Neuroendocrine Control of Growth Hormone Secretion

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Müller, Eugenio E., Vittorio Locatelli, and Daniela Cocchi. Neuroendocrine Control of Growth Hormone Secretion. Physiol. Rev. 79: 511–607, 1999.—The secretion of growth hormone (GH) is regulated through a complex neuroendocrine control system, especially by the functional interplay of two hypothalamic hypophysiotropic hormones, GH-releasing hormone (GHRH) and somatostatin (SS), exerting stimulatory and inhibitory influences, respectively, on the somatotrope. The two hypothalamic neurohormones are subject to modulation by a host of neurotransmitters, especially the noradrenergic and cholinergic ones and other hypothalamic neuropeptides, and are the final mediators of metabolic, endocrine, neural, and immune influences for the secretion of GH. Since the identification of the GHRH peptide, recombinant DNA procedures have been used to characterize the corresponding cDNA and to clone GHRH receptor isomers in rodent and human pituitaries. Parallel to research into the effects of SS and its analogs on endocrine and exocrine secretions, investigations into their mechanism of action have led to the discovery of five separate SS receptor genes encoding a family of G protein-coupled SS receptors, which are widely expressed in the pituitary, brain, and the periphery, and to the synthesis of analogs with subtype specificity. Better understanding of the function of GHRH, SS, and their receptors and, hence, of neural regulation of GH secretion in health and disease has been achieved with the discovery of a new class of fairly specific, orally active, small peptides and their congeners, the GH-releasing peptides, acting on specific, ubiquitous seven-transmembrane domain receptors, whose natural ligands are not yet known.
I. INTRODUCTION

The secretion of somatotrophin, or growth hormone (GH), like other anterior pituitary (AP) hormones, is regulated through a complex neuroendocrine control system that comprises two main hypothalamic regulators, GH-releasing hormone (GHRH) and somatostatin (SRH or SS), exerting stimulatory and inhibitory influences, respectively, on the somatotrope cell. Both GHRH and SS, which are subject to modulation by other hypothalamic peptides and by complex networks of neurotransmitter neurons, are the final mediators of metabolic, endocrine, neural, and immune influences for GH secretion in the pituitary.

In the last decade, the rather static scenario of GH control has been shaken by a host of advances, some resulting from molecular biology techniques. Of particular interest is cloning of the GHRH receptor isoforms in the rat, mouse, and human pituitary; discovery of distinct genes encoding a family of structurally related G protein-coupled SS receptors in brain and the pituitary and synthesis of new SS analogs with subtype specificity; identification of a novel class of fairly specific, orally active, hexa-hepta peptides, the GH-releasing peptides (GHRP) and their congeners, whose GH-releasing activity is greater than GHRH; better insight into the neurotransmitter control of GH; and the involvement of somatotropic dysfunction in a host of diseases and in the catabolic processes related to aging.

This review updates and focuses on aspects of the neural control of GH secretion, especially in the light of these advances. For comprehensive surveys of GH secretory control, the reader is referred to Frohman et al. (377), Müller (749), and Cronin and Thorner (274).

II. GROWTH HORMONE SECRETION PATTERN

The secretion of GH is pulsatile in all species studied so far, with its pattern not dissimilar in humans and male rodents. In the former, an ultrasensitive immunoradiometric assay (IRMA) (limit of detection, 20 ng/l) given to healthy men and women showed an ultradian rhythm with an interpulse frequency of ~2 h (1101), which compared favorably with an interpulse frequency of ~3 h in the male rat (1014) and plasma GH detectable at all time points. In these subjects, GH levels ranged over three orders of magnitude in both sexes.

Women of reproductive age had significantly higher overall GH levels, a higher pulse amplitude, and a higher baseline, but the pulse frequency was the same as in men (mean secretion rates: 327 ± 24 h for men and 392 ± 24 h for women; Ref. 1101). These figures are ~50% lower than previously assumed (see Ref. 1030). A new ultrasensitive GH chemiluminescence (CL) assay (limit of detection, 5 ng/l) detects serum GH concentrations in all samples collected at 10-min intervals for 24 h.

The Cluster method to estimate GH pulsatility and deconvolution analysis, which simultaneously resolves the endogenous hormone secretory rate and clearance kinetics (1063), showed that pulsatile GH secretion continued in all subjects in the awake and fed state, despite undetectable serum GH in daytime samples analyzed by a conventional IRMA, run for comparison. Interestingly, the CL assay also revealed for the first time a low rate of basal (constitutive) GH secretion mixed with pulsatile and nycotemeral variations (508).

Further studies were then done in healthy lean and obese subjects aimed at ascertaining how age, steroid sex hormones, and obesity, singly or jointly, influence the basal and pulsatile modes of GH release. Basal and pulsatile GH release were found to be regulated differently by adiposity and steroid hormones. Thus basal GH secretion rates were negatively correlated with the circulating estrogen (E2) concentration and with an interactive effect of age and body fat, whereas the GH secretory burst mass was strongly positively related to serum testosterone concentrations.

Concerning the biological consequences of GH secretion, basal and pulsatile GH release may influence serum insulin-like growth factor I (IGF-I), its main binding protein (BP), i.e., IGFBP-3, and IGFBP-1 in distinct ways (1064). In a study in healthy men, IGFBP-3 correlated negatively to basal GH secretion, whereas IGFBP-1 concentrations were positively correlated with this measure. Measurements of serum IGF-I concentrations indicated that pulsatile GH release was strongly positively correlated with the mean serum IGF-I level. Conversely, there was no association between basal GH release rates and serum IGF-I levels (1064). This observation is consistent with the theory that a pulsatile GH signal is important in acting on one or more major target tissues (e.g., the liver) to stimulate IGF-I production in the healthy human. These findings have major implications for the evaluation of GH control and secretion in different experimental and clinical conditions.

Present knowledge thus indicates that the secretory pattern of GH depends on the interaction between GHRH and SS at the somatotroph level, although we cannot overlook the existence of anatomic connections and functional peptide interactions within the hypothalamus (see sect. IIIA3) and the contribution of a still elusive endogenous GHRP system (see sect. IV).

The basis of the pulsatile release of GH, however, remains unknown. A host of nutritional, body composition, metabolic, and age-related sex steroid mechanisms, adrenal glucocorticoids, thyroid hormones, and renal and hepatic functions all govern pulsatile release in adults (see sect. II, A–C), but spontaneous elevations in GH secretion are more likely to result from the ability of hypotha-
lamic neuroendocrine cells themselves to promote and sustain phasic bursts of action potentials (34). Basal GH release may also be subject to neurophysiological regulation; slow removal of a pool of GH in plasma from trapping with a GH-binding protein seems less likely (508).

Hypothalamic GHRH and SS functions both appear essential for pulsatile secretion. In male rats, anti-GHRH serum inhibited spontaneous GH pulses (1090), reduced growth rate (207), and suppressed stimulated GH secretion (207, 548). Initial studies in male rats utilizing an anti-SS serum bolus injection showed that pulsatile GH secretion was preserved despite an increase in basal GH levels (350). However, when a highly potent anti-SS serum was infused for 6 h, it did inhibit pulsatile GH secretion (375). The most likely explanation for the different effects of anti-SS serum bolus injection versus continuous infusion is that the latter but not the former penetrates the region of the arcuate nucleus (ARC), where the inhibitory effects of SS on GHRH secretion occur (see sect. II.A3).

Supporting this view, electrolytic lesions in the medial preoptic area (MPOA) or anterolateral hypothalamic deafferentation in male rats, two techniques which eliminate SS immunostaining in the median eminence (ME), resulted in elevated plasma GH levels and no pulsatile GH secretion. Under these experimental conditions, basal and K⁺-stimulated GHRH release from the hypothalamic ME-ARC complex in vitro was significantly increased, although the hypothalamic GHRH content was decreased, suggesting an increased turnover of the peptide (548).

Overall, these results suggest that SS in the hypothalamus plays a tonic inhibitory role in the regulation of GHRH release and that GH hypersecretion in lesioned rats is due to a combination of secretion changes of both SS and GHRH. They are in line with the general model proposed by Tannenbaum and Ling (1013), in which GHRH release during troughs in a sinusoidal pattern of SS release induces the episodic release of GH, and the rise in SS suppresses baseline release. Direct measurement of GHRH and SS in the rat portal blood would validate this view by showing that each GH secretion episode started by pulsatile release of GHRH, which is preceded by or concurrent with a moderate reduction of the SS tone (853). These portal blood measurements in the rat, however, would require confirmation, since other studies have not been able to maintain GH pulsatility under the conditions reported by Plotsky and Vale (853) (see Ref. 895 for references). Moreover, this model would explain patterns of secretion in the male rat. Female rats show much more continuous irregular GH secretion and much more regular responses to GHRH (248, see also sect. II.A).

The proposed model of SS-GHRH interactions cannot be extrapolated to all the animal species so far investigated. In the sheep, portal GHRH and SS levels suggest regulation is more complex, although the majority of GH pulses coincided with or occurred immediately after a GHRH pulse, ~30% were completely dissociated (378). This variability was also seen in SS measurements, in the sheep, where pulses were not necessarily asynchronous with those of GHRH and did not correlate with GH troughs, nor SS troughs with GH pulses. In addition, active immunization of rams against SS did not change basal and pulsatile GH secretion, whereas in the anti-GHRH group, plasma GH levels were very low, and the amplitude of GH pulses was strikingly reduced (652). The redundant GHRH and SS pulsatility in the portal blood may reflect neuropeptide actions beyond pure GH release, e.g., trophic effects on the target somatotrophs (805).

It must be recalled, in addition, that the simple rat GHRH-SS model ignores all other alleged hypophysiotrophic factors, including the endogenous GHRP system (see sect. IV), additional modulators such as pituitary GHRH and SS receptors, feedback effects of GH and IGF-I, and other target gland hormones.

In humans, the neuroendocrine control of pulsatile GH secretion is also poorly understood. Because portal blood cannot be sampled directly in humans, most of our understanding of the regulation of GH secretion comes from either indirect human studies or extrapolation of animal data. In healthy young men, a single dose of a competitive GHRH antagonist blocked 75% of nocturnal GH pulsatility (521), supporting the hypothesis that pulsatile GH secretion in humans is driven by GHRH. The persistence of pulsatile GH secretion in normal and GH-deficient subjects during continuous GHRH infusion (473, 1056) and the ability of exogenous GHRH to release GH in the face of grossly elevated GHRH levels in the ectopic GHRH syndrome (74) are also in keeping with this suggestion.

Periodic falls in SS secretion might account for the persistence of pulsatile GH secretion in the above conditions, a proposal in line with GH secretory profiles determined by deconvolution analysis (460). Plasma GH secretory patterns were measured in normal adult men at baseline and during submaximal intravenous boluses of GHRH every 2 h for 6 days (522). No evidence was found of either acute or chronic desensitization; GH responses were seen at every GHRH bolus, and circadian patterns of GH secretion were similar at baseline and during the GHRH schedule, with maxima occurring during the early nighttime hours. This pattern was consistent with a model for the ultradian secretion of GH in which circadian SS secretion (reduction at night?) is superimposed on more rapid GHRH pulses.

A. Sex

Sex-related differences in GH control by GHRH and SS have been found particularly in the rat. They include different 1) responsiveness to exogenously administered
This strongly supports the proposal (246, 249) that in the body weight gain and increased pituitary GH stores (250). the intermittent infusion of SS, which also stimulated similar pulsatile GH secretory pattern was achieved with GHRH were much less effective (248). Interestingly, a stimulated body growth, whereas continuous infusions of rats, GHRH pulses delivered every 3 h changed the pattern possibly being continuous rather than cyclical, its release suggested for males. The episodic GHRH bursting, which does not appear to follow a specific rhythm, as in the male, superimposed on the fairly continuous SS release of hypothalamic neurons to produce the neuropeptide.

In male and female rats, passive immunization with specific antisera to both peptides was used to assess the physiological roles of GHRH and SS in the dimorphic secretion (802). An acute dose of an anti-SS serum to males raised the GH nadir, as expected, but did not alter other parameters of GH secretion, whereas in females it markedly increased plasma GH levels at all time points of the 6-h sampling period and there was, in addition, a significant increase in GH peak amplitude, GH nadir, and overall mean 6-h plasma GH levels. Anti-GHRH serum had no effect on the GH nadir in males but markedly raised the GH nadir in females.

These findings led to the conclusion that the secretory pattern of SS undoubtedly plays an important role in this sexual dimorphism, the female pattern of SS secretion possibly being continuous rather than cyclical, its level intermediate between the peak and troughs of SS release suggested for males. The episodic GHRH bursting, which does not appear to follow a specific rhythm, as in the male, superimposed on the fairly continuous SS release, would cause the erratic GH secretory profile, present in the peripheral plasma of female rats.

This pattern of low-amplitude GH release is reminiscent of that after continuous GH infusion and may be more effective in inducing specific biological changes, such as GH-binding protein in plasma and hepatic cytochrome P-450-containing enzymes (955). For the role of sex steroids in imprinting the sexually differentiated GH secretory pattern, see also section II A8b.

The pattern of GH secretion in rodents seems to be of considerable importance for optimal growth. In female rats, GHRH pulses delivered every 3 h changed the pattern of pituitary GH release toward that of the male and stimulated body growth, whereas continuous infusions of GHRH were much less effective (248). Interestingly, a similar pulsatile GH secretory pattern was achieved with the intermittent infusion of SS, which also stimulated body weight gain and increased pituitary GH stores (250). This strongly supports the proposal (246, 249) that in the rat the pulsatile pattern of GH is optimal for growth, irrespective of whether this pattern is achieved by a stimulator or inhibitor of endogenous GH secretion. The pulsatile delivery of SS would be vital in the generation of GH pulsatility not only for its opposing action to GHRH but also because it enables GHRH to be effective, because unopposed exposure to the releasing hormone leads to downregulation of GH responses.

Sex differences in the pattern of GH secretion are not so clear-cut in humans, but there are noticeable quantitative differences, with serum GH concentrations being definitely higher in women than in men (221, 474, 1101). In a small group of healthy middle-aged men and premenopausal women, highly sensitive immunofluorometric assays (sensitivity 0.011 μg/l) and blood sampling at 10-min intervals showed the 24-h GH secretion was three times higher in the women, the GH secretory episodes were larger and more prolonged, more GH was secreted per burst, and maximal plasma GH concentrations were higher than in men. The frequency of pulses, timing during the day/night estimates of baseline GH secretion, and the endogenous clearance rates for GH were the same in both sexes (1059) (Fig. 1).

A sex-related difference was also found in the serial regularity of GH release in a tissue series (848). Application of novel regularity statistics (approximate entropy) showed that the serial regularity of GH release was lower in female humans and rats (847).

Although E₂ is an obvious candidate for the mechanism that increases the mass of GH secreted per burst in women, it is not yet known at what level(s) in the GH axis it may act. Another question is whether the much higher daily GH output in women has biological significance for GH action, and what action this might have in middle age. In GH-deficient men and women, small but frequent intravenous boluses and continuous intravenous infusion of GH were equally effective in increasing circulating IGF-I (532), which implies that women do not secrete more GH than men simply to maintain the same IGF-I levels.

B. Age-Related Growth Hormone Secretion

The 24-h pattern of spontaneous GH release changes with age in experimental animals and humans. Plasma GH in the fetus is elevated compared with postnatal levels in a number of species, including humans and sheep (431). The rat, an altricial species, which is an animal model for the midgestational hypothalamic differentiation in humans, has a rise in plasma GH immediately before birth and a decline to near adult levels by ~18–20 days postnatally (1078). This is coupled with striking changes in pituitary susceptibility to GHRH, which at doses of 1–50 ng/100 g given subcutaneously in 10-day-old pups induces clear-cut plasma GH rises. However, in 25-day-old pups,
the dose of 500 ng/100 g given subcutaneously has this effect but not lower doses (205) (see also sect. III A9). Moreover, in rat pups but not in young adult rats, an in vivo and ex vivo 5-day GHRH treatment stimulated pituitary GH biosynthesis (205, 272). Consonant with these results, in monolayer cultures of rat pituitary cells, the stimulatory effects of GHRH and dibutyryl cAMP on GH secretion were inversely related to age (281).

Although differential developmental regulation of pituitary GHRH receptor gene expression is very likely involved in these events (572), maturational changes in the somatotroph responsiveness to SS may contribute to the pattern of circulating GH levels characteristic of early development. In fact, pituitaries from newborn rats are relatively resistant to the GH-suppressive effect of SS, and sensitivity increases with age (281, 889), although in rats brain SS mRNA is detectable by day 7 of embryonic life (1130a) and IR-SS has been detected in the fetal rat as early as 18.5 days of gestation (1130a).

In the human fetus, plasma GH peaks at mid-gestation, then GH levels fall in the third trimester and into the neonatal period (540). Excessive GHRH or deficient SS release from and function in the fetal hypothalamus, excessive somatotroph responsiveness to GHRH, deficient negative feedback control by IGF-I, or stimulation of GH secretion by paracrine factors may account for GH hypersecretion at mid-gestation.

Functional hypersomatotropism is still present in small for gestational age (SGA) newborn twins as revealed by higher baseline GH levels and stronger GH responses to GHRH than in appropriate gestational age (AGA) twins (302, 633). Interestingly, in these studies mean serum IGF-I was higher in SGA than in the AGA newborns, which would rule out any underlying effect of a defective IGF-I feedback mechanism or functional GH resistance (see also sect. VI). Growth hormone-releasing hormone appears in the rat and human hypothalamus by 18–20 days and 18–22 wk of gestation, respectively (292, 514, 748), and at late gestation and mid-gestation, respectively, rat and human fetal pituitary cells respond in vitro to GHRH or SS by increasing or reducing GH secretion (559, 748). Miller et al. (725) studied changes occurring after birth in pulsatile GH release of male and female term infants. There were significant differences between infants studied at ~28 h and later at ~80 h postnatally. Spontaneous pulsatile release was present in all infants; within the first 1–2 days of life, GH release was enhanced through the amplitude and frequency of pulses, then as the infant matured, there was a decrease in GH frequency, amplitude, and nadir. The decrease in GH levels in older infants very likely resulted from decreased pituitary secretion, secondary to enhanced IGF-I hypothalamo-pituitary feedback or, as shown in the postnatal rat, to changes in the pituitary sensitivity to SS or GHRH.

Considering that rats are born at the equivalent of 100 gestational days in the human (559), the ontogeny of neuroendocrine control and GH secretion in the human is chronologically consistent with that in the rat.

In initial studies of the 24-h pattern of spontaneous GH secretion, a small sample of prepubertal children
lacked waking GH peaks and secreted GH only during sleep (356). This observation, however, contrasted with many subsequent reports of GH peaks during waking hours as well as during sleep (726, 978). Deconvolution analysis depicted the pattern of GH secretory events subserving the changes in serum GH concentrations in normal boys at various stages of puberty and young adulthood (660). Late pubertal boys secreted more GH per 24 h than boys in any other pubertal group and triple that of prepubertal boys (1,800 vs. 600 \text{g/24 h}). After normalization of GH secretion for body surface area, late pubertal boys still had higher GH release than any other pubertal group. The primary neuroendocrine alteration responsible for the enhanced GH secretion was an increased mass of GH released per secretory burst, with no change in GH burst frequency, burst duration, or serum GH half-life.

In girls, GH responses to GHRH did not change throughout pubertal development, and the responses in boys at midpuberty were somewhat lower than either prepubertal or adult men (395). It cannot be excluded, however, that periodic release of SS might have obscured the genuine GH response to GHRH at puberty.

In young adults, total GH secretion was almost half that in late pubertal boys (900 \mu g/24 h), but the 24-h burst frequency was the same. This figure compares favorably with reports based on other techniques (574, 694). However, absolute GH secretion rates estimated by deconvolution analysis are dependent on the specific assay system, and the values reported here tend to overestimate GH secretion compared with the more recent and sensitive assays. Studies in which a large number of prepubertal and pubertal girls and boys were evaluated for GH secretion using the Pulsar program (713) found no correlation between GH secretion and age in the prepubertal children, supporting the proposal that gonadal hormones are not important for the regulation of growth and GH secretion during childhood, whereas at puberty there was a marked increase in GH secretion rates in both sexes (23). However, the change was sex specific, since the increase in GH secretion rate was an early event (stages 2–4) in puberty for girls but a late event (stages 3–4) in puberty for boys, parallel to the height velocity curves of girls and boys in puberty (1019). Shortly after cessation of linear growth in boys, the overall peripheral GH pulse pattern returned toward prepubertal levels so that the concentration profiles in young men were remarkably similar to those in prepubertal boys despite the rising testosterone concentrations (661).

The gonadal hormones, particularly circulating androgens, appear to play a central role in the GH pulse amplitude changes (619, 661). However, androgens alone do not seem to account entirely for the pubertal increase in GH pulse amplitude in humans, in view of the pattern in young men (see above), and although ample data in rats support it (see Ref. 970), the evidence that testosterone is involved in the control of GH secretion in humans is not so clear. To explain these discrepant findings, it would seem, however, that testosterone itself has little or no effect and that it is the estradiol derived from aromatization of testosterone that increases GH secretion (1065). This is suggested by the potent action of physiological amounts of \text{E}_2 in stimulating pulsatile GH secretion and the fact that nonsteroidal anti-\text{E}_2 inhibits it (see Ref. 557 for review).

However, apart from conversion to \text{E}_2, a role for androgens cannot be dismissed. In prepubertal boys with constitutional delayed growth, both testosterone and the nonaromatizable androgen oxandrolone raised the daily GH secretory rate by specifically increasing the total mass of GH released per pulse, with no effect on GH burst frequency or the half-life of endogenous GH (1045). Supporting these findings, an increase in GH in response to GHRH was suggested in one study of boys with constitutional growth delay treated with oxandrolone for 2 mo (633), and increased plasma \text{IGF-I} concentrations have also been reported (980). In boys with isolated hypogonadotropic hypogonadism, progressively higher testosterone doses, which affected plasma \text{E}_2 levels, increased the GH secretory burst number and amplitude and GH response to combined GHRH and arginine (427).

The effect of endogenous estradiol concentrations on total and pulsatile GH release in humans also emerges from a study that evaluated the separate and combined effects of age and sex in young adults and aged women and men (474). The integrated GH concentration was significantly higher in women than men and in the young than the old. A major finding was that estradiol but not testosterone concentrations correlated with indices of total and pulsatile GH release, i.e., integrated GH concentration (IGHC), pulse amplitude, number of large pulses, and fraction of GH secreted per pulse (FGHP) so that on removing the variability due to free estradiol, neither sex nor age influenced IGHC or mean pulse amplitude. The finding, however, that aging still affected the residual variability, i.e., large pulses and FGHP, indicated that other factors in addition to \text{E}_2 contribute.

That aging lowers GH secretion in mammals is almost a tenet of neuroendocrinology (754). In male and female rats, the amplitude of the GH pulses is significantly smaller, and in male rats, this results in lower mean secretion of GH to about one-third that in young rats. In both female and male old rats, the frequency of GH pulses does not change with age (970). In humans, the mean concentration of GH over 24 h is similar after the fourth decade of life to that in prepubertal children and is about one-quarter of that in pubertal youngsters (1120). In addition, approximate entropy analysis in healthy aging men revealed a progressive increase in the entropy of 24-h GH profiles, which signifies less orderliness or regularity of GH release with age (1064).
These findings complement earlier reports of substantial changes in GH secretion in the aging human (169). Despite evidence of a preserved pituitary GH pool (see sect. vB3) and the strong belief that, as in old rodents, the primary disturbance is hypothalamic, a low GH response has been clearly shown to many secretagogues, including GHRH (406, 478). In agreement with the GH hyposecretion, decreased plasma IGF-I levels have also been found (see sect. ivD1). However, investigations on this topic are not unanimous in reporting a decline in GH release with age, and it has been suggested that a significant decline occurs only in women (see above and Ref. 474). In a study in 142 healthy elderly people of both sexes, aged 60–90 yr, basal GH levels showed a very weak positive correlation with age, whereas IGF-I showed a highly significant negative correlation; basal GH and IGF-I did not correlate with each other (518).

It is likely that other confounding variables, such as institutionalization, body mass, nutritional status, and sex steroid hormone concentrations, exert either independent or combined effects on total and pulsatile GH release. To control some of the confounding variables, a group of normal adult males was studied using deconvolution analysis to clarify the specific features of GH secretion and clearance related to age and adiposity, alone or jointly (509). Age was a major negative statistical determinant of GH burst frequency and endogenous GH half-life; body mass index (BMI), an indicator of relative adiposity, correlated negatively with GH half-life and GH secretory burst amplitude. Age and BMI both correlated negatively with the daily GH secretion rate and together accounted for >60% of the variability in 24-h GH production and clearance rates. On average, it was estimated that for a normal BMI, each decade of increasing age reduced the GH production rate by 14% and the GH half-life by 6%. Each unit increase in BMI, at a given age, reduced the daily GH secretion rate by 6%.

Thus a logical corollary is that age, sex, pubertal status, BMI or other indices of adiposity, quality and quantity of sleep, medication use, and physical exercise are but a few of the confounding factors that must be considered in the design and interpretation of clinical studies.

For patterns of GH secretion in different pathophysiological contexts such as fasting, exercise, type I diabetes mellitus, obesity, see the corresponding sections.

C. Sleep

It has long been recognized that GH release increases during the night (498, 1003) and nocturnal blood sampling for GH has been proposed as a diagnostic alternative to pharmacological testing of GH secretory capacity (470, 560). Early studies indicated a consistent relationship between slow-wave sleep (SWS) and GH secretion during early sleep and later during the night (503). Subsequent studies, with 30-s sampling of plasma GH during sleep, showed mean GH concentrations and secretory rates were significantly higher during stage 3 and 4 sleep than during stages 1 and 2 and rapid-eye-movement (REM) sleep. Growth hormone secretory rates and peripheral GH concentrations were maximally correlated with sleep stage in a fashion suggesting that GH release is maximal within minutes of the onset of stage 3 or 4 sleep (486). The temporal correlation between pituitary GH secretion and SWS is consistent with a model in which altered cortical activity is transmitted through GHRH and SS centers to the hypothalamus, and further stimulation by these centers is conveyed to the pituitary.

These findings supported the concept that the daily GH secretory output is dependent on the quality and occurrence of sleep and were consistent with results of sleep reentry and SWS deprivation studies (923). However, a series of reports, e.g., pronounced nocturnal GH surges divorced from the presence of SWS (526), selective SW stage deprivation failing to suppress or delay the sleep-onset GH pulse (126), temporal disconnection between the effect of GHRH on SWS and on GH release (984), challenged this concept and suggested that GH and SWS are not causally related but are two independent outputs of a common hypothalamic GHRH mechanism. However, studies in human African trypanosomiasis (sleeping sickness) have shown that the association between GH secretion and SWS persisted despite disrupted circadian rhythms (871), further suggesting that sleep and stimulation of GH secretion are outputs of a common mechanism.

A careful study in healthy young men examined how normal and delayed sleep and time of day affected pulsatile GH secretion, analyzed by the deconvolution method and blood sampling at 15-min intervals (1054). During normal waking hours, the GH secretory rate was similar in the evening and morning, double during wakefulness at times of habitual sleep, and tripled during sleep even when it was delayed until 0400 h. Interestingly, the amount of GH secreted in response to sleep onset was closely correlated with the levels during wakefulness, with the hourly GH output in these young men being two to three times greater when asleep than when awake.

Although these results demonstrated that SWS facilitates GH secretion, they did not show that SWS is obligatory for nocturnal GH secretion to occur. Approximately one-third of the nocturnal GH pulses occurred in the absence of SWS, and approximately one-third of the SWS periods were not associated with increased GH secretion. Thus circadian timing, regardless of sleep, influences somatotrophic activity, although under normal conditions sleep occurs at the time of maximal propensity for GH
secretion, with circadian and sleep effects being superimposed.

In a sequel to these studies, GHRH or saline was administered intravenously to young healthy men at various times of day and stages of sleep. When injected during the waking hours, GHRH elicited a response whose magnitude was directly related to the amount of spontaneous GH secretion, negatively correlated with circulating levels of IGF-I and was not influenced by time of day. The response was greater when GHRH was given during SWS, whereas when GHRH was given during REM, it was similar to that observed during waking. This suggests that the two sleep stages may be associated with reduced and enhanced SS secretion, respectively. Awakenings during sleep consistently inhibited the secretory response to GHRH, which reappeared with resumption of sleep (1053).

Because the intersubject variability in GH responses was very wide but the response was quite reproducible for each subject, it would seem that there is no diurnal variation in the sensitivity to exogenous GHRH and that the GH response to GHRH is dependent on the sleep or waking condition, circulating levels of IGF-I and, possibly, genetic and life-style factors.

For the electrophysiological effects of GHRH and GHRP, see sections A10 and nC.

III. HYPOPHYSIOTROPIC HORMONES

A. Growth Hormone-Releasing Hormone

1. General background

The regulation of GH secretion has been the focus of research since Reichlin’s pioneering observation in 1960 (879) in the rat that surgical destruction of the ventromedial hypothalamus slows growth velocity. Parallel to the identification and sequencing of most of the other hypothalamic regulatory hormones at the end of the 1960s and during the 1970s was the discovery of the GH release inhibiting hormone SS, the principal modulator of GH secretion (141).

Although the possibility had been advanced that changes in SS secretion might account for all the hypothalamic influences on GH secretion, a hypothalamic stimulating hormone was needed. Growth hormone-releasing factor or GHRH was only identified and sequenced ~10 years after SS.

The identification of GHRH represented an exception to the rule, since this neuropeptide is unique among regulatory hormones in that it was first isolated (1121) and sequenced (447, 892) from human and not animal tissue, and, secondly, not from the hypothalamus but from pancreatic islet tumors, in which the ectopic secretion was associated with hypersecretion of GH and acromegaly (376).

The discovery of GHRH has been reviewed extensively (see Refs. 274, 749) and is only briefly alluded to here. Growth hormone-releasing hormone was isolated in 1982 from two patients in whom pancreatic tumors had caused acromegaly. From one of these tumors a 40-amino acid peptide designated human pancreatic growth hormone-releasing factor hpGRF-(1–40) was isolated (892), and three GHRH peptides, GHRH-(1–44)-NH₂, GHRH-(1–40)-OH, and GHRH-(1–37)-OH, were sequenced from the other tumor (450). Structure-activity relationships showed that the NH₂-terminal 29 residues of GHRH-(1–40)-OH have biological activity and indicated that although the NH₂-terminal portion of the molecule is involved in binding to the receptor, the COOH-terminal portion is critical in regulating the potency of GHRH.

Two of the three GHRH forms found in tumors were subsequently identified in human hypothalamus [GHRH-(1–44)-NH₂ and GHRH-(1–40)-OH] and differed only by the absence in the latter of the four COOH-terminal amino acid residues (613, 617). Identification of the GHRH sequence in pigs, goats, sheep, and cattle has shown the existence of the 44-amino acid form amidated at the COOH terminal (617). Rat hypothalamic GHRH (rGHRH) contains 43 amino acids with a free acidic group. In addition, rGHRH is structurally different from the other GHRH peptides [14 amino acid substitutions or deletions compared with GHRH-(1–44)] (see Refs. 756, 979).

