Endocrine, *vol. 14, no. 1, 35–43, February 2001* 0969–711X/01/14:35–43/$12.25

Growth Hormone–Releasing Hormone and Growth Hormone Secretagogue-Receptor Ligands

Focus on Reproductive System

Emanuela Arvat,[1] Laura Gianotti,[1] Roberta Giordano,[1] Fabio Broglio,[1] Mauro Maccario,[1] Fabio Lanfranco,[1] Giampiero Muccioli,[2] Mauro Papotti,[3] Andrea Graziani,[4] Ezio Ghigo,[1] and Romano Deghenghi[5]

Division of Endocrinology, Departments of [1]Internal Medicine, [2]Pharmacology, [3]Biomedical Sciences [4]Genetics and Biochemistry, University of Turin, Italy; and [5]Europeptide, Argenteuil, France.

Growth hormone–releasing hormone (GHRH) and somatostatin are the most important hypothalamic neurohormones controlling growth hormone (GH) secretion. Several neurotransmitters and neuropeptides also play an important role in the control of GH secretion, mainly acting via modulation of GHRH and somatostatin. In the past two decades, particular attention has been given to a new family of substances showing a strong GH-releasing effect: GH secretagogues (GHSs). GHSs increase GH secretion in a dose- and age-related manner after iv and even oral administration. The endocrine effects of GHSs, are not fully specific for GH; they show, in fact, prolactin- (PRL), adenocorticotropic hormone- and cortisol-releasing effects. Specific GHS receptors are present in both the central nervous system and peripheral tissues, where they mediate several extraendocrine effects of GHSs. The isolation of these "orphan" receptors suggested the existence of an endogenous GHS-like ligand that could be represented by a recently discovered gastric peptide, named ghrelin. The interaction between GHSs and GHRH at the central level and in the pituitary gland, but not at peripheral level, has clearly been shown. Because GHRH and GHS receptors share the same localization in some peripheral tissues, they may have some interactions even at this level.

Key Words: Growth hormone-releasing hormone; growth hormone secretagogues; prolactin; adenocorticotropic hormone; cortisol; central nervous system.

Author to whom all correspondence and reprint requests should be addressed: Ezio Ghigo, Division of Endocrinology, Department of Internal Medicine, University of Turin, Corso A.M. Dogliotti, 14-10126 Torino, Italy. E-mail: ezio.ghigho@unito.it

Introduction

Spontaneous growth hormone (GH) release is mainly regulated by the tight interplay between hypothalamic growth hormone–releasing hormone (GHRH), which stimulates both GH synthesis and release, and somatostatin, which inhibits somatotroph release and is aimed at modulating somatotroph function *(1–4)*. A central role of GHRH in the control of GH secretion, as shown by experimental models using passive immunization against GHRH, GHRH antagonists, and transgenic animal models, has clearly been shown *(1,2,5)*.

GHRH immunoreactivity has also been detected in several animal and human brain areas, such as the cortex, hippocampus, and amygdala, according to the evidence that GHRH also possesses extraendocrine central effects *(1,6)*. Moreover, GHRH has been identified in several extraneural tissues, including the gastrointestinal tract, kidney, lung, adrenal gland, heart, ovary, and testis *(7–12)*, which are likely to mediate the peripheral endocrine and nonendocrine tissue-specific GHRH activity *(13–17)*.

About 20 yr ago, a new class of synthetic, non-natural, small peptidyl molecules, denominated growth hormone–releasing peptides (GHRPs), was shown to possess a marked and dose-dependent GH-releasing effect *(18–22)*. Their stimulatory effect on somatotroph release was higher than that of GHRH, and a true synergistic effect between GHRPs and GHRH on GH secretion has clearly been reported *(18,20–22)*. Now, this class of molecules includes both peptidyl and nonpeptidyl analogs and is denominated the growth hormone secretagogue (GHS) family *(18–22)*. The activity of GHSs is not fully specific for GH; in fact, they also stimulate prolactin (PRL), adrenocorticotropic hormone (ACTH), and cortisol secretion in both animals and humans, at least after acute administration *(21,23)*. Moreover, GHSs also show both central and peripheral extrahormonal activities: they have been reported to influence food intake and sleep *(24–26)*, and evidence is

increasing about direct effects of GHSs on cardiac function *(27–29)*. Furthermore, recently, it has been shown that GHSs possess a direct antiapoptotic effect in cardiomyocytes and antimitogenic effects in some endocrine tumors *(30,31)*.

The endocrine activities of GHSs are mediated by specific receptors, located at both the pituitary and hypothalamic levels; however, specific GHS receptors (GHS-Rs) have also been identified in other central areas and in several peripheral tissues, where they are likely to mediate the extraendocrine actions of GHSs *(31–34)*. Recently, a gastric peptide named ghrelin has been isolated in both rats and humans, characterized, and proposed as the natural ligand of the "orphan" GHS-Rs *(35)*.

Based on these premises, the aim of this chapter is to focus on the endocrine, nonendocrine, central, and peripheral activities of GHRH and GHS-R ligands, with particular attention to human studies on the reproductive system.

Growth Hormone–Releasing Hormone

Central Localization and Neuroendocrine Effects

Mediobasal hypothalamus is the main site of GHRH immunoreactivity, in particular the ventromedial and arcuate nuclei *(36,37)*. Nerve fibers originating in these nuclei mainly project to the median eminence and terminate on the capillaries of the primary plexus of the hypothalamic-pituitary portal vascular system *(38–40)*. GHRH mRNA is expressed from a single GHRH gene, early during ontogeny *(41,42)*.

It is well known that hypothalamic GHRH has a crucial role in the neuroregulation of pituitary GH synthesis and secretion, while somatostatin has a time-dependent modulatory influence on somatotroph function *(1)*. In fact, somatostatin has an early inhibitory and a late stimulatory effect on GH secretion, which, in turn, induces hypothalamic somatostatin synthesis and release *(43–48)*. A tight interplay between GHRH and somatostatin is needed to generate pulsatile GH secretion in both animals and humans. GH pulses reflect GHRH pulses occurring at times of nadir of somatostatin release, while "through periods" are concomitant with high circulating somatostatin levels *(46,48)*. Time-dependent variations in endogenous somatostatin levels, which modulate the GHRH-induced GH rise, seem to account for the marked intrasubject variability in the GH response to GHRH recorded in both men and women *(49)*. It has been demonstrated that the stimulatory effect of GHRH on GH release is mediated by the activation of specific receptors on somatotroph cells and an increase in intracellular cyclic adenosine monophosphate *(1)*, whereas the opposite effect is exerted by somatostatin, which also binds to specific receptors on somatotroph cells *(1)*.

GHRH secretion and activity have been shown to vary during the life span of both animals and humans. GHRH shows its maximally GH-releasing effect in newborns, and the GH response to the neuropeptide is similar, although lower than in newborns, in children and adults, then decreases thereafter, and is very low in aging *(50,51)*. An age-related reduction in hypothalamic GHRH expression and release as well as impairment of pituitary GHRH receptor and postreceptor mechanisms have been demonstrated *(52–55)*. On the other hand, the existence of a concomitant hypothalamic somatostatin hyperactivity seems to have a main role in the reduced GH-releasing effect of GHRH in aging *(51,54)*.

