Understanding the Multifactorial Control of Growth Hormone Release by Somatotropes

Lessons from Comparative Endocrinology

Manuel D. Gahete, Mario Durán-Prado, Raúl M. Luque, Antonio J. Martínez-Fuentes, Ana Quintero, Ester Gutiérrez-Pascual, José Córdoba-Chacón, María M. Malagón, Francisco Gracia-Navarro, and Justo P. Castaño

Department of Cell Biology, Physiology and Immunology, University of Córdoba, Cordoba, Spain

CIBERobn Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Cordoba, Spain

Control of postnatal growth is the main, but not the only, role for growth hormone (GH) as this hormone also contributes to regulating metabolism, reproduction, immunity, development, and osmoregulation in different species. Likely owing to this variety of group-specific functions, GH production is differentially regulated across vertebrates, with an apparent evolutionary trend to simplification, especially in the number of stimulatory factors governing substantially GH release. Thus, teleosts exhibit a multifactorial regulation of GH secretion, with a number of factors, from the newly discovered fish GH-releasing hormone (GHRH) to pituitary adenylate cyclase-activating peptide (PACAP) but also gonadotropin-releasing hormone, dopamine, corticotropin-releasing hormone, and somatostatin(s) directly controlling somatotropes. In amphibians and reptiles, GH secretion is primarily stimulated by the major hypothalamic peptides GHRH and PACAP and inhibited by somatostatin(s), while other factors (ghrelin, thyrotropin-releasing hormone) also influence GH release. Finally, in birds and mammals, primary control of GH secretion is exerted by a dual interplay between GHRH and somatostatin. In addition, somatotrope function is modulated by additional hypothalamic and peripheral factors (e.g., ghrelin, leptin, insulin-like growth factor-I), which together enable a balanced integration of feedback signals related to processes in which GH plays a relevant regulatory role, such as metabolic and energy status, reproductive, and immune function. Interestingly, in contrast to the high number of stimulatory factors impinging upon somatotropes, somatostatin(s) stand(s) as the main primary inhibitory regulator(s) for this cell type.

Key words: **growth hormone; evolution; vertebrates; somatostatin; GHRH**

Origin and Evolution of Growth Hormone

Growth hormone (GH; somatotropin) is a protein hormone secreted by the anterior pituitary gland (adenohypophysis). This gland secretes a number of peptide hormones, which regulate a variety of physiological processes in vertebrates. The adenohypophysial hormones can be classified, on the basis of structural and functional similarity, into three groups: the proopiomelanocortin family, the glycoprotein hormone family, and the GH family. Each family is thought to have evolved from an ancestral

Address for correspondence: Dr. Justo P. Castaño, Department of Cell Biology, Physiology and Inmunology, Edificio Severo Ochoa, Planta 3, Campus de Rabanales, University of Córdoba, E-14014 Cordoba, Spain. Voice: +34 957 21 85 94; fax: +34 957 218634. justo@uco.es

Trends in Comparative Endocrinology and Neurobiology: Ann. N.Y. Acad. Sci. 1163: 137–153 (2009).
doi: 10.1111/j.1749-6632.2008.03660.x

gene by duplication and subsequent mutations.[1] Specifically, the GH family was classically comprised of GH and prolactin (PRL); yet, some years ago, somatolactin (SL) (a fish hormone) and related mammalian placental hormones [placental lactogens and prolactin-related proteins] were included in this family because of their similar tertiary structure and their functional overlapping in some species.[2] GH and PRL are present throughout the vertebrates[3] except the cyclostomes, where neither has been described,[2] whereas SL is found only in fish (including lungfish). The structural similarity between GH, PRL, and SL is well established,[4,5] and it appears that these proteins have evolved from a common ancestral gene, which was lost secondarily in the lineage leading to land vertebrates after the lungfish branched off.[6] Additionally, GH and PRL structurally related proteins are reported to be produced from fetal placenta of three groups of mammals, rodents, ruminant artiodactyls, and primates.[7] Bovine and ovine placental lactogens are structurally more similar to PRL than they are to GH.[8] Recently, a new superfamily has been proposed that includes these GH family peptides and a variety of cytokines, including many interleukins, colony stimulating factors, and erythropoietin, which are distantly related. All these peptides share a common structural fold (a four-helix bundle with an atypical topology) and a characteristic receptor type with a single membrane-spanning domain. The GH/cytokine superfamily presumably arose as the result of a series of gene duplications and subsequent divergent evolution.[9,10]

GH Production

Classically, GH was thought to be exclusively produced and secreted by somatotrope cells of the anterior pituitary, and no other site of GH production had been detected.[11] However, nowadays it is commonly accepted that there are sites of extrapituitary production of GH where this hormone can exert autocrine and paracrine actions,[12] although the regulation of GH production in these extrapituitary sites is not yet totally clear. GH gene expression and somatotrope development are strongly dependent on Pit-1 (pituitary-specific transcription factor-1), which was thought to be solely expressed in somatotrope cells.[13] Nevertheless, increasing evidence points to extrapituitary expression and additional functional roles for Pit-1.[14]

Regulation of GH Secretion

GH secretion shows a pulsatile pattern in all species studied to date. In particular, humans and rats exhibit a sexual dimorphic pattern of GH secretion. Specifically, in male rats, GH secretion occurs in discrete pulses with low interpeak levels. Conversely, GH release in female rats displays less pulsatility and the interpeak levels are higher.[15] In fish, diurnal variations of GH secretion have been described in rainbow trout,[16,17] Atlantic salmon,[18] goldfish,[19] and grass carp.[20] These circadian variations are characterized by the existence of several peaks throughout the day, being higher during the dark phase.[16,17,21,22] It is widely accepted that this pulsatility is primarily controlled by the hypothalamus. Additionally, this episodic secretion can be modulated by diverse factors residing in the target organ, the pituitary, other regions of the central nervous system, or factors arriving from peripheral organs/tissues. Hypothalamic control of GH secretion in mammals has long been considered as a classic paradigm of the "dual control" system of pituitary hormone secretion. Namely, two hypothalamic peptides with opposing roles, GH-releasing hormone (GHRH) and somatotropin release-inhibiting factor (SRIF; or somatostatin), directly regulate GH secretion by adenohypophysis.[23,24] However and despite recent evidence showing important GH-regulatory roles for additional hypothalamic peptides (see below), the

hypothalamic regulation of GH secretion appears to be more complex and heterogeneous in nonmammalian than in mammalian vertebrates. In fact, the regulation of GH secretion in fish differs significantly from other lower vertebrate groups because of the unique organization of the hypothalamopituitary axis in teleosts. Endocrine cells of anterior pituitary of teleosts show a zonal distribution[25] and are directly innervated by nerve fibers from the hypothalamus.[26] Consequently, as will be outlined later, a number of neuroendocrine factors can act directly at the pituitary level to regulate GH secretion. On the other hand, in amphibians and reptiles, three hypothalamic peptides [GHRH, SRIF, and pituitary adenylate cyclase-activating polypeptide (PACAP)] have been described as playing a major role in the regulation of pulsatile GH secretion. Therefore, regulation of somatotrope cell function in these animal groups seems more similar to that observed in mammals. Finally, in birds, regulation of GH secretion is primary, also, under a "dual control" system similar to mammals, where GHRH and SRIF represent the most important hypophysiotropic factors but PACAP would not play a decisive role. Thus, in spite of the obvious group-specific differences, it appears that most of the main hypothalamic, pituitary, and peripheral factors involved in the control of the somatotropic function exert a comparable role in different groups, whereas their relative importance has changed during evolution.

Neuroendocrine Control

GH Release Inhibitors

SRIF: SRIF was discovered in 1973 in Roger Guillemin's laboratory as a GH-secretion inhibiting neurohormone.[27] It is widely distributed in mammals where it also acts as a peripheral hormone, an autocrine or paracrine factor, and a neuropeptide.[28–30] Two different forms of mammalian SRIF (SRIF-14 and SRIF-28) are derived from a single precursor, preprosomatostatin I (PPSS-I), which contains SRIF-14 at its C terminus with an identical amino acid sequence in all species studied.[31] However, in fish, PPSS-I is believed to yield only SRIF-14.[32] In lower vertebrates (lampreys, teleost fish, and frogs), in addition to PPSS-I, other PPSS exist, which give rise to diverse forms of SRIF depending on the species (e.g., SRIF-22, 25, 28).[33] A third PPSS cDNA has been isolated from several species of fish,[34–36] frog,[37] chicken,[38] and mammals,[39] including humans.[40] These SRIF precursors were named PPSS-III in nonmammalian vertebrates and cortistatin (CST) in mammals.[39,41] Recently, it has been shown that the mammalian CST and zebrafish PPSS-III genes are orthologous.[42,43]

SRIF is the main negative regulator of GH secretion and its inhibitory function is conserved during vertebrate evolution. In teleost, SRIF-14 is a potent inhibitor of basal and stimulated GH secretion. Exogenous administration of SRIF-14 reduces basal GH secretion in goldfish,[44] rainbow trout,[45] tilapia,[46] and chinook salmon.[16] The inhibitory effect of SRIF-14 on GH secretion was clearly demonstrated in several *in vitro* experiments.[33,41,47] However, the function of the other SRIF forms is less clear. In other nonmammalian vertebrate phyla, SRIF action on GH release is slightly different to that exerted in teleost. In grass-frog (*Rana pipiens*), bullfrog (*Rana catesbeiana*), clawed toad (*Xenopus laevis*), and two species of terrapin (*Chrysemys picta* and *Pseudemys scripta*), SRIF by itself had no apparent effect on release of hormones but it inhibited thyrotropin-releasing hormone (TRH)-stimulated release of GH from both amphibian and reptilian pituitary glands *in vitro.*[48,49] In chicken, SRIF is able to inhibit basal and GHRH-stimulated GH secretion.[50,51] In mammals, SRIF is essential to establish and maintain pulsatility of GH secretion. In male rats, Tannenbaum *et al.* proposed a classic model wherein GHRH and SRIF are secreted alternatively to stimulate and inhibit, respectively, the secretion of GH.[52] This reciprocal relationship was supported later by Plotsky and Vale who measured both hormones in portal blood collected from anesthetized

rats.[53] Nevertheless, application of this model to other species or even to female rats does not appear a simple issue; measurements of portal GHRH and SRIF levels in sheep or pig do not fully support this notion but suggest a more complex relationship between these two peptides and additional factors.[24] In this scenario, it has been proposed that in humans the role of SRIF in the control of GH secretion seems to be mainly circumscribed to the adjustment of the magnitude of its basal and pulsatile release but not to regulate generation of GH pulsatility.[23] Thus, although SRIF is undoubtedly the main inhibitory signal for GH secretion in all vertebrate groups, its role, as will be discussed below, can be more complex than it was initially envisioned.