Growth hormone-releasing hormone belongs to an expanding family of brain-gut peptides that includes glucagon, glucagon-like peptide I (GLP-I), vasoactive intestinal polypeptide (VIP), secretin, gastric inhibitory peptide (GIP), a peptide with histidine as NH₂ terminus and isoleucine as COOH terminus (PHI), and pituitary adenylate cyclase-activating peptide (PACAP) (160).

2. Synaptic communication in the hypothalamus

The availability of GHRH-(1–40)-OH and GHRH-(1–44)-NH₂ means the topography of GHRH neurons could be established in humans and a number of animal species (see Ref. 756 for review). There are slight species differences in GHRH distribution. Growth hormone-releasing hormone IR is present mostly in the basal hypothalamus and is appropriate anatomically for release into the pituitary portal vasculature. In early studies, GHRH antiseras stained neuronal cell bodies in the ARC (infundibular nucleus) of squirrel monkeys and humans with

1 The conclusion that the material of hypothalamic origin was identical to the previously identified tumor-derived GRF avoided the need to keep the nomenclature human pancreatic GRF or the abbreviation “hpGRF”; the abbreviation GHRH will be used in the text and to refer to any of the active sequences. The term somatocrin was proposed to replace GHRH, but has not been widely accepted.
fibers projecting to the ME and ending in close contact with the portal vessels (114). Studies in macaques and squirrel monkeys revealed GHRH-IR neurons also in the ventromedial nucleus (VMN) (114), where GH release may be increased by electrical stimulation (664). These neurons were also seen in the rat hypothalamus (708 and see below).

In the rat, GHRH-positive perikarya were located in the following regions: ARC from the rostral portion as far caudally as the pituitary stalk, medial perifornical region of the lateral hypothalamus, lateral basal hypothalamus, paraventricular nucleus (PVN) and dorsomedial nucleus (DMN), and the medial and lateral borders of the VMN (708). Immunocytochemical studies in humans showed a similar distribution of GHRH-positive perikarya, mainly within, above, and lateral to the infundibular nucleus. In addition, some GHRH staining cells were found in the perifornical region and in the periventricular zone (114, 115).

Careful charting of the distribution of GHRH-IR in the brain of adults rats was consistent with at least two distinct GHRH-containing systems, one responsible for delivery of the peptide to portal vessels in the ME and one whose relationship to hypophyseotropic function was less direct (see sect. mA10).

Growth hormone-releasing hormone neurons innervating the external layer of the ME were centered in the ARC on each side of the brain, whereas the other GHRH-stained cells, very likely contributing to the extrahypophyseotropic GHRH-stained projections, were in the caudal aspect of the VMN. From here, GHRH fibers could be traced projecting to the periventricular region of the hypothalamus including the preoptic and anterior part, where SS-containing neurons are clustered (see below and sect. mB1). Other important terminal fields were found in discrete parts of the DMN, PVN, suprachiasmatic nucleus (SCN), and premammillary nuclei and the MPOA and lateral hypothalamic area. Beyond the hypothalamus, sparse projections were traced to the lateral septal nuclei, the bed nucleus of the stria terminalis, and the medial nucleus of the amygdala (926). Growth hormone-releasing hormone projections in the amygdala, a part of the visceral brain involved in emotion and responses to stress, may account for GH responses elicited by electrical stimulation of this area in rats (663) or humans (639) and suggest the amygdala in humans may be part of the pathways responsible for stress-induced GH release.

Immunohistochemical (292, 514) and RIA (525) measurements indicated that GHRH is first detectable at embryonic days 18–20 in the rat, reaching adult levels at postnatal day 30 (525), and in situ hybridization has detected GHRH mRNA 1 day earlier (896). In the human fetus, ontogenetic studies detected GHRH neurons between 18 and 29 wk of gestation (115, 143). These figures are on the whole consistent with the appearance of fetal pituitary somatotropes and GH storage and release in plasma (see sect. mB2).

Colocalization studies in the rat using radioimmunologic, immunohistochemical, and in situ hybridization techniques indicated that a subset of GHRH neurons in the ventrolateral part of the ARC contains a large number of molecules including tyrosine hydroxylase (TH), the key enzyme of catecholamine (CA) biosynthesis, GABA, choline acetyltransferase (ChAT), the ACh synthesizing enzyme, neurotensin (NT), and galanin (GAL) (see Refs. 697, 756 for review) (Fig. 2). From a functional viewpoint, particularly important colocalizations are with NT, ChAT, and GAL, in view of the clear involvement of these compounds in GH release (see sect. mB2). Other studies have shown colocalization of GHRH with neuropeptide Y (NPY), in neurons which do not project to the external layer of the ME but constitute a tubero-extra-infundibular system, not involved in direct control of the AP, but presumably having a neuromodulatory function (see Ref. 243 and sect. mB6).

Direct proof that cells in the ventrolateral ARC are likely to be the main source of GHRH-containing projections to the ME has been provided using a combination of retrograde tract-tracing and immunocytochemistry in normal and GH-deficient dwarf mice (902). Inferential evidence had been provided with the use of monosodium glutamate (MSG), a neurotoxic substance which destroys about 80–90% of the cell bodies in the ARC (794), without significantly injuring other hypothalamic regions or axons of passage. Injection of MSG into rats resulted in the virtually complete disappearance of GHRH-stained fibers in the ME (115), stunted skeletal growth, and severe obesity. Interestingly, these changes were combined with marked decreases in ME-stained fibers containing GAL, the GABA synthesizing enzyme, glutamic acid decarboxylase (GAD), dynorphin, and enkephalins, but not those containing SS and NPY, meaning these peptides do not project to the ME (NPY) or to the ME from another source (SS) (696, see also sect. mB1).

All in all, the localization of GHRH-IR neurons mainly in the ARC and in the capsule surrounding the VMN is consistent with the results of stimulation and ablation studies that implicate the medial basal hypothalamus (MBH) in the stimulatory control of GH secretion (see Ref. 749).

3. Growth hormone-releasing hormone-somatostatin interactions

The neuronal systems regulating GHRH and SS release receive a variety of neural inputs, many of which are described fully in the next few sections. Here it already seems appropriate to look at the anatomic and functional interactions occurring between GHRH and SS neurons, which are so important for understanding many aspects of GH control.
The organization of hypothalamic SS-containing neurons is essentially similar in all mammalian species studied so far, including humans (see sect. III B1). Most hypothalamic areas contain an extensive network of SS-containing fibers, and there are IR-SS perikarya in the anterior periventricular area and the parvocellular portion of the PVN and in the SCN, ARC, and VMN. Combined retrograde tracing and immunohistochemistry showed that up to 78% of periventricular neurons project to the external layer of the ME, and other hypothalamic neurons do not innervate this neurohemal organ (555). The SS axons and perikarya in the ARC and VMN, two areas from which GHRH neurons originate, indicate the existence of anatomic and functional peptide interactions. Immunocytochemical double-labeling studies located SS-producing neurons in the dorsomedial part of the rat ARC and found SS-IR axons in the lateral part of the nucleus, an area containing GHRH-synthesizing cells (622). Several somatostatinergic axon varicosities were clustered around single GHRH synthesizing cells, suggesting synaptic associations. In the external layer of the ME, axonal terminals immunolabeled for GHRH and SS had the same pattern of distribution and close proximity.

More morphological proof of SS-GHRH interactions in the ARC was provided by autoradiography studies showing a selective association of $^{125}$I-labeled SS binding sites with a subpopulation of cells, concentrated rostrocaudally in the lateral portion of the ARC; their distribution was remarkably similar to that of GHRH-IR neurons (338). That at least some SS receptors may be directly linked to GHRH-containing cells was demonstrated in colocalization autoradiography and immunohistochemistry studies in which $\approx 30\%$ of the IR-GHRH cells labeled with $^{125}$I-SS in sections adjacent to GHRH cells in extra-ARC regions did not show SS binding (690). Interestingly, most of the GHRH-SS colabeled cells were detected in the ventrolateral region of the ARC, where there is a dense fiber network of SS-nerve terminals (see above).

An ultradian pattern has also been reported in SS binding to ARC neurons in relation to the peaks and troughs of the GH rhythm (1010), although the changes were in the direction opposite those expected. Further
direct evidence of cellular colocalization of SS receptors and GHRH-producing cells within the ARC was provided by in situ hybridization studies showing that in the ventrolateral portion of the ARC GHRH mRNA labeled neurons had the same distribution as $^{125}$I-SS labeled cells (97) (Fig. 3). For the SS receptor subtypes involved, see section II.B2.

Two other cell types could be identified in these studies: GHRH perikarya, around the perimeter of the VMN not associated with pericellular SS binding sites, and very likely containing substance P and enkephalin-8 binding sites (282), and $^{125}$I-SS labeled cells not associated with GHRH perikarya, in the periventricular zone along the dorsal part of the third ventricle, probably associated with $\beta$-endorphin- (558) or TH-producing (484) cells.

With regard to GHRH inputs to SS-IR neurons, only a few of these neurons were closely approached by GHRH-IR fibers, indicating that SS neurons are only scantily innervated by GHRH cells (1098) and suggesting the relative independence of SS from GHRH.

The data reported provide strong morphological support for a direct hypothalamic control of GHRH neurons...
by SS and for the concept that the two hypophysisotropic peptides interact within the central nervous system (CNS) to modulate GH secretion (see also sect. ii). A wealth of physiological evidence bears witness to the interaction, including a rapid increase in plasma GH after intracerebroventricular administration of SS to anesthetized (2) or awake rats (641), depending at least in part on hypothalamic GHRH (762); increased GHRH concentrations in hypophysial portal blood after intracerebroventricular administration of SS antiserum (853); elevated basal GH levels, which can be reduced by anti-GHRH serum after lesions of the hypothalamic MPOA (548); and triggering of GH secretion through a mechanism involving GHRH, after acute withdrawal of endogenous (722) or exogenous SS (210, 244, 964). For the GHRH-SS interactions in ultrashort feedback mechanisms, see section iii.

4. Distribution outside the CNS

Like other neuropeptides originally described as primarily of hypothalamic origin, GHRH has also been found in peripheral tissue, although its physiological role as an extrahypothalamic hormone, in addition to stimulating GH secretion, is far from clear. Activity of GHRH outside the brain was initially only found in neoplastic tissues producing GHRH (see sect. iiA1). However, the hormone was then seen in the upper intestine, particularly in the jejunum, with smaller amounts in the duodenum. None was found in the ileum or colon. The peptide was confined to the epithelial mucosa, suggesting a possible endocrine role. In addition to the gastrointestinal tract, mRNA for GHRH was found in leukocytes, where GHRH inhibits human natural killer cell activity (819) and also affects human leukocyte migration (1126), in the testis (94), ovary (65), and placenta (657). In the rat testis, the estimated size of GHRH-like immunoreactivity (GHRH-LI) was 3.7 times that of synthetic GHRH (831), and it was found in the interstitial tissue (740) and in mature germ cells (831), its function being to amplify the action of GH on Leydig cells (steroidogenesis) and of follicle-stimulating hormone on Sertoli cells (cAMP formation) (240). In view of the existence of a blood-testis barrier and the abundance of GHRH in rat testis, it presumably acts as an autocrine or paracrine factor.

In the rat placenta, GHRH mRNA is distinct from that described from the hypothalamus, and placental GHRH is synthesized in the cytotrophoblast (657). Cells expressing GHRH are located differently in the mouse placenta (992). The GHRH peptide and mRNA have also been identified in the human placenta (95). Placental GHRH may have a role in regulating fetal pituitary somatotrophs (445) and placental growth factors in an autocrine and/or paracrine fashion (476). Initial studies in the somatotrophs found IR-GHRH in secretory granules and the nuclei (738), and others found it internalized in another intracellular compartment (705).

5. Gene structure and expression

Pancreatic tumors from which the GHRH peptide was purified provided the mRNA source for the construction of libraries from which cDNA encoding the human GHRH precursor could be isolated (444, 685). A rat GHRH cDNA was isolated directly from a hypothalamic library (681). Using cDNA to screen human genomic libraries resulted in the elucidation of the human GHRH gene (682), and the rat and mouse GHRH genes have also been identified in the genome (991).

Like most small peptides, GHRH is generated by the proteolytic processing of a larger precursor protein. This is a single copy in the human genome, and it has been mapped to the p12 band of chromosome 20 in the human (682) and chromosome 2 in the mouse (433). The gene includes five exons spanning ~10 kb of DNA, with clear segregation of the functional domains of the GHRH precursor into the five exons of the genes (for further details, see Refs. 377, 683).

The GHRH precursor proteins identified in the human, the mouse, and the rat range in length from 103 to 108 amino acids; their sequences are fairly homologous, with the amino acids being closest in the NH2-terminal portion of the mature GHRH peptide, the region crucial for GHRH binding and biological activity (160). The rat and mouse proteins differ completely from the human GHRH precursor near the COOH terminus. In the rat, although most of the 104 amino acids of the GHRH precursor protein are similar to the human precursor, the last 13 residues in the COOH-terminal domains are all different (678).

The cDNA probes for GHRH made it possible to examine gene expression in the mammalian hypothalamus. In situ hybridization analysis of GHRH mRNA in the rat brain showed without doubt that GHRH-expressing neurosecretory cells, as revealed by immunocytochemistry (see sect. iiA2), are an actual site of GHRH synthesis (89, 678, 1125). In agreement with RIA and immunohistochemical findings, most of the GHRH-expressing cells were on the ventral surface of the hypothalamus within the ARC, with additional cells extending into the DMN. No substantial expression was observed in brain regions outside the hypothalamus.

It is now clear that the hormonal and metabolic status of the animals dictates appropriate changes of GHRH mRNA expression in ARC neurons. The GHRH mRNA levels are higher in male than female rats, and testosterone is reported to increase the expression of GHRH mRNA in males and to prevent the decline due to castration. This latter effect is shared by dihydrotestosterone, suggesting direct activation of the androgen re-
The effects, i.e., Ca\(^{2+}\) release. Multiple second messenger pathways underlie activation of GABAergic function (63) impaired GHRH showed the releasing effects of K\(^{+}\) use. Such systems have been used to evaluate GHRH secretion. Such systems have been used to evaluate GHRH secretion (see sect. viC1). Streptozotocin-induced diabetic rats have diminished GH secretion, which can be restored by an anti-SS serum (1007). However, GHRH mRNA levels are low, and SS mRNA levels are high, indicating a metabolic regulation independent of GH (793) (see also sect. vi for discussion).

Hormonal feedback regulation through GH appears to be the most important factor in GHRH mRNA expression. Growth hormone deficiency induced by target organ removal (hypophysectomy) or selective abrogation of GHRH function is associated with increased GHRH mRNA levels, whereas GH treatment lowers them (237, 290, 678), although not always (202) (see sect. vii for discussion).

Levels of hypothalamic GHRH do not always change in parallel with GHRH mRNA (237, 386) because they also reflect a different rate of peptide increase and impairment of GHRH mRNA translation. Thus proper interpretation of changes in GHRH content requires independent information on GHRH mRNA levels and/or GHRH secretion.

The expression of GHRH mRNA in the placenta and other tissues is discussed above.

6. Secretion and metabolism

In vitro studies with the use of both long-term cultures of fetal rat hypothalamus and short-term static incubations of adult rat hypothalami with perfusion have been used to evaluate GH secretion. Such systems showed the releasing effects of K\(^{+}\)-induced depolarization (547) and the increased release after hypophysectomy (237) and thyroidectomy (324) in response to low glucose or intracellular glucopenia (64) and \(\alpha_2\)-adrenergic stimulation (536). Conversely, IGF-I (959), SS (1114), and activation of GABAAergic function (63) impaired GHRH release. Multiple second messenger pathways underlie these effects, i.e., Ca\(^{2+}\), phosphatidylinositol-protein kinase C, and adenylyl cyclase-protein kinase A (63, 277).

In vivo picomolar to nanomolar concentrations of GHRH have been reported in the rat and the sheep portal venous blood. In anesthetized, hypophysectomized rats, portal plasma levels of GHRH were \(\sim\)200 pg/ml with synchronized pulses up to 800 pg/ml (853). In freely moving, ovariectomized ewes, pulsatile GHRH secretion reached a mean of 20 pg/ml, with peak values from 25 to 40 pg/ml and a secretion rate of 13 pg/min. About 70% of spontaneously occurring GH peaks could be attributed to GHRH pulses, as indicated by simultaneous measurement of peripheral GH levels (378, see also sect. ii).

Unstimulated plasma levels of GHRH were in the range of 10–70 pg/ml in normal adults (827, 1032), with no apparent sex-related differences (924). Comparably low values have been reported in the cerebrospinal fluid (CSF) of children and adults of both sexes with no endocrine diseases (541). Growth hormone-releasing hormone is high in the cord blood from term human newborns (39), as are levels of GH (see sect. iiB). Normal children of both sexes between ages of 8 and 18 had levels five times higher at mid-puberty than prepuberty in girls and double in boys (41), supporting the central role of gonadal hormones (see also sect. iiB).

The source of GHRH-LI in human plasma is not known, but the comparable levels in normal subjects and patients with hypothalamic lesions (232) suggest that the major source is the hypothalamus (see also sect. iiA4). Food (972) or glucose injection (542) can raise GHRH-LI levels, and this also occurs in patients with hypothalamic lesions or in GH-deficient subjects. These observations add weight to the idea that GHRH produced in the periphery (stomach, pancreas, small intestine) (239) may also act as a paracrine or autocrine factor. The hypothalamic component of circulating GHRH-LI, however, is specifically stimulated by insulin-hypoglycemia (542, 909; but see also Ref, 1022) or levodopa (231, 542, 914), but not by clonidine or arginine (1085).

Other conditions that increase circulating GHRH levels include the initial slow stage of sleep (914) and the sauna bath in young subjects (605). For other details on GHRH levels in GH hypo- and hypersecretory states, see Reference 749.

Growth hormone-releasing hormone is metabolized in human plasma by dipeptidyl peptidase type IV (DPP-IV) and trypsinlike endopeptidases (375, 380). Degradation comprises an initial step whereby DPP-IV removes the NH\(_2\)-terminal dipeptide (Tyr-Ala) leaving metabolites [GHRH(3–44)-NH\(_2\) and GHRH(3–40)-OH] with no bioactivity. Independent cleavage by trypsinlike endopeptidase at residues 11–12 also inactivates the peptide. Because the half-life of GHRH(3–44)-NH\(_2\) in vivo is considerably longer (55 min) than the native molecule (15 min), most of the plasma GHRH-IR is in the biologically inactive form. Studies with bovine GHRH suggest metabolic disposal is similar to human GHRH (579).

7. Mechanism of action

Growth hormone-releasing hormone exerts its primary control of somatotrophic function by increasing GH
secretion, GH gene transcription and biosynthesis, and somatotroph proliferation. A host of intracellular second messenger systems including the adenylate cyclase-cAMP-protein kinase A, Ca\(^{2+}\)-calmodulin, inositol phosphate-diaclyglycerol-protein kinase C, and arachidonic acid-eicosanoid pathways are activated after binding to specific GTP-linked receptors in the plasma membranes (see also Ref. 683).

Earlier evidence that cAMP was involved as a second messenger in the stimulation of GH secretion rested on the demonstration that crude ectopic GRF preparations, cAMP analogs, and theophylline, a phosphodiesterase inhibitor, stimulated GH release (822, 938, 997). With GHRH in pure form, it was then shown that GH release was associated with a dose-dependent rapid stimulation of adenylate cyclase activity and cAMP production in the somatotrophs (102). Within 5 min of the addition of 1 nM GHRH to cultured rat pituitary cells, intracellular cAMP levels rose 6-fold, with a maximal response at 30 min; 10 times more GHRH was required to obtain half-maximal stimulation of cAMP production than GH secretion, suggesting that only partial receptor occupancy is required to elicit a maximal GH response (1066).

Growth hormone-releasing hormone-induced stimulation of cAMP production would occur in the manner typical of receptors coupled to GTP-binding proteins (106). The occupied GHRH receptor activates a heterotrimeric stimulatory G protein (G\(_s\)), composed of \(\alpha\), \(\beta\), and \(\gamma\)-subunits, by catalyzing the binding of the \(\alpha\)-subunit to GTP. Guanosine 5'-triphosphate binding leads to dissociation of the \(\beta\gamma\)-subunit complex from the \(G_s\) \(\alpha\)-subunit, and the latter then activates the catalytic subunit of adenylate cyclase. Thus adenylate cyclase stimulation by GHRH is dependent on GTP (769, 974), but nonhydrolyzable GTP analogs or a high concentration of GTP reduced GHRH receptor binding (988), and stimulation of adenylate cyclase (974), consistent with existing models for receptor-G protein interactions. Like GHRH, agents that bypass the receptor, such as cholera toxin, which decreases the GTPase activity of \(G_s\alpha\) (182) or forskolin, which directly activates the catalytic subunit of adenylate cyclase (944), potently stimulated cAMP accumulation and GHRH release (140, 275).

In the absence of GHRH or other hypothalamic peptides, somatotropes in culture have either a stable basal intracellular Ca\(^{2+}\)-concentration ([Ca\(^{2+}\)]\(_i\)) level (487) or asynchronous spontaneous [Ca\(^{2+}\)]\(_i\) transients (487) that result from Ca\(^{2+}\) entry through Ca\(^{2+}\)-permeable channels (487).

Extracellular Ca\(^{2+}\) is required for GHRH to stimulate cAMP accumulation in pituitary cell preparations (140), suggesting that Ca\(^{2+}\) is also a second messenger for GHRH and that Ca\(^{2+}\) acts beyond, or independently of, cAMP. On purified preparations of normal rat somatotrophs, GHRH elicited a rapid increase in [Ca\(^{2+}\)]\(_i\), detectable within 5 s (487, 643); this first phase was followed by a decline in [Ca\(^{2+}\)]\(_i\) to a plateau that was always higher than baseline and lasted 5 min (643).

In addition to GHRH, other GH secretagogues rapidly raised intracellular Ca\(^{2+}\) (371, 576). Incubation in low Ca\(^{2+}\) medium or in the presence of Ca\(^{2+}\) channel blockers or antagonists blocked or greatly diminished both the GHRH-dependent increase in [Ca\(^{2+}\)]\(_i\) and GH release, with no changes in the cytosolic Ca\(^{2+}\) levels (102, 140, 487, 644); this was taken to demonstrate that GHRH increases [Ca\(^{2+}\)]\(_i\) in somatotrophs by stimulating Ca\(^{2+}\) influx through L-type voltage-operated channels (VOCC). These effects are highly dependent on external Na\(^+\) and result from a cascade of voltage-sensitive currents (581). The inhibitory action of SS on basal and GHRH-induced GH release resulted from its ability to lower [Ca\(^{2+}\)]\(_i\) by inhibiting Ca\(^{2+}\) influx (643, 644). Somatostatin also inhibited the Ca\(^{2+}\) influx induced by other GH secretagogues, the only exception being high K\(^+\).

These studies led to a model in which a net influx of extracellular Ca\(^{2+}\) through VOCC is the primary source of the first phase of the GHRH-dependent increase in [Ca\(^{2+}\)]\(_i\); (222). Alternatively, the increase in cAMP accumulation might increase Na\(^+\) conductance directly or through protein kinase A-dependent phosphorylation, leading to depolarization and opening of the L-type VOCC (643). A more important role of Ca\(^{2+}\) mobilized from intracellular stores has also been suggested, based on a high correlation between Ca\(^{2+}\) efflux and GH release in perfused somatotrophs or the ability to stimulate GH secretion and Ca\(^{2+}\) efflux despite the absence of extracellular Ca\(^{2+}\) (343, 789).

With regard to the interaction between the two second messenger functions, there is some evidence of a direct relationship between cAMP and Ca\(^{2+}\). W-7, an antagonist of the Ca\(^{2+}\) binding protein calmodulin, inhibited GHRH stimulation of adenylate cyclase, cAMP accumulation, and GH release, whereas the calcium ionophore A-23187 stimulated GH secretion and cAMP production (714, 935).

Admittedly, Ca\(^{2+}\) effects occur together with a metabolic reaction, the hydrolysis of membrane phosphoinositides (PI), and these two processes are functionally connected (780). It was initially reported that GHRH increased 32P incorporation into PI of rat AP cells (165); however, this would be entirely separate from the PI catalysis involving the production of inositol 1,4,5-trisphosphate (IP\(_3\)) and diacylglycerol, since GHRH did not enhance IP\(_3\) (647). Other investigators too have found no early effect of GHRH on PI hydrolysis (260, 370, 875). Although GHRH does not induce PI hydrolysis, synthetic diacylglycerols and phorbol esters, which mimic the effect of the endogenous substrate by activating protein kinase C (184), dose-dependently stimulate GH secretion (535, 790). These compounds bind to the cell membrane.
and then induce translocation of the inactive enzyme from the cytosol to the cell membrane, resulting in a large increase in its affinity for Ca\(^{2+}\). Protein kinase C appears to act synergistically with Ca\(^{2+}\) mobilization to increase cell product release in various systems because low levels of Ca\(^{2+}\) ionophore can potentiate the protein kinase activation (535). This synergism may operate through several mechanisms, and no firm conclusion has been reached (535).

Whatever the underlying mechanism is, it is noteworthy that increased Ca\(^{2+}\) mobilization in the pituitary enhances the sensitivity of the somatotroph to protein kinase C activators (535). It would seem, however, that this mechanism is only marginal to the secretagogue action of GHRH and may better account for the action of GHRP and their analogs (see sect. \(\nu C2\)), and protein kinase apparently increases GH release by mechanism(s) different from those used by GHRH (790).

The fact that GHRH does not enhance IP\(_3\) production and may thus be unable to mobilize intracellular Ca\(^{2+}\) from the IP\(_3\)-sensitive Ca\(^{2+}\) pool implies only a minimal effect on fractional Ca\(^{2+}\) efflux. However, GHRH may affect Ca\(^{2+}\) efflux through the formation of arachidonic acid, derived from the direct activation of phospholipase A and hydrolytic cleavage of the fatty acid from membrane phospholipids or, alternatively, by hydrolysis of diacylglycerol by diacylglyceride. Arachidonate and its metabolites are known to mobilize Ca\(^{2+}\) (567, 765). Growth hormone-releasing hormone and Ca\(^{2+}\) efflux are discussed above.

Supporting a GHRH-arachidone interaction, arachidonic acid elicits a dose-dependent increase in GH secretion and GHRH stimulates arachidonic acid release in cultured rat pituitary cells (166, 534).

Prostaglandin (PG) \(E_1\), \(PGE_2\), and prostacyclin (PG\(_L\)) enhance GH secretion in the rat in vitro (716, 800) and in vivo (551, 800) as well as the production of cAMP (716, 938), probably by stimulation of adenylate cyclase through a G\(_s\) protein-coupled PG receptor (966). Growth hormone-releasing hormone stimulates PGE\(_2\) release from incubated pituitaries, and indomethacin and aspirin, two cyclooxygenase inhibitors, reduce GHRH-induced GH and PGE\(_2\) release (347).

8. GHRH receptor

A) CHARACTERIZATION, CLONING, AND STRUCTURE. A better understanding of GHRH action in promoting GH secretion and linear growth requires knowledge of the GHRH receptor (GHRH-R) structure and function. Previous studies in intact cells or cell membrane preparations of the rat described high-affinity low-capacity specific GHRH binding sites, using GHRH or GHRH analogs (5, 947, 1066). Two studies reported an additional class of low-affinity high-capacity sites (5, 6). Binding sites were also evidenced in bovine pituitaries (1066) and human pituitary tumor tissue (502). Binding of GHRH resulted in the activation of adenylate cyclase, whereas guanine nucleotides inhibited hormone binding, suggesting the involvement of G\(_s\) protein (see sect. \(mA7\) for details).

Initial approaches to identify GHRH-R activity based on functional expression of the receptor in Xenopus laevis oocytes injected with pituitary mRNA were unable to isolate an individual GHRH-R cDNA. More successful was a strategy based on the contention that the receptor, which utilized a G protein to transduce its signal, would predominantly have structural features of other G protein-coupled receptors (679). This led first to the expression cloning of the GHRH-related hormone secretin (513) and then to the recognition that this receptor and the related calcitonin and parathyroid hormone (PTH) receptors constituted a new subfamily of G protein-coupled receptors (see Ref. 683).

Three groups subsequently achieved the molecular cloning of AP receptors for GHRH in human, rat, and mouse (393, 612, 679). The isolated cDNA encoded a 423-amino acid protein containing seven putative transmembrane domains characteristic of G protein-coupled receptors (Fig. 4). Rat cDNA clones also had a 41-amino acid insert at amino acid 325. However, PCR analysis of rat pituitary mRNA only gave evidence of the shorter form (679). The two rodent receptors were closely related (94% identical) and were also homologous to the human receptor (82% identical). The GHRH-R colocalizes with GH cells (612), although studies about cell-specific expression of GHRH-R are not yet complete.

As mentioned in section \(mA7\), GHRH belongs to a family of brain-gut peptides, and a similar family of receptors has now been identified that includes the VIP receptor, the glucagon receptor, the GLP-1 receptor, the PACAP receptor, and the GIP receptor. These peptide receptors also show consistent homology with the GHRH receptor (see Ref. 945).

Transient expression of rat cDNA in COS cells induced saturable, high-affinity GHRH-specific binding and stimulated the accumulation of cAMP in response to physiological concentrations of GHRH, consistent with the predicted coupling of the GHRH-R to a G\(_s\) protein. Binding and second messenger responses were blocked by a specific GHRH antagonist, whereas ligands for related receptors (see above) did not effectively compete with GHRH for binding to its receptor (683).

Initial Northern analysis studies indicated that GHRH-R mRNA was mainly expressed in extracts of pituitary and not in other tissues. Two transcripts of ~2.5 and 4 kb were identified in rat pituitary, 2.0 and 2.1 kb in mouse, and 3.5 kb in ovine pituitary (393, 612, 689). Mouse studies showed that the GHRH-R is expressed in a temporal and spatial pattern corresponding to GH gene expression (612).
The identification of the pituitary-specific transcription factor, Pit-1, which is involved in developmental generation of somatotrophs (lactotrophs and thyrotrophs) and in the appropriate regulation of the GH gene (120, 507) and very likely also in direct regulation of the GHRH-R mRNA, has contributed to our understanding of GHRH-R biology. Growth hormone-releasing hormone plays a major stimulatory role in regulation of the Pit-1 mRNA concentration, although Pit-1 is preserved in the pituitaries of anencephalic fetuses (845), an effect mimicked by other GH secretagogues such as PACAP-38 but not by GHRP-6 (973). The regulation is apparently reciprocal, since GHRH-R is not expressed in the pituitary of dw/dw mice, which are impaired in functional Pit-1 gene expression (614). Thus GHRH-R would be dependent on Pit-1, and a deficiency of Pit-1 would result in a lack of GHRH-R gene expression and thus of somatotroph development.

B) FUNCTION. Information about GHRH-R regulation and function or dysfunction offers new insights into the control of GH secretion during development, maturity, and aging and helps explain GH deficiency states of animals or humans leading to dwarfism and metabolic dysfunction.

Pituitaries of fetal and neonatal mammals are highly responsive to the stimulatory effects of GHRH, compared with mature mammals (see sect. II B). Differences in pituitary responsiveness to GHRH may therefore contribute to the elevated plasma GH titers characteristic of the perinatal period and the subsequent decline in circulating GH occurring late in life. Studies of the ontogenic development of rat GHRH-R gene expression have in fact found the highest concentration on day 19.5 of gestation (ED 19.5), with a decline during the perinatal period to reach a nadir at 12 days of age. The GHRH-R mRNA levels increased at 30 days of age, corresponding to the onset of sexual maturation, and then declined later in life (572).