Regulation of GHRH synthesis and activity is modulated by central and peripheral factors. A short-loop autofeedback mechanism mediated by GH and somatostatin has been clearly demonstrated. In fact, GHRH has been found able to trigger the increase in hypothalamic somatostatin release, which, in turn, inhibits subsequent GHRH release from the hypothalamus, while GH inhibits and increases, respectively, hypothalamic GHRH and somatostatin synthesis and secretion *(1,56)*. Accordingly, in humans a GHRH-stimulated rise in GH induces a refractoriness to the GH response to a consecutive GHRH stimulus, which is not owing to GHRH receptor desensitization, while it is counteracted by pretreatment with substances inhibiting somatostatin release *(2,4)*. Also the long-loop negative GH autofeedback, mediated by insulin-like growth factor-1 (IGF-1), has been reported to involve hypothalamic GHRH. In fact, an inhibiting effect of IGF-1 on both GHRH expression and release has been shown, associated with the well-known stimulating effect on somatostatin release *(57,58)*.

Among the neurotransmitters and neuropeptides controlling GH secretion, catecholamines, through $\alpha 2$ receptors, galanin and opioids stimulate GH release by increasing GHRH secretion *(2,4)*. In addition, GHSs have been found to stimulate the activity of hypothalamic GHRH-secreting neurons, probably antagonizing the inhibitory effect of somatostatin at this level *(21)*. Among peripheral hormones, gonadal steroids have been shown to play an important role in the modulation of GH release, partially through GHRH-mediated mechanisms *(59–62)*. Spontaneous GH release has been found higher in young women than in age-matched men, positively related to estrogen levels, and a greater GH response to GHRH has been observed by some researchers *(63)* but not by others *(64,65)*. Moreover, thyroid hormones and adrenal steroids influence GH release through mechanisms involving the modulation of the activity of GHRH-secreting neurons *(3,62)*.

In addition to the hypothalamic localization, GHRH-like immunoreactivity has been detected in several other central areas, such as the cortex, hippocampus and amygdala, and extraendocrine central effects of GHRH have been shown *(1)*. GHRH increases food intake and influences sleep pattern in animals *(66)*. Similarly, in humans slow-wave sleep and rapid eye movement (REM) sleep significantly increased whereas time awake decreased after the administration of pulsatile GHRH *(25)*. As GH and GHRH have different effects on some sleep param-

eters, according to the existence of central extrahypothalamic receptors, a direct effect of GHRH on sleep has been suggested.

Peripheral Localization and Activity

It has been demonstrated that mRNA and specific immunoreactivity for GHRH were also present in several peripheral tissues in both animals and humans, such as the pancreas, duodenum, lung, heart, vascular system, adrenal gland, kidney, testis, ovary, and placenta *(6–12)*. The evidence of the evolutionary conservation of this organ-specific expression suggested that the GHRH-like peptide in peripheral tissues might be biologically important *(67)*. Among the various peripheral tissues, testis and ovary have been demonstrated as major sites of GHRH production, binding, and biologic activity.

Testis

Immunocytochemical studies in animals and humans demonstrated the presence of GHRH-like immunoreactivity in early spermatogenic cells as well Leydig cells, but neither in mature sperm nor Sertoli cells *(6,11,15,16,68–70)*. A GHRH-like peptide similar to the hypothalamic neuropeptide as well as a larger but equally active molecular form of GHRH have been detected in the testis *(11,68)*. Recently, high concentrations of the carboxyl-terminal peptide GHRH (GHRH-related peptide) has been identified in rat germ cells, with specific intratesticular actions *(71)*.

GHRH gene expression has been detected in all testicular cells positive for GHRH immunoreactivity *(11,67,69–72)*. The major testicular GHRH-like mRNA is larger than that in the hypothalamus, suggesting a tissue-specific alternative transcription or splicing of the GHRH gene *(11,67,69,70,72)*. The hypothesis of a specific role of local GHRH in testicular function was supported by the evidence that its mRNA expression is developmentally regulated. In fact, testicular GHRH mRNA is not expressed in rat fetal life: it becomes evident at birth, then increases at puberty, and reaches maximal concentrations in adult life *(67)*. The control of GHRH gene expression seems to be differentially regulated in different tissues; it has been supposed that gonadotropins, which are known to stimulate, at the testicular level, synthesis and release of other neuropeptides, such as corticotropin-releasing hormone (CRH), vasopressin, and β-endorphin, can also be mainly involved in the control of testicular GHRH *(15)*.

A specific activity of GHRH on male gonadal function has clearly been demonstrated. Considerable amounts of GHRH are secreted by Leydig cells under positive luteinizing hormone (LH) control *(15,16)*. Moreover, GHRH seems to act as a direct stimulator of Leydig cell function and facilitates LH/human chorionic gonadotropin-induced steroidogenesis *(15,16,71,73)*. GHRH has also been shown to increase both basal and follicle-stimulating hormone (FSH)-stimulated Sertoli cell functions *(15,16,71,73)*. All these effects are mediated by vasoactive intestinal polypeptide (VIP)/GHRH receptors present on the membranes of both Leydig and Sertoli cells *(15)*. All this evidence strongly suggests that local GHRH may be an important autocrine/paracrine agonist and synergistic factor potentiating the gonadotropin-induced hormonal and spermatogenetic function. Accordingly, it has been reported that treatment with exogenous GHRH improved sperm parameters in oligozoospermic patients *(74)*, through direct local action of GHRH and/or activation of the GH/IGF-1 axis *(15,75)*.

The interaction between GHRH and somatostatin to modulate the male reproduction function has been shown. Both somatostatin-14 (SS-14) and SS-28 immunoreactivity have been found in the testis, prostate, and semen *(76)*. Both stimulatory and inhibitory effects of somatostatin on testicular steroidogenesis have been shown in animals *(77,78)*, and the acute injection of a long-acting somatostatin analog has been demonstrated to induce a significant increase in testosterone levels in adult men, without affecting LH or FSH secretion *(79)*.

Ovary

Similar to that observed in the testis, ovaries are a site of GHRH synthesis, release, and action in both animals and humans. GHRH immunoreactivity has been detected in corpora lutea, granulosa cells, and follicular fluid *(7,13, 14,17)*. As in the testis, ovarian extracts contain both hypothalamic-like and larger GHRH molecules, which probably represent the GHRH precursor *(7,13,14)*.

GHRH gene is expressed in the ovary: GHRH mRNA at this level is larger than that detected in the hypothalamus, but similar to that in the testis, suggesting also in the ovary an alternative transcription initiation and splicing of the GHRH gene *(7,13,14)*.

Evidence that cultured rat granulosa cells secrete a GHRH-like substance first suggested a role of this peptide in the mediation of intercellular ovarian information and a direct effect on ovarian function *(17)*. It has been demonstrated that GHRH potentiates FSH-induced follicular maturation and amplifies, in the luteinizing granulosa cells, FSH-stimulated steroidogenesis and LH receptor expression *(13,17)*. These effects are mediated by VIP/GHRH receptors located on the membranes of the granulosa cells *(80,81)*. Because the expression of GHRH receptors in granulosa cells is increased by FSH, a positive autoregulatory action of GHRH on FSH-induced follicular maturation has been suggested *(81)*. In addition, somatostatin was detected in the rat granulosa ovarian cells *(82)* and a modulatory role, both inhibitory and stimulatory, on FSH-induced steroidogenesis has been indicated *(83–85)*.