In recent years, CST, the mammalian counterpart of PPSS-III, has been found to mimic the endocrine actions of SRIF, including its inhibitory effect on GH release both *in vivo* in human and *in vitro* in human pituitary adenomas and in porcine pituitary cells.[54–56] Nevertheless, the precise physiological relevance of those actions still remains to be fully elucidated.

Norepinephrine: Norepinephrine (NE) is a catecholamine synthesized from dopamine in neurons located in several regions of vertebrate brain. This neurotransmitter exerts its actions through binding to adrenergic receptors that are divided into α- and β-classes. NE inhibits the secretion of GH in different species, likely by activating α_2-adrenergic receptors directly in somatotrope cells,[24,57–62] although it cannot be considered an universal inhibitor of GH. In teleosts, catecholamines are particularly interesting in terms of regulation of GH release. In fact, pituitary gland of fish is innervated by adrenergic fibers located in isthmal tegmentum.[63] In goldfish, NE suppresses basal GH release from pituitary cells in a reversible and dose-dependent manner.[59,62] In contrast, NE is not able to decrease basal GH release from chicken pituitary, although high doses of this catecholamine reduces GHRH-stimulated GH release.[64] In mammals, the function of NE regulating somatotrope cell actions is not clear. On one hand, it is reported that NE decreases basal and GHRH-stimulated secretion of GH from ovine somatotropes.[61] On the other hand, activation of α_2-adrenergic receptors has no effect on bovine somatotropes.[58] In humans, almost all the doses of NE tested resulted in no significant and/or consistent changes in plasma concentration of GH,[60] suggesting that action of catecholamines on GH secretion from pituitary cells is mainly mediated by a dopaminergic mechanism.[65]

Serotonin: Serotonin or 5-HT (5-hydroxytryptamine) is an indoleamine synthesized from tryptophan in several regions of vertebrate brain. This neuropeptide exerts opposite actions on somatotrope cells in different vertebrate phyla. In fish, serotonin causes a dose-related inhibition of GH release from goldfish pituitaries at different sexual stages[66] via 5-HT_2 receptors.[67] In chicken, no direct actions of 5-HT on somatotrope cells have been reported, although this peptide reduces plasma level of GH.[68,69] However, all data reported in mammals strongly suggest a predominant stimulatory role of 5-HT on pituitary function. In rats, 5-HT induces release of GH directly from pituitary gland. The 5-HTR2B, 5-HTR7, and 5-HTR1B receptors mediate this response, although 5-HTR1D receptor could mediate an inhibitory response.[70,71] In ruminants, the role of serotonin on GH secretion is not consistent as 5-HT increases GH plasma levels in cattle[24,72] while it can inhibit release of GH from ovine pituitary.[24,73] However a stimulatory role of GH secretion has been assigned to serotonin in humans. Using specific 5-HT1A and 1D receptor agonists, two independent groups have reported that stimulation of these receptors can stimulate GH secretion, although 5-HT1D action could be mediated by the release of hypothalamic somatostatin.

GH Release Stimulators

GHRH: GHRH, also known as GH-releasing factor, is a 44-amino acid peptide hormone produced in the arcuate nucleus of the hypothalamus and initially identified

in 1982 from a pancreatic tumor causing acromegaly[74,75] and subsequently isolated and characterized in other species.[76] In contrast to most hypophysiotropic neurohormones, the primary structure of GHRH is highly variable.

Initial studies analyzing the potential role of GHRH in the control of GH release in fish failed to demonstrate a major stimulatory action, likely because of the fact that the peptides originally thought to correspond to fish GHRH were indeed homologues for PACAP-related peptides.[46,77–80] However, recent identification of GHRH and its receptors in goldfish has shed light into this issue by showing that this peptide is a potent stimulus for GH release in this species.[81] More experiments should be performed in other species to confirm a general stimulatory role of GHRH in GH secretion in teleost. In amphibians, it has been demonstrated that GHRH is able to stimulate GH secretion, and this effect is inhibited by SRIF in a dose-dependent manner.[49] Also, in reptiles, GHRH stimulates GH secretion by pituitary cells.[82] Finally, in birds and mammals, GHRH has been unequivocally proven as the main stimulatory neuropeptide in generating and maintaining GH secretion and pulsatility.[24,52,53,83]

PACAP: PACAP is a polypeptide with two molecular forms (PACAP27 and PACAP38) that was originally isolated from ovine hypothalamus based on its ability to stimulate adenylate cyclase activity in rat pituitary cells.[84] This peptide is a member of the vasoactive intestinal peptide/secretin/glucagon/GHRH/gastric inhibitory peptide superfamily and is highly conserved throughout evolution.[85,86] In fish, both PACAP27 and PACAP38 induce a robust stimulation of GH release from goldfish,[87] eel pituitary cells,[85] turbot,[88] and salmon.[89] Initial studies carried out in amphibians revealed that PACAP is able to increase free cytosolic calcium concentration ($[Ca^{2+}]_i$) in cultured frog somatotrope cells[90] and stimulate cAMP level in frog pituitary slices.[91] In 1994 it was reported that PACAP-stimulated GH secretion in the European green frog.[92] In birds, PACAP induces a dose-dependent stimulation of cAMP by chicken pituitary cells. However, GH secretion stimulated by PACAP is very weak compared with that produced by human GHRH.[93] In mammals, data concerning the effect of PACAP on GH release are controversial. Some studies found a stimulatory effect of PACAP in rat,[94–97] sheep,[98] swine,[99,100] and cattle,[101] while other reports indicated that PACAP has no effect on GH secretion.[84,96,102,103] In humans, PACAP is less potent than GHRH in stimulating GH release from somatotropic adenoma cells in primary culture,[104] and intravenous administration of PACAP does not modify plasma GH levels.[105] Interestingly, PACAP and GHRH are encoded by two closely related genes.[81] In spite of being two highly related peptides, their role on GH regulation across vertebrate evolution seems to have diverged. In fish, both PACAP and GHRH seem to play an important role; in amphibians and reptiles, both neuropeptides seem to exert equipotent actions on GH release; however, in birds and mammals, while GHRH is critical for GH release, PACAP seems to play only a secondary role in the regulation of the somatotropic axis.

TRH: TRH is a tripeptide synthesized in neurons of the paraventricular nucleus in most species that stimulates the release of thyroid-stimulating hormone and PRL by the anterior pituitary. Additionally, TRH has also shown to act as a stimulator of GH release by somatotrope cells in all vertebrate groups, although with species-specific differences. The somatotropinergic role of TRH is importantly conserved during early vertebrate evolution. In fish, TRH-stimulated GH release or activation of somatotropes has been reported in some teleost species, such as goldfish and carp,[47,106] but no effect has been observed in tilapia,[46] eel, and turbot.[88,107] Accordingly, direct action of TRH on somatotrope function has been reported in amphibians[108] and reptiles[48] as well as in birds. TRH seems to be equipotent to GHRH[109] in the case of chickens[110] but not in fowl.[110–113] In mammals, TRH has

been demonstrated to stimulate GH release in cattle[114] and in sheep.[73,115,116] In humans, the ability of TRH to induce GH release is maintained in tumor somatotropinoma cells, while this effect is less evident in normal subjects, thereby suggesting that, as for PACAP, TRH has lost, at least in part, its capacity to act as a primary stimulus for the somatotrope axis.[117–120]

GnRH: Gonadotropin-releasing hormone (GnRH) is a decapeptide with a similar structure in all vertebrate species. It is produced in the hypothalamus and plays a crucial role in regulation of reproduction, stimulating follicle-stimulating hormone and luteinizing hormone secretion. However, its role as stimulator of GH release seems to be restricted to some fish species. Consequently, GnRH directly induces GH secretion from pituitary cells of goldfish,[121,122] common carp,[123,124] and tilapia,[46,125] but no effect was demonstrated in catfish,[126] eel, and turbot.[88,107] No data have been reported in other vertebrate species.

Recently, a novel group of neuropeptides encoded by the *KISS-1* gene belonging to the RF-amide family of peptides, kisspeptins, which primarily act in the hypothalamus to stimulate GnRH neurons, have been found to act also on somatotropes from peripubertal rats to stimulate GH release[127] as well as in cattle.[128,129] However, the physiological relevance of this effect is still to be fully elucidated.

