should be recalled that GHRH is co-localized partly with galanin and/or dopamine (23, 34) and neuropeptide Y (35) in the ventrolateral arcuate nucleus hypothalamic neurons.

By conventional film autoradiography, SRIH receptors seem not very abundant in hypothalamic regions. However, the use of desaturation techniques allowed the amount of binding in most hypothalamic areas to increase considerably (36, 37). In accordance with the extensive network of SRIH terminals and fibers, this indicates that endogenous SRIH is likely to act tonically on hypothalamic receptors. Using emulsion autoradiography, SRIH receptors have been visualized in close apposition with a subpopulation of arcuate neurons (38), some of which were demonstrated later to correspond to GHRH neurons (39, 40). Neonatal monosodium glutamate treatment, which destroys all GHRH-containing neurons in the ventrolateral arcuate nucleus, only partially depletes SRIH binding on perikarya located more medially, closer to the wall of the third ventricle (41). This latter population might correspond to arcuate SRIH or neuropeptide Y inerneurons, which display such a distribution. Four of the five recently cloned SRIH receptor subtypes (SSTR1–5) are synthesized in the hypothalamus (42, 43). By in situ hybridization, SSTR1 mRNA-containing cells are visualized in all hypothalamic areas while SSTR2 mRNA-containing cells are restricted more to the arcuate nucleus, which is one of the most enriched regions of the entire brain for both SSTR1 and SSTR2 mRNA. It remains to be demonstrated which subtype is expressed in GHRH-containing neurons. Conversely, no information is available at present concerning the localization of GHRH receptors in the hypothalamus.

The regulation of SRIH and GHRH metabolism by various neuropeptides and neurotransmitters has been evaluated by several approaches. In situ hybridization of peptide mRNA levels allowed a specific effect of a given drug on a restricted cell population to be determined. For instance, GABA agonist and benzodiazepine strongly diminish SRIH mRNA levels in the hypophysiotropic periventricular neurons (44) but the stimulatory effects of excitatory amino acids are more evident on arcuate intrinsic neurons (45). Such an anatomical resolution cannot be achieved in release experiments. A number of different experimental models (slices, synaptosomes, whole hypothalamic or median eminence explants, embryonic cell culture) have been used to study SRIH and/or GHRH release in vitro (see Ref. 46 for review). Owing to the diversity of the hypothalamic SRIH neurons, most of these models do not permit changes in SRIH release to be attributed to a particular neuronal population. Nevertheless, release experiments from isolated median eminence fragments allowed us to conclude that a presynaptic action on the hypophysiotropic terminals arising from the periventricular perikarya and careful dissection of a hypothalamic region resulted, in some instances (adrenergic and cholinergic stimulations, somatostatin and opiate inhibitions), in the demonstration of an intrahypothalamic effect. In vivo measurements either on unanesthetized animals by a push–pull cannula located in the median eminence or by sampling of portal blood in anesthetized animals after icv injection allow us to detect whether a drug interacts with SRIH release from the median eminence. However, none of these techniques provide any clue to the direct or indirect nature of a given pharmacological effect. A good example is given by the stimulatory effect of GHRH on SRIH release from the median eminence, which is blocked by naloxone, thus suggesting a role for an opioid terminal as a relay in this effect (46). Conversely, dopamine stimulates both SRIH and GHRH release from the median eminence–arcuate complex but the GHRH-stimulating effect of dopamine is masked unless endogenous SRIH is immunoneutralized (47). Also, dopaminergic stimulation is more effective on somatostatin-28 than on somatostatin-14 (48). A further degree of complexity is achieved in the case of galanin stimulation of GHRH and SRIH release from median eminence fragments, which is mediated through dopaminergic mechanisms. In both SRIH and GHRH release D1 dopaminergic receptors are involved, but D2 receptors only mediate GHRH release stimulation (49). Finally, the localization of SRIH-binding sites on GHRH-containing arcuate neurons suggest that the inhibition of GHRH release as observed in vitro is mediated directly (50).