Although the timing of the decline in GHRH-R mRNA, at 12 days, did not precisely coincide with recorded changes in GH responsiveness to GHRH in vitro and in vivo (see sect. II B), there was a dissociation with the
FIG. 5. Changes in GH-releasing factor receptor (GRFR)-to-glycer-aldehyde 3-phosphate dehydrogenase (GAPDH) ratio by treatment with GH-releasing factor antibody (GRF-ab) and/or GH. GRFR/GAPDH were expressed as percent (mean ± SD of 4 independent experiments) of group I. Treatments to each group were as follows: group I, normal rabbit serum (control group); group II, GRF-ab; group III, normal rabbit serum and GH; group IV, GRF-ab and GH. *P < 0.01, analyzed by ANOVA. [From Horikawa et al. (489); Copyright The Endocrine Society.]

developmental pattern of GH mRNA (see Ref. 572 for discussion), and the maturational changes in GHRH-R mRNA abundance were, on the whole, consistent with a similar role in the regulation of somatotroph function during early development. The presence of abundant GHRH-R mRNA as early as ED 19.5 supports the idea that GHRH-R plays a role in mediating GHRH stimulation of somatotroph function by late fetal life and, ultimately, that GH secretion is also involved during this period (146, 1094). These data were consistent with reports that mice passively immunized with a GHRH antiserum (see Ref. 996) expressed similar mutations in GH deficiency syndromes in which the discovery of a mutation in GHRH-R (433) suggested similar mutations in GH deficiency syndromes in mouse and human leads to severe GH deficiency syndromes. The little mouse is an animal model for isolated GH deficiency type 1B (333), in which the discovery of a mutation in GHRH-R (433) suggested similar mutations in GH deficiency syndromes in the human population (see below).

Horikawa et al. (489) showed that in neonatal rats passively immunized with a GHRH antiserum (see Ref. 207), there was a >50% decrease in GHRH-R mRNA; GH treatment significantly reduced the pituitary GHRH-R expression in control rats but not in GHRH antiserum-treated rats (Fig. 5). The marked reduction of GHRH-R mRNA in GHRH-deprived neonatal rats was taken to indicate failure of the developmental induction of pituitary GHRH-R expression by GHRH itself. In control rats, GH acted through an initial decrease of hypothalamic GHRH synthesis induced by short-loop feedback (see sect. viA). A major reduction of pituitary GHRH-R mRNA is also observed in transgenic dwarf mice expressing a TH-human GH fusion gene in the hypothalamus (996).

Regulation of GH secretion by GHRH-R would also be important in the sexually dimorphic expression of GH secretion (see sect. viA) and would thus be of physiological significance. Normal female rats, in fact, had strikingly lower levels of pituitary GHRH-R mRNA than male rats; in addition, female rat hypothalami had a lower GHRH content and were less able to release GHRH than male rats (799). Although the sex difference in GH secretion of rats cannot be extrapolated as such to humans, these animal findings reinforce the view that hypothalamic GHRH secretion is mandatory to permit expression of its own pituitary receptor and show, in particular, that E₂ inhibits hypothalamic GHRH secretion and pituitary GHRH-R gene expression simultaneously.

Supporting these findings, castration raised the low GHRH-R mRNA levels of female rats two- to threefold, an effect counteracted by short-term E₂ implants. Estrogen also markedly suppressed GHRH-R mRNA in castrated male rats, although orchidectomy lowered GHRH-R mRNA only slightly, the opposite effect to ovariectomy. In both male and female rats, castration enhanced GHRH secretion in response to high K⁺ concentrations ~1.5-fold, and this effect too was reversed by E₂ treatment (798).

Various other steroid hormones also modulate GHRH-R mRNA expression. In primary cultures of rat AP cells, 100 nM dexamethasone for 12 h increased gene expression by fivefold; adrenalectomy and hormone replacement studies ex vivo showed corticosterone also stimulated GHRH-R mRNA expression in the intact rat pituitary gland (727). These findings, broadening previous observations in vivo with dexamethasone (584), provide a molecular mechanism to explain how glucocorticoids restore GHRH binding capacity in dispersed AP cells from adrenalectomized rats (947) and why dexamethasone induces an acute GH secretory response when administered to healthy humans (177) (see also sect. viA1).

The proposition that hypothalamic GHRH is mandatory for the expression of GHRH-R in the pituitary has now been strongly corroborated by more straightforward experiments. Treatment of AP cell cultures with 100 nM GHRH-R mRNA levels were 6-fold, and there was a 10-fold rise in GHRH-R mRNA after cell exposure to 100 mM dibutyryl cAMP for 12 h (727). For thyroid hormone action at GHRH-R, see section viA2.

The importance of pituitary GHRH-R for somatotroph function is illustrated by the fact that loss of function mutations in GHRH-R in mouse and human leads to severe GH deficiency syndromes. The little mouse is an animal model for isolated GH deficiency type 1B (333), in which the discovery of a mutation in GHRH-R (433) suggested similar mutations in GH deficiency syndromes in the human population (see below).

Functionally, pituitary glands of the little mouse are deficient in but not devoid of GH and are unresponsive to GHRH in vivo and in vitro (247). Somatic growth is increased by systemically administered GH.
In humans, some familial cases of isolated GH deficiency have been attributed to mutations in the GH gene itself, but GH deficiency is not always linked to this locus (799). The GHRH-R mutations account for other instances of GH deficiency in which the GH gene is normal. A nonsense mutation in the human GHRH-R gene has been reported that resulted in profound GH deficiency in two members of a consanguineous family, (1076). The Glu-72 stop mutation identified was close to the position of the little mutation (Asp-60-Gly), both occurring in the highly conserved region of the extracellular domain. Conformational polymorphism analysis applied to the coding region of the GHRH-R gene in 30 families with individuals suffering from GHD revealed a polymorphism changing codon 57 from GCG (alanine) to ACG (threonine) in the extracellular position of the receptor in 20% of the patients (836). However, the presence of this polymorphism did not seem to cause the GH deficiency in these patients; its frequency may be helpful to identify those patients in whom mutations in the GHRH-R gene are likely to be a cause of their GH deficiency.

A novel form of familial isolated GH deficiency linked to the locus for the GHRH-R has been described in a lower valley of Pakistan. A total of 18 dwarfs were discovered in a kindred with a high degree of consanguinity. Of several candidate genes probed by linkage analysis, only the GHRH-R locus on chromosome 7p14 was highly linked to the dwarfed phenotype. Amplification and sequencing of the GHRH-R gene in affected subjects brought to light an amber nonsense mutation (GAG→TAG; Glu 5→Stop) in exon 3, implying that this form too is a human homolog of the little mouse (78).

9. Conclusions

Molecular characterization of GHRH and GHRH-R is instrumental to a better understanding of the hypothalamic regulation of pituitary somatroph function (Fig. 6) (see Ref. 683 for details). In brief, the model of GHRH action proposes the interaction of the peptide with a seven-transmembrane domain Gs-coupled receptor on the somatotroph, leading to GH release from secretory granules, presumably mediated by a G protein interaction with ion channels and by stimulation of intracellular cAMP accumulation. In turn, elevated cAMP titers would lead to the phosphorylation and activation of cAMP response element binding protein (CREB), a transcription factor, by protein kinase A (434, 956). One target gene for CREB action is the pituitary-dependent transcription factor Pit-1 that is involved in the developmental generation of somatotrophs and in regulation of the genes for GH, prolactin and possibly for the β-chain of thyroid stimulating hormone. It provides the pathway whereby GHRH leads to increased GH synthesis.
in the pituitary but, in addition, is likely to directly regulate the synthesis of GHRH-R. This, in fact, is not expressed in the pituitary of dw/dw mice that lacks functional Pit-1 (612). In line with GHRH action through a cAMP-dependent pathway, treatment of primary cultures of AP cells with dibutyryl cAMP strikingly raises GHRH-R mRNA levels (727). The inhibitory peptide SS presumably interacts through G protein-mediated suppression of this cAMP pathway.

Disruption in several steps of the signaling pathway leading to GH secretion may result in GH deficiency. An inactivating mutation in the GHRH-R has been found in the little mouse, a model of isolated GH deficiency, and similar alterations in receptor function play a role in some human growth disorders.

10. In vivo animal studies

Detailed description of the GH-releasing activity of GHRH and its effects outside the GH axis, derived from animal studies, is beyond the scope of this review, so these topics are discussed here only briefly. For the clinical implications, the reader is referred to the excellent review by Cronin and Thorner (274).

Growth hormone-releasing hormone is active in vivo, stimulating GH secretion in anesthetized rats; conscious rats or dogs; rats bearing stereotaxic lesions of the VMN, ablation of the MBH, or selective destruction of the ARC; and in every vertebrate tested so far (see Ref. 1086).

Growth hormone-releasing hormone is needed for endogenous pulsatile GH secretion and optimal statural growth; subacute administration of an anti-GHRH serum to neonatal rats drastically slows growth (1089), impairs many aspects of somatotrophic function (207), and completely abolishes spontaneous GH release (1090). Once passive immunization ceases, no catchup growth occurs, and the immunized rats maintain a growth rate that is parallel to, but still lags behind, that of control rats (207). This growth pattern is virtually identical to the pattern in children with constitutional growth delay. The GH deprivation induced by the anti-GHRH serum delays sexual maturation in male rats (48), probably because of the low IGF-I titers (303).

Alterations in postnatal somatotropic function are also obtained when GH deprivation is induced in fetal rats on ED 16 by intra-amniotic injection of GHRH antibodies (212), a finding supporting the concept that GH exerts an important role in perinatal growth in the rat, as it is likely in humans (432, 1094). On the other hand, GHRH administered over several days to weeks enhances body growth and function in experimental animals (336, 1031). These effects are particularly evident in mice transgenic for GHRH, where excess GHRH exposure is associated with elevation of serum GH and stimulation of linear growth, increased pituitary mass, and mammosomatotroph hyperplasia, finally resulting in adenoma formation (see Refs. 684, 982).

There is an ontogenetic pattern in basal GH responses to exogenous GHRH; in the rhesus monkey, they decrease from postnatal days 1 to 28 (1086), whereas in the rat, GHRH does not raise GH levels up to postnatal days 5–10 (204), when GH pituitary responsiveness to the GH-releasing (205) and synthesizing (272) activities of the peptide is at its best (see also sect. μA). For the developmental pattern of the GHRH-R and its hormonal regulation, see section II μA.

In the rat, the GHRH system shows a strong sex dependency (40), and Spiliotis et al. (978) showed that GHRH mRNA level and GHRH secretion were higher in male than female rats and were reduced by orchidectomy but increased by testosterone in castrated male rats (1125); E2 had no effect on hypothalamic GHRH mRNA (653, 1125).

The sexually dimorphic organization of the GHRH system may account for the sex difference in GH secretion in the rat (see sect. μA), a major role also being attributed to sex-related differences in GHRH-R mRNA, although regulation by hypothalamic SS as well cannot be ignored (see sect. μB).

Consistent with the inhibitory influence of E2 on hypothalamic and pituitary GHRH-R function is the fact that pituitary responsiveness to GHRH is lowest in rats at proestrus (18). This pattern is somewhat different from humans, where GHRH’s effect does not change during the menstrual cycle (345, 396) and GH responsiveness to GHRH and GH secretion rate is higher in premenopausal women than young-adult men (590) (see sect. μA for discussion).

11. Extrapituitary central effects

Growth hormone-releasing hormone has few extrapituitary effects, as might be expected considering its limited distribution within the body. Huge doses (5–10 μg) of GHRH into the lateral brain ventricle of freely moving rats reportedly induced motor and behavioral effects and raised blood glucose levels (1008). However, dispute over the identity of the GHRH material used in this study (618, 1018) suggests caution in interpreting these findings.

More reliable are the findings that GHRH promotes sleep in animals and normal controls. Ehlers et al. (332) and Nisticò et al. (781) found a significant increase in SWS and a decrease in time to onset of SWS in rats after intracerebroventricular or hippocampal injections of GHRH. Non-REM sleep and REM sleep increased in both rats and rabbits after intrathecal injection of GHRH (782). Sleep was reduced in rats when GHRH action was inhibited by a specific antagonist (784) or by GHRH antibodies (785).
Four systemic boluses of GHRH between 22.00 and 1.00 h caused a significant increase in SWS in male controls which was evident throughout the entire sleep period (984). Entry of the peptide through the circumventricular organs and/or other CNS-permeable sites is the most likely explanation for these findings (543).

An important question is whether peripheral effects of GHRH, particularly the GH rise, are responsible for the increase in SWS. Apart from the observation that the SWS increase lasts considerably longer than the GHRH-induced GH rise (984), it is noteworthy that exogenous GH suppresses SWS (701).

The potential of GHRH and potent long-acting analogs (161) for improving the quantity and quality of sleep, particularly in obese patients and the elderly, where the endogenous GHRH drive is reduced (754), has not escaped attention (see also sect. iv).

Intracerebroventricular injection of picomolar doses of GHRH exerts another important effect, centrally mediated and independent of its GH-promoting properties, i.e., stimulation of food intake in rats and sheep. At higher doses, intravenous GHRH also stimulates food intake (893, 1007). Intracranial mapping studies have indicated that the SCN/MPOA region of the hypothalamus is a highly sensitive site for GHRH-induced feeding (1048) and that this effect depends on PVN for expression. The fact that the feeding effects of GHRH are exerted at sites remote from the hypophysial portal system is consistent with electrophysiological (1043) and anatomic (926) evidence focusing on the neurotransmitter-like properties of the peptide.

The GHRH signal would contribute to both the circadian pattern of feeding behavior and macronutrient intake. Microinjection of GHRH increased food intake during the light phase (behaviorally inactive) of the male rat’s cycle and either had no effect or reduced food intake during the dark (behaviorally active) period (349), when rats eat up to 80% of their daily food requirement.

In support of the view that GHRH is one signal contributing to the circadian pattern of food intake, injection of a specific GHRH antiserum into the SNC/MPOA, at four different periods during the light-dark cycle, suppressed feeding only at dark onset. This strongly suggested that GHRH action in the SCN/MPOA contributes to the feeding burst seen at dark onset (1047). The protein-selective nature of GHRH-induced feeding indicated a macronutrient-selective effect during the light-dark period. In rats habituated to two diets (carbohydrate-fat and protein-fat), microinjection of a GHRH antiserum into the SCN/MPOA blocked the increase in protein intake normally seen at dark onset but had no effect on carbohydrate intake (312).

Few data are available on the effects of GHRH on food intake in humans. Because the higher GH responsiveness to GHRH of subjects with anorexia nervosa (AN) (see sect. viC2) may also reflect altered sensitivity or activity of central GHRH, the compound was administered systemically to a group of AN patients at meal time, and caloric intake and macronutrient selection were determined (1049). Growth hormone-releasing hormone stimulated food intake (especially carbohydrates and fats) in AN patients but reduced it in anorectic/bulimic patients or normal controls, with a mechanism apparently not depending on the subjects nutritional status or the ability of the peptide to release GH. The authors’ conclusions that low levels of GHRH may contribute to the restricted eating pattern of AN patients were not consistent with experimental, although inferential, evidence pointing to an enhanced function of GHRH neurons in AN (751, 929).

For other potential extrapituitary effects of GHRH, see section smell.

B. Somatostatin

With the demonstration of stimulatory CNS influences over GH secretion, evidence accumulated of an inhibitory CNS control, based especially on observations in subprimate and primate species (749). In humans, elevated plasma GH levels were reported in the diencephalic syndrome of infancy, a CNS disturbance usually caused by a tumor of the optic chiasm and anterior hypothalamus (66, 360). More proof of a hypothalamic inhibitory influence for GH secretion was given by Kruilich et al. (578), who showed that in vitro certain fractions separated from ovine hypothalamic extracts had consistent inhibitory effects on the release of bioassayable and then RIA GH (see Ref. 3 for review). These authors were the first to suggest that GH secretion was regulated by a dual system, one stimulatory and the other inhibitory. The inhibitory factor was named GH-inhibiting factor (GHIF).

The isolation, identification, and synthesis several years later of a tetradecapeptide, GH release-inhibiting hormone or somatotrophin release-inhibiting hormone (SRIF or SS) should be credited to Guillemin and co-workers (141), who used a highly sensitive in vitro pituitary assay.

Somatostatin was first identified as a hypothalamic peptide involved in the physiological inhibitory control of GH and thyroid-stimulating hormone (TSH) secretion. It soon became clear, however, that SS was ubiquitous (see below) and inhibited the secretion of most hormones, under physiological and pathological circumstances, and could also inhibit exocrine secretions. Because targets of SS action were often the same tissues where the peptide was localized, the concept developed that, in addition to its endocrine effects, its actions were regionally limited as a neuroendocrine factor or in adjacent tissues, as an autocrine or paracrine regulator (880).
Many aspects of SS function have already been dealt with in earlier sections, so in this section we confine ourselves mainly to its neuroendocrine effects in relation to the hypothalamic-pituitary unit.

Somatostatin-14 (SS-14), the native form, belongs to a family of SS-like peptides including NH₂-terminal-extended SS (SS-28) (864), a fragment corresponding to the first 12 amino acids of SS-28 [SS-28-(1—12)], and still larger forms that vary in molecular size and in different species from 11.5 to 15.7 kDa.

In vertebrates, SS-14 and SS-28 have evolved from separate encoding genes in fish to a single gene encoding a common precursor that is differentially processed to generate tissue-specific amounts of the two peptides (816).

The SS-like peptides are multifunctional and are synthesized and located in most brain regions as well as in peripheral organs, especially the gastrointestinal tract of both vertebrate and invertebrate species (reviewed in Refs. 814, 1081). Somatostatin-14 is the predominant form and usually has the same sequence in different species. Both SS-14 and SS-28 have partially overlapping but distinct bioactions and act through different receptors (see below). In neural tissues, SS-14 predominates (ratio 4:1 in the hypothalamus). There is virtually no SS-28 in the stomach and the duodenum, but lower down the gut SS-28 is the most important molecular form (306).

Both SS-14 and SS-28 have similar potency and bind all cloned receptors with high affinity (see sect. **B3**). The biological significance of the two forms in relation to neuroendocrine function, however, remains to be determined.

1. Anatomic distribution

Somatostatin-producing cells are present throughout the central and peripheral nervous systems, the gut, the endocrine pancreas and, in small number, in the thyroid, adrenals, submandibular glands, kidneys, prostate, and placenta (813, 880). Within the hypothalamus, the majority of SS-positive nerve perikarya lie in the PeVN (360), but these are also seen in the ARC and VMN (see sect. **A3**). They are located close to the third ventricle, in a few layers parallel to the periventricular wall, within an area extending from the preoptic nucleus (PON) to the rostral margin of the VMN (357). Axons from these cells run caudally through the hypothalamus to form a discrete pathway toward the midline that enters the ME, where the nerve endings extend in a compact band throughout the zona externa.

Not all fibers from the PeVN take this route; a small proportion of them travels through the neural stalk to the neurohypophysis where they presumably play a role in the regulation of the secretion of neurohypophysial hormones, arginine vasopressin (AVP) and oxytocin. Other fibers project outside the hypothalamus to areas such as the limbic system; others interconnect, possibly through interneurons, with several hypothalamic nuclei, including the ARC where GHRH is synthesized, the PON, and the VMN, which are involved in regulating reproductive functions, and the SCN, which has a circadian pacemaker activity (see also sect. **A2**).

Somatostatin neurons in the anterior PeVN area interact with multiple populations of neurons such as galanin, NPY, GABA, 5-HT, endogenous opioid peptides (EOP), and others and receive a host of neurochemically defined terminals (96, 118). In addition to the anatomic interactions with GHRH, there is also a connection between SS and corticotropin-releasing hormone (CRH) (reviewed in Ref. 96).

Further details on the distribution of SS neurons and fiber systems are given in section **A3**.

2. Gene structure and expression

The SS gene in the rat and humans has a simple configuration, the coding region consisting of two exons separated by an intron (see Ref. 814 for review). The 5’-upstream region contains three regulatory elements, two promoters, and a cAMP-response element (enhancer). This latter was first identified in the SS gene and is present in a large number of genes regulated by cAMP (736). Most agents influencing SS secretion also alter SS gene expression. Activation of the AC-cAMP pathway plays an important role in the stimulation of SS secretion and mediates the effect of several agents physiologically regulating SS gene transcription (glucagon, GHRH).

Growth hormone secretion is also autoregulated through the stimulation of SS mRNA (see sect. **A4**). Gonadal hormones play a significant effect contributing to the sex-related differences in SS neuronal activity (see sect. **B3**).

3. Secretion from the hypothalamus

Somatostatin release from different in vitro prepara-
tions is enhanced by K⁺-induced membrane depolarization, mediated by Ca²⁺ influx (818), cAMP, dopamine (DA), glycocytopenia, IGF-I, GH, and GHRH (for these aspects, see sects. **A3** and **A4**). Somatostatin-14 is the main form of SS-IR released on K⁺ depolarization from slices of the rat hypothalamus, implying that this is the form responsible for hypothalamic neurotransmission (841).

Contradictory results have been reported on how different neurotransmitters affect SS release, a vital question for understanding their mechanism(s) of action (see sect. **A**). Factors related to the different experimental models used to study SS release in vitro (e.g., hypothalamic slices or explants, embryonic cell cultures, synaptosomes) may hinder evaluation of the neurotransmitter’s...
action in vivo. Comparison in vivo is already difficult because of the complex neuronal circuitry of hypothalamic-extrahypothalamic connections and accessibility of specific sites.

Acetylcholine has been reported to inhibit (888) or to have no effect (650) on SS-LI release from hypothalamic fragments and to enhance SS-LI release from dispersed cell cultures (834) and portal blood (228), whereas enhancement of the cholinergic tone in humans appears to suppress SS release (see sect. VA3 and Ref. 749 for discussion). Dopamine in micromolar concentrations can release SS-LI from incubated hypothalamic tissue (772) and synaptosomal preparations (1077) and raise the SS-LI concentration in the pituitary portal blood (228). However, DA did not have this effect when the whole MBH was exposed to physiological concentrations of the transmitter (339), a finding more in line with in vivo results (see sect. VA1n). Norepinephrine (NE) stimulated or had no effect on SS-IR release from the ME (650, 771), whereas in vivo an inhibitory role on SS function is suggested (see sect. VA1a). Finally, GABA inhibited SS-LI release from hypothalamic cells in cultures, an action blocked by the GABA receptor antagonist bicuculline (385), and in line with evidence obtained in vivo (see sect. VA5n). Somatostatin release in vivo is stimulated by a number of neuropeptides, including thyrotrophin-releasing hormone (TRH) (544), CRH (188, 730), and GHRH (42). Each of these peptides administered centrally inhibits its GH secretion, and these effects can be completely counteracted by pretreatment with SS antibodies.

In contrast to CA and ACh, intraventricular injection of 5-hydroxytryptamine (5-HT) did not affect SS-LI release into the portal blood (228), whereas centrally injected bombesin (1) or NT (3) was effective and EOP release from fetal brain cultures (352), a finding consistent with previous reports that ovariectomy increased the amount of IR-SS in the ME and that this effect was reversed by estradiol (439). Reduction of SS release would be related to the raised baseline GH levels observed in female rodents (524, see also sect. IA).

4. Receptors

Parallel to studies of the effect of SS and its analogs on endocrine and exocrine secretion were investigations into their mechanisms of action. Plasma membrane receptor binding of SS, and especially of analogs resistant to degradation, was demonstrated on all target tissues (151). Receptor binding was demonstrated in vitro to plasma membrane preparations and to tissue sections (586) and in vivo by radionuclide scanning (577).

Shortly after the discovery of SS-28 as a second endogenous ligand, the existence of two populations of SS receptors (sstr) was proposed, based on the different receptor binding potencies and actions of SS-14 and SS-28 in brain, pituitary, and islet cells (817, 979). The heterogeneity of sstr became increasingly apparent when cross-linked studies revealed a wide array of proteins of different molecular weights that bound the labeled peptide. Cloning of the sstr family disclosed the overall complexity of the system.

Cloning of five separate sstr genes confirmed the existence of molecular subtypes and indicated a greater heterogeneity in this receptor family than previously suspected (see Ref. 815 for review). Four of the human genes are intronless, the exception being sstr2, which gives rise to the spliced variants sstr2A and sstr2B that differ only in the length of the cytoplasmic COOH tail. There are thus six putative sstr subtypes highly conserved in size and structure, displaying the putative seven-transmembrane domain typology typical of G protein-coupled receptors; sstr1 and sstr4 show the highest degree of sequence iden-
of mRNA for sstr confirmed their wide tissue distribution

A further mechanism would be inhibition of Ca$^{2+}$ currents through induction of cGMP, activation of cGMP protein kinase, and phosphorylation-dependent inhibition of Ca$^{2+}$ channels (712).

A) TISSUE EXPRESSION OF RECEPTOR SUBTYPES. Expression of mRNA for sstr confirmed their wide tissue distribution with a particular pattern for each receptor type, which does not rule out overlap, because few tissues or cells have the mRNA for only one receptor type (151). Adult rat pituitary features all five sstr mRNA, and the adult human pituitary expresses the four subtypes 1, 2, 3, and 5 (286, 815). All five genes are expressed by the major pituitary cell subsets with high levels of sstr1 and sstr5 in rat somatotrophs and of sstr2 mRNA in rat thyrotrhops (786). The finding that sstr5 mRNA is more abundant than sstr2 mRNA in the pituitary is consistent with fact that SS-28 is more potent than SS-14 for inhibiting GH and TSH secretion.

All five sstr mRNA are variably expressed in the brain (151) (Table 1); sstr1 and sstr2 were found in the highest concentrations in cortex, amygdala, hypothalamus, and hippocampus, whereas the highest level of sstr5 was in the cerebellum, a unique finding given the relatively low receptor density in this area (151). Expression of sstr4 was observed throughout the CNS, but this isofrom was not found in the cerebellum. Sstr5 showed a unique pattern, occurring mainly in the hypothalamus and POA, which suggests it has a highly specialized role in the CNS (151).

Future attempts to correlate the regional distribution of sstr subtypes in the CNS with the functional properties of SS will call for the development of ligands that bind selectively and with high affinity to each subtype. Nevertheless, the high levels of sstr1, sstr2, sstr3, and sstr4 in such brain regions as cortex, hippocampus, hypothalamus, and amygdala suggest they are involved in processing integrative functions.

Of particular interest for our topic is the distribution of sstr and mRNA expression in the hypothalamus, where it provides evidence of multiple receptor subtypes. Conventional film autoradiography suggests sstr are not very abundant in hypothalamic regions, although desaturation...
techniques considerably increase the amount of binding (608). Of the five cloned sstr subtypes, four are synthesized in the hypothalamus, the expression of sstr5 being particularly abundant (151). In situ hybridization showed sstr mRNA-containing cells in all hypothalamic areas, whereas sstr mRNA cells were restricted mainly to the ARC, an area rich in both sstr1 and sstr2 mRNA (151).

The presence of sstr close to a subpopulation of ARC neurons, some corresponding to GHRH neurons, has been already discussed (see sect. mA3). They allow cross talk in the GH regulatory pathway between the PeVN and the ARC. Sstr1, sstr3, and sstr5 mRNA have been located in the ARC and may therefore be implicated in the modulation of GH secretion by SS (81, 948).

In addition, sstr1 mRNA in the ARC appears to be regulated by GH, which further implicates this receptor gene in the feedback regulation of GH secretion by the ARC (448, see also sect. viA4). In the reverse direction, the ARC sends projections that may contain galanin and proopiomelanocortin (POMC)-derived peptides to the PeVN (see sect. mB1). These feedback mechanisms involving SS neurons play an important role in driving the pulsatile secretion of GH.

Analysis of secreting and nonsecreting human pituitary tumors (monoclonal in origin) and rodent pituitary tumor cells provided evidence of several sstr genes and probably receptor proteins expressed in the same tumor cell. Both sensitive to octapeptide analogs, sstr2 and sstr5 are expressed most frequently, whereas the expression of sstr3 and sstr4 is much less common (815). The common presence of sstr2 and sstr5 in pituitary tumors helps explain their responsiveness to octreotide, for which these receptor subtypes have high affinity.

Somatostatin receptors are expressed not only in a variety of pancreatic and intestinal tumors (glucagonomas, insulinomas, pheochromocytomas), but also in most solid tumors; sstr1, sstr2, sstr5, and sstr4 have been shown on endocrine tumors, but sstr5 mRNA has not been reported (815).

It would seem, therefore, that sstr and subtype-specific analogs offer new opportunities for locating a variety of neoplasms and their metastases, inhibiting their secretory products, and providing access to the interior of the cells themselves, since SS analogs can be internalized. An antiproliferative effect has also been suggested for these analogs (481).

B) DESENSITIZATION. Patients undergoing long-term therapy with SS analogs develop tolerance to side effects such as inhibition of insulin and TSH secretion. However, the inhibitory effect on secretory tumors persists often for many years. This suggests that sstr in normal tissues are regulated differently from their antiproliferative effects on tumors (481).

Desensitization in normal cells is mainly due to agonist-dependent internalization of the receptors, as demonstrated in rat anterior pituitary and islet cells (31, 737). Agonist-dependent desensitization responses have been reported after SS pretreatment for 2 h or less. Longer agonist exposure, 24–42 h, upregulated sstr in GH4C1 cells and RIN 5f cells (866, 993). In CHO-K1 cells expressing all the five human sstr subtypes, sstr2a, sstr3, sstr4, and sstr5 induced rapid agonist-dependent internalization of the ligand over 60 min; sstr1 caused virtually no internalization. Prolonged agonist treatment led to differential upregulation of some of the sstr: after 22 h, the agonist upregulated sstr1 by 110%, sstr2 and sstr4 by 22–26%, whereas sstr3 and sstr5 showed no change (493).

C) REGULATION OF GENE EXPRESSION. Expression of sstr subtypes is regulated by hormones. Glucocorticoids up-regulate sstr1 and sstr2 mRNA after short-term exposure, whereas prolonged treatment inhibits transcription of both genes (1109). Estrogens upregulate mRNA expression of sstr3 and sstr5 in rat prolactinoma cells (1102) and in breast cancer cells (1110). Metabolic derangements such as starvation or diabetes lower sstr3 mRNA levels in the pituitary and sstr5 mRNA levels in the hypothalamus (150), but the significance of this is still obscure.

Four sstr promoters have been characterized and contain consensus sequences for a variety of transcription factors. Rat sstr1 gene shows AP2 and Pit-1 binding sites (464); human sstr2 and sstr4 contain several AP1 and AP2 sites that may confer cAMP responsiveness (438).

5. Pituitary and extrapituitary effects

The physiological role of SS in pituitary responsiveness to hypothalamically driven stimulatory influences, especially those of GHRH, and thus contributing to the pulsatile secretion of GH, has already been extensively discussed. Here, brief consideration is given to some in vivo inhibitory effects of SS on the pituitary, especially in conditions of stimulated GH release. These inhibitory effects have been shown in a number of species in addition to rodents, e.g., dogs, monkeys, and humans. In humans, SS prevented levodopa, exercise, hypoglycemia, and sleep-evoked GH secretion and also lowered GH levels in diabetic and acromegalic subjects (749).