NPY: Neuropeptide Y (NPY) is a 36-amino acid peptide, widely distributed throughout the brain, with highest density of neurons in the arcuate nucleus. In lower vertebrates, direct actions of NPY on GH release from somatotropes have been described in goldfish,[130–132] while no data have been reported in amphibians, reptiles, or birds. In mammals, similar to fish, NPY seems to be a stimulator of GH secretion. In fact, NPY has been reported to directly stimulate basal GH secretion in pig[133] and ruminants.[134–137] However, data reported in rats are controversial. In fact, Rettori *et al.* found that NPY could increase plasma levels of GH,[138] while Suzuki *et al.* reported inhibitory effects.[139] Surprisingly, some years later, it was reported that NPY had no direct actions on rat pituitary cells.[140] Therefore, although NPY seems to be a stimulator of GH secretion, acting directly on somatotropes, in some of the species studied, elucidation of its precise role in the control of somatotropes will require additional work.

CRH: Corticotropin-releasing hormone (CRH) is a 41-amino acid peptide derived from a 191-amino acid preprohormone. CRH is secreted by the paraventricular nucleus of the hypothalamus. It is the major hypothalamic factor mediating stress-induced adrenocorticotropin secretion by pituitary gland. This action and its sequence has been highly conserved during vertebrate evolution.[141] However, its action on GH release seems to be restricted to lower vertebrates. Specifically, although it has been reported that CRH can stimulate GH production in a primitive teleost, the European eel,[107] and in a reptile (the hatchling turtle),[82] no additional data have been reported to date in other vertebrate groups. Interestingly, two different groups have observed that CRH can paradoxically increase GH plasma levels in patients with acromegaly,[142,143] although a third group failed to confirm this effect.[144] Consequently, CRH, similar to GnRH, seems to act as a GH release stimulator only in lower vertebrates.

Pituitary Control

Growth Hormone

Several results support the existence of an ultra-short feedback by GH acting locally at the pituitary level via autocrine/paracrine mechanisms. Indeed, GH receptors are ubiquitously expressed in the anterior pituitary.[145] However, it is not yet clear if this regulatory feedback acts as a negative or a positive loop. In mammals, some reports suggest a negative effect of GH on its own secretion. Specifically, GH treatment can attenuate GH secretion in bovine pituitary cells.[146] These observations are consistent with recent reports that somatotropes of GH

receptor-lacking transgenic mice exhibit histological features typical of secretory hyperactivity.[147] However, GH is not able to alter basal GH release in rat pituitary cells[148] or in purified rat somatotropes.[149] In contrast, Wong *et al.* postulated a positive ultra-short loop in fish pituitary. Specifically, they demonstrated that GH treatment can elevate GH release in grass carp pituitary cells.[67] In humans, GH has been also postulated to inhibit its own secretion, although it is not clear if this is a direct or an insulin-like growth factor (IGF)-mediated effect.[150,151] Consequently, autoregulation of GH secretion seems to be group and even species specific.

Metabolic Control

IGF-I

IGF-I is a 70-amino acid hormone highly conserved throughout vertebrate evolution. It is expressed in a wide range of tissues, with the highest level being found in the liver where IGF-I is the major growth factor secreted under the control of GH. Circulating IGF-I is known to exert a long-loop feedback on GH secretion by acting at both the hypothalamus and pituitary level.[152–154] In teleosts, a direct effect of IGF-I on GH secretion by somatotropes has been reported in European eel,[155] turbot,[156] and striped bass.[157] This direct action has also been reported in fowl[158] and mammals (rat,[159,160] sheep,[161] and baboon[162]). In humans, IGF-I suppresses plasma GH concentrations by 50–80% in both sexes.[23] However, in women, IGF-I fails to suppress GHRH-stimulated GH levels, suggesting that, in contrast to men, this effect is exerted exclusively at hypothalamic level.[23] As expected, IGF-I exerts a direct inhibitory effect on GH release from pituitary cells in all species studied (long-loop feedback).

Ghrelin

Ghrelin is a 20- to 30-amino acid peptide, depending on species, predominantly produced by the stomach although expressed in many other tissues, including the pancreas, the cardiovascular system, and the hypothalamus.[163] It is produced as two different forms, an octanoylated form, which binds the GH secretagogue (GHS) receptors 1a (GHS-R1a), which is biologically active in terms of GH release, and a deoctanoylated form that does not bind to GHS-R1a and was previously thought to be inactive but shows several actions in both endocrine and nonendocrine tissues.[163] This peptide, as suggested earlier by studies on its synthetic analogs (GHSs), exerts several biological actions, including modulation of GH secretion and potent orexigenic functions.[164] In teleosts, ghrelin acts as a potent GH release stimulator directly from pituitary in several species (goldfish,[165] tilapia,[166,167] rainbow trout,[168] catfish,[169] or eel[170]). This peptide is able to stimulate GH release from somatotrope cells in amphibians.[171] In reptiles, ghrelin has also been characterized.[172] The stimulatory role of ghrelin has also been reported in chicken by acting directly in somatotropes.[109,173] Finally, in mammals, ghrelin has a stimulatory role in GH secretion, as reported, for example, in rats,[174] pigs,[175] primates[176] or humans,[23,177] and other species.[178] Consequently, ghrelin stimulates somatotrope activity in all species studied to date and could, thus, play a relevant role in the modulation of GH release, an effect that would have been conserved during vertebrate evolution. Notwithstanding, mice models lacking ghrelin or GHS-R do not show overt changes on GH release, and the regulatory function of ghrelin on somatotropes appears subtle; increasing evidence suggests that this orexigenic peptide would act as a pivotal link between metabolic status and growth.[178]

Leptin

Leptin is a class-I helical cytokine hormone, discovered by positional cloning of the murine obese gene and its human homologue.[179] Leptin is a circulating hormone mainly secreted, in mammals, by adipose tissue but also by few other tissues. It is widely accepted that leptin

secreted by adipocytes communicates the amount of stored energy (lipid) to the brain. This peptide has also been detected in lower vertebrates but, in contrast to mammals, fish leptin seems to be mostly produced in the liver.[180,181] Moreover, leptin-like inmunoreactivity has also been detected in stomach of amphibians[182] and in stomach, plasma, liver, and fat bodies of reptiles.[182,183] At least in mammals, leptin also acts as a neurotransmitter because this peptide and its receptors are expressed in hypothalamus and normal and adenomatous pituitary cells of mice, rats, sheep, and humans.[184–187] To our knowledge, no data have been reported regarding modulation of GH secretion by leptin in nonmammalian vertebrates. In contrast, several studies have shown that in mammals, leptin exerts a direct effect on somatotropes. In rats and cows, the effect of leptin on GH secretion is dependent on feeding status.[188,189] In mice and pigs, leptin acts directly on somatotropes, inducing GH secretion.[190–193] In humans, leptin might exert a positive effect on the hypothalamic–pituitary–adrenal axis,[194] although in GH-secreting adenomatous tissues leptin seems to exert a slight inhibitory effect on spontaneous GH secretion and a stimulatory effect on GHRH-stimulated GH secretion.[195] In conclusion, it seems clear that leptin has the capacity to modulate GH secretion from pituitary, at least in mammals, although this modulation seems to be species dependent and remains to be explored in lower vertebrates.

Evolutive Aspects of Regulation of GH Secretion

As stated above, GH secretion exhibits a pulsatile pattern in all the species studied. However, the frequency and amplitude of pulses are age and species dependent and show a deep sexual dimorphism. This pulsatility is crucial for GH to precisely regulate the basic function of a number of key physiological processes. As an obvious example, the pattern of GH release from somatotrope cells is important in determining growth rates in mammals.[196,197] In spite of important differences among vertebrate groups, in all the species, the main control of GH secretion essentially resides at the hypothalamus–pituitary unit. However, the hypothalamic hormones involved in this regulation, and more precisely their differential contribution, have changed during vertebrate evolution. Thus, in teleosts, SRIF, GHRH, and PACAP are the main regulators, while other neuropeptides display less potent but still significant inhibitory and stimulatory roles; in amphibians and reptiles, GHRH and PACAP seem to be equipotent in releasing GH, and SRIF acts as inhibitor; and in birds and mammals, PACAP does not have an obvious role, and GHRH and SRIF regulate GH secretion through a tight interplay. Moreover, depending on the vertebrate group or even the species considered, a variety of central, pituitary, and peripheral signals impinge upon the somatotrope to modulate GH secretion in order to finely tune its pattern and adjust it to punctual necessities of each species in each situation. In fact, it has been described that the secretory pattern of GH is influenced by feeding regimes,[22,198] temperature and photoperiod,[19] and development.[18] Clearly, lower vertebrate groups exhibit complex life cycles marked by continuous growth and drastic physiological changes imposed by metamorphosis or migrations and are therefore more exposed to environmental conditions.[199] That could be the reason why GH secretion in lower vertebrates is regulated by a high number of different molecules in contrast with higher vertebrates where only a few molecules exert a marked influence in somatotropes and are therefore able to affect the main regulation of GH release. In conclusion, available evidence supports the notion that there is an evolutionary reduction in the number of regulatory molecules, mainly stimulatory signals, implicated in the control of GH secretion, in agreement with the simplification of life cycles across vertebrate evolution.

Somatostatin: More than an Inhibitor?