Effects of peripheral hormones on intrahypothalamic networks

While thyroid hormones and corticosteroids are mostly active at the pituitary level (see Ref. 53 and regulation of GH gene expression, below), the sexual dimorphic pattern of GH pulsatility (higher but less frequent pulses and lower basal values in male as compared to female) is likely to be mediated through changes in the expression of SRIH and GHRH directly in hypophysiotropic periventricular and arcuate nucleus neurons, respectively. Such neurons do contain estrogen receptors and SRIH and GHRH expression is higher in male than in female rats (54). In addition, galanin co-expression in GHRH hypophysiotropic neurons is higher in male than in female rats (55).

There is considerable evidence that GH regulates its own rhythmic secretion through a reciprocal feedback
Fig. 1. Intrahypothalamic networks in the control of growth hormone (GH). Hypophysiotropic somatostatin (SRIH) and growth hormone-releasing hormone (GHRH) neuronal perikarya, located respectively in the anterior periventricular nucleus (Pev) and the arcuate nucleus, are connected indirectly through several arcuate interneurons containing SRIH, proopiomelanocortin (POMC) or galanin (not represented). Growth hormone, either directly or through insulin-like growth factor (IGF), controls SRIH and GHRH expression and release from the two neurohormonal systems.

on SRIH and GHRH hyophysiotropic neurons (see Ref. 56 for review). Indeed, SRIH mRNA and peptide levels, as well as release from the median eminence, increase in response to excess GH and decrease in response to GH deficiency. Conversely, GHRH mRNA and peptide levels, as well as peptide release, decrease under elevated GH conditions and increase under conditions of GH deficiency. The mechanism(s) by which GH mediates these central effects has not been demonstrated unequivocally. In particular, it is not yet clear whether these effects are direct or mediated through IGF-I. For instance, although GH receptor mRNA has been visualized in periventricular SRIH neurons (57) and short-term GH hypersecretion exerts negative feedback without modifying IGF-I plasma levels (58), SRIH expression in the periventricular nucleus of the GH-deficient Dw/Dw rat is independent of GH and regulated by IGF-I only (59). Conversely, in the same model, GHRH expression is dependent on GH but not on IGF-I. Alternatively, in rats rendered GH hyposecreting by immunoneutralization with GHRH antibodies, GH and IGF-I treatment were equally active in partially reversing increased GHRH mRNA levels while SRIH mRNA levels were affected only minimally (60).

Transduction from the cell surface to the nucleus of the hormonal signals regulating GH gene expression

The GH and PRL genes derive from duplication of a common ancestor gene and are present in all vertebrates (see Ref. 61 for review). The placental lactogene (PL) genes result from subsequent duplication of the GH gene in primates and of the PRL gene in rodents. Among the five genes of the hGH/PL family, only the hGH-N gene is expressed in the pituitary and will be discussed herein. The GH gene is expressed in a highly restricted cell-specific pattern in somatotrophs cells of the anterior pituitary. In keeping with this physiological observation, non-pituitary tumoral expression of GH is a very rare event in acromegaly (62). Growth hormone mRNA is detected in the developing pituitary on embryonic day 18 (ED18) in the rat and on ED15.5 in the mouse, following the expression of the pituitary-specific POU domain transcription factor Pit-1/GHF-1 (see below), which is detected on ED16 in the rat and ED13 in the mouse (63, 64).

The transcriptional regulation of gene expression represents the first step of eukaryotic gene expression and a main level of GH gene regulation. As for any eukaryotic gene, transcription of the GH gene depends upon cis-elements and trans-acting factors. These trans-acting factors are nuclear proteins (or transcription factors) that bind the cis-acting elements present in the promoter or enhancer of a given gene, thereby controlling the transcriptional activity of this gene.

Cell culture studies and transgenic mice experiments have shown that efficient pituitary specific gene expression of GH is mediated by sequences within 235 and 289 base pairs upstream of the transcription start site of, respectively, the rGH and hGH genes (65). This proximal part of the human and rat GH promoters contains two cis-acting elements important for tissuespecific expression, which bind the homeobox transcription factor Pit-1/GHF-1 (63, 64). Pit-1/GHF-1 is a potent transactivator of the GH promoter. This mechanism of transcriptional regulation of GH is well conserved in vertebrate evolution because sequences homologous to mammal Pit-1/GHF-1 binding sites are present also in the rainbow trout GHII gene promoter, and cloning of the rainbow trout Pit-1/GHF-1 gene revealed high homology with its mammal counterparts (66, 67).