Somatostatin may not merely inhibit GH secretion, but under appropriate conditions may also trigger GH release, through an intrahypothalamic site of action. Within the PeVN, SS in fact seems to operate an ultrashort feedback loop to inhibit its own secretion. The recent demonstration that the distribution of neurons expressing sstr1 mRNA in the hypothalamus overlaps the distribution of SS has led to the proposal that sstr1 may be the autoreceptors (816).

Furthermore, SS can directly act on AP thyrotrophs to inhibit TSH secretion or it may act on the hypothalamus to inhibit TRH secretion (880). Thyroid hormones can stimulate SS release, although this effect appears to
be indirectly mediated through a direct action to increase GH gene transcription (see sect. viA2). Somatostatin in the AP can also inhibit prolactin secretion from normal and adenomatous glands in humans and GH<sub>C<sub>1</sub></sub> tumor cells and ACTH secretion from human and mouse AtT-20 ACTH-producing tumors (817) (for interactions between the SS system and HPA axis, see sect. viA7). Pituitary sstr involved in the effects of SS or its analogs have already been discussed.

Somatostatin has no effect on basal levels of GH synthesis, gene transcription, mRNA, or somatotroph proliferation in rat or bovine pituitary cell cultures (73, 104, 381, 960, 1020) or on the elevation of rat GH mRNA induced by exogenous cAMP (963). It also does not influence GHRH-stimulated rat GH transcription, although it attenuated the GHRH-induced increase in somatotroph proliferation (104) and the expression of c-fos in the rat (103).

Limited data are available on how SS maintains normal GH synthesis and body growth in vivo. Despite AP concentrations of SS two to three times higher than any other body tissue, transgenic mice expressing a metallothionein-SS fusion gene grew at a normal rate (638) (for details, see Ref. 377).

The extensive localization of SS in the CNS indicates its neurotransmitter and/or neuromodulatory role. In recent years attention has focused especially on its function in certain CNS areas such as the cortex or the hippocampus, with clinical implications for cognitive functions and Alzheimer’s disease, and in the striatum, with implications for movement control in Parkinson’s disease (936).

Somatostatin also plays an important physiological role in the pancreas and gut (814, 941). It regulates the endocrine and exocrine functions of the stomach, intestine, and pancreas and the motor functions of the first two, partly by local or paracrine mechanisms and partly through the circulation as a true endocrine factor. Thus not only does SS exert its regulatory actions within organs that have SS-containing D cells, but it also ensures a more integrated control over the nutrient flux by acting on remote target organs containing no D cells (941).

IV. GROWTH HORMONE-RELEASING PEPTIDES

Many years before native GHRH was isolated and sequenced, a new series of peptides with strong GH-releasing properties had been identified (732). The size and scope of this new class, known jointly as GHRP or GH secretagogues (GHS) and comprising nonpeptide pharmacological analogs, have grown enormously. 2

The first GHRP were discovered in 1977 by Bowers et al. (133). They were derivatives of the pentapeptide Met-enkephalin but had no, or very little, opioid activity. The pentapeptide Tyr-D-Trp-Gly-Phe-Met-NH<sub>2</sub> had relatively weak potency as a GH secretagogue and was active only in vitro; however, it served as a model to design new molecules with much greater activity (Fig. 7).

As described by Momany et al. (733), the design approach consisted of using “structural concepts” derived from conformational energy calculations in conjunction with peptide synthesis and biological activity data. Unique, and the key to the success of this approach, was the insertion of structural data derived from conformational energy calculations into the design cycle, before synthesis of the peptide, as well as after obtaining experimental data. A number of structurally related GH-releasing peptides were designed, with greater GH-releasing activity. Growth hormone-releasing peptide-6 (Fig. 7) was considered particularly suited because of its potent GH-releasing activity in vitro and in vivo.

The GH-releasing activity of GHRP-6 was demonstrated in a range of species, i.e., monkeys, sheep, pigs, chicks, steer, and rats (134, 276, 322, 575, 655), as well as in humans (135, 504). Growth hormone-releasing peptide-6 was orally active in rats, dogs, monkeys, and humans (132, 1079), and interestingly, it was 5.3–30 times more effective in monkeys than in rats or dogs. In humans, 1 μg/kg GHRP-6 administered as a bolus injection released more GH than GHRH-44 given at the same dosage and by the same route (131, 135). In healthy humans, comparable amounts of GH were released after intravenous 1 μg/kg GHRP-6 or 300 μg/kg oral GHRP-6 (132). In healthy volunteers, clear-cut GH release also occurred after intranasal administration of 30 μg/kg GHRP-6; when 15 μg/kg GHRP-6 was administered intranasally every 8 h for 3 days, serum GH and IGF-I levels increased significantly (466). In these studies, the stimulation of GH release appeared to be specific and divorced from concurrent adverse effects, with the exception of occasionally mild sweating.

Growth hormone-releasing peptides are the most effective GHS in experimental animals; their action is greater than that of GHRH, with which it is synergistic, and is well preserved in aged rats when GHRP are administered combined with GHRH (1080). In the same model, chronic treatment with a GHRP-6 analog, hexarelin (296), maintained its GH-releasing effect for up to 2 mo, did not change circulating IGF-I levels, and, interestingly, reduced the hypothalamic somatostatin mRNA content to the levels of young controls (189). In senescent dogs, a 16-wk huge daily dose of hexarelin initially primed and then blunted the GH response to acute hexarelin, although this was easily restored after treatment withdrawal. Despite this effect, hexarelin raised the indices of spontaneous GH pulsatility, reduced bone resorption, and improved some biochemi-

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2 The acronyms GHRP and GHS are used interchangeably in the text.
cal and morphological muscular indices, although it did not change plasma IGF-I levels (210).

A. Structure-Activity Relationships

These peptides all need a very specific configuration of selected aromatic rings to specifically stimulate GH release. The native pentapeptide served as the starting model for the development of more potent GHRP. The prototype was GHRP-6, from which many different analogs, ranging from 7- to 3-amino acid residues (GHRP-1, GHRP-2, hexarelin, and so on) have been synthesized and shown to be active also in humans. Newer peptides with smaller molecular size and less complexity have now been synthesized in the hope of understanding the minimal tridimensional structure required (295, 1115).

Homologous desensitization occurs very rapidly in vitro after exposure to GHRP, whereas the cells remain sensitive to GHRH (335). Although peptidyl GHRP were initially introduced as specific GHS, small rises in serum cortisol and prolactin were seen in humans after intravenous infusion, bolus injection, or intranasal administration of these compounds (135). Stimulation of GH and prolactin secretion from primary pituitary cell cultures has also been reported (692). The real impact of these observations in situations of long-term peptide and nonpeptide GHS therapy is not known.

Despite their potential for affecting the secretion of more than one AP hormone (see also sect. IV E), nowadays GHRP are the most effective GHS known and could be profitably used in humans with GH hyposecretory disturbances to promote a pattern of GH secretion that mimics the physiological situation better than exogenous GH.

B. Nonpeptidyl Growth Hormone Secretagogues

The strategy of stimulating pituitary GH secretion by activating the endogenous machinery is an attractive idea that has been pursued with the synthesis of nonpeptidyl mimics of GHRP-6; this research has led to the discovery
of a series of new compounds, the benzolactam secretagogues, reviewed in Reference 965 (Fig. 7).

Structural modifications of the original molecule led first to the isolation of L-692,429, which was well tolerated in animals and humans, and then to the synthesis of new compounds culminating in the selection for clinical use of L-163,191 and its mesylate salt MK-677, which offers superior oral potency and duration of action (811). These new molecules are very effective in eliciting GH release, although in vivo they also stimulate the release of ACTH and prolactin. MK-677 also raises fasting blood glucose and insulin concentrations in humans (220, 400, 467). Although the oral bioactivity of this compound is greater than the peptidyl secretagogues, the clinical use of these molecules is currently hampered by our inadequate understanding of the pharmacodynamic profile required for optimal efficacy.

C. Site(s) and Mechanism of Action

Growth hormone-releasing peptide may act on the pituitary (407, 628), although experimental evidence indicates that they are also active in the hypothalamus (314, 335, 966, 1037). Specific binding sites for GHRP have been detected in the pituitary and hypothalamus in rats and pigs (261, 856, 953, 1062) and in rats also in several extrahypothalamic structures, including the substantia nigra, CA2 and CA3 subfields, dentate gyrus of the hippocampus, and medial amygdaloid nucleus. The membrane binding sites are specific, reversible, saturable, as well as time, temperature, pH, and concentration dependent.

Studies on pituitary and hypothalamic membranes suggest these may be multiple binding sites with different affinities for peptidyl and non-peptidyl GHRP. In the rat pituitary and hypothalamic membranes, MK-677 would bind preferentially to a high-affinity site (dissociation constant in the picomolar range), whereas medium-affinity (nanomolar range) and low-affinity (millimolar range) sites for the GHRP would primarily bind GHRP-6 (261, 856, 953). Hong et al. (488) have now directly demonstrated the existence of receptor subtypes for the GHRP using a photoactivable derivative of hexarelin. This subtype is apparently distinct from the recently cloned GHRP receptor.

Compounds such as bombesin, neurenomedin C, and substance P (SP) did not influence these binding sites, whereas GHRH-(1—29)-NH2 and SP were potent inhibitors of pituitary and hypothalamic GHRP-6 binding (261, 953, 1062). Competition by SP antagonists was consistent with the reported ability of the antagonists to decrease the release of GH by GHRP-6 in vitro and in vivo in a dose-dependent manner (107, 226). Because SP antagonists selectively blocked the GH release elicited by GHRP but not by GHRH and SP was ineffective in vivo and in vitro, these compounds presumably interact with GHRP receptors. More surprising was the inhibitory effect of the GHRH analog, since GHRP receptors are different from GHRH receptors, as suggested by the lower GH-releasing activity of GHRP in vitro, the additive effect of the two stimuli, and the fact that GHRP-6 and GHRH antagonists do not competitively antagonize each other’s responses, with the only exception being GHRP-2, whose action is inhibited by GHRH antagonists (1107).

The finding of specific binding sites for peptidyl-GHRP in humans has confirmed the highest specific binding in the hypothalamus and pituitary but also has shown their presence in the choroid plexus and the cerebral cortex (746). Specific binding sites in extrahypothalamic areas (see also above), coupled with the accessibility to the brain of different GHRP, even when administered peripherally (314, 315, 963), suggests these compounds may affect brain function. Accordingly, peripherally administered GHRP modified the sleep pattern in humans (267, 268, 372) and stimulated food intake in the rat (642) and humans (220).

Identification of the sites(s) of action of GHRP is of major importance; their much greater effectiveness in vivo than in vitro suggested a major hypothalamic site (136, 468, 624, 1037), although this view has to be tempered by the observation that after hypothalamo-pituitary disconnection, GHRP released GH in rats (164, 654) or sheep (364). In pigs, GHRP are effective in intact animals and those with a hypophysial transection (468), although in the latter GH release was reduced and depended on the interval from surgery.

In contrast to most of these findings, GHRP were unable to stimulate GH release in children and adults with pituitary stalk transection, despite their responsiveness to GHRH (465, 631, 861). Overall, these results suggest that the hypothalamus is the primary site of action of GHRP at least in humans and pigs.

In our studies in rats with complete surgical ablation of the medial basal hypothalamus, the GH release elicited by hexarelin was reduced 1 wk after surgery, whereas the response to GHRH was maintained. Thirty days postsurgery, the GH response to an acute challenge with hexarelin or GHRH was similar to that with hexarelin 7 days postsurgery (1037). It would seem, therefore, that the decreased pituitary GH content resulting from the chronic GHRH deprivation was not responsible for blunting the GH response to GHRP.

1. Hypothalamic mechanisms

Briefly reviewed here are the possible mechanisms underlying the GH-releasing activity of GHRP.

A) GHRH. In sheep, direct measurement of hypophysiotropic neurohormones released into the portal blood
showed that one GHRP, hexarelin, raised GHRH concentrations without altering SS concentrations (446). Supporting the involvement of GHRH was the finding that GHRP partially lost their ability to stimulate GH release in adult rats passively immunized against GHRH (88, 245, 266). Systemic or intraventricular doses of GHRP-6 or the nonpeptidyl analogs caused c-fos accumulation and electrical excitation of putative GHRH neurons in the ARC (313), and c-fos mRNA was also induced in GHRH and NPY ARC neurons after systemically administered KP-102 or GHRP-6 (315, 537, 963). More direct proof for GHRP-GHRH interactions was provided by autoradiographic in situ hybridization studies. They showed that in the rat MBH there was extensive overlap between GHRP-R and GHRH hybridizing cells in both the ARC and VMN nuclei (1011a).

However, other findings suggest GHRH is not necessarily involved. In dogs and humans, GHRH further increased the GH release induced by a maximal effective dose of GHRH (135, 823), and reportedly, the GH response to GHRP alone is considerably greater than to GHRH (129–131, 135). We showed in neonatal rats that removal of GHRH and SS by passive immunization did not affect the GH-releasing activity of GHRP-6 or hexarelin (624), and there is also clear-cut, although attenuated, GH release by administration of the same GHRP in 10-day-old pups passively immunized against GHRH since birth (624, 628). Supporting the existence of independent mechanisms of action for GHRP and GHRH, at least under some circumstances, is also the observation that a series of peptidyl and nonpeptidyl GHRP at low doses did not affect GHRH release from freshly removed rat hypothalamus and, at millimolar concentrations, even inhibited GHRH release, suggesting an ultrashort-loop feedback with GHRH (569).

Growth hormone-releasing hormone activation thus appears to be an intermediate, but not obligatory, step of GHRP action; an intact hypophysial stalk is mandatory for maximal GH response, and finally, the pituitary component of the action of these compounds is not of major importance.

b) SS. Somatostatin involvement in GHRP action is less controversial than that of GHRH. Somatostatin did not seem to be directly involved in the GHRP action in adult rats passively immunized against SS (266), and similar conclusions were reached in the infant rat (94). Other experiments indicate that GHRP may even stimulate SS secretion from hypothalamic fragments (452). However, SS may play an indirect role by functioning as a GHRP antagonist in the pituitary (245).

In conscious rats, continuous subthreshold GHRP-6 infusion together with repeated injections of GHRH induced GH responses that were uniform and greater in magnitude than those of rats given GHRH alone. Interestingly, between repeated GHRH boli, serum GH concentrations remained higher than baseline, suggesting that the GHRP-6 had reduced SS inhibitory influences on the pituitary. Consistent with these findings, in rats, intracerebroventricular octreotide completely blocked the GH release induced by GHRP-6 administered centrally 20 min later. Because intracerebroventricular octreotide did not affect the GH release stimulated by intravenous GHRH, SS was presumably acting as a functional antagonist of GHRP-6 at a central site. This was confirmed by Dickson and Luckman (315), who found the GHRP-6- and MK-677-induced c-fos protein expression was attenuated in the ARC after intravenous or intraventricular octreotide.

Data in humans also point to GHRP antagonism of SS effects. The GH-releasing effect of hexarelin was partly refractory not only to inhibition by compounds stimulating hypothalamic SS release but even to a dose of SS fully effective in suppressing the GH response to GHRH (51, 53, 645). Moreover, administration of GH abolished the GH response to GHRH, but only blunted the GH response to hexarelin (50, 674). In view of the GH autofeedback on hypothalamic release of SS (see sect. VII-A), this indicates that hexarelin had counteracted the GH-induced somatostatinergic hyperactivity. Results with oral MK-677 also suggest that nonpeptide GHS may functionally antagonize SS action (220).

c) THE UNKNOWN FACTOR (U FACTOR). Although many reports have clearly shown that GHRH may be necessary for GHRP to exert their full effect, none has clearly indicated a mechanism(s) that would account satisfactorily for the synergistic effect of GHRH and GHRP. Bowers et al. (136) postulated that GHRP stimulated an unknown endogenous hypothalamic factor (named U factor) which, in combination with GHRH, would stimulate GH release in rats with hypothalamic ablation (1037). However, the stimulatory effects of GHRP in rats with selective GHRH deficiency (624) also suggest a mechanism mediated by an endogenous hypothalamic non-GHRH factor, whose existence and nature are still elusive.

2. Pituitary mechanisms

A) EFFECTS ON GH RELEASE. Sufficient experimental evidence exists that GHRH and GHRP act on the pituitary through different mechanisms, and very likely through different receptors. Somatotroph cells that are maximally stimulated with GHRP can release more GH in response to GHRH, and vice versa (223, 436). Moreover, homologous but not heterologous desensitization is seen after continuous pituitary exposure to GHRH or GHRP (113, 219, 245, 1106).

It was thought initially that GHRP-6 acted on the GHRH receptor, since it did not elicit GH release in the lit/lit mouse, an animal model with a point mutation in the GHRH receptor (433) (see sect. iiA8i). However, investigations using cultures of human somatotrophino-
mas showed a GH responsiveness to GHRP-6 in all adenomas, whereas only one-half of them responded to GHRH (883). As an alternative, despite the mutually exclusive pituitary binding sites for GHRH and GHRP, the postreceptor activity triggered by GHRH would be instrumental to GHRP action (335).

In contrast to the marked synergy in vivo, only additive or mild synergistic effects were evident when GHRP and GHRH were coincubated in vitro (21, 62, 113, 134). It is widely recognized that GH release induced by GHRH is paralleled by an increase in cAMP titers in the pituitary, followed by the opening of VOCC (see sect. III A7). The ensuing rapid increase in \([Ca^{2+}]_i\) promotes GH release. In contrast, GHRP-6 had no such effect on cAMP (223, 1107), but there was a synergistic increase of cAMP levels when GHRP-6 and GHRH were administered together (223).

Although a precise definition of the molecular mechanism(s) of action of GHRP is still lacking, these compounds elicit an increase in \([Ca^{2+}]_i\), from an extracellular source, since it was blocked by the chelation of extracellular calcium or incubation with calcium channel blockers (21, 919). It has also been shown that GHRP-1 and GHRP-6 depolarize rat pituitary cell membranes, leading to opening of VOCC (857), and it has been proposed that GHRP-6 and L-692,429 may act, at least in part, through activation of the protein kinase C pathway (224, 225). Pong et al. (856) have described a specific binding site for GHRP in porcine and rat pituitary membranes distinct from that for GHRH and with the properties of a new G protein-coupled receptor. Van der Ploeg and co-workers (491) reported the cloning of a receptor for GHRP-6 and nonpeptidyl GHS.

All in all, there is abundant experimental evidence that the GHRP operate through common cellular mechanisms, involving the activation of a receptor(s) different from that of GHRH and of intracellular mechanisms different from the cAMP pathways. It would seem, however, from some studies that the second messengers activated by GHRP in somatotrophs may vary depending on the species considered (184a). Although the GHRP receptor or, most likely, one of its subtypes, has been cloned in humans, rats, and swine (491, 695), we still have nonconfirmation of the existence, and the nature, of the purported endogenous GHRP ligand. Figure 8 depicts schematically the hypothetical mechanisms underlying the GH-releasing properties of GHRP.

b) Effects on GH synthesis. The GH synthesis in the pituitary is under the positive control of GHRH (see sect. III A7), an effect which involves cAMP activation but appears to be independent from changes in \([Ca^{2+}]_i\) (73). It was therefore of interest to investigate whether GHRP plays a role in the control of GH biosynthesis. There are only very few reports about GHRP effects on GH mRNA levels. In our own studies, a five-day treatment with

**FIG. 8.** Mechanisms underlying GH releasing effects of GH-releasing peptide. Solid line refers to a probable mechanism; dotted line refers to an hypothetical (?) mechanism. Functional, reciprocal interaction between GHRH and somatostatin neurons are also indicated (see text for details).

**FIG. 9.** Effects of 3–10 days of treatment with hexarelin (HEXA) on GH mRNA levels in infant rats. Pups were treated as described in legend to Fig. 1. Because acute challenge with hexarelin had no effect on GH mRNA levels, in each experimental group data obtained from rats challenged with hexarelin and saline were pooled. Data are expressed as means ± SE of 6 determinations. NRS, normal rabbit serum; GHRH-Ab, GH-releasing hormone antibody. *P < 0.05 vs. indicated group. [From Torsello et al. (1038).]
hexarelin (150 μg/kg sc, twice daily) did not modify GH mRNA levels in the pituitary of normal adult male rats (1037). The same was true for a similar treatment schedule in rats with surgical ablation of the MBH or in intact rats with two ectopic pituitaries transplanted under the kidney capsule, two experimental models of reduced GH mRNA levels.

A further approach was to study the effect of GHRP in infant rats, in which both GHRP-6 and hexarelin are very effective stimulators of GH secretion (296, 624, 1037). In these pups 8–10 days of treatment with GHRP-6 or hexarelin consistently primed the pituitary to the GH response elicited by the acute GHRP challenge, but no effect was detected on pituitary GH mRNA levels. However, in the same pups treated since birth with an anti-GHRH serum, whose pituitary GH mRNA levels were significantly lower than controls, 5 days of treatment with GHRP-6 or hexarelin restored GH mRNA levels to control values (624, 1038) (Fig. 9). The same effects were seen in young adult male rats given an anti-GHRH serum for 15 days and receiving a 10-day treatment with hexarelin (1038).

Overall, these findings indicate that GHRP can raise GH mRNA levels independently of endogenous GHRH; this effect does not seem to be present in normal rats, although it may be masked by the overwhelming action of GHRH. The failure of GHRP to restore GH mRNA levels in the pituitary of rats with surgical disconnection of the hypothalmo-pituitary unit and in the ectopic pituitaries emphasizes the concept that GHRP act mostly on the hypothalamus and that the hypothalmo-pituitary unit must be anatomically and functionally intact.

D. Human Studies

Different peptidyl and nonpeptidyl GHRP are very effective to stimulate GH secretion in humans (135). Prolonged infusions of GHRP-6 enhanced pulsatile GH secretion and raised plasma IGF-I concentrations (466, 492, 521; see also below). A challenge with GHRP-6 at the end of the infusion elicited an attenuated GH response, not due to depletion of GH stores, since a challenge with GHRH stimulated GH release (492, 521). Maximally effective doses of GHRP were more effective than maximally effective doses of GHRH (135, 137, 403), and nonpeptidyl GHRP were clearly less potent than peptidyl GHRP (309, 335, 400, 692).

In line with the results of preclinical studies, experimental evidence in humans indicates that GHRP potentiate the GHRH-induced GH release (51, 135, 137, 422, 823) and, as maintained before, induce homologous but not heterologous desensitization (287, 409, 492, 521, 1035), confirming that also in humans GHRP and GHRH act on different receptors and activate different postreceptor pathways. Growth hormone responses indicating the development of a state of receptor downregulation were only observed in humans when GHRP were delivered by continuous infusion; repeated, i.e., three times daily, oral or intranasal doses of peptidyl GHRP for up to 15 days (405, 409), once daily oral dosing with nonpeptidyl GHRP for 4 wk (220), or intranasal peptidyl GHRP for up to 2 yr (843) induced no signs of receptor downregulation.

However, a 16-wk treatment with subcutaneous hexarelin, twice daily, in elderly subjects resulted in a partial and reversible attenuation of the GH response (871a).

The GH response to GHRP appears to have less intrasubject variability than the GH response to GHRH (409, 492, 686, 1055), probably in relation to its lower sensitivity to SS action (see above). Similarly, glucocorticoids, glucose, and exogenous GH, all stimuli allegedly triggering release of endogenous SS (see sects. v1, A1, and BI, and v4A), poorly inhibited the GH-releasing activity of GHRP, and the same occurred for pirenzepine, a muscarinic M1 receptor antagonist, or salbutamol, a β2-adrenoceptor agonist, and atropine, a nonselective muscarinic antagonist, which have in common the same mechanism (50, 167, 401, 645, 674, 823). The modulatory action of SS was not seen for stimuli allegedly inhibiting SS release such as arginine, atenolol, a selective β1-antagonist, and pyridostigmine, an inhibitor of acetylcholinesterase (AChE) (see sect. vB), which did not affect hexarelin’s action (407). These findings may explain why GHRP are more effective GH releasers than GHRH in vivo.

γ-Aminobutyric acidergic mechanisms appear to be involved in the GHRP stimulatory action, since pretreatment with the benzodiazepine alprazolam blunted hexarelin-induced GH release (54).

Another point brought to light by the human studies is the importance of viable hypothalamic connections for full expression of GHRP activity on GH secretion. Thus, in patients with pituitary stalk lesions, the GH-releasing activity of GHRP was severely blunted, also when these compounds were coadministered with GHRH (631, 855, 861, 976a).

The activity of the GHRP is independent of sex but clearly dependent on age (410). In contrast to GHRH, which is maximally effective as a GH releaser at birth, and then progressively loses effect with age (52, 403, 408), GHRP are less effective at birth than at puberty or in the young adult period, their GH-releasing activity starting to decline only with aging, although they remain more effective than GHRH (27, 52, 87, 403, 405, 408, 718). Gonadal steroids very likely play a role in the increased GH response to GHRP at puberty but are unable to act at later periods; in menopausal women, a 3-mo treatment with transdermal estradiol failed to restore the GH response to GHRP (53). However, with aging, a host of disturbing
factors may impair the GH response to most direct- or indirect-acting stimuli to GH release.

1. Aging

That acute administration of GHRP consistently stimulates GH secretion in healthy elderly subjects (27, 52, 403, 408, 718) holds promise for restoring their reduced GH secretion rate, which probably contributes for the increased central obesity, reduced muscle mass and bone mineral content, and loss of psychological well-being (904).

The response to GHRP persisted after repeated oral or intranasal doses of GHRP-6 or hexarelin (405), and after oral administration, there was a tendency toward higher IGF-I concentrations (408). In a randomized, double-blind, placebo-controlled trial in healthy elderly subjects, once daily oral MK-677 enhanced pulsatile GH release, significantly increased serum GH and IGF-I concentrations and, at a dose of 25 mg/day, restored serum IGF-I concentrations to levels normally seen in young adults. Desensitization to the action of MK-677 was not apparent after 4 wk of daily treatment (220).

It still remains to be established, however, whether prolonged treatment with GHS has favorable effects on body composition, functional capacity, and serum lipids, since impairment of glucose tolerance was observed particularly in subjects with risk factors. This might limit the therapeutic usefulness of nonpeptidyl GHS. A comparison of the GHRP is therefore mandatory to assess their safety because these side effects have not been reported with peptidyl GHS. Thus it seems inappropriate as yet to extrapolate the results with a single GHRP to all the other members of the family, even if they purportedly share a common mechanism of action.

2. GH-secreting tumors

Growth hormone-secreting adenomas do not have an altered GH response to GHRP in vivo (29, 241, 451, 860), and the marked GH release from somatotrophinoma cells induced in vitro by GHRP-6 or GHRP-2 (15) may well contribute to the GH response in vivo. In addition, tumors refractory to GHRH in vitro may respond to GHRP-6 by releasing GH (883).

Messenger RNA for the GHRP receptor has been found present in all human pituitary somatotrophinomas so far (14, 299) and in the GH3 tumor pituitary cell line of the rat (14). The receptor was also transcribed in some prolactin-secreting adenomas and, more impressively, in all of 18 ACTH-secreting pituitary adenomas studied (299).

3. Obesity

The GH response to GHRP in obesity is clear, although it is attenuated compared with lean controls (269–271, 561). The combination of GHRP and GHRH, however, markedly stimulated GH secretion in obese patients (131, 269–271, 630). Interestingly, addition to the peptide regimen of acipimox, a lipolytic inhibitor, further enhanced the GH response (269), stressing the role of circulating nonesterified fatty acids (NEFA) in the pathogenesis of the GH hyposecretion of obesity (see sect. viB3). In contrast, somatotroph responsiveness to coadministered hexarelin and GHRH was refractory to the inhibitory effect of glucose (443). This might reflect a selective refractoriness of hypothalamic SS to glucose in obese patients, since exogenous SS, like pirenzepine, abolished the GH response to either GHRH or arginine.

4. Catabolic states

Prolonged critical illness is characterized by protein catabolism and preservation of fat deposits, blunted GH secretion, elevated serum cortisol levels, and reduced IGF-I concentrations. In these conditions, prolonged infusion of GHRP-2, alone or combined with GHRH, strikingly increased pulsatile GH secretion and induced a robust rise in circulating IGF-I levels within 24 h, without affecting serum cortisol (1058). These findings open new perspectives for GHRP as potential antagonists of the catabolic state in critical care medicine.

5. GH deficiency states

A) Children. Although their exact mechanism of action has yet to be defined, the striking effectiveness of GHRP as GH releasers in humans prompted investigations on their use as diagnostic tools as well as therapeutic agents in children with GH deficiency. A number of studies have confirmed that GHRP are effective GHs in children with short stature and/or GH deficiency (416, 568, 594) but not in patients with anatomic disconnection of the hypothalamo-pituitary links or with perinatal stalk transection (630, 855, 861). The potential for these compounds as a rapid, safe, and economical test to identify hypopituitarism due to pituitary stalk transection is evident.

An open trial in children with GH deficiency showed that intranasal administration of hexarelin for up to 6 mo accelerated growth (594). Similarly, in children with GH insufficiency, GHRP-2, given subcutaneously at increasing doses every 2 mo (from 0.3 to 3 mg·kg⁻¹·day⁻¹) for 6 mo, raised IGF-I levels and growth velocity (709). In children with short stature, intranasal GHRP-2, twice daily for 3 mo then three times a day for up to 18–24 mo, induced a modest but significant rise in growth velocity (843). However, plasma IGF-I or IGF-BP3 concentrations, or acute GH responses to intravenous or intranasal GHRP-2 were not changed and only the GHBP significantly increased.

Despite some positive results, long-term and double-blind, placebo-controlled trials are mandatory before any
firm conclusions can be drawn on the therapeutic utility of GHRP in GH deficiency states.

B) ADULTS. Many studies have shown that prolonged parenteral, intranasal, or oral administration of GHRP enhances spontaneous pulsatile GH secretion in normal elderly people (405, 492), or in adults with GH deficiency (599). Growth hormone deficiency of adults has received little attention in the past and only recently, since the demonstration of altered body composition, increased prevalence of cardiovascular morbidity, and decreased life expectancy (278, 533), has been investigated more. The problem of GH deficiency is widespread because many patients develop life-long hormone deficiency due to lesions induced in the hypothalamo-pituitary unit by tumors or radiation. Growth hormone therapy has proven very effective in these patients (278); GHRP might offer an even better therapeutic approach for restoring a normal condition.

Growth hormone-releasing peptides would also be important for the diagnosis of GH deficiency in adults, which is more difficult than in children, because of the confounding effects of many factors such as obesity, sex, and age. A combined test of GHRP and GHRH, which obviates many of the factors that normally inhibit GH secretion (137, 270, 631), might be a safe and powerful tool for the diagnosis of GH deficiency, allowing the rationale as therapeutic use of GHRP.

E. Effects on Other Pituitary Hormones

Although predominant, the activity of GHRP is not fully specific for GH release. Reportedly, peptidyl GHRP in vitro elicit a small but unequivocal release of prolactin (692) and in vivo of prolactin, ACTH, and cortisol in humans, dogs, pigs, and rats (628). In vivo, the main site of action for stimulation of ACTH and cortisol is neither the pituitary (225, 335) nor the adrenals because these endocrine effects were abolished by stalk transection in the pig (468) or hypophysectomy in the dog (937), thus indicating a hypothalamic mechanism. Interestingly, hexarelin was shown to release AVP from freshly prepared rat hypothalamic tissues (569).