In contrast to the diversity of stimulatory molecules involved in regulation of GH secretion, SRIF (or, perhaps, somatostatins) seems to be the only major inhibitor of GH release consistent and preserved throughout vertebrate evolution. Despite the important feedback loops involved in GH regulation, exerted mainly by IGFs but also by gonadal steroids and GH itself, SRIF has been reported as the only peptide that is able to inhibit GH secretion from somatotrope cells in all the species studied. In this scenario it comes as a surprise to our earlier observation that SRIF can paradoxically stimulate GH secretion from a subpopulation of pig somatotropes (high-density subpopulation).[200] Subsequently, low concentrations of SRIF (10^{-15} mol/L) were found to stimulate pig GH release from the two major somatotrope subpopulations from pig pituitary and also in intact pituitary cultures, whereas a high SRIF concentration (10^{-7} mol/L) inhibited GHRH-induced GH secretion. These data suggested that SRIF could play an unsuspected dual stimulatory/inhibitory role in the control of GH secretion in this species.[201,202] Analysis of second messenger pathways revealed that cAMP is the main signal conveying the stimulatory effects of low-dose SRIF.[203–205] This peptide also exerts a distinct, dose-dependent regulation of the expression of three of its receptor subtypes (sst1, sst2, and sst5) at the pituitary.[206] Indeed, acute *in vitro* treatment with a high SRIF dose increased mRNA levels of all three subtypes, whereas a low SRIF concentration only increased that of sst5. Moreover, short-term treatment with GHRH or ghrelin reduced the expression of sst5 and not that of sst1 and sst2. Interestingly, the stimulatory effect of SRIF on GH release was reported to be mediated by sst5.[207] To date, the dual stimulatory/inhibitory action of SRIF has not been reported to occur in other species. However, in 1997, Chen *et al*. reported that SRIF was able to induce a paradoxical increase in $[Ca^{2+}]_i$ or to have no effect on $[Ca^{2+}]_i$ in a small proportion of somatotrope cells coming from human pituitary adenomas.[208] These data suggest that SRIF may not act just as a mere inhibitor in GH release since it is able to stimulate GH secretion from pig pituitaries. As mentioned earlier, the paradoxical action of low-dose SRIF has only been reported in pig, but further studies will be needed to determine if this is a species-specific phenomenon or is also present in other species.

Conclusion

In conclusion, fine regulation of GH secretion is crucial to maintain correct metabolism in vertebrates since GH is involved in key physiological processes. However, factors implicated in this regulation have changed during vertebrate evolution, perhaps because of group-specific life styles. Lower vertebrates exhibit, in contrast to higher vertebrates, complex cycles of life and a deep environmental dependence. Accordingly, regulation of GH secretion has evolved from a multifactorial control (in fish, a remarkable number of molecules act directly on pituitary cells) to a primary dual control exerted by GHRH and SRIF in order to adequate the patterns of GH secretion to precise necessities of each species. In contrast with the large number of stimulating factors capable of regulating GH secretion, SRIF has conserved its primary role as the most important inhibitor throughout vertebrate evolution. Nevertheless, data reported in pig suggest that SRIF, under some conditions, can also act as a stimulator of GH secretion.

Acknowledgments

This work was supported by BIO-139 and CTS-1705 (Plan Andaluz de Investigación, Junta de Andalucía, Spain) and BFU2007-60180/BFI (Ministerio de Educación y Ciencia, Spain/Fondo Europeo de Desarrollo Regional).

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Kawauchi, H. & S.A. Sower. 2006. The dawn and evolution of hormones in the adenohypophysis. *Gen. Comp. Endocrinol.* **148:** 3–14.
2. Forsyth, I.A. & M. Wallis. 2002. Growth hormone and prolactin–molecular and functional evolution. *J. Mammary Gland Biol. Neoplasia* **7:** 291–312.
3. Kawauchi, H. 1989. Evolutionary aspects of pituitary hormones. *Kitasato Arch. Exp. Med.* **62:** 139–155.
4. Ono, M. *et al*. 1990. cDNA cloning of somatolactin, a pituitary protein related to growth hormone and prolactin. *Proc. Natl. Acad. Sci. USA* **87:** 4330–4334.
5. Rand-Weaver, M. *et al*. 1991. Isolation and characterization of somatolactin, a new protein related to growth hormone and prolactin from Atlantic cod (Gadus morhua) pituitary glands. *Biochemistry* **30:** 1509–1515.
6. Fukamachi, S. & A. Meyer. 2007. Evolution of receptors for growth hormone and somatolactin in fish and land vertebrates: lessons from the lungfish and sturgeon orthologues. *J. Mol. Evol.* **65:** 359–372.
7. Forsyth, I.A. 1994. Comparative aspects of placental lactogens: structure and function. *Exp. Clin. Endocrinol.* **102:** 244–251.
8. Anthony, R.V. *et al*. 1995. The growth hormone/prolactin gene family in ruminant placentae. *J. Reprod. Fertil. Suppl.* **49:** 83–95.
9. Kossiakoff, A.A. & A.M. De Vos. 1998. Structural basis for cytokine hormone-receptor recognition and receptor activation. *Adv. Protein Chem.* **52:** 67–108.
10. Wallis, M. 1992. The expanding growth hormone/prolactin family. *J. Mol. Endocrinol.* **9:** 185–188.
11. Karin, M. *et al*. 1990. Tissue-specific expression of the growth hormone gene and its control by growth hormone factor-1. *Recent Prog. Horm. Res.* **46:** 43–57; discussion 57–58.
12. Harvey, S. & K.L. Hull. 1997. Growth hormone. A paracrine growth factor? *Endocrine.* **7:** 267–279.
13. Bodner, M. & M. Karin. 1987. A pituitary-specific trans-acting factor can stimulate transcription from the growth hormone promoter in extracts of non-expressing cells. *Cell* **50:** 267–275.
14. Gil-Puig, C. *et al*. 2005. Pit-1 is expressed in normal and tumorous human breast and regulates GH secretion and cell proliferation. *Eur. J. Endocrinol.* **153:** 335–344.
15. Giustina, A. & J.D. Veldhuis. 1998. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr. Rev.* **19:** 717–797.
16. Le Bail, P.Y. *et al*. 1991. Development and validation of a highly sensitive radioimmunoassay for chinook salmon (Oncorhynchus tshawytscha) growth hormone. *Gen. Comp. Endocrinol.* **83:** 75–85.
17. Gomez, J.M. *et al*. 1996. Characterization of growth hormone nycthemeral plasma profiles in catheterized rainbow trout (Oncorhynchus mykiss). *J. Exp. Zool.* **274:** 171–180.
18. Bjornsson, B.T. *et al*. 2000. Photoperiod regulation of plasma growth hormone levels during induced smoltification of underyearling Atlantic salmon. *Gen. Comp. Endocrinol.* **119:** 17–25.
19. Marchant, T.A. & R.E. Peter. 1986. Seasonal variations in body growth rates and circulating levels of growth hormone in the goldfish, Carassius auratus. *J. Exp. Zool.* **237:** 231–239.
20. Zhang, W.M. *et al*. 1994. Episodic growth hormone secretion in the grass carp, Ctenopharyngodon idellus (C. & V.). *Gen. Comp. Endocrinol.* **95:** 337–341.
21. Boujard, T. 1995. Diel rhythms of feeding activity in the European catfish, Silurus glanis. *Physiol. Behav.* **58:** 641–645.
22. Boujard, T. *et al*. 1992. Description of a modular, low cost, eater meter for the study of feeding behavior and food preferences in fish. *Physiol. Behav.* **52:** 1101–1106.
23. Goldenberg, N. & A. Barkan. 2007. Factors regulating growth hormone secretion in humans. *Endocrinol. Metab. Clin. North Am.* **36:** 37–55.
24. McMahon, C.D. *et al*. 2001. Neuroregulation of growth hormone secretion in domestic animals. *Domest. Anim. Endocrinol.* **20:** 65–87.
25. Doerr-Schott, J. 1980. Immunohistochemistry of the adenohypophysis of non-mammalian vertebrates. *Acta. Histochem. Suppl.* **22:** 185–223.
26. Gorbman, A. 1995. Olfactory origins and evolution of the brain-pituitary endocrine system: facts and speculation. *Gen. Comp. Endocrinol.* **97:** 171–178.
27. Brazeau, P. *et al*. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* **179:** 77–79.
28. Krantic, S. *et al*. 2004. Novel modalities of somatostatin actions. *Eur. J. Endocrinol.* **151:** 643–655.
29. Patel, Y.C. 1999. Somatostatin and its receptor family. *Front Neuroendocrinol.* **20:** 157–198.
30. Moller, L.N. *et al*. 2003. Somatostatin receptors. *Biochim. Biophys. Acta.* **1616:** 1–84.
31. Pierotti, A.R. & A.J. Harmar. 1985. Multiple forms of somatostatin-like immunoreactivity in the hypothalamus and amygdala of the rat: selective