Based on evidence of an in vitro positive effect of GHRH on ovarian function, some clinical studies have suggested the efficacy of the combined administration of exogenous GHRH and gonadotropin in the treatment of female infertility *(17)*.

GHS-R Ligands

The first GHSs were synthesized in 1977 *(86)*, well before the isolation and characterization of GHRH, in 1982 *(1)*; they were synthetic and nonnatural peptidyl molecules with a strong GH-releasing effect.

During the last 20 yr, many peptidyl and nonpeptidyl GHs have been developed *(18–22)*. In addition to GHRP-6, the first GHRP showing a strong GH-stimulatory activity after iv, sc, intranasal, and even oral administration, the GHSs most studied in humans include peptidyl analogs, such as GHRP-1, a heptapeptide; GHRP-2 and hexarelin, two hexapeptides; Tyr-ala-hexarelin, an octapeptide; ipamorelin, a pentapeptide; some tetra- and pseudo-tripeptides; as well as nonpeptidyl GHRP mimetics, such as MK-677, a spiroindoline that showed marked bioavailability and a long-lasting effect after oral administration *(18–22, 87,88)*.

The activity of GHSs is not fully specific for GH. In fact, they also possess PRL- and ACTH/cortisol-releasing effects, at least after acute administration *(21,23)*. Moreover, GHSs have central and peripheral extraendocrine effects: they stimulate food intake, influence sleep pattern, exert cardiotropic effects, and even influence apoptosis and tumorigenesis, independently of the endocrine activity *(24–31)*.

The effects of GHSs are mediated by specific receptors subtypes that are mainly present at the pituitary and hypothalamic levels, but also in other central areas and in peripheral tissues, suggesting the existence of a natural GHS-like ligand *(31–34)*.

In this regard, an endogenous ligand specific for the "orphan" GHS-Rs, named ghrelin, has recently been isolated from rat and human stomach *(35)*. It is a 28 amino acid peptide showing a unique structure with an *n*-octanoyl modification at its third serine residue, essential for its marked GH-releasing effect *(35)*. In fact, ghrelin has been shown to strongly stimulate GH secretion in rat somatotroph cells as well as in rat in vivo *(35)*, in a dose-dependent manner *(89)*. Human ghrelin is homologous to rat ghrelin apart from two amino acids *(35)* and circulates in human blood at considerable plasma concentrations *(90)*. The ghrelin mRNA as well as the immunoreactivity for this peptide have also been found in the brain, in particular in the hypothalamic arcuate nucleus, suggesting a neuroendocrine role of this substance in the regulation of GH secretion *(35)*. On the other hand, in addition to being localized in the stomach, ghrelin transcripts have been detected in several peripheral tissues, such as the small and large intestines, pancreas, liver, kidney, heart, lung, bone, adipose tissue, and placenta *(91–94)*, suggesting actions of this peptide other than the GHS one.

Central and Peripheral Human GHS-Rs

A specific human GHS-R has been cloned. It is encoded by a rare mRNA with a predicted open reading frame of 366 amino acids with a transmembrane topography typified by the G-protein-coupled receptor family. The receptor sequence does not show significant homology with other receptors known so far, and mRNA for this receptor is expressed in the pituitary and hypothalamus *(22,32)*.

As in animals *(18)*, the human hypothalamus and pituitary gland show the highest specific GHS binding, which, otherwise, is also present in other central areas, such as the cerebral cortex, hippocampus, medulla oblongata, and choroid plexuses, but not in the cerebellum, thalamus, striatum, substantia nigra, and corpus callosum *(31,95)*.

The existence of GHS-R subtypes at both the pituitary and hypothalamic levels has been shown, which may mediate the different endocrine and extraendocrine central GHS activities *(33,96,97)*. There is evidence that human GHS-Rs are age but not gender dependent. They have been demonstrated in fetal human pituitary, according to the finding that GHSs stimulate GH release from human fetal pituitary in vitro as well as in newborns *(98,99)*. Moreover, we have recently demonstrated that GHS-R density does not vary as a function of sex in the pituitary, hypothalamus, and other areas of human brain, while advancing age significantly decreases hypothalamic GHS-R numbers *(100)*. Central and pituitary GHS-R expression is modulated by several hormonal factors; both gonadal steroids and glucocorticoids increase mRNA expression for GHS-R in the pituitary whereas GH reduces rat hypothalamic GHS-R mRNA expression *(101–103)*.

Our recent studies demonstrate that GHS-Rs are also present in peripheral tissues, such as the adrenal gland, heart, vascular system, ovary, testis, lung, and skeletal muscle, and are even more remarkable or overlapping in the pituitary and the hypothalamus *(31)*. Significant binding for peptidyl GHSs was also found in the kidney, epiphysis, and thyroid gland but not in the smooth muscle, pancreas, parotid gland, and spleen *(31)*. Note that MK-677 but not peptidyl GHSs has specific binding in the pancreas *(22,104)*. All these findings indicate the existence of different GHS-R subtypes also at the peripheral level, some specific for peptidyl and others for nonpeptidyl GHSs.

The functional significance of peripheral GHS-Rs is mostly unknown. Interestingly, there is increasing evidence showing that GHRPs have GH-independent cardiotropic activities in both animals and humans *(27–30)*. Moreover, recent data suggest a modulatory role of GHRPs on cellular apoptosis as well as on tumorigenesis *(30,105,106)*.

Endocrine Activities of GHSs in Humans

GH-Releasing Activity

The GH-releasing effect of GHSs is dose dependent after iv, sc, intranasal, and oral administration *(18–22)*. After iv injection, GHSs show good intraindividual reproducibility, differently from GHRH *(21)*.

The GH-stimulatory effect of GHSs is higher in vivo than in vitro, in humans than in animals *(18–22)*. Although

they stimulate GH secretion from pituitary somatotroph cells, data in humans and animals indicate that the most important action of GHSs takes place at the hypothalamic level *(18–22)*.

GHSs and GHRH have a synergistic effect and even a very low GHS dose has been found able to strongly potentiate a GHRH-induced GH rise, indicating that these peptides act, at least partially, via different mechanims of action, in agreement with data in animals *(18,20–22)*. Nevertheless, GHSs need GHRH activity to fully express their GH-releasing effect. In fact, in humans the GH response to GHSs is strongly inhibited by GHRH antagonists and hypothalamopituitary disconnection, as well as in patients with GHRH receptor deficiency *(107–111)*.

It has been hypothesized that GHSs could act as functional somatostatin antagonists at both the pituitary and hypothalamic levels. In agreement with this assumption, in humans the GH response to GHS is not modified by substances acting via somatostatin inhibition, which truly potentiate a GHRH-induced rise in GH *(21,112)*. Moreover, the GH responsiveness to GHSs is only slightly blunted, differently from that to GHRH, by substances acting via stimulation of hypothalamic somatostatin, acting directly on somatotroph cells, and is even partially refractory to the inhibitory effect of exogenous somatostatin *(21,112)*. Interestingly, the effect of GHSs on GH release is also partially refractory to the negative GH autofeedback *(113,114)*, while showing peculiar sensitivity to the negative IGF-1 feedback action *(115)*.