Some GHRP maintained their prolactin-releasing effect on mammosomatotroph adenomas both in vitro (13) and in vivo (241). This was not the case for hexarelin, which does not stimulate prolactin release in patients with idiopathic hyperprolactinemia, thus implying a partial resistance to GHRP in this condition (241). The precise mechanism(s) underlying these effects is still unknown.

The lack of specificity of GHRP is of concern if these agents are to be used in clinical trials. To address this issue, Massoud et al. (675) constructed dose-response relationships for GH, cortisol, and prolactin and investi-
tion has been sorted out, and despite recognized differences (see below), the two systems can no longer be considered entirely separate entities. The same compound may act differently as a transmitter (via strictly local short-lived synaptic responses), as a neuromodulator (modulating the subsynaptic actions of a neurotransmitter-coupled event), and as a neurohormone (acting at a distance from the site of release with no synaptic contact with the synthesizing neurons), depending on the engagement of specific receptors (117).

Another reason why the demarcation between these messenger molecules has become blurred is the recognition of their existence in the same neuron in different CNS areas, as well as in the MBH. The functional role of the costored neurotransmitters and neuropeptides remains to be fully elucidated, although demonstration of costorage within nerve terminals at the ME suggests corelease at this site. These events are of particular interest for the control of GH secretion. Because GHRH neurons of the ARC also contain enzymes for neurotransmitter biosynthesis, neurotransmitters and neuropeptides (see sect. III A2) and GHRH-induced GH release may be influenced by some of these.

B. Characteristic Features

A detailed description of the mechanisms of biosynthesis, release, and metabolic disposal of principal neurotransmitters and neuropeptides and of the CNS-acting compounds that interfere with the different metabolic steps or with pre/postsynaptic receptors is beyond the scope of this review, and the readers are referred to Müller and Nistico (756) and Bloom (117). Here only key neurobiological features are given and a brief mention of the regional distribution of the main neuronal systems in the CNS.

Neurotransmitters are small molecules synthesized in a short series of steps from precursor amino acids in the diet. They are found in the brain in concentrations of nanograms to micrograms per gram and show high affinity for specific receptor sites.

FIG. 10. Synthesis pathways of principal neurotransmitters. See text for definitions.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Metabolic path</th>
<th>Method of inactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>TH → Dopa → AADC → DA → DBH → NE → PNMT → E</td>
<td>Reuptake, MAO, COMT</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Trp-H → 5-HTP → AADC → 5-HT</td>
<td>Reuptake, MAO, aldehyde dehydrogenase</td>
</tr>
<tr>
<td>Choline</td>
<td>CAT → Acetylcholine → ACh-ase → Choline</td>
<td>Reuptake</td>
</tr>
<tr>
<td>Histidine</td>
<td>HD → Histamine</td>
<td>MAO, histaminase</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Glutaminase → Glutamate → GAD → GABA</td>
<td>GABA-T and reuptake</td>
</tr>
</tbody>
</table>

Many drugs are capable of inhibiting the functional activity of neurotransmitter neurons. Biosynthesis inhibitors act on the different enzymatic steps, and there are depletors of the granular pool and neurotoxic agents that destroy neurotransmitter neurons. Neurotransmitter pre-
neuropeptides and agonists or antagonists for D1, D2, and D4 receptors. No major differences have been described in how these subclasses of receptors affect GH secretion.

The 5-HT system arises from cell bodies in the mesencephalic and pontine raphe and sends axons especially to the hypothalamus, ME, POA, limbic system, septal area, striatum and cerebral cortex. On the basis of the action of 5-HT receptor agonists and antagonists, four subgroups of 5-HT receptors have been characterized: 5-HT1-like, 5-HT2, 5-HT3 and 5-HT4. To date, 5-HT1A-F, 5-HT2A-C, 5-HT3, and 5-HT4–7 subtypes have been described.

Antibodies specific for the ACh-synthesizing enzyme CAT, coupled with the use of AChE histochemistry, ligand binding, or in situ hybridization studies, have made it possible to outline cholinergic neurons and trace their pathways. The enzymatic activities are present in most hypothalamic nuclei, including the ARC and the ME. Because only small changes have been detected in CAT concentrations in the MBH after mechanical separation of this area, and no change at all was found in the ME, an intrahypothalamic cholinergic pathway, similar to the TIDA pathway, has been envisaged. This may be important in some effects of the cholinergic drugs on somatotropic function (see below), also considering the similar distribution of somatostatinergic and cholinergic neurons in the lateral region of the external layer of the ME (954).

There are two classes of ACh receptors, the muscarinic and the nicotinic. Five subtypes of muscarinic cholinergic receptors (M1 to M5) have been detected by molecular cloning; all five are found in the CNS. The regional localization of histamine in the brain and in individual nuclei of the hypothalamus has been studied in rodents and primates. In the monkey and human hypothalamus, the highest concentrations are found in the mammillary bodies, SON, VMN and ventrolateral nucleus, and ME. After deafferentation of the MBH in rats, levels of histamine do not decrease significantly in the ARC, VMN, DMN, and ME, suggesting that histamine is present in the posterior two-thirds of the hypothalamus in cells intrinsic to this area. Three subtypes of histamine receptors have been described, H1, H2, and H3. Unlike the monoamine and amino acid transmitters, there does not appear to be any active histamine reuptake. In addition, no direct evidence has been obtained for release of histamine from neurons either in vivo or in vitro.

Specific antibodies raised against GAD have made it possible to precisely locate GABAergic neurons in the hypothalamus. A dense network of GAD-positive nerve fibers is seen in different hypothalamic nuclei of the rodent and cat brain. In the ME, a dense immunofluorescent plexus is found in the external layer, extending across the entire mediolateral axis from the rostral part to the pituitary gland. The hypothesis of an intrinsic, hypothalamic TI-GABAergic pathway projecting from the ARC to the
ME is supported by deafferentation experiments of the MBH. γ-Aminobutyric acid receptors are divided into two main types: 1) GABA_A, which is bicuculline sensitive and has multiple subtypes, interacts directly with and is the site of action of many neuroactive drugs (benzodiazepines, barbiturates), anesthetic steroids, volatile anesthetics, and alcohol; and 2) GABA_B, which is bicuculline insensitive and is present in both the CNS and the periphery, although mainly at the presynapses, at least peripherally. Specific agonists and antagonists of GABA_A and GABA_B receptors are now available.

Glutamate and aspartate are found in very high concentrations in different brain areas such as discrete hypothalamic nuclei (ARC, VMN, PeVN), with nerve terminals projecting to the ME. The widespread distribution of these two dicarboxylic amino acids in the CNS and their role in the intermediary metabolism tended to exclude any action as transmitters, but it is now acknowledged that both amino acids exert extremely powerful excitatory effects on neurons in virtually every region of the CNS. Many receptor subtypes have been characterized pharmacologically. They fall into two main groups: the ionotropic and metabotropic receptors. Ionotropic receptors can be subdivided into N-methyl-D-aspartate (NMDA), kainate, and D,L-α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA); metabotropic receptors are activated most potently by quisqualate.

Neurotransmitter-hypophysiotropic peptides, e.g. GHRH and SS, interact in many ways. These include direct action of neurotransmitters transported into hypophysial vessels (DA, E and GABA), the still elusive neurotransmitter-neurotransmitter, peptide-peptide and neurotransmitter-peptide interactions due to colocalization in and corelease from the same neuron of two or more molecules (Fig. 2) (457, 484, 699). Consequently, drugs that act as agonists or antagonists at neurotransmitter receptors or alter different aspects of neurotransmitter function can be useful for studying the physiology and pathophysiology of GH secretion and as diagnostic and therapeutic tools.

Detailed mapping of the principal brain neuropeptides is beyond the scope of this review, and the reader is referred to References 457 and 484. Briefly, however, several hypothalamic peptides such as oxytocin, AVP, POMC, gonadotropin-releasing hormone (GnRH), and also GHRH all tend to be synthesized by single large clusters of neurons that give off multibranched axons to several distant targets. Others, such as systems that contain cholecystokinin, enkephalins, and also SS, have many forms, with patterns varying from moderately long connections to short axons, local-circuit neurons that are widely disseminated throughout the brain.

D. Neurotransmitters

1. Catecholamines

A) α2-Adrenoceptors. Although formulated more than 25 years ago and based on the pituitary “depletion” method, subsequently shown to be fraught with inconsistencies, the concept that NE is a synaptic transmitter that releases GRF (758) is essentially valid today. Proper evaluation of neurotransmitter function became possible with RIA methods for the measurement of GH in rat plasma, the awareness of the labile and episodic function of GH secretion in the rat, and hence the adoption of chronically cannulated rats for blood sampling (666).

Since then, evidence has accumulated that central adrenergic pathways acting through α2-adrenoceptors stimulate GH secretion in humans and rats (756). Instrumental to this conclusion were experiments in rats with an intratral cannula, in which blockade of CA synthesis by α-methyl-p-tyrosine, depletion of hypothalamic CA stores by reserpine, and injection of 6-hydroxydopamine, a CA neurotoxin, markedly suppressed the episodic GH secretion; these effects were counteracted by the α2-adrenoceptor clonidine, but not the DA agonist apomorphine (759). More selective inhibition of NE and E synthesis by blockers of DA-β-hydroxylase, the enzyme that converts DA to NE and E (see Fig. 10), also inhibited spontaneous GH bursts, this effect again being counteracted by clonidine (549, 578, 771). Epinephrine was presumably the main endogenous CA active on α2-adrenoceptors, since selective blockade of E synthesis by phenylethanolamine N-methyltransferase (PNMT) inhibitors reduced GH secretion in freely moving rats, and the effect was reversed by clonidine (1028). There was concomitantly a reduction of E levels in the hypothalamus with no changes in DA and NE stores. A peripheral E synthesis blocker had no effect on GH secretion, indicating that inhibition of brain rather than adrenomedullary E was responsible (1028).

The importance of α-adrenergic mechanisms in GH secretion was substantiated by the finding that clonidine given acutely to 10-day-old rats induced a clear-cut rise in plasma GH, although the effect was not dose related; short-term administration of clonidine to 5-day-old rats also raised plasma GH levels and pituitary GH content (204). A sound interpretation of these findings was that in infant rats clonidine, in addition to stimulating GH release, had elicited GH synthesis, as confirmed by measurement of pituitary [3H]leucine incorporation into GH of infant rats treated ex vivo with the drug (272). Because similar results were obtained in infant rats with GHRH (272), it appeared likely that clonidine acted at least partially, through the release of endogenous GHRH. Clonidine did not affect GH release in rats with hypothalamic destruction or from cultured AP cells (552).
In keeping with this conclusion, studies in the rat had shown that clonidine-induced GH release was abolished by passive immunization with anti-GHRH serum (206, 721), whereas it was not affected by anti-SS antibodies (331). The drug was reported to stimulate GRF release in vitro from perfused rat hypothalamus (536). It was subsequently shown that a single dose of clonidine to adult male rats significantly lowered GHRH content, leaving GHRH mRNA levels unaltered and raising plasma GH while hypothalamic SS mRNA and pituitary GH mRNA levels remained unchanged. In contrast, in rats treated with clonidine for 1 and 3 days, hypothalamic GHRH content fell, and there was a significant reduction in GHRH mRNA levels. In these rats, pituitary GH content and mRNA levels increased significantly, whereas hypothalamic SS content and mRNA remained unaltered. In 6-day clonidine-treated rats, hypothalamic GHRH content and mRNA was still significantly reduced, plasma GH levels were increased, although less than in 1- and 3-day clonidine-treated rats, and pituitary GH content and mRNA had returned to control values (294).

It would seem that in these experimental conditions, clonidine-induced \(\alpha_2\)-adrenergic receptor stimulation led directly, or through inhibition of hypothalamic SS release (see below), to increased GHRH release from the hypothalamus and enhanced GH biosynthesis and secretion and that as a result of greater exposure to clonidine, circulating GH (and IGF-I) fed back to hypothalamic GHRH neurons, thus finally reversing pituitary somatotropic hyperfunction.

Apparently, SS neurons were refractory to circulating GH or IGF-I under these experimental conditions. In similar studies, Gil-Ad et al. (418) detected a decrease in the hypothalamic content of SS only after acute clonidine, and not after a 7-day treatment. Additional, although inferential, evidence in favor of a GHRH-mediated effect is the fact that either compound induces sedation and sleepiness and stimulates food intake (see Ref. 479 and sect. mA10) and that specific \(\alpha_2\)-adrenergic nerve terminals have been detected in the arcuate nucleus (620).

None of the above observations, however, excludes the possibility that clonidine may also act through inhibition of SS pathways. In fact, in rats, clonidine failed to induce GH release when directly instilled into the VMN, an area of GHRH neurons (see sect. mA2), but was effective when injected into the SS-rich MPOA (515). Later studies in humans showed that pretreatment with GHRH abolished the GH response to subsequent administration of the peptide but did not alter the GH response to clonidine (1052).

Investigations in humans and animals were then started, to clarify the precise mechanism of action of clonidine, the possibility being considered that GH responsiveness to GHRH may be closely dependent on the functional status of the hypothalamic somatotroph rhythm (HSR) (308), and that in previous in vivo studies clonidine had disrupted the spontaneous surges of GH release in male rats (see sect. i).

In normal adult volunteers, clonidine administered 60 or 120 min, but not 180 min, before a GHRH bolus increased the GH response to GHRH; the close relationship between pre-GHRH plasma GH and GHRH-induced GH peaks, not present with clonidine alone, was also lost after pretreatment with this drug. Clonidine-induced disruption of the intrinsic HSR suggested that \(\alpha_2\)-adrenergic pathways exert an inhibitory effect on SS release (307). Similarly, in conscious rats, clonidine at all doses tested failed to stimulate GH release when administered at the time of a spontaneous peak, i.e., when the intrinsic SS tone would be minimal. In contrast, clonidine injected at a trough time, functionally the opposite condition, raised plasma GH. In addition, clonidine administered during a GH trough period before GHRH challenge potentiated the GH response to GHRH (591).

Acute and short-term clonidine studies in dogs gave similar findings. In old conscious beagles given GHRH and clonidine together, twice and once daily for 10 days, treatment significantly raised the frequency of spontaneous bursts of GH secretion, the mean GH peak amplitude, and the total peak area evaluated in 6-h blood samples taken at 10-min intervals (207). These effects, paradoxically, were not as marked when clonidine and GHRH were given twice rather than only once daily. This was presumably because the \(\alpha_2\)-adrenergic agonist downregulates its own receptors in the ARC at a higher dose (208).

The most parsimonious interpretation of these findings is that a dual mechanism of action is exploited by \(\alpha_2\)-agonism in enhancing GH secretion, e.g., stimulation of hypothalamic GHRH and inhibition of SS neuronal function. Light microscopic and electron microscopic evidence supports this. Immunocytochemical double-labeling and dopamine \(\beta\)-hydroxylase (DBH) and PNMT immunolabeling for tracing the CA system (620) revealed a similar pattern of distribution with juxtaposition of catecholaminergic fibers and GHRH neurons in the ARC. Electron microscopic examination showed axodendritic and axosomatic synaptic specialization between these systems (620).

However, in addition to these morphological findings suggesting that the CA system stimulates GH release through GHRH, other neuroanatomic evidence indicates that SS structures in the anterior PeVN nucleus of the rat hypothalamus are innervated by PNMT-immunoreactive axons (621). These can be usefully viewed in connection with the existence of SS synapses on GHRH neurons (see sect. ui, A3 and B). With the assumption that they originate from hypophysiotropic SS neurons, it would seem that afferent neuronal systems to SS cells influence GH production either by regulating SS secretion into the portal circulation (235) or by transmitting their influence to the GHRH neurons through SS axons (622). This complex
mechanism may be used to synchronize these hypotha-
lamic pathways to achieve precise coordination between
stimulatory and inhibitory systems (853).

The GH-releasing effect of clonidine, as expected,
was antagonized in humans and rodents by the selective
$\alpha_2$-adrenergic antagonists yohimbe (158) and flupar-
oxan (529). However, in rats metergoline, a 5-HT$_1$/5-HT$_2$
antagonist, and mesulergine, ritanserin, and mianserin,
5-HT$_1$/5-HT$_2$ selective antagonists, all reduced clonidine-
induced increases in GH levels, without affecting the
decrease in locomotor activity. These findings suggest
that clonidine stimulates the release of GH by activating
$\alpha_2$-adrenergic heteroreceptors on 5-HT nerve terminals
which, in turn, enhance 5-HT activity by stimulating
postsynaptic 5-HT receptors. In the same study, clonidine
failed to increase GH levels in the fawn-hooded rat, a
model of altered serotonergic function (60).

1) Diagnostic applications. The fact that clonidine
stimulates GH secretion in a variety of species, including
humans (749), has led to $\alpha_2$-adrenergic stimulation being
used as a diagnostic test in children of short stature (419,
589). A single oral dose of clonidine stimulated GH re-
lease in healthy children and adolescents but not in hy-
popituitary children. Clonidine proved to be a more effect-
ive stimulant of GH secretion than either insulin
hypoglycemia or arginine infusion; there were, however,
false-negative responses to clonidine even in healthy sub-
jects and wide variability in the GH release in endocrino-
logically short children (76). Today, the clonidine test is
still widely used in pediatric endocrinology, but it has
been largely supplanted by more recently diagnostic tests
(see sect. VI.A). For a more thorough discussion of this
topic, see Reference 755.

2) Therapeutic implications. The suggestion that a
dysfunction of neurons regulating the release of GHRH
from GHRH-secreting structures may occur in some chil-
dren with idiopathic GH deficiency (231, 541) prompted
efforts to stimulate the purportedly depressed neuro-
transmitter function, initially with a DA precursor or ag-
tonist drugs, and then with clonidine.

Promising results with dopaminergic therapy (497,
498), and the possibility that some effects, e.g. those of
levodopa, might be due at least in part to its conversion to
NE in the hypothalamus (1108), suggested the use of clonidine in children with growth retardation. Oral clonidine for 3 mo to 1 yr stimulated linear growth in the majority of children, the most responsive being those in general with low pretreatment growth velocity and marked bone age delay. There was, however, a tendency for growth velocity to slow with time, i.e., after the first 6 mo or 1 yr, a pattern which might be due to $\alpha_2$-adreno-
ceptor desensitization (185, 388, 739, 851). However, in a
subsequent placebo-controlled study, clonidine had no
effect on the growth of short children with normal GH
responses to provocative stimuli. Unlike other studies, the
children in this study had higher pretreatment growth
velocity (832). Negative results were also reported by
Allen et al. (26). In a later controlled study, clonidine did
accelerate the growth of short children with normal GH
secretion, although, at the dose used, it was not as effec-
tive as GH (1073).

We do not know whether the effect of clonidine
observed in some of these studies will result in better final
height. Certainly, the negative results of one of the con-
trolled studies (832) discouraged further investigations on
a topic that, for its scientific interest and potential clinical
benefits, warrants closer attention (35).

It is noteworthy that continuous intravenous
clonidine to patients with alcohol abuse, for prevention of
alcohol withdrawal syndrome, significantly improved
their nitrogen balance and counteracted the decline of
plasma IGF-I and IGF-BP-3 seen in untreated patients
after resection of an esophageal cancer (715).

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3) $\alpha_1$-ADRENOCEPTORS. The availability of clonidine and
its ability to stimulate GH secretion in many animal spe-
cies allowed the characterization of the NE receptor sub-
types mediating its neuroendocrine effect. In dogs, pre-
treatment with the $\alpha_1$-adrenoceptor antagonist prazosin
left the clonidine-induced GH rise unaltered, whereas
blockade of $\alpha_2$-adrenoceptors by yohimbine completely
prevented it (215). However, prazosin partially sup-
pressed the GH secretory response to clonidine in rats,
which may indicate a mixed $\alpha_1$/$\alpha_2$-mechanism in the GH-
stimulating effect of the drug (578). However, in both rats
and dogs, $\alpha_1$-adrenoceptors might also mediate inhibitory
influences to GH release, indicating a dual NE mechanism
of control (214, 578). Overall, the results supported the
idea that $\alpha_1$-adrenoceptors in the CNS inhibited both tonic
and stimulated GH secretion in rats and dogs. Apparently,
the underlying mechanism involved the activation of SS
release. Thus the ability of methoxamine, an $\alpha_1$-adreno-
ceptor agonist, to suppress GH levels in infant rats was
completely abolished in animals pretreated with a SS
antiserum. However, the clear-cut GH rise induced by
prazosin was not further increased by pretreatment with the
antiserum (206).

$\alpha_1$-Noradrenergic inhibition would be mediated
through receptor sites in the PVN, and electrolytic lesions
of these in the rat double the amplitude of the GH curve
and the area under the curve, while blocking the GH inhi-
bitory effect of locally instilled methoxamine (744). This
is closely comparable to the obtained pattern under
similar conditions by bilateral destruction of the locus
coeeruleus (119). Because both the PVN and the soma-
tostatinergic hypothalamic PeVN are innervated by locus
coeeruleus NA$_1$ afferents (28), a pathway stimulatory of SS
function, presumably through PVN-CRH neurons, has
been envisaged.

Contrasting the clear-cut inhibitory effects of $\alpha_1$-ad-
renoceptor stimulation in dogs and rats are reports in
humans of either a small increase in GH secretion during intravenous infusions of \(\alpha_2\)-adrenergic agonists (506) or no effect on GH secretion, with a small, not significant, decrease after methoxamine (24). Although CA are clearly involved in the GH response to hypoglycemia, prazosin did not affect this response in humans (1024), suggesting that both stimuli share the same mechanism, i.e., inhibition of hypothalamic SS function. Although there is no information on how \(\alpha_2\)-antagonists affect the GH responses to more physiological stimuli, it would appear that endogenous CA acting on \(\alpha_2\)-adrenoceptors are unlikely to play a role in the secretion of human GH; this inhibitory action of CA is better accomplished through \(\beta\)-adrenoceptors.

c) \(\beta\)-Adrenoceptors. The inhibitory role of \(\beta\)-receptor activation on GH release has been known for many years. The nonspecific \(\beta\)-antagonist propranolol has no effect on GH secretion under basal conditions in Caucasians (110), whereas it does enhance the GH response to indirect-acting CA agonists, such as amphetamines and desipramine (582, 877). This effect is achieved through propranolol-induced suppression of inhibitory influences mediated by \(\beta\)-adrenoceptors, on which the synaptically released CA act. The same mechanism would explain why intravenous infusion of E had no effect on GH secretion in humans, whereas E combined with propranolol stimulated GH secretion (673). It would seem that E infusion activates both \(\alpha_2\) and \(\beta\)-adrenoceptors, with propranolol unmasking the opposite actions of these two receptors which, very likely located in the ME, are accessible to infused CA (917).

It has since been shown that the \(\beta_2\)-adrenergic agonist salbutamol inhibits the GH response to GHRH in humans (412). This is best explained by the possibility of \(\beta_2\)-agonism through stimulation of the secretion of SS; inhibition of \(\beta\)-receptors would inhibit SS release.

This view fits well with the marked enhancing effect of propranolol on the GHRH-induced GH rise in children (234) and of the selective \(\beta_1\)-receptor antagonist atenolol in adult humans (677) and short children (658). Moreover, acute administration of atenolol to GH-deficient children receiving long-term GHRH increased integrated GH secretion (659). These findings led to investigation of whether chronic treatment with atenolol might augment the therapeutic potential of GHRH. In a double-blind placebo controlled study, atenolol and GHRH coadministered to a group of GH-deficient children increased growth velocity during the first year, to a greater extent than treatment with GHRH alone. However, during the second year of therapy, there was no significant difference in growth velocity between the two groups, and the mean 24-h concentration, that had been higher in the atenolol group during the first year, was lower in this group during the second year (183).

\(\beta\)-Adrenergic agonism and its clinical implications especially concern salbutamol, a \(\beta_2\)-adrenergic agonist extensively used as a bronchodilator in the treatment of asthma. Inhibition of GH release after GHRH treatment and during overnight GH testing was described in type I diabetic patients after short-term oral administration of this drug (668). Evaluation of the short- and long-term effects of oral salbutamol in asthmatic short children showed that 24 h of drug treatment led to a decrease in the overnight IC-GH concentrations and peak GH levels after GHRH; however, after 3 mo of therapy, GH levels were no different from baseline and rose normally after GHRH (588). It would seem, therefore, that oral salbutamol produces no chronic deleterious changes in GH secretion and, hence, height velocity in children, although larger long-term studies are needed to confirm this.

Whereas in vivo activation of CNS \(\beta\)-adrenoceptors inhibits GH secretion in vitro, activation of these receptors stimulates GH secretion from rat pituitary cells (830). This mechanism is presumably not evident in vivo because it is a minor effect compared with the stimulant action of \(\beta\)-adrenoceptors on SS secretion, which causes overall inhibition of GH secretion.

d) Dopamine. Dopaminergic pathways are involved in the control of GH secretion, although their role appears to be largely ancillary. In rats, dogs, and monkeys, systemic administration of the DA precursor levodopa or direct-acting DA agonists stimulated GH release (see Ref. 756 for details). However, an \(\alpha\)-adrenergic component is also involved, as shown by the fact that \(\alpha\)-adrenoceptor antagonists partially or completely antagonize the stimulatory effect of DA in monkeys (985) and humans (159). Moreover, an inhibitory component seems inherent to DA's ability to affect GH secretion. Thus there is evidence that monkeys have DA receptors inhibitory to GH release in the blood-brain barrier (BBB) (985, 1036).

In healthy humans, although direct DA agonists such as apomorphine and the ergot derivates bromocriptine, lisuride, cabergoline, or indirect DA agonists such as amphetamine, nomifensine, or methylphenidate cause acute GH release and increase GH release in response to GHRH (1057), they blunt the GH response to insulin-induced hypoglycemia, levodopa, and arginine, administered by infusion (69, 1105). Bromocriptine (69) stimulates GH release in children with low baseline GH, but it has a rebound effect (inhibition, followed by rebound stimulation) or a clear-cut inhibitory effect in children with elevated baseline GH (79). These findings are best explained by the ability of DA to release both GH and SS from the rat hypothalamus (562) and by the different sensitivity of SS and GHRH neurons to dopaminergic stimulation in relation to the endogenous DA tone. This puzzling feature is further complicated by the fact that DA and its agonists inhibit GH release from rat (273) and human (656) pituitaries in vitro and inhibit GH release in conditions of
pathological hypersecretion, a topic which is beyond the scope of this review and for which the reader should refer to Reference 1034.

In the rat, the dopaminergic hypothalamo-pituitary axis appears to be involved in the differentiation, proliferation, and survival of pituitary cells during development. A DA transporter (DAT), which terminates DA action by amine reuptake into nerve terminals, is presumably expressed on hypothalamic dopaminergic neurons (698), but its relevance in calibrating DA overflow to the anterior pituitary remains unclear.

In a mouse strain with deletion of the DAT gene, it was confirmed that DA reuptake through the transporter plays a vital physiological role regulating hypothypothalamic dopaminergic function. These mice have an altered spatial distribution, and a dramatic reduction in the numbers of somatotroph (and lactotroph) cells, are unable to lactate and show dwarfism. The effects of DAT deletion on pituitary function result from elevated DA levels that strikingly reduce hypothalamic GHRH function and downregulate the lactotrope D₂ DA receptors. Dopamine-induced suppression of GHRH function then downregulates the Pit-1/GHF, a transcription factor that commits the pluripotent pituitary stem cell to generate specific cell types (128).

2. Serotonin

Serotonergic pathways in the brain contribute stimulatory influences for GH release in rodents (45). The situation is less clear in humans, where different drugs that either inhibit the functional activity of serotonergic neurons or potentiate neurotransmitter mechanisms did not induce the expected changes (108, 233, 702; see also Ref. 756 for review).

Data implicating 5-HT in raising the concentrations of circulating GH in the rat come from studies in which inhibition of tryptophan (Trp) hydroxylase by p-chlorophenylalanine significantly inhibited GH pulsatile secretion in unanesthetized male rats (665), or blockade of 5-HT receptors with nonspecific 5-HT antagonists was associated with suppressed GH levels (see Ref. 756). Conversely, the 5-HT precursor L-Trp, administered systemically, increased brain Trp and 5-HT levels and induced sustained release of GH (45, 1070); the same was true for intracerebroventricular injections of 5-HT or the direct 5-HT agonist quipazine (1070). The immediate 5-HT precursor 5-hydroxytryptophan (5-HTP) given systemically to unanesthetized rats also potently stimulated GH secretion, an effect prevented by the nonspecific 5-HT inhibitor cyproheptadine (967). However, after administration of 5-HTP, 5-HT may not be formed exclusively in 5-HT neurons but also in CA-containing neurons, leading ultimately to CA release (see Ref. 756).

In keeping with this in infant rats, the brisk rise in plasma GH induced by 5-HTP was not counteracted by two 5-HT receptor antagonists but by blockade of dopaminergic or α-adrenergic receptors or central sympathectomy induced by 6-OHDA (255). A further note of caution on the early 5-HT studies regards the low specificity of the 5-HT receptor antagonists often used, i.e., methysergide and cyproheptadine. The latter also has antihistaminic, anticholinergic, antidopaminergic, and protein synthesis blocking properties, and the former shows paradoxical stimulating activity on 5-HT receptors (756).

The lack of reasonably discriminating agonists and selective antagonists for most 5-HT receptor subtypes certainly accounts for some of the early controversial findings (749). Now that specific receptor ligands are available, studies have been designed to elucidate the involvement of specific 5-HT receptor subtypes. In freely moving conscious male rats, activation of 5-HT₁A receptors by systemic administration of the specific agonist hydroxy-2-(di-n-propylamidotetralin) or ipsapirone, or the less specific 5-HT₁B and 5-HT₁D agonist 1-(m-trifluoromethyl-phenyl)piperazine failed to affect plasma GH levels at any dose tested (311).

The roles of different 5-HT receptors have been somewhat clarified by studies on the mechanisms underlying α₂-adrenoceptor-induced GH stimulation. Among various 5-HT receptor antagonists, only metergoline, a non-selective 5-HT₁/₂ antagonist, and mesulergine, a 5-HT₁A/5-HT₂ selective antagonist, almost completely suppressed clonidine-induced increases in GH levels, whereas two other 5-HT₁/₅-HT₂ antagonists, ritanserin and mianserin, only partially attenuated clonidine’s effect. In these studies, a 5-HT₃ receptor antagonist, MDL 7222, spiperone, a 5-HT₁A/5-HT₂ antagonist, and propranolol, a β-adrenergic/5-HT₁D antagonist, were completely unable to antagonize clonidine (60). Overall, the pharmacological profile of these compounds and their rank order of potency suggest that 5-HT₁C rather than 5-HT₂ receptors are involved in the α₂-adrenoceptor stimulation-induced GH increase in the rat. However, studies of short-term maternal separation in preweanling rats, a model for the syndrome of maternal deprivation in humans, which encompasses growth retardation, weight reduction, and social withdrawal (32), once termed psychosocial dwarfism (863), have led to the conclusion that 5-HT₂A and 5-HT₂C receptors were certainly involved in GH hyposecretion (553).