Transcriptional regulation by Pit-1/GHF-1 is crucial for GH expression. This is suggested by the temporal pattern of Pit-1/GHF-1 expression during pituitary ontogeny (63, 64). Furthermore, Jackson and Snell dwarf mice, which carry, respectively, a recessive rearrangement or point mutation of the Pit-1/GHF-1 gene, develop a dramatic somatotroph deficiency (68). These mice exhibit pituitary hypoplasia with impairment of GH, PRL and TSH expression. This alteration of three different pituitary cell types is linked to the fact that Pit-1/GHF-1 binding sites have been identified also in the PRL and β-TSH promoters (the activity of these promoters is stimulated by Pit-1/GHF-1). Deleterious mutations of the Pit-1/GHF-1 gene also have been reported in patients with similar combined GH, TSH and
Fig. 2. Theoretical model of transduction from the cell surface to the nucleus and specific gene expression in somatotrophs. The diagram summarizes some of the pathways controlling somatotroph specific gene expression known to be important for cell differentiation and proliferation. Growth hormone-releasing hormone (GHRH) and somatostatin (SRIH) binding to their respective seven transmembrane receptors lead to activation of a heterotrimeric (α, β and γ subunit) G protein (Gα for GHRH and Gβ γ for SRIH). Activation of Gα protein leads to dissociation of its α-subunit from the β, γ complex and therefore stimulation of adenylate cyclase (AC). Intracellular cAMP stimulates cAMP-dependent protein kinase A (PKA), leading to dissociation of its catalytic (C) subunits from the regulatory (R) subunits. The catalytic subunit will in turn activate expression of the GH and GHRH receptor (GHRH-R) genes and also stimulate expression of its own gene. The dashed lines represent transduction of the activin signal. Activin receptors are members of the transmembrane serine/threonine kinase family. Activin has been shown to inhibit GHRH-stimulated cAMP production and transactivation by Pit-1. However, the transduction mechanisms of activin signals mediating these effects remain to be determined.

PRL pituitary deficiency with growth retardation (69–71). Pit-1/GHF-1 also can bind its own promoter, leading to positive autoregulation, as observed frequently for transcription factor involved in terminal differentiation.

Cyclic AMP (cAMP, controlled mainly by GHRH and SRIH in the somatotrophs), thyroid hormones, glucocorticoids hormones, and activin are among the factors known to regulate GH gene expression, in particular at the transcriptional level.

Growth hormone-releasing hormone and SRIH: regulation by cAMP (Fig. 2).

Cyclic AMP is known to stimulate both GH secretion and GH gene expression. In somatotrophs GHRH and SRIH exert opposite effects on the intracellular level of cAMP. Upon activation by its ligand the seven transmembrane GHRH receptors stimulate adenylate cyclase through coupling with a heterotrimeric Gα protein (72). Following this activation, intracellular
cAMP levels increase, leading to stimulation of the cAMP-dependent protein kinase A (PKA), which in turn phosphorylates numerous cytoplasmic and nuclear substrates (73). One of the best characterised PKA nuclear substrates is the transcription factor CREB (cAMP-responsive element binding protein), which stimulates transcription of cAMP-responsive genes after its phosphorylation by PKA (74). Growth hormone-regulating hormone regulates positively the level of GH mRNA by stimulation of GH gene transcription (75). Interestingly, the expression of GHRH and its receptor coincides with activation of the GH gene (72, 76). Conversely, SRIH decreases adenylate cyclase activity through seven transmembrane receptors coupled to Gi proteins (51), and inhibits the GHRH-dependent positive regulation of PKA activity (73). The stimulatory effect of cAMP on the rGH and hGH genes is mediated by the proximal 5′-flanking region. This cAMP-responsive region has been mapped between −104 and +11 bp of the transcription start site in the rGH promoter and within 212 bp upstream of the transcription start site in the hGH promoter (77, 78). Nevertheless, the canonical cAMP-responsive element (CRE: TGACGTCTA) is not found in these regions (74). This proximal part of the promoter contains binding sites for Pit-1/GHF-1 and this transcription factor has been implicated in the positive cAMP regulation of the GH gene. This regulation could occur through the well-documented cAMP stimulation of Pit-1/GHF-1 transcription, mediated by two canonical CREs present in the rat Pit-1/GHF-1 promoter and/or by PKA post-translational modification of Pit-1/GHF-1 (79). Although, Pit-1/GHF-1 binding sites are important, additional elements seem to be required for full cAMP stimulation of the GH promoter (77, 78). In the hGH promoter two partial CREs (CGTCA) located at nucleotides −187/−183 and −99/−95 have been shown to mediate cAMP stimulation of hGH promoter in cooperation with a Pit-1/GHF-1 binding site located at −123/−112 (78). These two partial CREs bind members of the CREB/ATF1-related family of transcription factors known to play a central role in cAMP regulation of gene expression (80).