The GH-releasing effect of GHSs is generally gender independent, with the exception of the pubertal period *(100)*. On the other hand, the GH response to GHSs undergoes marked age-related variations, different from those recorded after GHRH. In fact, while the rise in GH after GHRH is maximal in newborns and then progressively decreases with aging, that after hexarelin is low at birth, strikingly increases at puberty, persists similar in adulthood and decreases thereafter, being in middle age already similar to that in elderly subjects *(96)*. However, the reduction in the GH response to GHSs alone and combined with GHRH has been found by some researchers *(55,116,117)* but not by others *(118)*.

The mechanisms underlying the age-related variations in the GH-releasing activity of GHSs are, in turn, age-related. The GH response to hexarelin in prepubertal girls and boys is similar, whereas at puberty girls release more GH in response to hexarelin than boys *(119)*. The evidence that in prepubertal children the GH response to hexarelin is clearly increased by estradiol and testosterone but not by oxandrolone, a nonaromatizable androgen *(120)*, indicates a critical role of estrogens in enhancing the somatotroph sensitivity to the activity of GHSs during puberty. On the other hand, the low GH response to hexarelin in postmenopausal women is not modified by treatment with transdermal estradiol *(121)*, suggesting that the reduced activity of GHSs in aging does not seem to depend on the decline in gonadal steroid levels.

The most important mechanism accounting for the reduced GH-releasing activity of GHSs in aging is probably represented by the age-related hypoactivity of GHRH- and hyperactivity of somatostatin-secreting neurons, respectively *(51)*. In fact, the reduced somatotroph responsiveness to hexarelin alone as well as combined with GHRH is fully restored by arginine, which probably acts via inhibition of hypothalamic somatostatin release *(55,116)*. Finally, the existence of an impaired secretion and activity of the endogenous GHS-like ligand in aging cannot be ruled out *(55)*.

PRL-Releasing Activity

The stimulatory effect of GHSs on PRL secretion in humans is slight and dose dependent, remaining within the normal range of basal levels, and markedly lower than that recorded after the administration of thyrotropin-releasing hormone, metoclopramide, or arginine *(20,21)*. The lactotroph responsiveness to GHSs is not dependent on gender and age *(100)*, in contrast to what is observed for the somatotroph responsiveness (*see* before). The mechanism underlying the PRL-releasing activity of GHSs does not seem to be mediated by opioidergic, serotoninergic, and histaminergic pathways, which mainly regulate PRL release, but it may be mediated by direct stimulation of somatomammotroph cells *(20,21)*.

ACTH- and Cortisol-Releasing Activity

A stimulatory effect of GHSs on the activity of the hypothalamo-pituitary-adrenal (HPA) axis in humans has been shown. In fact, GHSs possess ACTH- and cortisol-releasing effects similar to that of $(Arg)^8$vasopressin or naloxone and are even similar to that of CRH *(122,123)*. The effect of GHSs seems, however, to be an acute neuroendocrine effect, being lost during prolonged treatment *(117)*.

The ACTH responsiveness to GHSs is not dependent on gender but shows peculiar age-related variations. It is present in childhood, significantly increases at puberty, then shows a reduction in adulthood, and, again, a trend toward increase in aging *(124)*. The age-related modulatory influence of GHSs on ACTH release is different from that of GH, suggesting that GHSs are likely to act at different levels and on different receptor subtypes. The increased effect at puberty could depend on estrogens and the rebounded effect in aging agrees with evidence showing HPA hyperactivity owing to neuroendocrine changes in the aging brain *(125)*.

The stimulatory effect of GHSs on cortisol secretion is owing to their ACTH-releasing activity, which, in turn, mainly depends on CNS-mediated mechanisms, at least under physiologic conditions. In fact, the stimulatory effect of GHSs on the HPA axis is abolished by hypothalamo-pituitary disconnection, and GHSs do not stimulate ACTH release from both rat and human normal pituitary *(109,126)*.

In humans, the ACTH-releasing activity of GHS seems, at least partially, independent of CRH or AVP, although recent data indicate an AVP-mediated action of GHSs *(123,127)*. Neuropeptide Y– and γ-aminobutyric acid–ergic pathways are likely to be involved in the mediation of GHS-induced ACTH release, but there is evidence that neither serotoninergic nor histaminergic pathways are involved in it *(21,128,129)*.

The ACTH responsiveness to GHSs is generally sensitive to the negative cortisol feedback mechanism under physiologic conditions, being inhibited by dexamethasone and enhanced by metyrapone *(129,130)*. In agreement with these data, the ACTH-releasing effect of hexarelin is absent in patients with adrenal cortisol-secreting adenoma *(131)*, whereas it is enhanced in patients with Addison disease *(130)*. Interestingly, in patients with Cushing syndrome, owing to pituitary ACTH-secreting adenoma, the ACTH responsiveness to hexarelin is exaggerated and clearly higher that that to hCRH, in spite of their hypercortisolism *(131)*. Because specific GHS-Rs in human pituitary and ectopic ACTH-secreting tumors have been demonstrated, a direct action of GHSs on ACTH release in patients with Cushing disease has been suggested *(95,132,133)*.

Central and Peripheral Extraendocrine Activities in Humans

In addition to neuroendocrine effects, GHSs also possess pure central actions. In young adults, the iv administration of GHRP-6 has been found able to increase stage D2 sleep, and prolonged oral MK-677 treatment significantly increased REM sleep and decreased REM latency in aged subjects *(25,134)*. Moreover, there is evidence in animals of a stimulatory effect of GHSs on food intake and in humans increased appetite has occasionally been reported after both acute and chronic administration *(24,26,135)*.

In agreement with the existence of specific GHS-Rs in peripheral tissues (*see* above), peripheral biologic activities of these compounds have been demonstrated. A cardiovascular activity of hexarelin has extensively been studied in animals and humans. It has been shown that prolonged treatment with hexarelin and other GHRPs dramatically protects against cardiovascular damage in aged rats as well as in GH-deficient rats with post-ischemic ventricular dysfunction, via a mechanism of action independent of GH-releasing activity and probably mediated through cardiac and endothelial receptor activation *(27,28,31)*. In humans, hexarelin, but not rhGH, has been shown to possess inotropic effects in normal young volunteers as well as in hypopituitaric patients with severe GH deficiency in the absence of any variations in mean blood pressure, heart rate, or catecholamines, indicating once again a direct, endocrine-independent peripheral action *(29–31)*.

Recent data also demonstrate a direct antiapoptotic effect of hexarelin on human cardiomyocytes *(105)*, mediated by specific GHS-Rs, and a modulatory role of both natural and synthetic GHRPs on tumoral growth has recently been suggested *(30,106)*. It has been shown, in fact, that they possess a strong antiproliferative effect in malignant follicular-derived thyroid tumors as well as in breast cancer *(30,106)*.