Whatever the real contribution of the different 5-HT receptor subtypes, the close functional relations between adrenergic and serotoninergic pathways involved in GH release, and the idea that brain 5-HT changes are implicated in the etiology of affective illness and the mode of action of antidepressants and antimanic drugs (700), might explain why several clinical studies found blunted GH responsiveness to clonidine in depressed patients compared with controls (756). The ability of moclobemide, an antidepressant drug and selective reversible inhibitor of type A monoamine
oxidase (MAO-A), to markedly increase 5-HT function and stimulate GH secretion in depressed subjects should be evaluated in this context (766).

In Hollstein steers, systemically administered quipazine, a nonspecific 5-HT_{1B/2A/2C} agonist and, possibly, a 5-HT_{1B} antagonist, increased GH secretion, whereas cyproheptadine lowered it. These drugs had this effect regardless of whether cattle ate ad libitum or were food deprived, but they did not influence SS concentrations in plasma after feeding, suggesting that 5-HT receptor-stimulated GH secretion is mediated within the CNS and is involved in stimulation of pulsatile GH secretion (394). In the monkey, however, 5-HT microinjected into the VMN did not raise plasma GH (1036), although a significant response was reported after systemic infusion of 5-HTP (216).

In dogs, 5-HT seems to have an inhibitory role in the canine GH (cGH) response to insulin hypoglycemia (626, 761). Other studies in dogs aimed to establish whether a primary 5-HT effect on cGH secretion could be distinguished from stress-related cGH stimulation. They were prompted by the observation that while low doses of 5-HTP produced brisk cGH responses, with no signs of distress or changes in plasma corticosteroids, high 5-HTP doses, although able to release cGH, were associated with behavioral and endocrine signs of stress (320). Pentobarbital sodium anesthesia abolished the rise in cGH and corticosteroids induced by insulin-hypoglycemia, but not an earlier increment in plasma cGH, unaccompanied by stimulation of corticosteroid release, induced by systemically administered 5-HTP (321).

Although these elegant studies imply that the 5-HT mechanism of GH stimulation in the dog differs from the stress-related mechanisms of cGH release, they are biased by the use of 5-HTP as a specific activator of 5-HT neurotransmission, as pointed out previously. In anesthetized dogs, the effect of 5-HTP was abolished by the adrenergic receptor antagonist phenoxybenzamine (1130), and the same was true for healthy volunteers (768).

Further evidence that in some animal species activation of central 5-HT neurotransmission inhibits GH release comes from experiments in sheep where tianeptine, a 5-HT uptake enhancer, increased GHRH release into the hypophysial portal vessels and raised GH secretion (186).

In humans, using a variety of 5-HT receptor agonists and antagonists, it has been seen that 5-HT pathways stimulate, inhibit, or do not alter GH secretion (see Ref. 756 for references). Sumatriptan, a recent selective 5-HT_{1D} receptor agonist with no activity on 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{3} receptors, and other neurotransmitter receptors, has permitted closer assessment of 5-HT’s effects on basal and stimulated GH secretion. In normal prepubertal children, sumatriptan raised basal GH levels and the GH responses to GHRH; however, sumatriptan pretreatment did not modify the GH response to clonidine or pyridostigmine, an inhibitor of AChE, but increased the GH response to arginine. In a group of obese children, sumatriptan did not alter baseline GH levels, but still enhanced the effect of GHRH (743). A cautious interpretation of these findings is that sumatriptan was active in normal and obese subjects through inhibition of the somatostatinergic tone, with the only reservation in this reasoning resting on the enhanced GH response to arginine which allegedly operates through the same mechanism. A CNS mechanism of action for sumatriptan is suggested by its lack of effect on GH secretion in acromegaly (279).

In a group of nondepressed male alcoholics with 1 or 2 yr of abstinence from alcohol, sumatriptan failed to increase GH secretion (1069), implying a persistent, selective loss of 5-HT_{1D} receptor function (see also sect. vB5a2).

Other studies of 5-HT involvement in GH regulation in humans have used 5-HT_{2} receptor antagonists. In healthy subjects, ketanserin did not modify the GH response to insulin hypoglycemia (865), whereas its companion drug ritanserin did (1027), very likely due to its higher affinity for the 5-HT_{2} receptor and better penetration of the BBB.

These findings further complicate the already contradictory data in the literature. Quipazine, a nonsselective 5-HT receptor antagonist, given orally, did not alter plasma GH in normal subjects or patients with neurological disorders (809). The elusive nature of serotoninergic modulation of GH secretion also emerges from studies with fenfluramine, a potent 5-HT releaser and inhibitor of 5-HT reuptake. In healthy subjects, the drug inhibited the GH release induced by the combination of L-dopa and propranolol but not when arginine was used as a GH stimulant. The drug alone slightly lowered baseline GH levels (180), although it did not do so in another study (610). Regardless of the interpretation of these findings, overall they do not support a serotoninergic stimulatory component in human GH secretion.

Fenfluramine has also been used to probe the function of central 5-HT neurotransmission in obese subjects, in whom defective function has been postulated (604). In a group of moderately obese subjects, GHRH induced a low GH response, which further decreased after 9 wk of treatment with fenfluramine (325). These data were taken to indicate that a serotoninergic defect was not involved in the low basal GH concentration or the blunted response to GHRH in obese subjects. Results were similar in experiments in which the GH release induced by GHRH was assessed in obese subjects after 7 days of treatment with fluoxetine, a 5-HT reuptake inhibitor (844).

3. ACh

The initial observation that in normal subjects atropine blockade of muscarinic cholinerge receptors had no effect on GH release elicited by insulin hypoglycemia
(111) for many years hindered further studies on the actual role of ACh in the neural control of GH secretion. Thus only in 1978 did Bruni and Meites (147) show that in conscious male rats intracerebroventricularly injected ACh bromide raised plasma GH and that the same effect was achieved by systemic injection of pilocarpine or eserine, a muscarinic agonist and an AChE inhibitor, respectively. The increase in GH release induced by pilocarpine was antagonized by atropine, which per se did not alter serum GH concentrations. Parallel studies in chronically cannulated, unrestrained rats showed that systemically administered atropine inhibited episodic GH release (665).

In dogs, ACh neurotransmission appeared to have a stimulatory role in GH release, through muscarinic receptors inside the BBB (173). The importance of cholinergic mediation in the GH-releasing mechanism(s) was emphasized by the observation that in dogs (174) and humans (825) atropine was the most effective antagonist of the GH release induced by EOP (see sect. vB8).

Further evidence that cholinergic transmission is involved in GH accumulated from human studies. Mendelson et al. (703) reported a striking suppression of the sleep-associated increase in GH secretion and a more subtle blunting of the GH response to insulin-induced hypoglycemia after administration of methscopolamine, a muscarinic antagonist unable to cross the BBB. The same group found that in humans infusion of piperidine, a cholinergic nicotinic agonist, left resting GH levels unaltered but slightly enhanced sleep-related and insulin-induced GH secretion; they were led to conclude that both nicotinic and muscarinic mechanisms were involved in these aspects of stimulated GH release (704).

The observation that methscopolamine, which does not cross the BBB, blunted the stimulated GH release in humans indicated a “peripheral” site of action for cholinergic stimulation of GH release. This was borne out by the reported ability of edrophonium and pyridostigmine, two irreversible AChE inhibitors and quaternary ammonium compounds, to release GH in normal subjects (609, 672). Cholinergic muscarinic receptors have been described in the anterior pituitary (747, 931) and the ME (954), and ACh is a direct GH secretagogue on bovine (1120) and rat pituitary (170).

The increasing awareness of the role played by ACh neurotransmission in the mechanism(s) subserving GH release led to fresh investigations of how cholinergic function interacted with the metabolically and neurotransmitter-driven GH release. In normal men, pirenzepine, a selective antagonist of M1 receptors that barely crosses the BBB, blocked the GH rise induced by DA and \(\alpha_2\)-adrenoceptor stimulation (301), and atropine suppressed that after arginine, clonidine, and physical exercise (178). In line with the methscopolamine findings, atropine or scopolamine completely abolished SWS-re-}

lated GH release in normal adults or children (835, 1025). Pirenzepine and atropine also completely blocked the GH response to GHRH, whereas conversely, blockade of AChE by pyridostigmine potentiated the GH response to GHRH (672) and normalized the blunted somatotroph responsiveness to repetitive GHRH boluses (671). Finally, paradoxical GH responses to TRH, which occur in some patients with insulin-dependent diabetes, endogenous depression, or liver cirrhosis, could be suppressed by pirenzepine (759).

Overall, the fact that M1 cholinergic receptors inhibited the GH response to most of the hypothalamic and pituitary GH-releasing stimuli was consistent with the idea that the restraint arose “downstream” of the chain of events leading to GH release. Enhanced release of hypothalamic SS and the ensuing inhibition of somatotroph function might explain these findings.

In vitro studies addressing the mechanism(s) whereby ACh neurotransmission affects GH secretion provided support for this concept. Low concentrations of ACh inhibited the release of SS-LI from rat hypothalami in culture, an effect shared by neostigmine and blocked by atropine, but not by hexamethonium, a nicotinic antagonist (888). These observations were in contrast to findings that a series of muscarinic cholinergic agonists raised secretion of SS-LI from fetal rat hypothalami in culture, an effect antagonized by atropine (834), and that intracerebroventricularly injected ACh in rats released SS-LI into hypophysial blood (228). These latter findings, however, must be interpreted with caution, since anesthesia is mandatory in these in vivo experiments and some anesthetics already induce SS release.

Proof that abolition of GHRH-induced GH release by cholinergic antagonists implies activation of SS release was provided by in vivo experiments in conscious male rats. Like in humans, pirenzepine or pilocarpine, respectively, reduced or potentiated the increase in plasma GH induced by GHRH. These modulatory effects were not seen in two experimental models of depletion of hypothalamic SS (629). These data indicated that in rats cholinergic modulation on GH release is through SS, in keeping with the high density of muscarinic binding sites in the ME (911) and their location in the lateral region of the external layer (954), to which most of the SS nerve terminals also project (see sect. viB). Cholinergic neurons would also be instrumental to the autoregulatory modulation of SS tone, in rats (1039) or humans (907) (see also sect. viA). Cholinergic receptor agonists and antagonists added to pituitary cells in vitro did not alter the GHRH-induced GH release (629).

Although the relationship between SS function and the hypothalamic cholinergic system is now generally established, some observations still challenge it. For instance, the hypoglycemia-induced increase in GH appears
to be almost totally (702) or partially (344) refractory to cholinergic blockade, but not to the action of SS. This and a series of other observations in animal (37) and human models of caloric restriction (see below) suggest an extra SS component in the modulatory action of cholinergic drugs on GH release. Thus, in subjects with AN, pyridostigmine failed to potentiate the release of GH induced by GHRH, whereas arginine, another GH secretagogue allegedly acting by inhibiting release (22), fully enhanced the GHRH-induced release of GH in these patients (409). In sheep, a single dose of neostigmine stimulated GHRH release into hypophysial portal vessels without changing SS concentrations (651), and interestingly, in the rat ARC ACh coexists in GHRH- but not in SS-containing neurons (689).

In sum, it cannot be ruled out that GHRH is involved in the modulatory action of cholinergic drugs on GH release as also inferred by the fact that a competitive GHRH antagonist suppressed the increase in GH induced by an AChE inhibitor (520), or by the type of GH pulse profiles evoked by a short pyridostigmine treatment in humans (373). The dual mechanism of cholinergic agonists explains why they do not completely reverse the defective GH release associated with obesity (415, 634) more convincingly than a mechanism relying exclusively on inhibition of hypothalamic SS. Instead, in obesity, the GH hyposecretion is completely reversed by the combination of GHRH and GHRP-6, which provides an appropriate pituitary-directed GHRH and anti-SS stimulation (270; see Ref. 752 for further discussion).

A) CLINICAL IMPLICATIONS. The availability of a class of compounds purportedly suppressing hypothalamic SS influences, i.e., muscarinic cholinergic agonists, prompted studies to see whether they could enhance the diagnostic power of GHRH. When used alone, GHRH does not discriminate between normal and GH-deficient subjects; wide inter- and intraindividual variability is frequent even in normal subjects, and there may be no GH response to the maximally effective dose (686, 1055). The effects of pyridostigmine on basal and GHRH-induced GH secretion were investigated in children with familial short stature, constitutional growth delay, and GH deficiency, and the results were compared with those following insulin-induced hypoglycemia or GHRH alone (416) or, in another study, with those of normal children undergoing a series of provocative tests (405). In normal children, the pyridostigmine-GHRH tests, unlike other GH secretagogues, gave no false-negative responses; the minimal normal GH response was ~20 ng/ml. The test therefore detected a pathological response in a much wider interval than the other tests; in patients with organic GH deficiency, peak GH responses were lower than 6 ng/ml, whereas in patients with idiopathic GH deficiency or constitutional growth delay, peak GH responses were 7–19 ng/ml. Thus the pyridostigmine-GHRH test and also the arginine-

GHRH test (405) are currently the most reliable for assessing GH secretory status in children, because they probe the maximal secretory potential of the pituitary. A normal GH response to the combined pyridostigmine or arginine-GHRH test does not exclude the existence of a GH hyposecretory state due to hypothalamic dysfunction, calling for 24-h monitoring of GH secretion.

The clear GH-releasing effect of cholinergic muscarinic agonists led to their use in the treatment of short stature. Briefly, in a group of short children, with hormonal features peculiar to hypothalamic dysfunction, long-term oral pyridostigmine (60 mg 3 times a day for 6 mo) did not significantly alter height velocity, mean GH concentration, and plasma IGFI-I levels, except in one child (755). Results were similarly negative with a combination of cholinergic agonists and GHRH in the treatment of short stature (908).

Muscarinic cholinergic agonists could also be used to investigate the hypothalamic SS (and GHRH?) system in different physiological or pathological conditions. In rats aged between 10 days and 29 mo, administration of GHRH alone led to an age-related decline in the responsiveness of GH, starting from 8 mo of age. Pretreatment with pilocarpine potentiated the GH response to GHRH during the entire life span, with the only exception being 10-day-old rats in which the drug had no effect. Interestingly, at this age, the hypothalamic SS tone is very low (see sect. uB). Pilocarpine rejuvenated the GH response to GHRH in the older rats (18 and 29 mo old) to the concentrations of that found in 15-mo-old rats (807). Similarly, in a group of normal elderly humans, pyridostigmine potentiated GH response to GHRH, although it was still significantly lower than in younger people (413).

These findings support the idea that in aged mammals the reduced response of GH to GHRH is only partly due to an intrinsic defect of the somatotrophs and that hypothalamic and/or extrahypothalamic inhibitory influences under cholinergic control are also involved. It is common knowledge that central cholinergic function can be impaired in the elderly brain (828), and cholinergic agonists per se induce a blunted GH response in aged animals (208) and humans (872).

At variance with the partial restoration of the GH response to GHRH by pyridostigmine is the complete restoration by pretreatment with arginine so that the increase in GH induced by arginine-GHRH cotreatment was similar in young and old subjects, and arginine had greater potentiating effect in elderly than in young subjects (414). These findings, while reinforcing the idea that the pool of releasable GH is essentially preserved in the human pituitary even during aging (see also sect. uB), illustrate the irreversible, age-related alteration of the cholinergic function coupled with a reversible SS hyper-function.

Attempts at using cholinergic blockade in GH hyper-
secretory states have not been successful. Insulin-dependent diabetics (IDDM) may present exaggerated nocturnal GH swings, and GH release is primarily responsible for the dawn phenomenon, i.e., decreased sensitivity of hepatic glucose production to insulin (754). Suppressing of GH secretion in IDDM may improve metabolic control and prevent the development of diabetic late complications.

After a few promising results with short- or medium-term pirenzepine (57, 670), in further studies, atropine and propantheline, another muscarinic cholinergic antagonist, had no effect on indices of somatotrophic function in IDDM patients with active proliferative retinopathy (500). These results, which recall the inability of pirenzepine to suppress 24-h GH secretion in tall children (469), put doubt on the effectiveness of long-term treatment muscarinic antagonists, probably because of the early development of tolerance (916).

4. Histamine

The role of histamine on GH release in the rat is still debated, but most of the evidence points to inhibition by H1 receptors. In conscious rats, intracerebroventricular administration of histamine or a series of H1-receptor agonists reduced GH release induced by morphine; mepyramine, a H1-receptor antagonist, had no effect by itself but prevented the inhibitory action of 2-methylhistamine, a H1-receptor agonist. The H2-receptor agonists and antagonists also inhibited morphine-induced GH release but apparently with a nonspecific mechanism (776). Similarly, nanomolar intracerebroventricular doses of histamine or amodiaquine, an inhibitor of histamine catabolism, caused a dose-related suppression of pulsatile GH secretion (775).

At odds with these findings, in E2 and progesterone-primed anesthetized male rats, a H1-receptor antagonist blocked the rise in GH induced by morphine, neurotensin, and SP, with a mechanism that could not be related simply to the hypotensive properties of these substances (891).

The inhibitory effect of histamine neurotransmission on GH release has since been illustrated in neonatal and adult rats. In the former, blockade of histamine synthesis with a-fluoromethylhistidine (a-FMH) significantly raised plasma GH and potentiated the GH response to an enkephalin analog; GH and SS mRNA were significantly higher in a-FMH-treated pups, whereas GHRH mRNA levels were unaltered. In young adult male rats, acute administration of a-FMH did not change baseline GH levels but potentiated the enkephalin-induced stimulation of GH secretion. Repeated intracerebroventricular doses of a-FMH did not alter the hormone levels, significantly reduced hypothalamic GHRH mRNA, and left SS and GHmRNA unchanged (439). Overall, these findings suggest an inhibitory histamine tone on GH secretion in neonatal and adult young rats, the failure of a-FMH to increase GH secretion in the latter being probably due to upregulation of histamine receptors after brain histamine depletion (862).

Data in freely moving dogs point instead to a facilitatory role on GH secretion. Of the three H2-receptor antagonists used, only clemastine, which reportedly has no antiserotonergic, antiadrenergic, and anticholinergic action, blunted hypoglycemia-induced GH release (626), whereas diphenhydramine, clemastine, and the H2-receptor antagonist cimetidine, respectively, suppressed or blunted the cGH release induced by an opioid peptide (174) or by cholinergic stimulation (100).

Histamine also appears to be involved in stimulated GH release. In normal humans, two H1-receptor antagonists significantly reduced the GH response to arginine infusion, but neither drug affected the secretion after insulin hypoglycemia (859). In healthy volunteers, diphenhydramine, which also has anticholinergic activity, completely suppressed the GH response to an opioid peptide, FK 33–824 (825). The H2-receptor antagonists have given different results depending on the dose, length of treatment, and route of administration. Thus cimetidine at oral therapeutic doses for 1 mo or more to patients with peptic ulcers (990) or normal subjects (1051) did not alter baseline or stimulated GH release but reduced mean nocturnal GH secretion (1051). The drug also reduced GH response to insulin hypoglycemia when repeatedly administered (858) and blunted the GH response to levodopa when given as an intravenous bolus injection (1122). Higher peak plasma concentrations of cimetidine after a single intravenous bolus than after chronic oral administration explain these results.

5. Amino acids

A) EXCITATORY AMINO ACIDS. A glutamatergic control of GH secretion was first suggested a long time ago when it was shown that MSG given neonatally, at doses damaging the ARC neurons, impaired the rhythmic GH secretion in postweaning rats (774). This compound acted similarly in adult male rats at doses causing no neurotoxic lesions. However, two agonists of glutamate receptors, N-methyl-D,L-aspartic acid (NMA) or kainic acid, increased GH secretion in adult male rats, implying that NMDA and non-NMDA receptor subtypes were involved in GH-secreting mechanism(s) (670, 850). In the same experiments, ibotenic acid or quinolinic acid did not alter AP hormone secretion (670).

The GH-releasing properties of systemically administered NMA were confirmed in prepubertal male rhesus monkeys. Injection of the amino acid analog elicited a prompt increase in GH secretion to three times the pre-injection values. N-methyl-D,L-aspartic acid maintained its
ability to release GH when given systemically to castrated male sheep which, unlike rodents, do not respond to the amino acid in terms of LH release (342). A dose-related increase of plasma GH levels after NMA was also reported in ovariectomized ewes (341) and in intact ewes (323).

Unlike the gonadotropin system (242), NMDA seems to stimulate GH release to much the same extent in neonatal and adult rats, as shown by its ability to increase plasma GH in 2-day-old rats (8). Consistent with a stimulatory role of excitatory amino acid (EAA) on GH secretion in either infant or adult rats was the observation that blockade of NMDA receptors by the noncompetitive antagonist MK-801 in immature female rats caused long-lasting reduction of growth rate, GH pituitary content, basal and GHRH-stimulated GH release, and plasma IGF-I levels. This effect was coupled with delayed puberty and reduced gonadotropin secretion, which mimics a condition frequent in humans (1068).

Stimulation of GH release by NMDA posed the question of the site(s) and mechanism(s) of action of EAA. Neural loci at which systemically administered NMDA may affect GH release comprise areas of the brain lacking a distinct BBB, especially the circumventricular organs, and including the ARC (868). When NMDA was tested on pituitary fragments from neonatal or young adult rats in static incubation, it showed no GH-releasing effect (8, 260), and stimulation of GH release was scant after incubation of pig pituitary cells with high concentrations of glutamate or aspartate (71). Results were different, however, on isolated perfused somatotrophs, where low concentrations of NMDA induced sustained GH release that was abolished by the concomitant addition of competitive and noncompetitive NMDA receptor antagonists (615). The possibility that isolation of the somatotrophs from the other pituitary cells was responsible for the enhanced responsiveness to NMDA in the perfusion system was unlikely; NMDA caused dose-related GH stimulation in a mixed population of cultured pituitary cells, as assessed by the reverse hemolytic plaque assay (777). The same GH-releasing effect was shared by L-glutamate or kainic acid and was completely counteracted by selective NMDA and non-NMDA receptor antagonists (777). The rat somatotrophs thus appeared to have both NMDA and non-NMDA ionotropic receptors.

In line with these findings, non-NMDA type of receptors were demonstrated in the anterior lobe of the rat pituitary by immunohistochemistry (563). Other studies have also detected the metabotropic type of EAA receptor (1117). The demonstration of the NMDA R3 receptor subunit, in the AP and its colocalization in many cell types, including the somatotrophs, indicate the importance of a direct effect in the pituitary (101).

Deafferentation of the MBH reportedly leads to a drop of glutamic acid concentrations in the MBH itself, and in both lobes of the pituitary (869). This implies that the amino acid is not manufactured in the pituitary gland and that EAA receptors are stimulated by hypothalamic-derived glutamate transported to the target sites by the portal vessels.

The stimulatory effect on GH secretion of hypothalamic injection of EAA strongly suggested a major CNS site of action. It was also shown that NMDA did not release GH in ARC-lesioned adult rats (8), indicating that its effect was mediated by substances released from this area. That pretreatment with a GHRH antisera reduced the GH-releasing effect of NMDA in neonatal rats (8) was in keeping with the idea that a GHRH deficiency was involved. Similar results were obtained in prepubertal gilts in which aspartate was a more potent GH secretagogue than glutamate, but neither compound had any effect in the GHRH antisera-pretreated animals (71). More proof of this mechanism was provided in male rats at weaning by evaluating the effect of 10 days of treatment with MK-801 on the hypothalamic content and gene expression of GHRH and SS. The drug impaired the growth rate and reduced GH secretion with significant lowering of the hypothalamic GHRH content and GHRH mRNA. Hypothalamic SS content and mRNA were unaltered (260).

This finding is seemingly in contrast to a report that the NMDA receptor antagonist counteracted NMDA-stimulated SS release from cultured diencephalic neurons (1022), but this might be due to differences in neuronal organization in fetal (1022), prepubertal (260) hypothalamus. In our hands too, however, quinolinic acid, an NMDA receptor agonist, strikingly increased SS release from in-vitro hypothalamic fragments, this effect being completely reversed by coincubation with MK-801 (260). It cannot be ruled out, therefore, that MK-801 may induce changes in selective groups of hypothalamic SS-producing neurons, whose involvement might perhaps be evidenced by a more refined approach, e.g., in situ hybridization.

Although there are reports that NMDA receptor activation is required for the expression of c-fos in GnRH neurons of female rats (602), no specific studies have been done on c-fos induction in neurons containing GH-regulatory neurohormones, although intraventricular injection of NMDA induced c-fos expression around the PVN and in the ARC, where GHRH and SS-containing cell bodies are located (see sect. mA2). An increase in c-fos-IR after NMDA was evident in noradrenergic cells of the locus coeruleus (914), and this would be relevant for the stimulatory effect of EAA on GH secretion, in view of the major role played by NE neurons in this context.

That excitatory amino acid action depends on sex steroids was shown in adult male rats orchidectomized 1 wk before. Systemic administration of kainic acid elicited a larger rise in plasma GH than in sham-operated controls; the GH-releasing properties of kainic acid completely dis-
appeared in male rats estrogenized at birth, a model of permanent decrease of plasma testosterone (850).

The results so far strongly suggest that EAA are positive modulators of GH secretion, mainly acting through at GH neuroregulatory hormones. It would appear that this effect is mediated by ionotropic NMDA and non-NMDA (AMPA/kainate) receptors, with metabotropic glutamate receptors not being involved. A direct action of EAA on somatotrophs is unlikely, in view of the controversial data on in vitro GH secretion, and the biochemical evidence of the presence of metabotropic but not ionotropic glutamate receptors in the pituitary. Hypothalamic GHRH neurons would be the privileged target of EAA action, with somatostatinergic neurons playing only an ancillary role.

From a physiological point of view, EAA seem to be involved in the central mechanisms triggering the endocrine events related to puberty, including the enhanced GH secretion that accelerates body growth (see also sect. III A and Ref. 139 for review). It is tempting to suggest, therefore, that the frequent association of delayed puberty and short stature (729) may share a common etiopathogenetic denominator, namely, reduced glutamatergic function at either the GnRH- or GHRH-producing neurons in the ARC. Were this the case, NMDA receptor antagonists would be useful for the treatment of precocious puberty, although the inhibitory effect of noncompetitive NMDA receptor antagonists on GH secretion would constitute an adverse event. Unlike MK-801, CGP-39551, a competitive antagonist of NMDA receptors, delayed puberty in female rats but had no effect on the rate of body growth (253). Compounds of this type would be a better choice for the treatment of precocious puberty than noncompetitive NMDA receptor antagonists, although further studies are needed to substantiate this in humans, who appear to be less sensitive than animals to the (acute) stimulatory effects of EAA on GH secretion. In eight Caucasian males, a MSG dose 25 times the normal daily intake had no effect on the secretion of GH and other pituitary hormones (353).

B) INHIBITORY AMINO ACIDS. The actual role of GABA on GH secretion has been a source of considerable controversy, and both stimulatory and inhibitory influences have been reported. Briefly, intracerebroventricular injection of GABA or systemic administration of amino-oxyacetic acid, an inhibitor of GABA-T, or γ-hydroxybutyrate (GHB), a physiological metabolite of GABA, induced a prompt dose-related increase in serum GH in conscious and anesthetized male rats and in ovariecotomized, steroid-replaced female rats (1071). Opposing the stimulatory effect of centrally administered GABA and its analog was the GH-lowering effect of of two GABA-T inhibitors, ethanolamine-O-sulfate and γ-acetylenic GABA (GAG), administered systemically, in conscious or pentobarbital sodium-anesthetized rats. Conversely, reducing GABAergic activity with 3-mercaptopropionic acid or with bicuculline raised plasma GH concentrations (359). In freely moving rats, systemic administration of muscimol, a direct agonist of GABAA receptors, or GAG inhibited the GH secretory episode, whereas bicuculline methiodide, a compound which barely crosses the BBB, induced powerful but short-lived stimulation of GH release when injected at the nadir of basal hormone levels (358).

A likely explanation of these and other findings (see Ref. 748 for more details) is that GABA may play a dual role in the control of GH secretion in the rat depending on the animals physiological state. γ-Aminobutyric acid would inhibit either the SS- or the GHRH-secreting neurons, or both, also in view of its alleged inability and that of muscimol to act directly on cultured AP cells (358) (but see below). That GABAergic neurotransmission inhibited SS neurons was borne out by the fact that muscimol and bicuculline injected into the POA, an area where SS-containing perikarya are located, stimulated and inhibited, respectively, GH secretion (1007). The GABA-SS interaction was confirmed by the fact that GABA inhibited SS release into the rat hypophysial portal vessels (998) and from hypothalamic cells in culture (387) and the discovery of GABA-IR synapses on SS-IR perikarya in the PeV hypothalamus (1007). Benzodiazepines, which reportedly act through GABA-mediated mechanisms, also inhibited SS release from rat diencephalic cells in vitro (989).

These and other findings led to the proposition that GABA and its analogs only affect the PeV brain areas, including the SS elements, when injected centrally, without impinging on more distant brain structures in which the GHRH-secreting neurons are located (see sect. III A2). These areas, which have no effective BBB, can be reached by drugs such as systemically injected GABA and its analogs or antagonists, which normally do not cross the BBB. Therefore, GABA-mimetic lower GH by inhibiting GHRH-secreting structures (see Ref. 748 for further details).

γ-Aminobutyric acid acts directly on the rat pituitary during the neonatal period, when sensitivity to hypophysiotropic factors is at its highest. Systemically injected GABA in newborn rats significantly increased plasma GH levels (9). This effect was very likely due to the fact that GABA and muscimol, in micromolar concentrations, elicit GH release from the entire pituitary of 2-day-old rats, an effect subsiding after the ninth postnatal day. In this system, bicuculline methiodide and picrotoxin, a known chloride ionophore antagonist, counteracted the effect of GABA, although at concentrations exceeding that of the agonist by 10-fold; diazepam stimulated, but baclofen, a stimulant of GABAB receptors, had no effect on GH secretion (9).

In further studies, a superfusion system was used to assess the GABA effect and to provide evidence of its mediation by specific GABA receptors. Muscimol was...
~10 times as potent as GABA in eliciting a prompt increase in GH secretion, and the effect of GABA was antagonized by picrotoxin and bicuculline, implying the involvement of a GABA_2 receptor. Continuous exposure of neonatal pituitaries to GABA resulted in a state of refractoriness to the action of muscimol, indicating receptor desensitization (10). Overall, these data suggested that GABA might be one of the factors involved in maintaining the high circulating GH levels of the early postnatal period. The mechanism by which GABA stimulates GH secretion from preloaded neonatal rat pituitaries is beyond the scope of this review, and the reader is referred to Acs and co-workers (11, 12) and Horvath et al. (490).

The use of a superfusion system demonstrated that both GABA and muscimol induced a large, transient stimulation of GH secreted from adult AP, which were sensitive to inhibition by bicuculline. Baclofen did not stimulate GH secretion, whereas the effect of muscimol was potentiated by benzodiazepines and barbiturates (33).

That GABA or GABA agonists act directly on the pituitary is supported by the presence of low- and high-affinity GABA and muscimol receptors in the rat (36) and human (437) pituitary, although their function appears mainly to involve an inhibitory control on prolactin secretion.

A series of studies has shown that in healthy, psychiatric, or neurological subjects, gram amounts of GABA or a few milligrams of muscimol induced clear-cut GH increments in plasma with a peak after 60–90 min (1004; see Ref. 96 for further details). Similarly, γ-aminohydroxybutyric acid, a GABA derivative, did not raise plasma GH when injected subcutaneously into normal volunteers but did when given intrathecally to cerebrovascular disease patients (998). Growth hormone release was also stimulated by intravenous diazepam or oral doses of other benzodiazepines (see Ref. 533).