localization of somatostatin-28 in the median eminence. *J. Endocrinol.* **105:** 383–389.
32. Duguay, S.J., Mommsen, T.P. 1994. Molecular aspects of pancreatic peptides. In *Fish Physiology*. N.M. Sherwood & C.L. Hew, Eds.: 225–271. Academic Press. San Diego.
33. Sheridan, M.A. & J.D. Kittilson. 2004. The role of somatostatins in the regulation of metabolism in fish. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **138:** 323–330.
34. Linn, C.L. & A.C. Gafka. 1999. Activation of metabotropic glutamate receptors modulates the voltage-gated sustained calcium current in a teleost horizontal cell. *J. Neurophysiol.* **81:** 425–434.
35. Trabucchi, M. *et al*. 1999. Molecular cloning of the cDNAs and distribution of the mRNAs encoding two somatostatin precursors in the African lungfish Protopterus annectens. *J. Comp. Neurol.* **410:** 643–652.
36. Trabucchi, M. *et al*. 2002. Polygenic expression of somatostatin in the sturgeon Acipenser transmontanus: molecular cloning and distribution of the mRNAs encoding two somatostatin precursors. *J. Comp. Neurol.* **443:** 332–345.
37. Tostivint, H. *et al*. 1996. Occurrence of two somatostatin variants in the frog brain: characterization of the cDNAs, distribution of the mRNAs, and receptor-binding affinities of the peptides. *Proc. Natl. Acad. Sci. USA* **93:** 12605–12610.
38. Trabucchi, M. *et al*. 2003. Characterization of the cDNA encoding a somatostatin variant in the chicken brain: comparison of the distribution of the two somatostatin precursor mRNAs. *J. Comp. Neurol.* **461:** 441–451.
39. de Lecea, L. *et al.* 1996. A cortical neuropeptide with neuronal depressant and sleep-modulating properties. *Nature* **381:** 242–245.
40. Fukusumi, S. *et al*. 1997. Identification and characterization of a novel human cortistatin-like peptide. *Biochem. Biophys. Res. Commun.* **232:** 157–163.
41. Lin, X. & R.E. Peter. 2001. Somatostatins and their receptors in fish. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **129:** 543–550.
42. Tostivint, H. *et al*. 2005. Linkage mapping of the [Pro2]somatostatin-14 gene in zebrafish: evolutionary perspectives. *Ann. N. Y. Acad. Sci.* **1040:** 486–489.
43. Tostivint, H. *et al*. 2004. Chromosomal localization of three somatostatin genes in zebrafish. Evidence that the [Pro2]-somatostatin-14 isoform and cortistatin are encoded by orthologous genes. *J. Mol. Endocrinol.* **33:** R1–R8.
44. Cook, A.F. & R.E. Peter. 1984. The effects of somatostatin on serum growth hormone levels in the goldfish, Carassius auratus. *Gen. Comp. Endocrinol.* **54:** 109–113.
45. Very, N.M. *et al*. 2001. Somatostatin inhibits growth of rainbow trout. *J. Fish Biol.* **59:** 157–165.
46. Melamed, P. *et al*. 1995. Hypothalamic and thyroidal regulation of growth hormone in tilapia. *Gen. Comp. Endocrinol.* **97:** 13–30.
47. Peng, C. & R.E. Peter. 1997. Neuroendocrine regulation of growth hormone secretion and growth in fish. *Zool. Stud.* **36:** 79–89.
48. Hall, T.R. & A. Chadwick. 1984. Effects of synthetic mammalian thyrotrophin releasing hormone, somatostatin and dopamine on the secretion of prolactin and growth hormone from amphibian and reptilian pituitary glands incubated in vitro. *J. Endocrinol.* **102:** 175–180.
49. Jeandel, L. *et al*. 1998. Effects of the two somatostatin variants somatostatin-14 and [Pro2, Met13]somatostatin-14 on receptor binding, adenylyl cyclase activity and growth hormone release from the frog pituitary. *J. Neuroendocrinol.* **10:** 187–192.
50. Piper, M.M. & T.E. Porter. 1997. Responsiveness of chicken embryonic somatotropes to somatostatin (SRIF) and IGF-I. *J. Endocrinol.* **154:** 303–310.
51. Spencer, G.S. *et al*. 1986. The effect of immunization against somatostatin on growth rates and growth hormone secretion in the chicken. *Comp. Biochem. Physiol. A.* **85:** 553–556.
52. Tannenbaum, G.S. *et al*. 1989. Paradoxical enhancement of pituitary growth hormone (GH) responsiveness to GH-releasing factor in the face of high somatostatin tone. *Endocrinology* **124:** 1380–1388.
53. Plotsky, P.M. & W. Vale. 1985. Patterns of growth hormone-releasing factor and somatostatin secretion into the hypophysial-portal circulation of the rat. *Science* **230:** 461–463.
54. Broglio, F. *et al*. 2008. Endocrine actions of cortistatin: In vivo studies. *Mol. Cell Endocrinol.* **286:** 123–127.
55. Luque, R.M. *et al*. 2006. Cortistatin mimics somatostatin by inducing a dual, dose-dependent stimulatory and inhibitory effect on growth hormone secretion in somatotropes. *J. Mol. Endocrinol.* **36:** 547–556.
56. Gahete, M.D. *et al*. 2008. Are somatostatin and cortistatin two siblings in regulating endocrine secretions? In vitro work ahead. *Mol. Cell Endocrinol.* **286:** 128–134.
57. Buonomo, F.C. *et al*. 1984. Catecholamine involvement in the control of growth hormone secretion in the domestic fowl. *Gen. Comp. Endocrinol.* **54:** 360–371.
58. Gaynor, P.J. *et al*. 1996. 5-hydroxytryptaminergic receptor-mediated regulation of growth hormone secretion in Holstein steers occurs via alpha

2-adrenergic-dependent and -independent mechanisms. *Proc. Soc. Exp. Biol. Med.* **212:** 355–361.
59. Lee, E.K. *et al*. 2000. Norepinephrine regulation of growth hormone release from goldfish pituitary cells. I. Involvement of alpha2 adrenoreceptor and interactions with dopamine and salmon gonadotropin-releasing hormone. *J. Neuroendocrinol.* **12:** 311–322.
60. Mileikowsky, G.N. *et al*. 1988. Influence of norepinephrine administration upon pituitary hormone secretion in normal men. *J. Endocrinol. Invest.* **11:** 641–645.
61. Soyoola, E.O. *et al*. 1994. Neurotransmitter receptor agonists regulate growth hormone gene expression in cultured ovine pituitary cells. *Proc. Soc. Exp. Biol. Med.* **207:** 26–33.
62. Yunker, W.K. *et al*. 2000. Norepinephrine regulation of growth hormone release from goldfish pituitary cells. II. Intracellular sites of action. *J. Neuroendocrinol.* **12:** 323–333.
63. Kah, O. *et al*. 1986. The dopaminergic innervation of the goldfish pituitary. An immunocytochemical study at the electron-microscope level using antibodies against dopamine. *Cell Tissue Res.* **244:** 577–582.
64. Donoghue, D.J. *et al*. 1990. Influence of catecholamines, prostaglandins and thyroid hormones on growth hormone secretion by chicken pituitary cells in vitro. *Domest. Anim. Endocrinol.* **7:** 35–42.
65. Ishibashi, M. & T. Yamaji. 1984. Direct effects of catecholamines, thyrotropin-releasing hormone, and somatostatin on growth hormone and prolactin secretion from adenomatous and nonadenomatous human pituitary cells in culture. *J. Clin. Invest.* **73:** 66–78.
66. Somoza, G.M. & R.E. Peter. 1991. Effects of serotonin on gonadotropin and growth hormone release from in vitro perifused goldfish pituitary fragments. *Gen. Comp. Endocrinol.* **82:** 103–110.
67. Wong, A.O. *et al*. 2006. Feedback regulation of growth hormone synthesis and secretion in fish and the emerging concept of intrapituitary feedback loop. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **144:** 284–305.
68. Hall, T.R. *et al*. 1983. Mechanism of serotonin effects on prolactin and growth hormone secretion in domestic fowl. *Acta. Endocrinol. (Copenh)*. **104:** 266–271.
69. Rabii, J. *et al*. 1981. Role of serotonin in the regulation of growth hormone and prolactin secretion in the domestic fowl. *J. Endocrinol.* **90:** 355–358.
70. Lopez, F. *et al*. 1986. Serotonin stimulates GH secretion through a direct pituitary action: studies in hypophysectomized autografted animals and in perifused pituitaries. *Acta. Endocrinol. (Copenh)*. **113:** 317–322.
71. Papageorgiou, A. & C. Denef. 2007. Stimulation of growth hormone release by 5-hydroxytryptamine (5-HT) in cultured rat anterior pituitary cell aggregates: evidence for mediation by 5-HT2B, 5-HT7, 5-HT1B, and ketanserin-sensitive receptors. *Endocrinology* **148:** 4509–4522.
72. Radcliff, R.P. *et al*. 2003. Thyrotropin-releasing hormone mediates serotonin-induced secretion of GH in cattle. *Domest. Anim. Endocrinol.* **24:** 137–153.
73. Spencer, G.S. *et al*. 1991. Neuroendocrine regulation of growth hormone secretion in sheep. III. Serotoninergic systems. *Domest. Anim. Endocrinol.* **8:** 383–391.
74. Guillemin, R. *et al*. 1982. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* **218:** 585–587.
75. Rivier, J. *et al*. 1982. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature* **300:** 276–278.
76. Malagón, M.M. *et al*. 2006. Growth hormone-releasing hormone. In *The Handbook of Biologically Active Peptides*. A.J. Kastin, Ed.: 663–672. Elsevier. Amsterdam.
77. Montero, M. *et al*. 1998. Distribution, characterization, and growth hormone-releasing activity of pituitary adenylate cyclase-activating polypeptide in the European eel, Anguilla anguilla. *Endocrinology* **139:** 4300–4310.
78. Blaise, O. *et al*. 1995. Role of IGF-I in the control of GH secretion in rainbow trout (Oncorhynchus mykiss). *Growth Regul.* **5:** 142–150.
79. Luo, D.S. *et al*. 1990. In vitro responses of rainbow trout (Oncorhynchus mykiss) somatotrophs to carp growth hormone-releasing factor (GRF) and somatostatin. *Gen. Comp. Endocrinol.* **80:** 288–298.
80. Vaughan, J.M. *et al*. 1992. Isolation and characterization of hypothalamic growth-hormone releasing factor from common carp, Cyprinus carpio. *Neuroendocrinology* **56:** 539–549.
81. Lee, L.T. *et al*. 2007. Discovery of growth hormone-releasing hormones and receptors in nonmammalian vertebrates. *Proc. Natl. Acad. Sci. USA* **104:** 2133–2138.
82. Denver, R.J. & P. Licht. 1990. Modulation of neuropeptide-stimulated pituitary hormone secretion in hatchling turtles. *Gen. Comp. Endocrinol.* **77:** 107–115.
83. Bertherat, J. *et al*. 1995. Neuroendocrine regulation of growth hormone. *Eur. J. Endocrinol.* **132:** 12–24.
84. Miyata, A. *et al*. 1989. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem. Biophys. Res. Commun.* **164:** 567–574.