Conclusion

The crucial role of GHRH in the control of neuroendocrine GH release, as well as the need for a tight interplay between GHRH and somatostatin to generate the pulsatile GH secretion, is widely accepted. On the other hand, the discovery of synthetic GHSs and their receptors, leading to the isolation of ghrelin, an endogenous GHS-R ligand, has allowed the hypothesis of the existence of a third major neuroendocrine pathway mainly controlling somatotroph function. It must be emphasized, however, that both GHRH and GHSs possess central and peripheral extraendocrine effects in humans and that Ghrelin was first isolated in a peripheral tissue.

GHRH is present in several extrahypothalamic areas of the brain and extraneural tissues, including the ovary and testis. In both male and female gonads, GHRH seems to represent an important factor modulating gonadotropin-induced hormonal and germinal function, suggesting a paracrine/autocrine, tissue-specific action of this neuropeptide.

On the other hand, the existence of specific GHRP binding sites in central extrahypothalamic and peripheral human tissues has been shown. GHRP binding activity has clearly been demonstrated in both the ovary and testis, although its biologic role at the gonadal level is, at present, unknown. However, the possibility that GHRPs and GHRH have some interactions even at the gonadal level in the regulation of reproductive function cannot be ruled out.

Acknowledgments

We wish to thank first Prof. F. Camanni, Dr. M. F. Boghen, and R. Deghenghi, and second, Dr. L. DiVito, C. Ghè, B. Maccagno, J. Ramunni, and A. Benso for their collaboration on our studies. Personal studies reported in this work were supported by CNR, SMEM Foundation, and Europeptides.

References

1. Frohman, L. A. and Jansson, J. O. (1986). *Endocr. Rev.* **7,** 223–231.
2. Muller, E. E. and Nisticò, G. (1989). In: *Brain messengers and the pituitary.* Muller, E. E. and Nisticò, G. (eds.). Academic: San Diego.
3. Casanueva, F. F. (1992). In: *Endocrinology Metabolism Clinics of North America,* Melmed, S. (ed.). Saunders: Philadelphia.
4. Ghigo, E. (1992). In: *Regulation of growth hormone and somatic growth.* De la Cruz (ed.). Elsevier Science: Amsterdam.