To the list of GABAergic compounds that stimulate GH release in humans one must also add baclofen (573), which has been used as a neuroendocrine probe of the GABA system in psychiatric studies (735, 787). There is, however, wide individual variability in the GH response to baclofen, with ~30% of false-negative responses in normal subjects (285).

The GH-releasing effect of GABA in humans may occur through activation of dopaminergic pathways, i.e., GABA would activate DA release at a site inside the BBB (194). Because peripherally administered GABA does not easily enter the brain, this CNS site must be incompletely covered with a BBB, e.g., the GHRH-secreting neurons (see above).

In sharp contrast to the above findings and consistent with an inhibitory component of GABA action are observations related to stimulated GH release. Premedication for a few days with either GABA (194) or baclofen (193) inhibited hypoglycemia- and arginine-induced GH secretion and blunted the GH response to levodopa (580). Stimulation of GABAergic neurotransmission also inhibits GH release during physical exercise (981), an effect which would be counteracted by activation of the opioidergic function (262), which might involve stimulation of GHRH release or, alternatively, inhibition of SS secretion (see sect. vB).

To explain some of the seemingly paradoxical findings reported, we can propose that inhibition of the GH release stimulated by GABA and its ability to raise baseline GH share the same basic mechanism, i.e., an action through dopaminergic neurons. Continuous stimulation of CNS-DA receptors by GABA mimetics through DA release (see above) would ultimately lead to a state of partial refractoriness to DA-mediated events (insulin hypoglycemia and levodopa).

The involvement of the GABAergic system in the GH-secreting processes would include pathological states such as IDDM. Reported, this disease is characterized by GH hypersecretion and an exaggerated GH response to GHRH very likely due to a spontaneous decrease in hypothalamic SS tone (754, see sect. vi). Insulin-dependent diabetes mellitus is well recognized as an autoimmune disease (334), and the decreased SS tone may reflect damage by an autoimmune process either directly to SS neurons or indirectly to the brain neurotransmitters controlling SS release. GAD, the key enzyme of GABA biosynthesis, has been identified as a major autoantigen of IDDM, being a target of both humoral and cell-mediated autoimmunity (127).

In a study investigating the possible influence of GAD autoimmunity on GH secretion, most of the patients with elevated serum GAD antibody levels had significantly higher serum GH after administration of GHRH than controls or diabetics with low GAD levels; pretreatment with pyridostigmine, which allegedly reduces SS tone, significantly enhanced the GH response to GHRH in patients with low GAD and in controls, but not in patients with high GAD levels (423). It would seem, therefore, that autoimmunity plays a major role in the exaggerated GH response to GHRH in IDDM, through a reduction of a GABAergic tone that stimulates SS production in the hypothalamus.

γ-Hydroxybutyric acid is a breakdown product of GABA that reportedly reduces ethanol consumption and suppresses the ethanol withdrawal syndrome (346, 384). In human volunteers, GHB stimulates the secretion of GH (999), and this finding has been confirmed (398). A GABA-mediated mechanism for GHB was suggested by the fact that flumazenil, an antagonist of benzodiazepine receptors, counteracts the GH response to an oral dose of the compound (398) and was consistent with the idea that GHB acts on a subpopulation of the GABA complex in close relation with benzodiazepine receptors (952). The finding that bicuculline antagonized the stimulatory effect
of GHB on GH secretion (1071) was consistent with a GABA_A-receptor mediated mechanism. Some data, however, contradict the GABAergic action, such as its inability to modify the chloride function coupled with the GABA_A receptor (951); action through a postsynaptic receptor has not been proven (968).

Contrasting findings have been reported on how GHB influences monoaminergic pathways (382). The interaction of GHB with the serotonergic system (976) appears interesting, considering the alleged functional deficiency of the latter in ethanol-prone subjects (598), who are detoxified by GHB (see above). Inferential support for this mechanism is provided by observations that the 5-HT receptor antagonist metergoline in male healthy volunteers blunted the GH response to GHB (397), and there was a persistent loss of 5-HT_1D receptor and GHB-mediated neurotransmission in long-term abstinent alcoholics, as shown by their inability to make a GH secretory response to sumatriptan or GHB (1069).

Table 2 lists the stimulatory or inhibitory influences on GH secretion of brain neurotransmitters studied so far, as derived from experimental evidence reviewed in section v.A.

### E. Growth Hormone-Releasing and -Inhibiting Neuropeptides

In addition to the specific hypothalamic regulatory hormones for GH secretion, GHRH and SS, whose physiological role is now established, and the still elusive ligand for the GHRP receptor, a host of CNS neuropeptides, some of them originally identified in the gut or peripheral nervous system, have marked stimulatory or inhibitory effects on GH secretion. The list of traditional neuropeptides has recently been enriched by the inclusion of immunomodulators, especially cytokines, and a neurotransmitter gas nitric oxide (NO).

Some of these compounds are found in specific nuclei of the hypothalamus, where they often coexist with hypophysiotropic neuropeptides or classical neurotransmitters, and at times they are detected in the hypophysial portal blood; this and the recognition of specific binding sites in the target pituitary gland suggest a neurohormonal role and a hypophysiotropic function. However, these peptides can alter endocrine function not only as hormones but also as neurotransmitters or neuromodulators, not excluding a role as autocrine or paracrine factors in the pituitary acting on GH secretion or somatotroph proliferation.

Because the physiological role of these peptides has yet to be unequivocally established and their effects on GH secretion are often unmasked by pathological situations, it seems appropriate to examine these compounds separately from the classical hypophysiotropic neurohormones.

1. Thyrotrophin-releasing hormone and gonadotropin hormone-releasing hormone

Thyrotrophin-releasing hormone, the first hypophysiotropic peptide to be isolated and synthesized chemically, was so named because its primary function is to stimulate TSH secretion from the pituitary gland (773). Apart from its ability to stimulate prolactin release, TRH releases GH in animals and humans, although only in pathological conditions (see Refs. 257, 462 for reviews). The TRH-induced GH response which, since we are unable to provide a meaningful explanation, has been termed “paradoxical,” appears to be mediated by various mechanisms, some of which are mentioned below.

One postulated mechanism encompasses expression of anomalous TRH receptors on somatotroph cells, probably in the pituitaries of acromegalic patients where the presence of TRH-binding sites has been related to a positive individual GH response to TRH in vivo (600). Consistent with this view, TRH also stimulates GH release from rat somatotroph lines such as GH_3 (124) and GH_4C_1 cells (20).

There is almost general agreement on the clinical significance of the TRH-induced GH release in acromegaly. It is frequently seen with prolactin-containing somatotrophinomas. Accordingly, TRH responders generally had higher baseline levels of prolactin than TRH nonresponders (1082). A likely interpretation was that adeno-
matous cells containing mixed GH/prolactin cells or/and mammosomatotroph cells possess many of the properties of normal lactotrophs including receptors for both TRH and DA. In contrast, acromegalic patients with pure somatotrophinomas are usually unresponsive to both the TRH and the GH-lowering effect of dopaminergic agonists (see Ref. 1082 for review).

The anomalous GH response to TRH might, alternatively, also result from an impairment of the inhibitory hypothalamic control of GH secretion (257, 749), as implied by the fact that TRH induces GH release from ectopically transplanted rat pituitary glands, pituitaries incubated in vitro, pituitaries from rats with lesions of the ME or passively immunized against SS (see Refs. 257, 462 for reviews). Thyrotrophin-releasing hormone is a strong stimulus to GH secretion in fetal, neonatal, and prepubertal animals in which the hypothalamic control of GH secretion is presumably functionally immature (see Refs. 257, 462 for review), and the paradoxical response may also occur in the elderly, whose hypothalamic control of pituitary function is diminished (75, 236).

The observation that passive SS immunization triggered the paradoxical GH response to TRH in intact rats (805) and that in acromegaly, or other pathophysiological conditions (rats with transplanted pituitaries), it was blocked by infusion of SS links the anomalous GH response to SS dysfunction (462).

A further possibility is that the GH response to TRH may be a consequence of GHRH-induced expression of TRH receptors on the somatotroph, in agreement with the facilitated GH release after TRH in acromegalic patients (921) or from rat or sheep pituitaries after exposure to GHRH (125, 597).

Thyrotrophin-releasing hormone receptors on the somatotrophs would also be unmasked when thyroid hormones are lacking or low, as in hypothyroid rats (233, 995) and humans (265).

As already mentioned, TRH-induced GH secretion is generally absent in normal subjects, although a subpopulation of normal people may respond to TRH. Careful studies with appropriate controls to exclude the confounding effect of GH peaks derived from spontaneous pulsatile release showed that in healthy young men the incidence of GH responsiveness to TRH was lower than 10%. Interestingly, in tests repeated at night, when reportedly the somatostatinergic tone is decreased (1054), 90% of the nonresponders during the day became GH responders to TRH (172). The way the paradoxical GH response to TRH is related to rest and inactivity might account for its being found in hospital patients and psychiatric cases who have altered biorhythms.

In addition to its stimulatory action, probably in the pituitary, TRH inhibits GH secretion, acting at the hypothalamus. In healthy subjects, TRH reduced or blocked the GH response to a variety of GH releasers. This inhibitory effect was present in a number of animal species (for review, see Refs. 462, 749).

The most likely mediator of the inhibitory effect of TRH on GH secretion is the somatostatinergic system. Immunohistochemical studies indicate that more than 95% of SS-IR perikarya in the preoptic-anterior hypothalamic area and in the PVN appear to be contacted by one or more TRH-IR terminals (1100). Furthermore, TRH causes SS release from the hypothalamus (544, see also sect. mB3), enhances plasma concentrations of SS (565), and upregulates pituitary sstr (940).

Thyrotrophin-releasing hormone might also affect neurotransmitter systems upstream of the SS neurons, although it is not known how the cholinergic system would be excluded, since enhancement of its function by pyridostigmine in the dog did not modify the inhibitory effect of TRH on GHRH-induced GH release (38).

In a group of cirrhotic patients, who reportedly include a high proportion of TRH responders, infusion of DA before the TRH stimulus increased baseline GH levels (decrease of SS tone?), and GH rise after TRH peaked sooner (760).

In addition to TRH, other hypophysiotropic hormones also stimulate GH secretion in acromegalic subjects or patients with CNS disturbances. Like TRH, GnRH (912) induces GH release from the pituitaries of rats anatomically and/or functionally disconnected from the CNS (804), and in human disorders in which TRH is also an effective GH releaser (749).

2. CRH, AVP, and oxytocin

Among the several systems of CRH neurons, the PVN-ME pathway is responsible for most of the hypophysiotropic actions of the peptide. A second series of CRH-LI stained neurons in the basal telencephalon, hypothalamus, and brain stem are interconnected by fibers of the medial forebrain bundle and the PeV system (756). These neurons are thought to play an important role in autonomic and neuroendocrine responses to stress, inducing, when activated, all the homeostatic changes that follow the stressful event (see Ref. 756).

Corticotropin-releasing hormone reduces basal or stimulated GH release in the rat (545, 796), an effect very likely mediated by SS release since it was abolished by pretreatment with an anti-SS serum (545). In addition, in vivo, CRH raised portal concentrations and secretion rates of SS (730) and extracellular release of SS from hypothalamic neurons (188). These findings, which are substantiated by the demonstration of direct synaptic connections between CRH and SS neurons (471), point to CRH as the mediator of the stress-induced suppression of GH secretion in rodents.

In the rat, CRH is also needed for the regulation of spontaneous GH release because continuous intraventric-
ular infusion of a CRH antagonist markedly increased GH peak amplitude without affecting either trough levels or the number of GH peaks (745). The same treatment significantly lowered GHRH mRNA levels in the ARC, without affecting SS mRNA in the PeVN and ARC. It would seem, therefore, that CRH, through regulation of ARC-GHRH neurons, also modulates endogenous GH secretion. Because connections between CRH and GHRH neurons have yet to be demonstrated, the inhibition of GHRH mRNA levels by CRH might result indirectly from enhanced inhibition of GHRH neurons by SS (see sect. III A3).

Controversial results have been reported on the effect of systemic CRH on GH release in humans. Barbarino et al. (72) found CRH dose-dependently inhibited the GHRH-induced GH release in healthy adult men and women; this effect was not confirmed in another study, which reported a tendency toward higher GH levels after a similar cotreatment (901). Corticotropin-releasing hormone dose-dependently inhibited GHRH-induced GH release in children (417). It must be recalled, however, that there is a major biological difference between rodents and humans, with stress being inhibitory on GH release in rodents and stimulatory in humans.

Neurons containing AVP and oxytocin not only project from the SON and PVN to the neurohypophysis but also send their axons to other CNS areas, such as the ME. High concentrations of AVP and oxytocin are thus found in the hypophysial portal blood (1129), suggesting that these peptides may also regulate anterior pituitary hormone secretion. This is borne out by the direct inhibitory effect of oxytocin on both basal and GHRH-stimulated GH secretion in dispersed rat AP cells (494). This inhibition would be tonic, since intracerebroventricular infusions of anti-oxytocin, but not anti-AVP serum, raised plasma GH in ovariectomized rats (368).

Oxytocin, therefore, by interfering with the action of GHRH at the AP might be one of the factors involved in the generation of pulsatile GH secretion, a function which could be important when oxytocin secretion is enhanced, e.g., in pregnancy.

Rats with hereditary AVP deficiency (Brattleboro strain) have normal GH levels and a normal GH response to hypothalamic electrical stimulation (mentioned in Ref. 664), thus excluding a role of AVP on GH secretion. The converse, however, is not true, since a group of GHRP increased AVP release in an in vitro rat hypothalamic incubation system (569).

3. Neurotensin

Growth hormone-releasing hormone is found in at least 40% of the NT-positive neurosecretory cells in the ventrolateral area of the ARC (779; see sect. III A); in the external layer of the ME, fibers containing both NT-LI and GHRH are close to hypophysial portal vessels (699) (Fig. 2).

Despite unequivocal neuroanatomical evidence, the effect of NT on GH release varies, depending on either the route of administration or the experimental model used. Centrally administered NT lowered plasma GH levels in anesthetized male rats (649), a finding consistent with its ability to increase SS concentrations in hypophysial portal blood (3) or SS release from hypothalamic slices incubated in vitro (957). In contrast, in unanesthetized ovariectomized rats, centrally injected NT increased GH secretion (910).

To further illustrate the complexity of NT’s role in GH regulation, after intracerebroventricular injection, an anti-NT serum and NT exerted reciprocal effects on plasma GH levels in ovariectomized female rats but, unexpectedly, similar inhibitory effects in male rats. As in humans (112), in ovariectomized female rats, plasma GH levels were unaltered by systemically administered NT but increased after systemic administration of an anti-NT serum.

The most likely explanation lies in the possibility that SS may have opposite effects on GHRH function, acting at different hypothalamic sites. This might account for the seemingly paradoxical effects of NT and anti-NT serum on GH secretion if its effects are mediated by SS neurons. Although it has not been demonstrated, one may infer that NT participates in the reciprocal regulation between GHRH and SS-producing neurons (see sect. III A3). In this connection, some hypothalamic regions containing SS neurosecretory cells, e.g., the PeVN, also contain NT receptors. An extensive review on the neuroendocrine effects of NT has been published (910).

4. VIP and PHI

Both VIP and PHI are products of the same precursor and belong to the glucagon-secretin family of peptides, structurally related to GHRH (86). They are synthesized in the rodent pituitary where they act as autocrine or paracrine factors (44). The pituitary cell that synthesizes them is not the somatotroph, as shown by the significantly lower levels of VIP mRNA and peptide in the AP gland of GHRH transgenic mice despite somatotroph hyperplasia (499). Several VIP-containing neurons project to the hypothalamus, including the ME, and VIP has been detected in high concentrations in portal blood (913).

In the rat, intracerebroventricular injections of VIP induced a prompt and sustained rise in plasma GH levels (see Ref. 749), whereas in rats (see Ref. 749) and in ewes (925), systemic injections had no such effect. These findings suggest a hypothalamic site of action for VIP, but in vitro findings clearly demonstrate that the peptide can release GH, also acting on the pituitary. In cultured bovine adenohypophysial cells, VIP, but not PHI, increased basal...
and GHRH-stimulated GH release (969). Previous data obtained in superfused rat pituitary cell reaggregates pretreated with dexamethasone had shown that VIP, like PHI, strongly stimulated GH release starting from a concentration as low as 0.1 nM. In this experiment, GH secretion in cells not pretreated with dexamethasone was unchanged, suggesting that VIP interacted with GHRH receptors, which were upregulated by the steroid (304) (see sect. II.A).

In contrast to the stimulatory effect in animals, in normal humans VIP blunted the GH peak occurring at night without modifying the time of occurrence of the physiological GH surge (764).

Vasoactive intestinal polypeptide stimulated GH release in subjects believed to have somatotrophinomas; in the few patients where VIP had no effect on basal GH release, it antagonized the inhibitory effect of DA agonists (230). However, screening a large group of acromegalics, Watanobe et al. (1082) observed that the positive GH response to TRH was combined with a high sensitivity to the inhibitory effect of DA agonists, whereas GH responsiveness to VIP and, possibly, to GnRH, existed with no or low sensitivity to dopaminergic agents. It would seem, therefore, that the first group of tumors had the functional characteristics of PRL-containing somatotroph adenomas, and the latter of pure somatotrophinomas.

Subsequently, Watanobe et al. (1083) found the paradoxical GH response to VIP in hyperprolactinemic patients with or without a prolactinoma. Because the GH response to VIP is not causally related to the presence of a pituitary tumor, it may be surmised that hyperprolactinemia itself played a role in inducing VIP receptors on normal or previously normal somatotrophs.

5. PACAP

Pituitary adenylate cyclase-activating peptide is a 38-amino acid peptide homologous to porcine VIP. Six of the 10 NH₂-terminal residues are identical to those of GHRH (731). The cDNA for PACAP encodes a PACAP precursor that gives rise to PACAP-38, PACAP-27, and PACAP-related peptides. Since its isolation, its activity has been amply described in a variety of tissues including pituitary, brain, adrenal, testis, gut, and lung. Several reviews have discussed its action in a variety of cell types and the characteristics of the three PACAP receptors cloned to date, two of which it apparently shares with VIP (43, 874). Although most studies have found no staining for PACAP-IR in the AP, one did describe PACAP-containing fibers in this tissue, mingling with the secretory cells (723). In rats, normal somatotrophs express the PVR3 receptor, which preferentially binds the peptide helodermin and is linked to the stimulation of cAMP production and a subsequent rise in Ca²⁺ concentrations. Differently from normal rodent cells, human GH-producing tumor cells express PVR1, more specific for PACAP than VIP (see Ref. 874 for review).

In vivo PACAP stimulates the release of GH, although this effect has been demonstrated in rats but not in sheep or humans (see Ref. 874 for review). In rats, the in vivo GH-releasing effect of PACAP is fairly marked, but whether this derives from a direct action on the somatotrophs is uncertain, since in vitro PACAP has a weak stimulatory effect on GH synthesis and release. In fact, its GH-stimulatory effect on rat, frog, and bovine pituitary cells was much lower than that evoked by GHRH, and GH release peaked at different intervals (458, 1002). The delayed GH-releasing effect of PACAP was attributed to indirect action on other cell types [i.e., stimulation of interleukin (IL)-6 release from folliculostellate cells] (458).

In addition to stimulating GH release, PACAP also raised GH mRNA levels during short- and long-term incubation in a somatotroph-enriched population of female rat pituitary cells (1067).

Similar to GHRH, but different from GHRP, PACAP-38 increases both the number of somatotrophs and the amount of GH released from each cell over a period of 30 min in dispersed preparations from male rat pituitaries (436). This suggests that PACAP and GHRH regulate GH secretion through similar mechanisms, most likely by activating the cAMP/protein kinase A system and Ca²⁺ influx.

Investigations into the physiological role of PACAP on GH secretion are limited by the lack of specific antagonists. Yamaguchi et al. (1111) showed that PACAP-(6−38), the NH₂ terminal deleted PACAP-38 analog, inhibited the GH secretory effect of the native peptide. Pituitary adenylate cyclase-activating peptide-(6−38) also abolished 5-HTP-induced GH release in conscious rats, suggesting that hypothalamic PACAP is involved not only in the physiological control of GH secretion but also in the GH release following serotonergic stimulation (see also sect. V.A2).

Although PACAP does not release GH in healthy humans (648), it stimulates GH release from in vitro cultured somatotrophinomas. This action, which is coupled to cAMP production, can only be observed in adenomas without gsp mutations, the somatic mutation within the gene that produces a constitutively active Gₛ protein (13).

In summary, it is interesting that PACAP acts as a GH releaser, sharing the same intracellular signaling pathway
as GHRH. However, the fact that it is much less potent than GHRH in activating GH secretion and the apparent restriction of this action to the rat makes uncertain how important it is in the physiological regulation of GH secretion and its therapeutic use.

6. NPY

Neuropeptide Y, a member of the pancreatic polypeptide family of peptides, shares significant sequence homology with pancreatic polypeptide and peptide YY. Neuropeptide Y is the most abundant peptide in the CNS (26). Within the hypothalamus, NPY is synthesized in neurons of the ARC, which mainly project to the PVN (305) (Fig. 2).

In addition to being involved in the control of food intake and energy and substrate metabolism, NPY affects the release of various hormones, including GH (1095). In the rat, intraventricular injection of NPY inhibited GH secretion (691), and a NPY antiserum led to elevated plasma GH levels (886) and partially restored GH secretory pulses in food-deprived rats (791). The latter effect is consistent with the increased levels of NPY in the PVN after food deprivation in rats (603), which might contribute to the reduction of GH secretion commonly seen in fasting rodents (1017). A similar alteration has been reported in obese rats (918) that have a striking reduction of the somatotrophic axis (see sect. viA).

Chronic infusion of NPY in the lateral ventricle of the brain, a treatment ultimately reproducing all the metabolic features of obesity, inhibited GH and IGF-I secretion in intact female (191) and male (842) rats, strongly suggesting a causal link between excessive NPY and reduced GH function in obesity.

The inhibitory action of NPY on GH secretion is mediated by stimulation of SS release (886), as supported by the demonstration of synaptic connections between NPY-containing axons and periventricular SS neurons in the anterior hypothalamus (472; see also sect. viA). A direct inhibitory effect of NPY on GH release, through the pituitary, was unlikely, since the peptide stimulated GH secretion from rat perfused pituitary cells (691) or goldfish AP (826). Neuropeptide Y inhibited basal and GHRH-stimulated GH secretion in human pituitary tumors (16).

For the involvement of NPY neurons in the autoregulation of GH secretion, see section viA.

7. Galanin

Galanin is a 29-amino acid neuropeptide that does not belong to any known peptide family and has a number of pharmacological properties in whole animals and isolated tissues (84). It is widely distributed in the CNS, highly concentrated in the hypothalamus, and partly co-localized with GHRH neurons in the ARC and nerve terminals in the ME (see sect. viA2) (Fig. 2). It is also present in the AP (see Refs. 96, 280 for reviews).

In rodents and humans, galanin increased GH secretion (77, 801) and appeared to play a role in the generation of pulsatile GH release, since in rats injection of a specific antiserum led to a dramatic alteration of the pulsatile secretory pattern (96). In healthy humans, in addition to eliciting GH secretion when given alone, galanin enhanced the GH response to GHRH (425).

In contrast, galanin lowered basal GH secretion in most acromegallic patients, an effect also observed in pituitary adenomas in vitro (429). The GH-inhibitory effect of galanin, which can be added to the list of paradoxical GH responses in acromegaly, reflects the presence of adenomatous somatotrophs, since acromegallic patients cured after surgery had a normal GH stimulatory response to the peptide. In rodent AP incubated in vitro, galanin had an elusive, apparently age-dependent effect (1040), with a GH-releasing effect only during the perinatal period. In adult life, galanin inhibited GHRH-stimulated GH release.

In perfused pituitaries from young male calves, galanin behaved as an effective stimulant of GH secretion (70), and its action was enhanced by concomitant perfusion of the pituitary with the hypothalamus, suggesting that the peptide exerts its optimal action when the integrity of the GHRH-pituitary axis is maintained. This study suggests that, at least in calves, galanin-mediated GH release occurred independently of hypothalamic SS, since the GH response to exogenous GHRH was not modulated by galanin.

Galanin together with GHRH in the same ARC neurons (707; see sect. viA2) may be the anatomic substrate for the functional interaction between the two peptides. Evidence for stimulation of GHRH release by galanin was provided in vitro using incubated hypothalamic slices (562, 707).

Consistent with a GHRH-mediated mechanism, in infant rats pretreatment with a GHRH antiserum abolished the potent GH-releasing effect of galanin (203). However, it would seem that galanin does not act directly on GHRH-secreting structures but requires the intervention of CA neurons. In neonatal rats, selective inhibition of E synthesis abolished the GH-releasing effect of galanin, implying that E provides the link between galanin-secreting and hypophysiotropic neurons for GH control (203).

Human studies suggest galanin’s action depends on SS. Like cholinomimetic drugs, which restrain the function of SS-producing neurons (see sect. viB3), pretreatment with galanin enhanced GHRH-induced GH release, an effect counteracted by salbutamol, a β2-adrenergic agonist (51), and restored the blunted GH response to GHRH during GH treatment in children with constitutional growth delay (920). Conscious adult rats had a sluggish response to galanin after pretreatment with
neostigmine, cysteamine, or a SS antiserum, all aimed at lowering the endogenous somatostatinergic tone (1021). In rat pups, pretreatment with the same antiserum significantly reduced the GH increase elicited by galanin (203). It is apparent from both animal and human studies that galanin plays a significant role in the regulation of GH secretion.

8. EOP

Studies in rats on the neuroendocrine effects of EOP showed that these compounds, as well as opiates such as morphine, stimulated GH secretion when administered either systemically or ivt (148, 259). In humans, however, some conflicting data have been reported. Thus, although FK 33–824, an allegedly potent and selective ligand of brain 𝛹-receptors proved to be a strong GH releaser, morphine and 𝛽-endorphin did not have any such effect (see Ref. 208 for review). Despite these inconsistencies, the effects of these compounds on GH release are specific, being completely abolished by pretreatment with the potent but nonselective EOP receptor antagonist naloxone.

In the rat, pretreatment with a SS antiserum did not influence the EOP-induced GH rise (328), whereas passive immunization with a GHRH antiserum completely blocked the GH rise induced by 𝛽-endorphin, morphine (1088), or FK 33–824 (721). These data indicated that opioids stimulate pituitary GH secretion through hypothalamic GHRH release and not through inhibition of SS release, although a small but distinct inhibition of SS release from rat hypothalamic fragments in vitro has been reported (326). In humans, it has been reported, though only inferentially, that FK 33–824 can also act by inhibiting SS release (302).

These findings disproved the direct effect of EOP on the pituitary (539, 890). The theory was supported by the observation that EOP antagonists, which are unable to cross the BBB (i.e., naloxone methyl bromide), did not affect the GH release induced by morphine (803).

Subsequently, Fodor et al. (367) suggested that opioid neurons might be implicated in the interactions between SS- and GHRH-containing neurons in the ARC. In fact, SS receptors are present on cells containing GHRH mRNA and POMC mRNA, and SS perikarya in the PeVN are innervated by POMC-derived peptide-containing neurons (see sect. ivB2a).

Previous studies suggested that the EOP acted mainly on the 𝛽-type opioid receptors, since in rats pretreatment with 𝛽-receptor antagonists abolished the GH rise induced by morphine or 𝛽-endorphin, while naloxone or β-funaltrexamine, a selective 𝛼-receptor antagonist, did not (566). It has since been demonstrated that multiple opioid receptor subtypes are activated in the regulation of GH secretion by 𝛽-endorphin (523). In this study, selective blockers of 𝛼-, 𝛽-, and 𝜋-sites inhibited GH secretion after intracerebroventricular injection of 𝛽-endorphin.

In humans, the stimulatory effect of opioids on GH secretion seems to be mediated by 𝛼-receptors (442). In contrast, in normal volunteers, deltorphin, the most potent and selective 𝛽-opioid receptor agonist known, blunted the GH response to several GHS, including galanin and arginine, whereas it did not affect the GH response to GHRH (297). These findings suggest that, in contrast to rats, in humans 𝛽-opioid receptors inhibit GH release, through modulation of hypothalamic release of SS. According to some authors (566), in rodents GH secretion is inhibited by activation of 𝜋-receptors, although as already discussed, 𝜋-receptors also had a stimulatory effect in adult (523) and immature rats (329).

With regard to the potential modulatory action of classic brain neurotransmitters, in the dog and in humans, muscarinic cholinergic, histaminergic, and 𝛼2-adrenergic receptor antagonists suppressed or blunted the GH rise induced by FK 33–824 (see Ref. 208 for review). The only discordant finding was that yohimbine did not inhibit the FK 33–824-induced GH rise in humans (441) and was effective in the dog (213).

Despite the wealth of information, the physiological role of EOP in the regulation of GH secretion is still unclear. An acute dose of a 𝛽-endorphin antiserum to freely moving rats (1016) or of naloxone to human volunteers (724) did not alter plasma GH levels. In contrast, chronic treatment with the long-lasting EOP receptor antagonist naltrexone did not modify basal GH secretion but significantly reduced the GH peak after GHRH (1052). These results suggest that chronic blockade of central EOP receptors enhances the function of SS neurons.

9. Cytokines

In recent years, evidence has accumulated of common peptide signals, receptors, and functions in cells of the immune and neuroendocrine systems, and this has provided the logical basis for a regulatory loop between the two (98). Receptors for IL-1α and the corresponding mRNA have been identified in rat and mouse pituitary, mainly in the AP. Receptors or binding sites for IL-6 and IL-2 have also been found in the pituitary, while many types of receptors for several cytokines have been described in the CNS (see Ref. 98 for review).

Stimulation of the HPA axis by IL-1 was the first and most extensively studied example of how cytokines influence endocrine function. Concerning GH, in conscious rats low intracerebroventricular doses of IL-1 (820, 885) or tumor necrosis factor-α (887) increased GH secretion, as did IL-2 therapy in cancer patients (58).

In another study, intracerebroventricular or ARC injection of IL-1 lowered GH levels in rats (640), suggesting an
underlying inhibition of hypothalamic GHRH and/or stimulation of SS release. Wada et al. (1075) demonstrated a significant inhibition of spontaneous GH secretion after intravenous infusion or intraventricular injection of IL-1α and in conscious rats. No effect was observed after IL-2 or IL-6.

Numerous in vitro studies indicated that IL-1, as well as IL-2 and IL-6 (975), stimulate GH secretion in pituitary cultures. More contradictory results were obtained with TNF-α, whereas interferon-γ (IFN-γ) was shown to inhibit the release of GH (see Ref. 98 for review).

The stimulatory effect of IL-1 on GH release repeatedly observed in vitro and the fact that it reduces GH pulsatile release in vivo suggest an intermediary factor may be responsible for the latter effect. The most likely candidates are glucocorticoids whose circulating concentrations are elevated due to IL-1-induced activation of the HPA axis and which reportedly inhibit spontaneous GH secretion in the rat (see sect. VI A).