85. Montero, M. *et al*. 2000. Molecular evolution of the growth hormone-releasing hormone/pituitary adenylate cyclase-activating polypeptide gene family. Functional implication in the regulation of growth hormone secretion. *J. Mol. Endocrinol.* **25:** 157–168.
86. Vaudry, D. *et al*. 2000. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol. Rev.* **52:** 269–324.
87. Wong, A.O. *et al*. 2000. Pituitary adenylate cyclase activating polypeptide as a novel hypophysiotropic factor in fish. *Biochem. Cell Biol.* **78:** 329–343.
88. Rousseau, K. *et al*. 2001. Pituitary growth hormone secretion in the turbot, a phylogenetically recent teleost, is regulated by a species-specific pattern of neuropeptides. *Neuroendocrinology* **74:** 375–385.
89. Parker, D.B. *et al*. 1997. Exon skipping in the gene encoding pituitary adenylate cyclase-activating polypeptide in salmon alters the expression of two hormones that stimulate growth hormone release. *Endocrinology* **138:** 414–423.
90. Gracia-Navarro, F. *et al*. 1992. Pituitary adenylate cyclase-activating polypeptide stimulates calcium mobilization in amphibian pituitary cells. *Endocrinology* **131:** 1069–1074.
91. Yon, L. *et al*. 1993. Neuroanatomical and physiological evidence for the involvement of pituitary adenylate cyclase-activating polypeptide in the regulation of the distal lobe of the frog pituitary. *J. Neuroendocrinol.* **5:** 289–296.
92. Martinez-Fuentes, A.J. *et al*. 1994. Effect of frog pituitary adenylate cyclase-activating polypeptide (PACAP) on amphibian pituitary cells. In *Vasoactive Intestinal Peptide, Pituitary Adenylate Cyclase-Activating Polypeptide and Related Regulatory Peptides: from Molecular Biology to Clinical Applications*. G. Rosselin, Ed.: 376–380. World Scientific. Singapore.
93. Peeters, K. *et al*. 1998. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) on cAMP formation and growth hormone release from chicken anterior pituitary cells. *Ann. N. Y. Acad. Sci.* **865:** 471–474.
94. Goth, M.I. *et al*. 1992. Pituitary adenylate cyclase activating polypeptide, growth hormone (GH)-releasing peptide and GH-releasing hormone stimulate GH release through distinct pituitary receptors. *Endocrinology* **130:** 939–944.
95. Hart, G.R. *et al*. 1992. Effects of a novel hypothalamic peptide, pituitary adenylate cyclase-activating polypeptide, on pituitary hormone release in rats. *J. Endocrinol.* **134:** 33–41.
96. Jarry, H. *et al*. 1992. Contrasting effects of pituitary adenylate cyclase activating polypeptide (PACAP) on in vivo and in vitro prolactin and growth hormone release in male rats. *Life Sci.* **51:** 823–830.
97. Wei, L. *et al*. 1993. Pituitary adenylate cyclase activating polypeptide-induced desensitization on growth hormone release from rat primary pituitary cells. *Biochem. Biophys. Res. Commun.* **197:** 1396–1401.
98. Sawangjaroen, K. *et al*. 1997. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on hormone secretion from sheep pituitary cells in vitro. *J. Neuroendocrinol.* **9:** 279–286.
99. Martinez-Fuentes, A.J. *et al*. 1998. Pituitary adenylate cyclase-activating polypeptide (PACAP) 38 and PACAP27 activate common and distinct intracellular signaling pathways to stimulate growth hormone secretion from porcine somatotropes. *Endocrinology* **139:** 5116–5124.
100. Martinez-Fuentes, A.J. *et al*. 1998. Pituitary adenylate cyclase-activating polypeptide (PACAP) 38 and PACAP27 differentially stimulate growth hormone release and mRNA accumulation in porcine somatotropes. *Life Sci.* **62:** 2379–2390.
101. Hashizume, T. *et al*. 1994. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP), prostaglandin E2 (PGE2) and growth hormone releasing factor (GRF) on the release of growth hormone from cultured bovine anterior pituitary cells in vitro. *Domest. Anim. Endocrinol.* **11:** 331–337.
102. Culler, M.D. & C.S. Paschall. 1991. Pituitary adenylate cyclase-activating polypeptide (PACAP) potentiates the gonadotropin-releasing activity of luteinizing hormone-releasing hormone. *Endocrinology* **129:** 2260–2262.
103. Sawangjaroen, K. & J.D. Curlewis. 1994. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) on prolactin, luteinizing hormone and growth hormone secretion in the ewe. *J. Neuroendocrinol.* **6:** 549–555.
104. Adams, E.F. *et al*. 1994. Effect of pituitary adenylate cyclase-activating polypeptide on human somatotrophic tumours in cell culture. *Endocrine. Journal* **2:** 75–79.
105. Chiodera, P. *et al*. 1996. Effects of intravenously infused pituitary adenylate cyclase-activating polypeptide on adenohypophyseal hormone secretion in normal men. *Neuroendocrinology* **64:** 242–246.
106. Trudeau, V.L. *et al*. 1992. Interactions of estradiol with gonadotropin-releasing hormone and thyrotropin-releasing hormone in the control of growth hormone secretion in the goldfish. *Neuroendocrinology* **56:** 483–490.
107. Rousseau, K. *et al*. 1999. Evidence that corticotropin-releasing hormone acts as a growth hormone-releasing factor in a primitive teleost, the

European eel (Anguilla anguilla). *J. Neuroendocrinol.* **11:** 385–392.
108. Gracia-Navarro, F. *et al*. 1991. Subcellular responsiveness of amphibian growth hormone cells after TSH-releasing hormone stimulation. *Gen. Comp. Endocrinol.* **84:** 94–103.
109. Kuhn, E.R. *et al*. 2005. The release of growth hormone (GH): relation to the thyrotropic- and corticotropic axis in the chicken. *Domest. Anim. Endocrinol.* **29:** 43–51.
110. Ahene, C.A. *et al*. 1991. Thyrotrophin-releasing hormone-induced growth hormone (GH) secretion in anaesthetized chickens: inhibition by GH-releasing factor at central sites. *J. Endocrinol.* **128:** 13–19.
111. Harvey, S. *et al*. 1990. Central action of thyrotrophin-releasing hormone on growth hormone secretion in domestic fowl. *J. Endocrinol.* **126:** 83–88.
112. Lea, R.W. *et al*. 1990. Short-loop inhibition of thyrotrophin-releasing hormone-induced growth hormone secretion in fowl. *J. Endocrinol.* **126:** 237–244.
113. Lea, R.W. & S. Harvey. 1990. Feedback effects of growth hormone on anterior pituitary function in chickens. *J. Endocrinol.* **125:** 409–415.
114. Convey, E.M. *et al*. 1973. Bovine prolactin, growth hormone, thyroxine and corticoid response to thyrotropin releasing hormone. *Endocrinology* **92:** 471–476.
115. Blanchard, M.M. *et al*. 1987. Effects of hypothalamic hormones (GRF, TRH, somatostatin) and insulin-like growth factor I on growth hormone secretion from prepubertal male lamb pituitary cultures. *Reprod. Nutr. Dev.* **27:** 471–480.
116. Fletcher, T.P. & I.J. Clarke. 1994. Effects of hypothyroidism on the growth hormone axis in sheep. *J. Endocrinol.* **140:** 495–502.
117. Okinaga, H. *et al*. 2005. Mechanisms of TRH-induced GH release (paradoxical response) in human somatotroph adenoma cells. *Endocr. J.* **52:** 763–767.
118. Giustina, A. *et al*. 1995. Effect of glucocorticoids on the paradoxical growth hormone response to thyrotropin-releasing hormone in patients with acromegaly. *Metabolism* **44:** 379–383.
119. Giustina, A. *et al*. 1997. Inhibitory effects of galanin on growth hormone (GH) release in cultured GH-secreting adenoma cells: comparative study with octreotide, GH-releasing hormone, and thyrotropin-releasing hormone. *Metabolism* **46:** 425–430.
120. Somjen, D. *et al*. 1999. TRH is a tonic secretagogue in growth hormone secreting but not in non-functioning pituitary tumors. *J. Endocrinol. Invest.* **22:** 104–108.
121. Klausen, C. *et al*. 2001. The effect of gonadotropin-releasing hormone on growth hormone and gonadotropin subunit gene expression in the pituitary of goldfish, Carassius auratus. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **129:** 511–516.
122. Marchant, T.A. *et al*. 1989. Evidence that gonadotropin-releasing hormone also functions as a growth hormone-releasing factor in the goldfish. *Endocrinology* **124:** 2509–2518.
123. Li, W.S. *et al*. 2002. Effects of gonadotropin-releasing hormone on growth hormone secretion and gene expression in common carp pituitary. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **132:** 335–41.
124. Lin, X.W. *et al*. 1993. Growth hormone and gonadotropin secretion in the common carp (Cyprinus carpio L.): in vitro interactions of gonadotropin-releasing hormone, somatostatin, and the dopamine agonist apomorphine. *Gen. Comp. Endocrinol.* **89:** 62–71.
125. Melamed, P. *et al*. 1996. Differential effects of gonadotropin-releasing hormone, dopamine and somatostatin and their second messengers on the mRNA levels of gonadotropin II beta subunit and growth hormone in the teleost fish, tilapia. *Neuroendocrinology* **64:** 320–328.
126. Lescroart, O. *et al*. 1996. A radioimmunoassay for African catfish growth hormone: validation and effects of substances modulating the release of growth hormone. *Gen. Comp. Endocrinol.* **104:** 147–155.
127. Gutierrez-Pascual, E. *et al*. 2007. Direct pituitary effects of kisspeptin: activation of gonadotrophs and somatotrophs and stimulation of luteinising hormone and growth hormone secretion. *J. Neuroendocrinol.* **19:** 521–530.
128. Kadokawa, H. *et al*. 2008. Kisspeptin-10 stimulates the secretion of growth hormone and prolactin directly from cultured bovine anterior pituitary cells. *Anim. Reprod. Sci.* **105:** 404–408.
129. Whitlock, B.K. *et al*. 2008. Interaction of estrogen and progesterone on kisspeptin-10-stimulated luteinizing hormone and growth hormone in ovariectomized cows. *Neuroendocrinology* **88:** 212–215.
130. Peng, C. *et al*. 1993. Neuropeptide-Y stimulates growth hormone and gonadotropin-II secretion in the goldfish pituitary: involvement of both presynaptic and pituitary cell actions. *Endocrinology* **132:** 1820–1829.
131. Peng, C. *et al*. 1990. Neuropeptide Y stimulates growth hormone and gonadotropin release from the goldfish pituitary in vitro. *Neuroendocrinology* **52:** 28–34.
132. Peng, C. *et al*. 1993. Actions of goldfish neuropeptide Y on the secretion of growth hormone and