5. Tannenbaum, G. S. (1993). *J. Pediatr. Endocrinol.* **6,** 273–282.
6. Brar, A. K., Brinster, R. L., and Frohman, L. A. (1989). *Endocrinology* **125,** 801–809.
7. Bagnato, A., Moretti, C., Ohnishi, J., Frajese, G., and Catt, K. J. (1982) *Endocrinology* **130,** 1097–1102.
8. Baird, A., Wehrenberg, W. B., Bohlen, P., and Ling, N. (1985). *Endocrinology* **117,** 1598–1601.
9. Bruhn, T. O., Mason, R. T., and Vale, W. (1985). *Endocrinology* **117,** 1710–1712.
10. Thorner, M. O., Vance, M. L., Evans, W. S., Ho, K., Rogol, A. D., Blizzard, R. M., Furlanetto, R., Rivier, J., and Vale, W. (1986). *Acta Endocrinol.* **(Suppl.)** (Copenh.). **276,** 34–40.
11. Berry, S. A. and Pescovitz, O. H. (1988). *Endocrinology* **123,** 661–663.
12. Matsubara, S., Sato, M., Mizobuchi, M., Niimi, M., and Takahara, J. (1995). *Endocrinology* **136(9),** 4147–4150.
13. Moretti, C., Fabbri, A., Gnessi, L., Forni, L., Fraioli, F., and Frajese, G. (1989). In: *Reproductive Medicine: Medical Therapy.* Proceeding of the Second International Symposium on Reproductive Medicine. Frajese, G., Steinberg, F., and Rodriguez-Rigau, L. J. (eds.). Elsevier: New York.
14. Moretti, C., Fabbri, A., Gnessi, L., Bonifacio, V., Bolotti, M., Arizzi, M., Nazzicone, Q., and Spera, G. (1990). *J. Endocrinol. Invest.* **13,** 301–305.
15. Ciampani, T., Fabbri, A., Isidori, A., and Dufau, M. L. (1992). *Endocrinology* **131,** 2785–2792.
16. Fabbri, A., Ciocca, D. R., Ciampani, R., Wang, J., and Dufau, M. L. (1995). *Endocrinology* **136,** 2303–2308.
17. Artini, P. G., de Micheroux, A. A., and D'Ambrogio, G. (1996). *J. Endocrinol. Invest.* **19,** 763–779.
18. Bowers, C. Y., Veeraragavan, K., and Sethumadhavan, K. (1993). In: *Growth hormone II, basic and clinical aspects.* Bercu, B. B. and Walker, R. F. (eds.). Springer-Verlag: New York. 203–222.
19. Deghenghi, R. (1993). In: *Growth hormone secretagogues.* Bercu, B. and Walker, R. (eds.). Serono Symposia: New York.
20. Korbonits, M. and Grossman, A. B. (1995). *Trends Endocrinol. Metab.* **6,** 43–49.
21. Ghigo, E., Arvat, E., Muccioli, G., and Camanni, F. (1997). *Eur. J. Endocrinol.* **136,** 445–460.
22. Smith, R. G., Van der Ploeg, L. X. T., Howard, A. D., Feighner, S. D., Cheng, K., Hickey, G. J., Wyvratt, M. J., Fisher, M. H., Nargund, R. P., and Patchett, A. A. (1997). *Endocr. Rev.* **18,** 621–645.
23. Ghigo, E., Arvat, E., and Camanni, F. (1998). *Growth Horm. IGF Res.* **8,** 145–148.
24. Locke, W., Kirgis, H. D., Bowers, C. Y., and Abdo, A. A. (1995). *Life Sci.* **56,** 1347–1352.
25. Frieboes, R. M., Murck, H., Maier, P., Schier, T., Holsboer, F., and Steiger, A. (1995). *Neuroendocrinology* **61,** 584–589.
26. Torsello, A., Luoni, M., Schweiger, F., Grilli, R., Guidi, M., Bresciani, E., Deghenghi, R., Muller, E., and Locatelli, V. (1998). *Eur. J. Pharmacol.* **360,** 123–129.
27. De Gennaro Colonna, V., Rossoni, G., Bernareggi, M., Muller, E. E., and Berti, F. (1997). *Eur. J. Pharmacol.* **334,** 201–207.
28. Berti, F., Muller, E., De Gennaro Colonna, V., and Rossoni, G. (1998). *Growth Horm. IGF Res.* **8,** 149–152.
29. Bisi, G., Podio, V., Valetto, M. R., Broglio, F., Bertuccio, G., Del Rio, G., Arvat, E., Boghen, M. F., Deghenghi, R., Muccioli, G., Ong, H., and Ghigo, E. (1999). *J. Endocrinol. Invest.* **22,** 266–272.
30. Cassoni, P., Papotti, M., Catapano, F., Ghè, C., Deghenghi, R., Ghigo, E., and Muccioli, G.(2000). *J. Endocrinol.* **165,** 139–146.
31. Muccioli, G., Broglio, F., Valetto, M., Ghè, C., Catapano, F., Graziani, A., Papotti, M., Bisi, G., Deghenghi, R., and Ghigo, E. (2000). *Ann. Endocrinol.* (Paris) **61(1),** 27–31.
32. Howard, A. D., Feighner, S. D., Cully, D. F., et al. (1996). *Science* **273,** 974–977.
33. Ong, H., McNicoll, N., Escher, E., Collu, R., Deghenghi, R., Locatelli, V., Ghigo, E., Muccioli, G., Boghen, M., and Nillson, M. H. L. (1998). *Endocrinology* **139,** 432–435.
34. Bodart, V., Bouchard, J. F., McNicoll, N., Escher, E., Carriere, P., Ghigo, E., Sejlitz, T., Sirois, M. G., Lamontagne, D., and Ong, H. (1999). *Circ. Res.* **85,** 796–802.
35. Kojima, M., Hosada, H., Data, Y., Nakazato, M., Matsuo, H., and Kankawa, K. (1999). *Nature* **402,** 656–660.
36. Frohman, L. A., Bernardis, L. L., and Kant, K. J. (1969). *Science* **162,** 580–585.
37. Martin, J. B. (1972). *Endocrinology* **91,** 107–111.
38. Bloch, B., Brazeau, P., Ling, N., Bohlen, P., Esch, F., Wehrenberg, W. B., Benoit, R., Bloom, F., and Guillemin, R. (1983). *Nature* **301,** 607–611.
39. Brazeau, P., Bloom, F., and Ling, N. (1983). *Neurosci. Lett.* **37,** 23–26.
40. Jacobowitz, D. M., Schulte, H., Chrousos, G. P., and Loriaux, D. L. (1983). *Peptides* **4,** 521–524.
41. Mayo, K. E., Cerelli, G. M., Lebo, R. V., Bruce, B. D., Reosenfeld, M. G., and Evans, R. M. (1985). *Proc. Natl. Acad. Sci. USA* **62,** 63–65.
42. Bresson, J. L., Clacequin, M. C., Fellman, D., and Bugnon, C. (1984). *Neuroendocrinology* **39,** 68–71.
43. Carlson, H. E., Mariz, I. K., and Daughday, W. H. (1974). *Endocrinology* **94,** 1709–1711.
44. Stachura, M. E . (1976). *Endocrinology* **99,** 678–681.
45. Tannenbaum, G. S., Epelbaum, J., Colle, E., Brazeau, P., and Martin, J. B. (1978). *Endocrinology* **102,** 1909–1912.
46. Tannenbaum, G. S. and Ling, N. (1984). *Endocrinology* **115,** 1952–1956.
47. Hindmarsch, P. C., Brain, C. E., Robinson, I. C. A. F., Matthews, D. R., and Brook, C. G. D. (1991). *Clin. Endocrinol.* **35,** 353–360.
48. Plotsky, P. M. and Vale, W. (1985). *Science* **230,** 461–466.
49. Mazza, E., Ghigo, E., Goffi, S., Procopio, M., Imperiale, E., Arvat, E., Bellone, J., Boghen, M. F., Muller, E. E., and Camanni, F. (1989). *J. Endocrinol. Invest.* **12,** 795–798.
50. Corpas, E., Hartman, S. M., and Blackman, M. R. (1993). *Endocr. Rev.* **16,** 686–715.
51. Ghigo, E., Arvat, E., Gianotti, L., Ramunni, J., Di Vito, L., Maccagno, B., Grottoli, S., and Camanni, F. (1996). *J. Pediatr. Endocrinol. Metab.* **9,** 271–278.
52. Ceda, G. P., Valenti, G., Butturini, U., Hoffman, A. R. (1986). *Endocrinology* **118,** 2109–2114.
53. Coiro, V., Volpi, R., Cavazzini, U., Bertoni, P., Corradi, A., and Bianconi, L. (1991). *J. Gerontol.* **46,** M155–M158.
54. Muller, E. E., Cella, S. G., Parenti, M., Deghenghi, R., Locatelli, V., and De Gennaro Colonna, V. (1995). *Horm Res.* **43,** 39–45.
55. Arvat, E., Ceda, G. P., Di Vito, L., Ramunni, J., Gianotti, L., Broglio, F., Deghenghi, R., and Ghigo, E. (1998). *Pituitary* **1,** 51–58.
56. Muller, E. E., De Gennaro Colonna, V., Cella, S. G., Torsello, A., Ghigo, E., Loche, S., Arce, V., Cocchi, D., and Locatelli, V. (1991). In: *Molecular and clinical advances in pituitary disorders.* Melmed, S. and Robbins, R. (eds.). Blackwell Scientific, Oxford.
57. Berelowitz, M., Szabo, M., Frohman, L. A., Firestone, S., Chu, L., and Hintz, R. L. (1981). *Science* **212,** 1279–1288.
58. Ceda, G. P. (1995). *J. Endocrinol. Invest.* **18,** 734–737.
59. Ho, K. K. and Weissberger, A. J. (1990). *Horm. Res.* **33,** 7–11.
60. Wehrenberg, W. B. and Giustina, A. (1992). *Endocr. Rev.* **13,** 299–308.
61. Vedlhuis, J. D. (1996). *J. Pediatr. Endocrinol. Metab.* **9,** 237–253.
62. Ghigo, E., Arvat, E., Gianotti, L., Maccario, M., and Camanni, F. (1999). In: *The endocrine response to acute illness.* Jenkins, R. C. and Ross, R. J. M. (eds.). Front. Horm. Res. Karger: Basel, Switzerland.