**Thyroid hormones (T3) and retinoic acid (RA)**

Multiples studies suggest that T3 is an important positive regulator of the rat GH gene (81). Triiodothyronine is known to regulate transcription by binding of its nuclear receptor to a cis-element termed thyroid hormone response element (TRE). The thyroid hormone response is mediated by sequences located between bases −208 and −164 in the rat GH promoter (77,82). A very potent TRE has been described also in the third intron of the rGH gene (83). The TRE and the cAMP-responsive region cooperate to stimulate the activity of the rGH promoter and a synergistic effect of forskolin, an activator of adenylate cyclase, and T3 have been observed (82, 84). However, the sequence of the rat TRE is not conserved in the human GH promoter. Triiodothyronine treatment does not change GH mRNA levels in primary culture of human somatotroph adenomas and an inhibition of hGH promoter activity by T3 in transfection experiments in rat tumoral somatotroph cells even has been observed (85, 86).

The vitamin A metabolite retinoic acid also stimulates GH expression, and its receptor (RAR) is able to bind a CRE from the rGH promoter (87). Interestingly, a binding site for the RAR also has been identified in a distal enhancer located 10,000 base pairs upstream of the transcription start site of the Pit-1/GHF-1 rat gene (88).

**Glucocorticoids (GC)**

Glucocorticoids exert a positive control of GH gene expression by transcriptional and post-transcriptional mechanisms varying among species. Transcriptional regulation seems of little importance for rGH gene expression regulation by GC. Although the proximal part of the rGH promoter can mediate transactivation by GC in the context of the heterologous RSV promoter, up to 1800 base pairs of the 5′-flanking region of the rGH promoter alone does not confer GC regulation (89). On the other hand, GC responsive elements able to bind the GC receptor have been identified in the proximal part (−245 to −206) and the first intron of the hGH gene (90, 91). Therefore, the stimulation of the hGH mRNA levels by GC observed in primary culture of GH-secreting adenomas (85) could be regulated at least in part at the transcriptional level. Post-transcriptional mechanisms have been implicated in the positive regulation of both hGH and rGH mRNA by GC (92, 93).

**Activin**

Activin originating from the gonadotrophs exerts a paracrine control of GH secretion and synthesis. Activin decreases GH mRNA levels and inhibits the activity of the rGH promoter as well as somatotroph proliferation (94). This inhibition is mediated by a decrease of Pit-1/GHF-1 binding to the GH promoter. Decreased synthesis and stability of Pit-1/GHF-1 as well as phosphorylation of Pit-1/GHF-1 have been suggested to mediate this effect of activin on GH biosynthesis. Activin also inhibits GHRH-stimulation of cAMP intracellular levels. Although phosphorylation of activin receptor type II (ACR-II) by activin has been observed in rat mammo-somatotroph tumoral cells MtTW15, the transduction mechanisms of the activin signal from cell surface to nucleus remains to be elucidated (95).

**Insulin-like growth factor I**

As with the hypothalamic peptides SRIH and GHRH, the peripheral hormone IGF-I participates in feedback
regulation of GH secretion and gene expression (96). Insulin-like growth factor I inhibits GH mRNA levels in primary culture of rat anterior pituitary and somatotrophs adenomas. In particular, IGF-I inhibits basal as well as GHRH-stimulated transcription of rGH gene. Transfection experiments of hGH gene in the JEG3 human choriocarcinoma cell line suggest that 500 bp of the 5'-flanking region of hGH gene and/or intronic sequences could mediate this transcriptional regulation by IGF-I. The IGF-I receptor is a member of the tyrosine kinase class and has been shown to phosphorylate the nuclear protein c-Jun/AP-1 in epidermoid cells (see Ref. 97 for a review). In somatotrophs, autophosphorylation of the IGF-I receptor as well as phosphorylation of endogenous substrates in response to IGF-I have been demonstrated (96). However, the mechanisms of IGF-I signal transduction to the nucleus, as well as the trans-factors and cis-elements mediating inhibition of GH gene transcription by IGF-I, remain to be determined.