gonadotropin-II in female goldfish. *Gen. Comp. Endocrinol.* **90:** 306–317.
133. Barb, C.R. *et al*. 2005. Leptin: a metabolic signal affecting central regulation of reproduction in the pig. *Domest. Anim. Endocrinol.* **29:** 186–192.
134. Garcia, M.R. *et al*. 2004. Leptin attenuates the acute effects of centrally administered neuropeptide Y on somatotropin but not gonadotropin secretion in ovariectomized cows. *Domest. Anim. Endocrinol.* **26:** 189–200.
135. Morrison, C.D. *et al*. 2003. Luteinizing hormone and growth hormone secretion in ewes infused intracerebroventricularly with neuropeptide Y. *Domest. Anim. Endocrinol.* **24:** 69–80.
136. McMahon, C.D. *et al*. 1999. Neuropeptide Y restores appetite and alters concentrations of GH after central administration to endotoxic sheep. *J. Endocrinol.* **161:** 333–339.
137. Thomas, M.G. *et al*. 1999. Injection of neuropeptide Y into the third cerebroventricle differentially influences pituitary secretion of luteinizing hormone and growth hormone in ovariectomized cows. *Domest. Anim. Endocrinol.* **16:** 159–169.
138. Rettori, V. *et al*. 1990. Physiological role of neuropeptide Y (NPY) in control of anterior pituitary hormone release in the rat. *Endocrinol. Exp.* **24:** 37–45.
139. Suzuki, N. *et al*. 1996. Inhibitory effect of neuropeptide Y on growth hormone secretion in rats is mediated by both Y1- and Y2-receptor subtypes and abolished after anterolateral deafferentation of the medial basal hypothalamus. *Regul. Pept.* **65:** 145–151.
140. Baranowska, B. *et al*. 1999. Effects of neuropeptide Y (NPY), galanin and vasoactive intestinal peptide (VIP) on pituitary hormone release and on ovarian steroidogenesis. *Neuro. Endocrinol. Lett.* **20:** 385–389.
141. Lovejoy, D.A. & R.J. Balment. 1999. Evolution and physiology of the corticotropin-releasing factor (CRF) family of neuropeptides in vertebrates. *Gen. Comp. Endocrinol.* **115:** 1–22.
142. Nishida, S. *et al*. 1988. Provocation of a paradoxical growth hormone response to corticotropin-releasing hormone by pretreatment with metoclopramide in patients with acromegaly and normal subjects. *Endocrinol. Jpn.* **35:** 783–789.
143. Utsumi, A. *et al*. 1992. Plasma growth hormone (GH) responses to corticotropin-releasing hormone in patients with acromegaly—the effect of dexamethasone pretreatment and the comparison with GH responses to thyrotropin-releasing hormone, gonadotropin-releasing hormone and GH-releasing hormone. *J. Endocrinol. Invest.* **15:** 167–171.
144. Watanobe, H. *et al*. 1991. Failure to confirm a growth hormone-releasing activity of corticotropin-releasing hormone in acromegaly: comparison with the effects of other hypothalamic hormones. *Acta. Endocrinol. (Copenh).* **125:** 487–490.
145. Fraser, R.A. & S. Harvey. 1992. Ubiquitous distribution of growth hormone receptors and/or binding proteins in adenohypophyseal tissue. *Endocrinology* **130:** 3593–3600.
146. Rosenthal, S.M. *et al*. 1991. Exogenous growth hormone inhibits bovine but not murine pituitary growth hormone secretion in vitro: evidence for a direct feedback of growth hormone on the pituitary. *Neuroendocrinology* **53:** 597–600.
147. Asa, S.L. *et al*. 2000. Evidence for growth hormone (GH) autoregulation in pituitary somatotrophs in GH antagonist-transgenic mice and GH receptor-deficient mice. *Am. J. Pathol.* **156:** 1009–1015.
148. Richman, R.A. *et al*. 1981. Regulation of growth hormone release: evidence against negative feedback in rat pituitary cells. *Endocrinology* **108:** 2287–2292.
149. Kraicer, J. *et al*. 1988. Failure of growth hormone (GH) to feed back at the level of the pituitary to alter the response of the somatotrophs to GH-releasing factor. *Endocrinology* **122:** 1511–1514.
150. Hashimoto, Y. *et al*. 2000. Exogenous 20K growth hormone (GH) suppresses endogenous 22K GH secretion in normal men. *J. Clin. Endocrinol. Metab.* **85:** 601–606.
151. Beckers, A. *et al*. 1990. Placental and pituitary growth hormone secretion during pregnancy in acromegalic women. *J. Clin. Endocrinol. Metab.* **71:** 725–731.
152. Daughaday, W.H. 2000. Growth hormone axis overview–somatomedin hypothesis. *Pediatr. Nephrol.* **14:** 537–540.
153. Le Roith, D. *et al*. 2001. The somatomedin hypothesis: 2001. *Endocr. Rev.* **22:** 53–74.
154. Le Roith, D. *et al*. 2001. What is the role of circulating IGF-I? *Trends Endocrinol. Metab.* **12:** 48–52.
155. Rousseau, K. *et al*. 1998. Long-term inhibitory effects of somatostatin and insulin-like growth factor 1 on growth hormone release by serum-free primary culture of pituitary cells from European eel (Anguilla anguilla). *Neuroendocrinology* **67:** 301–309.
156. Duval, H. *et al*. 2002. Cloning, characterization, and comparative activity of turbot IGF-I and IGF-II. *Gen. Comp. Endocrinol.* **126:** 269–278.
157. Fruchtman, S. *et al*. 2002. Characterization of pituitary IGF-I receptors: modulation of prolactin and growth hormone. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283:** R468–R476.
158. Buonomo, F.C. *et al*. 1987. Effects of insulin-like growth factor I (IGF-I) on growth hormone-releasing factor (GRF) and thyrotropin-releasing hormone (TRH) stimulation of growth hormone