63. Lang, I., Schernthaner, G., Pietsschman, P., Kurz, R., Stephenson, J. M., and Templ, H. (1987). *J. Clin. Endocrinol. Metab.* **65,** 535–539.
64. Gelato, M. C., Pescovitz, D. H., Cassorla, F., Loriaux, D. L., and Merriam, G. R. (1984). *J. Clin. Endocrinol. Metab.* **59,** 197–203.
65. Smals, A. E. M., Pieters, G. F. F. M., Smals, A. G. H., Benraad, T. J., van Laafhoven, J., and Kloppenborg, P. W. C. (1986). *J. Clin. Endocrinol. Metab.* **62,** 336–340.
66. Vaccarino, F. J., Bloom, F. E., Rivier, J., Vale, W., and Koob, G. F. (1985). *Nature* **314,** 167–171.
67. Berry, S. A. and Pescovitz, O. H. (1990). *Endocrinology* **127,** 1404–1411.
68. Pescovitz, O. H., Berry, S. A., Laudon, M., Ben-Jonathan, N., Martin-Myers, A., Hsu, S., Lambros, T. J., and Felix, A. M. (1990). *Endocrinology* **127,** 2336–2342.
69. Srivastava, C. H., Collard, M. W., Rothrock, J. K., Peredo, M. J., Berry, S. A., and Pescovitz, O. H. (1993). *Endocrinology* **133,** 83–89.
70. Srivastava, C. H., Monts, B. S., Rothrock, J. K., Peredo, M. J., and Pescovitz, O. H. (1995). *Endocrinology* **136,** 1502–1508.
71. Breyer, P. R., Rothrock, J. K., Beaudry, N., and Pescovitz, O. H. (1996). *Endocrinology* **137(5),** 2159–2162.
72. Berry, S. A., Srivastava, C. H., Rubin, L. R., Phipps, W. R., and Pescovitz, O. H. (1992). *J. Clin. Endocrinol. Metab.* **75,** 281–284.
73. Srivastava, C. H., Breyer, P. R., Rothrock, J. K., Peredo, M. J., and Pescovitz, O. H. (1993). *Endocrinology* **133(3),** 1478–1481.
74. Moretti, C., Fabbri, A., Gnessi, L., Bolotti, M., Giovenco, P., and Frajese, G. (1989). *Endocrinology* **124,** 335–339.
75. Spiteri-Grech, J. and Nieschlag, E. (1992). *Horm. Res.* **38(1),** 22–27.
76. Sasaki, A. and Yoshinaga, K. (1989). *J. Clin. Endocrinol. Metab.* **68,** 996–999.
77. Oh, S. S., Khardori, R., Kopplin, D. K., and Amador, A. G. (1995). *Rev. Esp. Fisiol.* **51(4),** 187–192.
78. Gerendai, I., Csaba, Z., and Csernus, V. (1996). *Life Sci.* **59(10),** 859–866.
79. Vasankari, T., Kujala, U., Taimela, S., Torma, A., Irjala, K., and Huhtaniemi, I. (1995). *J. Clin. Endocrinol. Metab.* **80(11),** 3298–3303.
80. Moretti, C., Bagnato, A., Solan, N., Fratese, G., and Catt, K. J. (1990). *Endocrinology* **127,** 2117–2126.
81. Bagnato, A., Moretti, C., Fratese, G., and Catt, K. J. (1991). *Endocrinology* **128,** 2889–2894.
82. McIntyre, H. D., Marechal, D. J., Deby, G. P., Mathieu, A. G., Hezee-Hagelstein, M., and Franchimont, P. P. (1992). *Acta Endocrinol.* (Copenh.). **126(6),** 553–558.
83. Andreani, C. L., Lazzarin, N., Pierro, E., Lanzone, A., and Mancuso, S. (1995). *Hum. Reprod.* **10(8),** 1968–1973.
84. Holst, N., Jacobsen, M. B., Haug, E., Tanbo, T., and Abyholm, T. (1995). *Hum. Reprod.* **10(6),** 1363–1366.
85. Mimuro, T., Smith, H., Iwashita, M., and Illingworth, P. J. (1998). *Hum Reprod.* **13(1),** 150–153.
86. Bowers, C. Y., Chang, J., Momany, F., and Folkers, K. (1977). In: *Molecular endocrinology.* Macintyne, I. (ed.). Elsevier/ North Holland Biochemical: Amsterdam.
87. Raun, K., Hansen, B. S., Johansen, N. L., Thogersen, H., Madsen, K., Ankersen, M., and Andersen, P. H. (1998). *Eur. J. Endocrinol.* **139,** 552–561.
88. Arvat, E., Di Vito, L., Lanfranco, F., Broglio, F., Giordano, R., Benso, A., Muccioli, G. P., Deghenghi, R., and Ghigo, E. (1999). *J. Endocrinol. Invest.* **22,** 91–97.
89. Balthasar, N., Carmignac, D. F., Mathers, K., Bennet, P. A., Le Tissier, P. R., Magoulas, C., and Robinson, I. C. A. F. (2000). 82nd Annual Meeting of the Endocrine Society, Toronto, June 2000 (abstract 645).
90. Kangawa, K., Kojima, M., and Matsuo, H. (2000). 82nd Annual Meeting of the Endocrine Society, Toronto, June 2000 (abstract 172).
91. Nakazato, M., Date, Y., Kojima, M., Kangawa, K., and Matsukura, S. (2000). 82nd Annual Meeting of the Endocrine Society, Toronto, June 2000 (abstract 692).
92. Mori, K., Yoshimoto, A., Hosoda, K., Sawai, K., Yokoi, H., Yoshiota, T., Nagae, T., Fujinaga, Y., Makino, H., Suganami, T., Yahata, K., Mukoyama, M., Sugawara, A., Hosoda, H., Kojima, M., Kangawa, K., and Nakao, K. (2000). Annual Meeting of the Endocrine Society, Toronto, June 2000 (abstract 1124).
93. Iwakura, H., Hosoda, K., Son, C., Sagawa, N., Itoh, H., Yura, S., Matsuda, J., Akamizu, T., Fujii, S., Hosoda, H., Kojima, M., Kangawa, K., and Nakao, K. (2000). Annual Meeting of the Endocrine Society, Toronto, June 2000 (abstract 1125).
94. Komatsu, I., Yasoda, A., Sakuma, Y., Miura, A., Tanaka, K., Hosoda, H., Kojima, M., Kangawa, K., Hosoda, K., and Nakao, K. (2000). Annual Meeting of the Endocrine Society, Toronto, June 2000 (abstract 1126).
95. Korbonits, M., Ciccarelli, E., Ghigo, E., and Grossman, A. B. (1999). *Growth Horm. IGF Res.* **9,** 93–99.
96. McKee, K. K., Tan, C. P., Palyha, O. C., Liu, J., Feighner, S. D., Hreniuk, D. L., Smith, R. G., Howard, A. D., and Van der Ploeg, L. H. T. (1997). *Genomics* **46,** 426–434.
97. Tan, C. P., McKee, K. K., Qingyun, L., Palyha, O. C., Feighner, S. D., Hreniuk, D. L., Smith, R. G., and Howard, A. D. (1998). *Genomics* **52,** 223–229.
98. Bartolotta, E., Bellone, J., Aimaretti, G., Arvat, E., Benso, L., Deghenghi, R., Camanni, F., and Ghigo, E. (1997). *J. Pediatr. Endocrinol. Metab.* **10,** 491–497.
99. Shimon, I., Yan, X., and Melmed, S. (1998). *J. Clin. Endocrinol. Metab.* **83,** 174–178.
100. Arvat, E., Giordano, R., Gianotti, L., Broglio, F., Muccioli, G. P., Camanni, F., and Ghigo, E. (1999). In: *Sex-steroid interactions with growth hormone.* Veldhuis, J. D. and Giustina, A. (eds.). Springer-Verlag: Serono Symposia USA.
101. Kamegai, J., Wakabayashi, I., Kineman, R. D., and Frohman, L. A. (1999). *J. Neuroendocrinol.* **11,** 299–306.
102. Tamura, H., Kamegai, J., Sugihara, H., Kineman, R. D., Frohman, L. A., and Wakabayashi, I. (2000). *J. Neuroendocrinol.* **12,** 481–485.
103. Bennet, P. A., Thomas, G. B., Howard, A. D., Feighner, S. D., van der Ploeg, L. H. T., Smith, R. G., Robinson, I. C. A. F. (1997). *Endocrinology* **138,** 4552–4557.
104. Guan, X., Yu, H., Palyha, O. C., McKee, K. K., Feighner, S. D., Sirinathsinghji, D. J. S., Smith, R. G., Van der Ploeg, L. H. T., and Howard, A. D. (1997). *Mol. Brain Res.* **48,** 23–29.
105. Graziani, A., Fubini, A., Filigheddu, N., Baldanzi, G. L., Pucc,i A., Ong, H., Muccioli, G., Bussolino, F., and Ghigo, E. (1999). *J. Endocrinol. Invest.* **22(Suppl. 4),** 16.
106. Papotti, M., Ghé, C., Cassoni, P., Catapano, F., Deghenghi, R., Ghigo, E., and Muccioli, G. (2000). *J. Clin. Endocrinol. Metab*. **85,** 3803–3707.
107. Pombo, M., Barreiro, J., Penalva, A., Peino, R., Dieguez, C., and Casanueva, F. F. (1995). *J. Clin. Endocrinol. Metab.* **80,** 3180–3184.
108. Popovic, V., Damjanovic, S., Micic, D., Djurovic, M., Dieguez, C., and Casanueva, F. F. (1995). *J. Clin. Endocrinol. Metab.* **80,** 942–947.
109. Hickey, G. J., Drisko, J., Faidley, T., Chang, C., Anderson, L., Nicolich, S., McGuire, L., Rickes, E., Krupe, D., Feeney, W., Friscino, B., Cunningham, P., Frazier, E., Chen, H., Leroque, P., and Smith, R. G. (1996). *J. Endocrinol.* **148,** 371–380.
110. Pandya, N., De Mott-Friberg, R., Bowers, C. Y., Barkan, A. L., and Jaffe, C. A. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1186–1189.
111. Maheshwari, H. G., Rahim, A., Shalet, S. M., and Baumann, G. (1999). *J. Clin. Endocrinol. Metab.* **84,** 956–959.