Other regulatory elements of the GH promoter
In addition to the previously described cis-elements of the GH promoter, which mediate specific hormonal transcriptional regulation, binding sites for ubiquitous transcription factors have been described on the proximal region of the GH promoter. A binding site for the factor Sp1 is present in both rGH and hGH promoter, overlapping the distal Pit-1/GHF-1 binding site (98); Sp1 can stimulate transcription from the GH promoter and seems to be important for its full activity. The activator protein AP-2 can bind hGH promoter (89). DNase I footprinting experiments have identified a region between nucleotide −239 and −219 of the rGH promoter that binds mainly GHF3 and seems to be important for basal activity of the promoter (99). Furthermore, an evolutionary conserved site located −110−95 in the rGH promoter and called Z box is functionally important. A novel DNA binding protein (Zn-15) of the zinc finger family, representing the major binding protein of the Z box, also is able to activate the GH promoter in synergy with Pit-1/GHF-1 (100).

Implication of the factors controlling GH gene expression in somatotroph proliferation and in the pathophysiology of acromegaly
Interestingly, cAMP and Pit-1/GHF-1 are important not only for GH gene expression but also for controlling critical events in the development and growth of the somatotroph lineage. The study of the cAMP pathway, from the cell surface to the nucleus, and its interactions with Pit-1/GHF-1 support its important role in somatotroph development and proliferation, as well as in the pathophysiology of GH-secreting tumors.

At the cell surface level cAMP is stimulated by GHRH and inhibited by SRIH, as mentioned already. Growth-hormone releasing hormone was purified first from a pancreatic tumor responsible for acromegaly: acromegalics patients with GHRH-secreting tumors usually exhibit somatotroph hyperplasia (101, 102), although documented somatotroph adenomas have been reported (103). Growth hormone-releasing hormone also is expressed in somatotroph adenomas and could play an autocrine role in these tumors (104, 105). It induces somatotroph proliferation by a cAMP-dependent mechanism that can be inhibited by SRIH in primary culture of rat anterior pituitary (106). The proliferative effects of GHRH could be mediated by the protooncogene c-fos, which is stimulated at the transcriptional level by GHRH (107). In transgenic mice, overexpression of GHRH or pituitary-targeted expression of the adenylate cyclase activator cholera toxin induce pituitary hyperplasia with GH hypersecretion and gigantism (108, 109). A somatic point mutation of the Gα protein α-subunit gene (which is coupled to the GHRH receptor) is present in about 40% of the somatotroph adenomas (110). The Gαs mutation (termed Gsp) leads to constitutive adenylate cyclase activation, with an increased intracellular level of cAMP. Functional expression of Gsp in rat pituitary cell lines stimulates PRL promoter activity (111). CREB phosphorylation by PKA and CREB-dependent transcription (Bertherat, unpubl.). On the other hand, the genetically transmitted dwarfism observed in the little (lit/lit) mice with anterior pituitary hypoplasia due to loss of GH-expressing cells is explained by an inactivating point mutation of the GHRH receptor gene (112, 113). This point mutation alters a single amino acid (Asp 60 to Gly) in the putative extracellular N-terminal domain of the GHRH receptor, leading to a defective receptor unable to elicit a cAMP response to GHRH. Furthermore, transgenic mice overexpressing an inactive CREB mutant (unphosphorylatable by PKA) targeted to the pituitary exhibit a selective depletion of GH-expressing cells with pituitary hypoplasia and dwarfism (114). Despite such an observation, it should be noted that in vivo deletion of the CREB gene in transgenic mice does not lead to pituitary hypoplasia, probably because up-regulation of the related transcription factor CREM in these animals rescues CREB deficiency (115). Taken together, these data suggest that not only cAMP can stimulate somatotroph proliferation but an intact cAMP pathway from the cell surface (GHRH receptor) to the nucleus (CREB) (74) is necessary for somatotroph proliferation and/or survival. In most tissues, cAMP usually induces cell differentiation and does not stimulate (or even inhibits) cell proliferation. Cyclic AMP proliferative effects are, in fact, quite restricted to a few tissues, such as the pituitary and the thyroid (116). Along with c-fos, another potential relay for the positive effect of cAMP in somatotroph proliferation could be Pit-1/GHF-1.