(GH) secretion in the domestic fowl (Gallus domesticus). *Gen. Comp. Endocrinol.* **66:** 274–279.
159. Morita, S. *et al*. 1987. Insulin-like growth factor I action on rat anterior pituitary cells: effects of intracellular messengers on growth hormone secretion and messenger ribonucleic acid levels. *Endocrinology* **121:** 2000–2006.
160. Yamashita, S. & S. Melmed. 1987. Insulinlike growth factor I regulation of growth hormone gene transcription in primary rat pituitary cells. *J. Clin. Invest.* **79:** 449–452.
161. Fletcher, T.P. *et al*. 1995. IGF feedback effects on growth hormone secretion in ewes: evidence for action at the pituitary but not the hypothalamic level. *J. Endocrinol.* **144:** 323–331.
162. Luque, R.M. *et al*. 2006. Examination of the direct effects of metabolic factors on somatotrope function in a non-human primate model, Papio anubis. *J. Mol. Endocrinol.* **37:** 25–38.
163. Van Der Lely, A.J. *et al*. 2004. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr. Rev.* **25:** 426–457.
164. Kaiya, H. *et al*. 2008. Ghrelin: a multifunctional hormone in non-mammalian vertebrates. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **149:** 109–128.
165. Unniappan, S. & R.E. Peter. 2004. In vitro and in vivo effects of ghrelin on luteinizing hormone and growth hormone release in goldfish. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286:** R1093–R1101.
166. Kaiya, H. *et al*. 2003. Identification of tilapia ghrelin and its effects on growth hormone and prolactin release in the tilapia, Oreochromis mossambicus. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **135:** 421–429.
167. Riley, L.G. *et al*. 2002. Rat ghrelin stimulates growth hormone and prolactin release in the tilapia, Oreochromis mossambicus. *Zoolog Sci.* **19:** 797–800.
168. Kaiya, H. *et al*. 2003. Peptide purification, complementary deoxyribonucleic acid (DNA) and genomic DNA cloning, and functional characterization of ghrelin in rainbow trout. *Endocrinology* **144:** 5215–5226.
169. Kaiya, H. *et al*. 2005. Purification, cDNA cloning, and characterization of ghrelin in channel catfish, Ictalurus punctatus. *Gen. Comp. Endocrinol.* **143:** 201–210.
170. Kaiya, H. *et al*. 2003. Amidated fish ghrelin: purification, cDNA cloning in the Japanese eel and its biological activity. *J. Endocrinol.* **176:** 415–423.
171. Kaiya, H. *et al*. 2001. Bullfrog ghrelin is modified by n-octanoic acid at its third threonine residue. *J. Biol. Chem.* **276:** 40441–40448.
172. Kaiya, H. *et al*. 2004. Structural determination and histochemical localization of ghrelin in the red-eared slider turtle, Trachemys scripta elegans. *Gen. Comp. Endocrinol.* **138:** 50–57.
173. Baudet, M.L. & S. Harvey. 2003. Ghrelin-induced GH secretion in domestic fowl in vivo and in vitro. *J. Endocrinol.* **179:** 97–105.
174. Yamazaki, M. *et al*. 2002. Regulational effect of ghrelin on growth hormone secretion from perifused rat anterior pituitary cells. *J. Neuroendocrinol.* **14:** 156–162.
175. Malagon, M.M. *et al*. 2003. Intracellular signaling mechanisms mediating ghrelin-stimulated growth hormone release in somatotropes. *Endocrinology* **144:** 5372–5380.
176. Kineman, R.D. & R.M. Luque. 2007. Evidence that ghrelin is as potent as growth hormone (GH)-releasing hormone (GHRH) in releasing GH from primary pituitary cell cultures of a nonhuman primate (Papio anubis), acting through intracellular signaling pathways distinct from GHRH. *Endocrinology* **148:** 4440–4449.
177. Lengyel, A.M. 2006. From growth hormone-releasing peptides to ghrelin: discovery of new modulators of GH secretion. *Arq. Bras. Endocrinol. Metabol.* **50:** 17–24.
178. Dimaraki, E.V. & C.A. Jaffe. 2006. Role of endogenous ghrelin in growth hormone secretion, appetite regulation and metabolism. *Rev. Endocr. Metab. Disord.* **7:** 237–249.
179. Zhang, Y. *et al*. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* **372:** 425–432.
180. Huising, M.O. *et al*. 2006. Increased leptin expression in common Carp (Cyprinus carpio) after food intake but not after fasting or feeding to satiation. *Endocrinology* **147:** 5786–5797.
181. Kurokawa, T. *et al*. 2005. Identification of cDNA coding for a homologue to mammalian leptin from pufferfish, Takifugu rubripes. *Peptides* **26:** 745–750.
182. Muruzabal, F.J. *et al*. 2002. Immunocytochemical detection of leptin in non-mammalian vertebrate stomach. *Gen. Comp. Endocrinol.* **128:** 149–152.
183. Paolucci, M. *et al*. 2001. Leptin presence in plasma, liver and fat bodies in the lizard Podarcis sicula: fluctuations throughout the reproductive cycle. *Life Sci.* **69:** 2399–2408.
184. Couce, M.E. *et al*. 1997. Localization of leptin receptor in the human brain. *Neuroendocrinology* **66:** 145–150.
185. Dyer, C.J. *et al*. 1997. Leptin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed-restricted ewes. *Domest. Anim. Endocrinol.* **14:** 119–128.
186. Malik, K.F. & W.S. Young, 3rd. 1996. Localization of binding sites in the central nervous system for

leptin (OB protein) in normal, obese (ob/ob), and diabetic (db/db) C57BL/6J mice. *Endocrinology* **137:** 1497–1500.
187. Morash, B. *et al*. 1999. Leptin gene expression in the brain and pituitary gland. *Endocrinology* **140:** 5995–5998.
188. Carro, E. *et al*. 1997. Regulation of in vivo growth hormone secretion by leptin. *Endocrinology* **138:** 2203–2206.
189. Zieba, D.A. *et al*. 2003. Effects of leptin on basal and GHRH-stimulated GH secretion from the bovine adenohypophysis are dependent upon nutritional status. *J. Endocrinol.* **178:** 83–89.
190. Baratta, M. *et al*. 2002. Leptin regulates GH gene expression and secretion and nitric oxide production in pig pituitary cells. *Endocrinology* **143:** 551–557.
191. Luque, R.M. *et al*. 2007. Effects of leptin replacement on hypothalamic-pituitary growth hormone axis function and circulating ghrelin levels in ob/ob mice. *Am. J. Physiol. Endocrinol. Metab.* **292:** E891–E899.
192. Ramsay, T.G. *et al*. 2004. Peripheral leptin administration alters hormone and metabolite levels in the young pig. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **138:** 17–25.
193. Saleri, R. *et al*. 2004. Leptin stimulates growth hormone secretion via a direct pituitary effect combined with a decreased somatostatin tone in a median eminence-pituitary perifusion study. *Neuroendocrinology* **79:** 221–228.
194. Ghizzoni, L. *et al*. 2001. Leptin, cortisol, and GH secretion interactions in short normal prepubertal children. *J. Clin. Endocrinol. Metab.* **86:** 3729–3734.
195. Giusti, M. *et al*. 2002. In vitro effect of human recombinant leptin and expression of leptin receptors on growth hormone-secreting human pituitary adenomas. *Clin. Endocrinol. (Oxf.)* **57:** 449–455.
196. Butler, A.A. & D. Le Roith. 2001. Control of growth by the somatropic axis: growth hormone and the insulin-like growth factors have related and independent roles. *Annu. Rev. Physiol.* **63:** 141–164.
197. Robinson, I.C.A.F. & P.C. Hindmarsh. 1999. Feedback regulation of growth hormone secretion. In *Handbook of Physiology. Hormonal Control of Growth*, Vol. V. J.L. Kostyo & H.M. Goodman, Eds.: 329–395. Oxford University Press. New York.
198. Reddy, P.K. & Leatherland, J.F. 1995. Influence of combination of time of feeding and ration levels on the diurnal hormone rhythms in rainbow trout. *Fish Physiol. Biochem.* **14:** 25–36.
199. Rousseau, K. & S. Dufour. 2008. Endocrinology of migratory fish life cycle in special environments: the role of metamorphoses. In *Fish Life in Special Environments*. P. Sébert, D.W. Onyango & B.G. Kapoor, Eds.: 193–231. Science Publishers. Enfield, NH.
200. Castano, J.P. *et al*. 1996. Somatostatin increases growth hormone (GH) secretion in a subpopulation of porcine somatotropes: evidence for functional and morphological heterogeneity among porcine GH-producing cells. *Endocrinology* **137:** 129–136.
201. Ramirez, J.L. *et al*. 1998. Somatostatin at low doses stimulates growth hormone release from intact cultures of porcine pituitary cells. *Horm. Metab. Res.* **30:** 175–177.
202. Ramirez, J.L. *et al*. 1997. Somatostatin plays a dual, stimulatory/inhibitory role in the control of growth hormone secretion by two somatotrope subpopulations from porcine pituitary. *J. Neuroendocrinol.* **9:** 841–848.
203. Luque, R.M. *et al*. 2005. Differential contribution of nitric oxide and cGMP to the stimulatory effects of growth hormone-releasing hormone and low-concentration somatostatin on growth hormone release from somatotrophs. *J. Neuroendocrinol.* **17:** 577–582.
204. Ramirez, J.L. *et al*. 2002. Somatostatin stimulates GH secretion in two porcine somatotrope subpopulations through a cAMP-dependent pathway. *Endocrinology* **143:** 889–897.
205. Ramirez, J.L. *et al*. 1998. Differences in second messengers (Ca2+ and cAMP) suggest a dual role for SRIF in regulating GH release from porcine somatotropes. *Ann. N. Y. Acad. Sci.* **839:** 375–377.
206. Luque, R.M. *et al*. 2004. Homologous and heterologous in vitro regulation of pig pituitary somatostatin receptor subtypes, sst1, sst2 and sst5 mRNA. *J. Mol. Endocrinol.* **32:** 437–448.
207. Luque, R.M. *et al*. 2006. Identification of the somatostatin receptor subtypes (sst) mediating the divergent, stimulatory/inhibitory actions of somatostatin on growth hormone secretion. *Endocrinology* **147:** 2902–2908.
208. Chen, Z.P. *et al*. 1997. Intracellular calcium ion responses to somatostatin in cells from human somatotroph adenomas. *Clin. Endocrinol. (Oxf.)* **46:** 45–53.