112. Arvat, E., Broglio, F., Giordano, R., Muccioli, G., Maccario, M., Camanni, F., and Ghigo, E. (1999). In: *Growth hormone secretagogues.* Ghigo, E., Boghen, M., Casanueva, F. F., and Dieguez, C. (eds.). Elsevier Science. Amsterdam.
113. Massoud, A. F., Hindmarsh, P. C., and Brook, C. G. (1995). *Clin. Endocrinol.* **43,** 617–621.
114. Arvat, E., Di Vito, L., Gianotti, L., Ramunni, J., Boghen, M. F., Deghenghi, R., Camanni, F., and Ghigo, E. (1997). *Metabolism* **46,** 83–88.
115. Ghigo, E., Gianotti, L., Arvat, E., Ramunni, J., Valetto, M. R., Broglio, F., Rolla, M., Cavagnini, F., Muller, E. E. (1999). *J. Clin. Endocrinol. Metab.* **84,** 285–290.
116. Arvat, E., Gianotti, L., Grottoli, S., Imbimbo, B. P., Lenaerts, V., Deghenghi, R., Camanni, F., and Ghigo, E. (1994). *J. Clin. Endocrinol. Metab.* **79,** 1440–1443.
117. Chapman, I. M., Bach, M. A., Van Cauter, E., Farmer, M., Krupa, D., Taylor, A. M., Schilling, L. M., Cole, K. Y., Skiles, E. H., Pezzoli, S. S., Hartman, M. L., Veldhuis, J. D., Gormley, G. J., and Thorner, M. O. (1996). *J. Clin. Endocrinol. Metab.* **81,** 4249–4257.
118. Micic, D., Popovich, V., Doknic, M., et al. (1998). *J. Clin. Endocrinol. Metab.* **83,** 2569–2572.
119. Bellone, J., Aimaretti, G., Bartolotta, E., Benso, L., Imbimbo, B. P., Lenhaerts, V., Deghenghi, R., Camanni, F., and Ghigo, E. (1995). *J. Clin. Endocrinol. Metab.* **80,** 1090–1094.
120. Loche, S., Colao, A., Cappa, M., Bellone, J., Aimaretti, G., Farello, G., Faedda, A., Lombardi, G., Deghenghi, R., and Ghigo, E. (1997). *J. Clin. Endocrinol. Metab.* **82,** 861–864.
121. Arvat, E., Gianotti, L., Broglio, F., Maccagno, B., Bertagna, A., Deghenghi, R., Camanni, F., and Ghigo, E. (1997). *Eur. J. Endocrinol.* **136,** 483–487.
122. Korbonits, M., Trainer, P. J., and Besser, G. M. (1995). *Clin. Endocrinol.* **43,** 365–371.
123. Arvat, E., Maccagno, B., Ramunni, J., Di Vito, L., Broglio, F., Deghenghi, R., Camanni, F., and Ghigo, E. (1997). *Neuroendocrinology* **66,** 432–438.
124. Arvat, E., Ramunni, J., Bellone, J., Di Vito, L., Baffoni, C., Broglio, F., Deghenghi, R., Bartolotta, E., and Ghigo, E. (1997). *Eur. J. Endocrinol.* **137,** 635–642.
125. Sapolsky, R. M., Krey, L. C., and McEwen, B. (1986). *Endocr. Rev.* 284–301.
126. Elias, K. A., Ingle, G. S., Burnier, J. P., Hammonds, R. G., McDowell, R. S., Rawson, T. E., Somers, T. C., Stanley, M. S., and Cronin, M. J. (1995). *Endocrinology* **136,** 5694–5699.
127. Korbonits, M., Kaltsas, G., Perry, L. A., Putignano, P., Grossman, A. B., Besser, G. M., and Trainer, P. J. (1999). *J. Clin. Endocrinol. Metab.* **84,** 2489–2495.
128. Dickson, S. L. and Luckman, S. M. (1997). *Endocrinology* **138,** 771–777.
129. Arvat, E., Maccagno, B., Ramunni, J., Di Vito, L., Gianotti, L., Broglio, F., Benso, A., Deghenghi, R., Camanni, F., and Ghigo, E. (1998). *Neuroendocrinology* **67,** 310–316.
130. Arvat, E., Ramunni, J., Maccagno, B., Giordano, R., Broglio, F., Deghenghi, R., Boscaro, M., and Ghigo, E. (1999). *Neuroendocrinology* **70,** 200–206.
131. Ghigo, E., Arvat, E., Ramunni, J., Colao, A., Gianotti, L., Deghenghi, R., Lombardi, G., and Camanni, F. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2439–2444.
132. De Keyzer, Y., Lenne, F., and Bertagna, X. (1997). *Eur. J. Endocrinol.* **137,** 715–718.
133. Jansson, J. O., Svensson, J., Bengtsson, B. A., Frohman, L. A., Ahlman, H., Wangberg, B., Nilsson, O., and Nilsson, M. (1998). *Clin. Endocrinol.* (Oxf.). **48,** 243–250.
134. Copinschi, G., Leproult, R., Van Onderbergen, A., Caufriez, A., Cole, K. Y., Schilling, L. M., Mendel, C. M., De Lepeleire, I., Bolognese, J. A., and Van Cauter, E. (1997). *Neuroendocrinology* **66,** 278–286.
135. Svensson, J., Lonn, L., Jansson, J. O., Murphy, G., Wyss, D., Krupa, D., Cerchio, K., Polvino, W., Gertz, B., Boseaus, I., Sjostrom, L., and Bengtsson, B. A. (1998). *J. Clin. Endocrinol. Metab.* **83,** 362–369.