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because it is stimulated transcriptionally by cAMP and involved in somatotroph proliferation (68, 117). Pit-1/GHF-1 also would be able to control the cAMP pathway in somatotrophs by stimulation of GHRH receptor gene expression. This suggest a positive feedback of the cAMP pathway on its own activity, through a complex loop regulated mainly at the transcriptional level (Fig. 2). Pit-1/GHF-1 is indeed expressed in GH-, PRL- and TSH-secreting pituitary adenomas and could be overexpressed in some tumors (118). Nevertheless, a clear alteration in Pit-1/GHF-1 gene expression has not been demonstrated in such tumors (119). Also, c-fos overexpression has been reported in pituitary tumors but is not observed frequently (120). This suggests that other nuclear targets of the cAMP/CREB pathway remain to be identified in somatotrophs and could play a role in the pathophysiology of acromegaly.

Although no other somatic mutation than the G50 gene has been demonstrated consistently in somatotroph adenomas (see Ref. 121 for a review), it remains to be demonstrated that activation of the cAMP pathway alone is sufficient to cause somatotroph proliferation leading to pituitary adenoma. Transgenic animal studies clearly demonstrate in mice that GHRH excess can induce pituitary adenomas (122). But, in human pathology, GHRH-induced adenoma vs hyperplasia in acromegaly is still debated (101–103). At any rate, it is tempting to speculate that for GH-secreting tumors, as for other types of neoplasia, several mutational events would be observed in a given tumor. Expression of the G50 mutant Gsp in Swiss 3T3 cells has suggested that concomitant alteration of G50 and another component of the cAMP signaling pathway or a different mitogenic signaling pathway would be required for abnormal growth (123). Study of the hormonal control mechanisms of somatotroph activity could help to identify these other alterations. Such alterations are likely to be genetic modifications at different levels of the transduction pathway, from the cell surface to the nucleus, mimicking increased stimulatory or decreased inhibitory endocrine, paracrine or autocrine factors controlling somatotroph activity. In thyroid tumors, point mutations causing hyperactivation of the cAMP pathway have been reported in genes encoding the G50 protein as well as the TSH receptor (116). Alteration of adenylate cyclase inhibition by dopamine has been observed in GH adenomas (121). Somatostatin is able to abolish the proliferation effects of GHRH and activin inhibits somatotroph proliferation (114). Furthermore, treatment of GH-secreting adenomas by SRIH analogs often effectively reduces GH plasma levels and also in some cases, tumor size (124). One might postulate that abnormal somatotroph proliferation could occur through loss of such inhibitory activity. In this respect, the resistance to SRIH analogs observed in about 20–30% of acromegalic patients, despite the persistance of SRIH receptors on these tumors, might be explained by alterations of SRIH receptor function and/or transduction mechanisms (125).

Conclusion

Studies of the neuroendocrine control of GH, leading ultimately to a correct pulsatile secretory pattern required for harmonious growth, have revealed the complexity of this regulation. Several factors taking part in this control, from a multicomponent neuronal network to a complex transcriptional machinery regulating GH gene expression, have been characterized. The coordinate actions of all this factors appear crucial for somatotroph development and adequate function in the mature pituitary. Interestingly, the basic knowledge acquired from these studies provides a better understanding of pituitary pathophysiology, as illustrated by GHRH purification from a pancreatic tumor causing acromegaly, the description of activating G50 mutations in somatotroph adenomas and the report of Pit-1 gene-inactivating mutations in some families presenting with pituitary deficiency. The somatostatin analogs now used in the treatment of acromegaly are also a clinical application resulting from the study of the hormonal control of GH. Future progress will certainly provide additional clues to pituitary physiopathology and offer improved treatment.

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