A common finding in juvenile rats and humans is the reduction of somatic growth during infectious states. In juvenile rats, activation of IL-1 by endotoxin lipopolysaccharide markedly reduced GH secretion, by stimulating CRH and SS release from the hypothalamus (821).

These findings suggest that hypothalamic IL-1 in the rat plays a role in inhibiting GH secretion. Were the underlying mechanism shared by humans, selective therapeutic reductions of SS tone might be helpful in the treatment of chronic diseases of childhood. The precise role of other cytokines in these processes remains to be elucidated.

10. NO

Nitric oxide has now been shown to be a very important intracellular and intercellular messenger in most organs of the body, involved in the control of a wide range of physiological events. This free radical is rapidly degraded to nitrate and nitrite and is produced by NO synthase (NOS) from arginine (687, 734). Of the three known forms of NOS, the constitutive neural isoenzyme is widely distributed within the cells of the hypothalamus, especially in the PVN and SON, which send axons to the neurohypophysis and the ME (142). This has raised the intriguing possibility that NO may act as a neuroendocrine regulator in the hypothalamus and pituitary.

Studies with NOS inhibitors such as Nω-nitro-L-arginine methyl ester (L-NAME) and NO donors such as sodium nitroprusside (SNP) or S-nitrosoacetylpenicillamine (SNAP) have demonstrated that NO influences the release of GHRH (884) and SS (17). The mechanism by which NO stimulates the release of neurosecretory hormones is through diffusion into the neurons and activation of guanylate cyclase and cyclooxygenase to synthesize cGMP and PG, respectively (689).

Microinjected into the third ventricle of conscious, freely moving rats, L-NMMA dramatically reduced GH pulsatility, primarily by cutting the height of the GH pulses (884). Nitric oxide release should also be involved in the mechanisms through which GHRH neurons modulate the output of SS into the portal blood (17).

In the pituitary, NO secreted from folliculostellate cells and gonadotrophs (195) modulates GHRH-induced GH release. In freshly dissociated male rat pituitary cells, Kato (550) showed that SNP blocked the GH-releasing effect of GHRH, without affecting the GH release evoked by K+ excess.

Recently, a careful series of studies has reevaluated the involvement of NO in the control of GH secretion. Endogenous NO deprivation induced by pretreatment with the NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) significantly attenuated GHRH, GHRP-6, and NMDA-induced GH secretion in prepubertal male and female rats. In vitro, neither SNP, L-NAME, nor cGMP modified baseline GH secretion by dispersed AP cells, but L-NAME completely blocked GHRH-induced GH release (1026). These findings suggest that endogenous NO plays a permissive role in the control of stimulated GH secretion and is an important factor in GH secretion in prepubertal rats.

Similar data have been obtained in the dog, in which systemically administered L-NAME completely blocked and the lipophilic NO donor erythritol tetrinitrate potentiated the GH-releasing effect of hexarelin (A. Rigamonti, unpublished results). Studies with NOS inhibitors in humans are hindered by the toxicity of these substances on the cardiovascular system. Low doses of L-NAME blocked hypoglycemia-induced ACTH release but did not change either the basal or stimulated GH release (1072). Interestingly, L-arginine is a potent GH secretagogue in humans (711); however, its effect does not appear to be linked to NO production, since other NO donors such as molsidomine in humans (570) and erythritol tetrinitrate in dogs (Rigamonti, unpublished results) did not modify basal GH secretion and the GH-releasing effect of arginine was not affected by pretreatment with L-NAME.

The effects on the hypothalamus and pituitary of the peptides evaluated so far are summarized in Table 3.

VI. PERIPHERAL HORMONAL AND METABOLIC SIGNALS OR CONDITIONS MODULATING GROWTH HORMONE SECRETION

A. Hormones

1. Glucocorticoids

Glucocorticoids play a crucial role in somatotroph differentiation, which only occurs when the steroids reach a critical concentration (1029). Incubation of GH₃...
cells with cortisol led to an increase in the number of somatotrophs and a decrease of lactotrophs, implying that the steroid modulates the functional heterogeneity of the GH3 cell line (124).

Glucocorticoids amplify the GHRH responsiveness of the somatotrophs, an effect linked to enhanced binding capacity of GHRH receptors, with no change in their affinity (946, 1002; see also sect. III A8B). In vitro exposure to glucocorticoids (487, 939), most likely secondary to a receptor effect of SS by downregulating the number but not the affinity of sstr (487, 939), increased the density of GHRH receptors, with no change in their affinity (946, 1002; see also sect. III A8B). In vivo data showing that dexamethasone for 3 or 8 days dose-dependently reduced gene expression of pituitary GH3 cells (124), may contribute to the tran-
sient rises in plasma GH observed in humans after an acute corticosteroid dose (318, 1029).

Acute administration of corticosteroids not only elicits a GH rise but potentiatates GHRH-induced (177), but not the pyridostigmine-induced (176), GH release in humans. Because indirect cholinergic agonists are currently thought to release GH mainly by inhibiting SS release (see sect. vA3), it was assumed that glucocorticoids act through a similar mechanism. The fact that dexamethasone did not raise plasma GH in patients with AN was taken to indicate that the functional activity of SS neurons was decreased in these patients (928; see also sect. vA3). Supporting the idea of a suprapituitary component in the stimulatory action of glucocorticoids was the inability of dexamethasone to elicit GH secretion in children with GH deficiency due to an idiopathic hypothalamic defect (669).

Unlike a single dose, sustained delivery of supra-physiological amounts of glucocorticoids reduces somatic growth (636) and spontaneous GH secretion and blunts the GH response to a variety of physiological and pharmacological stimuli in rodents (1092) or humans (369, 554). These effects occur in addition to the well-known steroid-induced peripheral catabolic effects and reduction of circulating IGF-I concentrations (1029).

The inhibitory effects on GH secretion can be mainly attributed to a glucocorticoid-mediated enhancement of hypothalamic SS release. In rats, huge doses of dexamethasone increased hypothalamic SS mRNA (767), and exposure of fetal hypothalamic cultures to high concentrations of corticosterone raised the SS content, whereas GHRH content was lowered (351).

These results agreed with the findings of experiments with β-adrenergic blockade and passive immunization with SS antibodies in suggesting that glucocorticoid exces enhances hypothalamic SS function in humans and rats (612, 1087).

Corticosteroids may act indirectly, i.e., through an action on β-adrenoreceptors, which inhibit GH secretion (see sect. vA1c). In vitro exposure to glucocorticoids increased the density of β-receptors on smooth muscle cells (264); were a similar effect to occur on SS neurons, this would increase the inhibitory hypothalamic influences on GH secretion.

In humans, however, pyridostigmine does not completely counteract glucocorticoid-induced GH inhibition (300), which therefore cannot result exclusively from increased hypothalamic SS function. One possibility is that it is due to permanent alterations of pituitary SS and GHRH receptors. However, contrasting this would be the finding that GHRH receptor mRNA levels in the rat AP increased, not decrease, after short-term dexamethasone treatment (584, see also sect. mA8B).

Although glucocorticoids reportedly enhance the number and function of GHRH receptors in the rat AP, they seem to affect hypothalamic GHRH neurons nega-
tively, especially at high doses and with sustained treatments (585). The GHRH content in and release from fetal rat hypothalamic cells both decreased after incubation with high doses of corticosterone (351), and the same was true for the hypothalami of rats treated ex vivo with a huge dose of dexamethasone (770).

In conclusion, glucocorticoids have a dual effect on the somatotrophic axis, one short-lived and stimulatory and the other inhibitory, after prolonged administration of pharmacological doses. This probably results from an action on either the pituitary, through regulation of GH transcription, GHRH and SS receptors, or the hypothalamus, where they can affect the function of the two hypothalamic hormones differently. A further mechanism may be the reduction of circulating IGF-I concentrations and the ensuing inhibition of autofeedback mechanism(s) (see sect. VII A8). The reader is referred.

2. Gonadal steroids

The modulatory action of E2 and androgens on GH secretion in animals and humans, and the underlying mechanisms, have already been discussed in other sections of the review (sects. II A and II A8a), to which the reader is referred.

3. Thyroid hormones

The effects of thyroid hormones on GH secretion and their mechanisms of action are very similar in humans and laboratory animals (see Ref. 429 for review). Physiological concentrations of circulating thyroid hormones are necessary to maintain normal GH secretion, owing to direct stimulation of the AP. Growth hormone mRNA levels and rate of transcription increase after 3,3',5-triiodothyronine (T3) exposure, by direct action on the genome level (see Ref. 429). Conversely, in infant (288, 290) and adult (662) rats, pituitary GH content drops steeply with experimentally induced hypothyroidism.

Early studies on how perturbations of the pituitary-thyroid axis affected central GH regulatory mechanisms reported that in hypothyroid rats hypothalamic SS content and release were diminished and that replacement therapy with T3 restored both to normal (92). Subsequent studies, however, found hypothalamic SS content and release within normal limits in rats 10 days to 5 wk after thyroidectomy (see Ref. 429 for review). Long-term induction of hypothyroidism in the rat markedly lowered hypothalamic GHRH, which was restored to normal by thyroxine replacement therapy (546).

In our own studies in neonatal and infant rats made hypothyroid by giving the dams propylthiouracil, we found no functional alterations in the GHRH hypothalamic machinery with respect to euthyroid rats, despite the lower pituitary and plasma GH concentrations (290). These findings indicate that in neonatal rats, deprivation of thyroid hormones primarily depresses pituitary somatotroph function and that possible changes in GHRH function are only a later event probably linked to GH deprivation and the ensuing annulment of GH autofeedback mechanism(s) (see sect. VII A4). Consistent with this view, in adult rats induction of hypothyroidism enhanced both basal and K+-stimulated GHRH secretion from incubated hypothalamic fragments 5 wk later (719), as a sort of compensatory mechanism to reverse the hyposomatotrophism of this condition.

Thyroid hormones also regulate GH secretion by modulating somatotroph responsiveness to GHRH. Anterior pituitary cells from hypothyroid animals have a decreased response to GHRH in vitro (662). This reduced GH responsiveness probably results from the diminished pool of GHRH receptors after abrogation of thyroid function (571) (see also sect. II A8a).

In humans, as in other species, hypothyroidism severely impairs postnatal growth, reduces spontaneous nocturnal GH secretion, and is correlated with impaired circulating IGF-I levels (227), the GH response to a variety of pharmacological stimuli being blunted (see Ref. 429). Rodent findings suggest the decreased pituitary GH pool due to impaired GH synthesis might also account for the low GH secretion of hypothyroid humans.

The reciprocal hormone condition, i.e., hyperthyroidism in rats, is coupled to a reduced function of hypothalamic GHRH (531) and SS neurons, as suggested by the suppression of K+-stimulated SS release from in vitro incubated hypothalami (833).

As in hypothyroidism, hyperthyroid patients also have reduced GH secretion, which is restored to normal by antithyroid drugs (355, 922). Production of IGF-I increases in the hypothalamus after T3 administration (105), an effect which may contribute to the diminished GH secretion because of activation of SS function. There is inferential evidence of an enhanced SS activity in hyperthyroid states (see Ref. 429 for review), suggesting this may be the mechanism underlying the hyposomatotrophic function.

B. Metabolic Signals

1. Glucose

Glucose is an important regulator of GH secretion, although GH responses to hypo- or hyperglycemia differ among animal species. Hypoglycemia stimulates GH secretion in humans, and insulin-induced hypoglycemia is used clinically as a test to provoke GH secretion in GH-deficient children and adults (144), although it is poorly reproducible and gives some false-negative responses (477, see also sect. II A3). In contrast, in rats, insulin-induced hypoglycemia or intracellular glucopenia inhibits pulsatile GH secretion (1014). This inhibitory effect oper-
ates through stimulation of SS release, as shown by studies of incubated rat hypothalami (90) and the fact that hypothalamic SS mRNA levels are increased after ex vivo administration of insulin (763). Even severe hypoglycemia does not alter pulsatile GH secretion in mice (1003), in line with the reported resistance of mouse hypothalamic SS release or mRNA levels to intracellular glucopenia induced in vitro (924) or ex vivo (1003).

The same species differences can be observed in hyperglycemic states (see Ref. 930 for review). In humans, acute administration of glucose inhibits GH secretion, although there is a rebound release 3–4 h later. In the chronic hyperglycemia of IDDM (type 1), GH secretion is often increased, particularly in poorly managed patients, although basal GH levels can be normalized with proper metabolic control (see Refs. 759, 985 for review). Because GH is diabetogenic, the increased secretion of the hormone can induce adverse effects (971) (see also sect. v.A3).

In rats, acute hyperglycemia scarcely affects GH release, whereas diabetic rats have impaired GH secretion (see Refs. 428, 985 for review). The involvement of either the hypothalamus or the pituitary appears to be important. In streptozotocin-induced diabetic rats, SS antisem restored GH secretion (1007), implying that enhanced SS release was responsible for the reduction. Hypothalamic SS (793) and SS mRNA concentrations (808) were instead unaltered. However, in the face of low circulating GH and IGF-I levels, this “normal” hypothalamic SS function might in fact have been inappropriately elevated. Supporting this was the lower number of SS receptors in pituitary membranes from diabetic rats than in controls (793), very likely resulting from downregulation. Hypothalamic SS release into the portal blood or from hypothalamic fragments is increased in streptozotocin-diabetic rats, but not until GH secretion had been suppressed for several days (527).

Hypothalamic GHRH mRNA levels were low in diabetic rats compared with normal controls (793), despite the unaltered content of the peptide, suggesting that GHRH neuronal function was diminished. The fact that diabetic rats are unresponsive to clonidine (625), which releases GH partly through a GHRH-mediated mechanism (see sect. v.A.1A), confirms this. Also relevant is the finding that somatotrophs from diabetic rats have a blunted GH response to GHRH, although this difference disappeared when the data were normalized to account for differences in basal GH release (793).

For the pituitary GH content of diabetic rats, reduced (770) or preserved (625, 1007) pituitary stores have been reported. Unlike in vitro, in vivo GH responsiveness to GHRH was significantly enhanced in streptozotocin-diabetic rats (627). In all these studies, lack of agreement may rest on differences in the strain of rats or length and severity of the disease.

Secretion of GH is reportedly enhanced in IDDM patients. They have higher peak frequency and interpeak GH concentrations, despite elevated blood glucose, an exaggerated GH response to physiological and pharmacological stimuli, and paradoxical GH secretion after TRH (754). Indirect evidence suggests that enhanced GH secretion and responses to GHRH are likely to be related to a decreased SS tone, perhaps subsequent to damage of SS neurons by autoimmune processes (see sect. v.A5n1 for discussion).

In IDDM, high circulating GH levels would therefore be unable to feed back normally on SS-producing neurons (see sect. v.A). In fact, GH pretreatment does not inhibit the GH response to GHRH (420) and pyridostigmine, which reduces SS activity (see sect. v.A.3), is unable to enhance the GH response to GHRH after GH pretreatment, as it does in normal controls (424). If hypothalamic SS function is really defective in IDDM patients, the brisk rise of plasma GH levels elicited by clonidine must be attributable to stimulation of GHRH neurons (see sect. v.A1A).

Different from IDDM, non-insulin-dependent diabetic (NIDDM) patients, have impaired responsiveness to GHRH, not only when they are obese (see below) but also in lean subjects (421).

2. Amino acids

Amino acids are a potent stimulus for GH secretion, either as selected nutrients or when included in a protein-rich meal (90, 283, 516). Parenteral administration of an amino acid solution raises GH secretion by acting on pulsatility and pulse amplitude, an effect presumably mediated by increased GHRH secretion (791). Arginine is the most striking stimulant, although lysine, ornithine, tyrosine, glycine, and tryptophan are all effective GH releasers (564). An oral mixture of arginine and lysine evokes a sevenfold increase of plasma GH levels (516), and a similar effect is observed after arginine aspartate (99, 163).

Arginine-induced GH secretion in humans is blocked by antagonists of α-adrenergic and cholinergic neurotransmission (153, 178), a carbohydrate-rich diet (710), hyperglycemia (711) and NEFA, anti-E₂ (878). Estrogens enhance arginine-induced GH secretion in men or women (711).

The effect of arginine on GH secretion appears to be exerted through suppression of hypothalamic SS release, as suggested by neuropharmacological studies (22, 413, 414). Alternatively, the effect of arginine on GH and other pituitary hormones (56, 366) may depend on its conversion to NO, a gaseous neurotransmitter (734). Arginine is in fact a more effective GH releaser than muscarinic cholinergic agonists (414) which inhibit SS release (629; but see sect. v.B10 for discussion).
3. NEFA

Nonesterified fatty acids are important among the peripheral factors controlling GH secretion. Pharmacological reduction of circulating NEFA levels raises GH (510), but conversely, NEFA elevation induced by the combination of exogenous Intralipid plus heparin markedly reduces spontaneous GH secretion in different animal species (109, 342) and abolishes GH responses to various stimuli (175, 181, 342).

How NEFA act is not clear. In rats, NEFA’s inhibitory effect is directed at the pituitary (30, 175), but a hypothalamic site of action has also been suggested, since their in vivo inhibitory effect on GHRH-stimulated GH release was abolished by passive immunization with SS antiserum (505). Similar conclusions were drawn from human studies (824).

At variance with these findings, experiments using monolayer cultures of fetal hypothalamic neurons showed that exposure to NEFA increased GHRH secretion and inhibited SS output, lowering SS mRNA content (949). The differences in vivo and in vitro may result from disruption of the normal organization in cultured dispersed neurons or the use of fetal tissue.

A pituitary site of action for NEFA was suggested again by Alvarez et al. (30), who showed that their inhibitory effect on GHRH-stimulated GH release was also present in normal rats pretreated with a SS antiserum, or with hypothalamic ablation, and in hypophysectomized rats with two AP transplanted under the kidney capsule.

The inhibitory effect of NEFA on GH secretion at the pituitary is rapid (within minutes), dose and time dependent, and closely related to the chemical structure of the NEFA tested (175). Although this point has not been completely clarified, the most likely mechanism is a reduction of \([\text{Ca}^{2+}]\), \(\text{cis}\)-unsaturated NEFA, such as oleic acid, at physiological concentrations, suppressed the TRH-induced increase in \(\text{Ca}^{2+}\) in primary cultures of pituitary cells and in \(\text{GH}_3\) and \(\text{GH}_4\text{C}_1\) cells. In the same cells, NEFA abolished the TRH-induced \(\text{Ca}^{2+}\) efflux, through plasma membrane \(\text{Ca}^{2+}\) pumps, suggesting they perturbed the function of integral membrane proteins (829). In summary, the inhibitory influence of NEFA on GH secretion appears to be mainly exerted in the pituitary.

4. Leptin

Leptin, the product of the \(ob\) gene, is a recently discovered hormone secreted by adipocytes (1128) that regulates food intake and energy expenditure (162). Because GH secretion is markedly influenced by body weight, and, in particular, adiposity (see also below), leptin may act as a metabolic signal functionally connecting the adipose tissue with the GH/IGF-I axis. Obese mice lacking the \(ob\) gene (1128) or obese rodents with point mutated receptors for leptin show reduced function of the somatotrophic axis.

Circulating leptin concentrations, which are positively correlated with abdominal and total body fat (361), are inversely related with serum GH concentrations (1041, 1042). In subjects with GH deficiency, low-dose GH replacement lowered plasma leptin, with no changes in BMI (365). The effect of GH on leptin secretion was probably indirect, since addition of GH to cultured mature white adipocytes did not affect leptin secretion (453).

Leptin, however, does modify GH secretion, which decreases after administration of a leptin antiserum in freely moving rats; intraventricular-injected leptin reverses the inhibitory effect of fasting on GH secretion (172).

The effect of leptin on GH secretion is presumably mediated by the long form of the \(ob\) receptor, which is primarily located in the hypothalamus (ARC, lateral, VM, DM nuclei) (706, 943). Preliminary results from our laboratory suggest that in the rat leptin increases GHRH function, concomitantly reducing SS activity (254).

The inhibitory effects of the \(ob\) protein on NPY gene expression and secretion (942, 987) suggested that inhibition of NPY function was a major mechanism of action, and this was shown to be valid by the action on feeding behavior (see Ref. 162). Schwartz and co-workers (942, 943) showed that intracerebroventricular injection of leptin into lean rats decreased NPY gene expression in the ARC, while increasing CRH gene expression in the PVN. Recalling the effects of the two peptides on GH secretion (see sect. v, \(E2\) and \(E6\)), it appears highly likely that inhibition of NPY function accounts for leptin GH-stimulatory action.

Erickson et al. (340), by breeding the mutant NPY allele onto the \(ob/ob\) background, generated mice defective in both leptin and NPY. In the absence of NPY, \(ob/ob\) mice were less obese because they ate less and expended more energy; they were also less severely affected by diabetes, sterility, and somatotrophic defects. In adult fasted rats, intraventricular leptin prevented the disappearance of GH pulsatile secretion by reducing the fasting-induced enhancement of hypothalamic NPY gene expression (1074).

The GH stimulation by leptin is hard to reconcile with the presence of high circulating levels of the protein and impaired somatotrophic axis of obese subjects (see below). One possible mechanism involves the development of hypothalamic leptin receptor desensitization, as demonstrated in obese rodents.

C. Physiopathology of Nutritional Excess or Deficiency

Discussion of the many GH deficiency and hypersecretory states is beyond the scope of this review, and the
reader is referred to Müller and co-workers (755, 759). We confine ourselves here to aspects of GH control in two pathological conditions of humans, obesity and food deprivation, where some of the hormonal and metabolic factors alluded to previously are profoundly involved.

1. Obesity

A) Animal Studies. Obese subjects have diminished GH secretion in response to a variety of GH secretagogues (317, 755). Because GH is a lipolytic hormone (601), impaired secretion may help perpetuate obesity.

The mechanisms underlying the defective GH secretion have yet to be fully elucidated, although the reversibility of the defect after successful weight reduction (256, 1005) strengthens the view that the impaired GH secretion is a metabolic consequence of obesity rather than a primary disturbance.

Information from our own and other studies (19, 253, 1012) in animal models of genetic obesity point to a striking impairment of the somatotrophic axis. Obese male rats of the Zucker strain have reduced pulsatile secretion of GH (1012) and pituitary responsiveness to GHRH, both in terms of GH release and of activation of adenylate cyclase (253). Moreover, in these obese rats, pituitary GH gene expression and content are significantly reduced (19) and so are hypothalamic GHRH mRNA and GHRH content, whereas hypothalamic SS content is low but not mRNA (19, 253).

Thus the hyposomatotropism of genetically obese male rats is associated with a primary reduction in the function of hypothalamic GHRH-producing neurons, which may well be the event responsible for the attenuation of GH gene expression and the diminution of circulating plasma GH.

The picture in these rats overlaps that of aged rodents, which also have reduced GH secretion (1110) and a lower in vitro and in vivo responsiveness to GHRH both in terms of GH and activation of adenylate cyclase (810). As for obese and aged rats, and quite likely, humans too (296a), the main cause of the defective GH secretion would be a defective function of GHRH neurons (293), leading to unresponsiveness of GHRH receptors to the endogenous ligand. In aged (6) but not in obese rats (7), this is coupled with a reduction in the number of pituitary GHRH receptors. The reduced genomic expression of GHRH, both in terms of GH release and of activation of adenylate cyclase (258), is much reduced (188, 189, 882). Rats made obese by feeding a hypercaloric diet, although their in vivo responsiveness to GHRH was strikingly reduced, had no differences from controls in pituitary GH content and gene expression and hypothalamic concentrations of GHRH and SS mRNA (188, 189).

These findings underline the peripheral nature of hyposomatotropic function in diet-induced obese rats, as opposed to the “central” impairment of GH regulation of the Zucker obese rats, which have a primary alteration of GHRH function. Among the different endocrine and metabolic indices, low plasma testosterone in males (188) and high NEFA in females (189) play a primary role in the impairment of GH secretion. Either a defect of androgens (849, 1088) or an excess of NEFA (see sect. VI.B3) reportedly inhibits GHRH-stimulated GH secretion.

B) Human Studies. As expected, obese people have impaired 24-h GH secretion and blunted GH responses to provocative stimuli, both reversed by massive weight loss (873). Different from rodents, an alteration in the tonic secretion and/or phasic withdrawal of SS may underlie this GH dysfunction. Reduced GH response to a variety of stimuli is a characteristic feature of obesity, although the pituitary can still make at least a partial secretory response to a direct secretagogue (284, 1096). The GH response to GHRH is reportedly reduced in obese children and adults but was significantly enhanced by pretreatment with pyridostigmine, so the mean baseline GH and the GH responses to pyridostigmine and GHRH overlapped those of lean subjects after GHRH alone. However, in lean subjects, combined administration of pyridostigmine and GHRH evoked a greater rise in plasma GH than in the obese people (415, 634). These findings indicate a role for SS, and hence SS disinhibition, in dictating the extent of GH responsiveness to GHRH in obese subjects, but they also imply some chronic background suppression of somatotroph function unrelated to SS.

Normal people, like obese individuals, may show increased GH responsiveness to acute nutrient deprivation, as indicated by the fact that a short fasting enhances GH responses to GHRH (556). Because in fasting conditions the reported increase of spontaneous GH secretory burst frequency has been inferentially linked to functional
activation of the GHRH system (1033), the effect of fasting in increasing GH secretion in obesity suggests that in humans, like in rodents, this pathological condition is at least partially related to a GHRH neuronal defect.

2. Food deprivation

In rats, prolonged (24–72 h) food deprivation inhibits episodic GH secretion (1017) without altering the ultradian rhythm (791, 792). An intravenous dose of anti-SS serum restored large GH pulses in food-deprived rats, suggesting SS is involved (1012). However, hypothalamic SS mRNA levels were unaltered in 72-h food-deprived rats, whereas GHRH mRNA levels were markedly reduced (149), indicating that decreased GHRH secretion plays an important role in the inhibition of episodic GH secretion in food-deprived rats.

Feeding after prolonged food deprivation caused a rapid and dramatic increase in GH pulse frequency and amplitude, and the characteristic ultradian rhythm was restored (791, 792). This was related to caloric intake rather than specific macronutrients, although ingestion of an amino acid solution, but not of glucose and lipids, to fasted rats rapidly raises the pulse frequency and amplitude of GH secretion (795). In rats with sustained food deprivation, calorie intake restored GH pulse frequency and amplitude even in the absence of endogenous SS (SS antiserum or anterolateral deafferentation of the hypothalamus), suggesting this effect was mediated by activation of GHRH-producing neurons (994).

Dietary protein intake is an important factor in GH secretion, particularly in the early postnatal period, as illustrated by studies (456) showing that in rat pups 3 wk of protein restriction stunted growth and caused a permanent reduction of pulsatile GH secretion in the postweaning period.

In humans, the importance of protein intake for normal GH secretion is evident from the profound disturbance in GH homeostasis of diseases involving protein malnutrition. Fasting GH levels were high in children with kwashiorkor (underweight, edema, hypalbuminemia, and dermatosis) or marasmus (<60% expected weight for age, no edema), and these levels were not normally reduced by induced hyperglycemia, did not correlate with fasting blood glucose, or dropped when an adequate carbohydrate but protein-free diet was given (846), indicating a state of GH resistance. Fasting GH and glucose-induced suppressibility returned to normal with oral protein. Levels of GH in patients before and after amino acid or albumin infusions correlated inversely with some plasma branched chain amino acids (leucine, isoleucine, and valine) (846).

Limited growth in this malnourished children, despite elevated GH, depends on the defective secretion of IGF-I, which is closely related to nutritional status (840, 1046) and responds to protein and energy intakes (517) and to the quality of protein in the diet (252).

In humans, unlike rodents, GH levels are significantly raised by fasting (475), even before the decline in serum IGF-I starting within 48 h of beginning the fast (251). Two days of fasting in healthy men induced a fivefold increase in the GH production rate, by an account of an increase in the number and amplitude of GH secretory bursts. An increase in the frequency of GHRH release with prolonged periods of reduced SS secretion was surmised from the increased frequency of constituent individual secretory bursts with prolonged intervolutary nadirs. Serum IGF-I levels were still unchanged after 56 h of fasting (461) in the presence of increased levels of IGFBP-1 (1123). This may reduce the bioavailable portion of IGF-I, thus limiting IGF-I negative feedback on GH secretion before total serum levels of IGF-I change (see also sect. viB1).

These findings may have some relevance when assessing the GH hypersecretion seen in patients with AN, a disease marked by self-imposed starvation in exaggerated attempts to maintain a thin body, and by a phobia for weight gain and fat (389). Resting GH levels are reportedly elevated in some patients with AN (145 587) but return to normal with recovery. This fall is unrelated to body weight but is closely tied to the patient’s caloric intake (390). It appears, therefore, that the elevated GH in AN is at least partly secondary to starvation and plays an important adaptive role.

However, an analysis by Cluster algorithm of pulsatile GH secretion at night in a group of AN patients disclosed a GH secretory profile somewhat different from that of sustained fasting and reminiscent of that in poorly controlled IDDM (see sect. viB1). The enhanced nocturnal GH secretion was largely due to an increase in the nonpulsatile component, although the pulsatile component was also significantly enhanced because of the larger number of secretory episodes. The lack of correlation between parameters of GH release and BMI indicated a more complex hypothalamic dysregulation of GH release than simple disinhibition of the IGF-I autofeedback mechanism subsequent to malnutrition (929).

In AN, despite exaggerated GH response to GHRH (138, 676, 898), not confirmed in other studies (175, 713), there is little or no GH response to insulin-induced hypoglycemia and dopaminergic stimulation which, in all, suggests hypothalamic dysfunction.

Somatostatin function may be reduced in the hypothalamus of AN patients, in view of the failure of glucose, a stimulus which would inhibit GH secretion through release of SS (867, see also sect. viB1), to counter the GHRH-induced GH rise (900), and the lack of the GH-releasing effect of short-term glucocorticoid treatment which, in fact, is attributed to inhibition of SS secretion (see sect. viA1) (928). In line with this view, pirenzepine, a drug thought to act by stimulating SS function, was only
partially effective in blocking the GHRH-induced GH secretion in AN patients, whereas it completely suppressed it after recovery, as it did in controls (899, 1001). Similarly, in dogs in a chronic state of caloric restriction, a standard dose of pirenzepine failed to prevent the GHRH-induced GH rise, and double the dose was needed (37). Conversely, pyridostigmine, a GH secretagogue allegedly acting through inhibition of SS release (see sect. V A3), had no effect on the GHRH-induced GH rise in either AN patients (409) or in calorically restricted dogs (37), while clearly enhancing it in controls.

However, in AN patients, arginine, another GH secretagogue allegedly acting through inhibition of SS release (see sect. VI B2), strikingly enhanced the GHRH-induced GH rise, like in controls, and blunted the GH response to the second of two consecutive boluses of GHRH (409), indicating that a SS-mediated autocrine mechanism was preserved.

In all, animal and human experiments showing that cholinergic drugs do not affect the GHRH-induced GH rise do not provide evidence that SS function is defective in states of caloric deprivation, but rather indicate the existence of (primary?) hypothalamic cholinergic hyperfunction. In rats under caloric restriction with reduced titers of circulating IGF-I, IGF-I receptors in the ME are upregulated (121). This may be a homeostatic mechanism to suppress GH secretion by maintaining SS secretion during food shortages or other metabolic conditions involving low plasma IGF-I levels (see sect. VII B1).

VII. GROWTH HORMONE AUTOFEEDBACK MECHANISM

Evidence has accumulated over many years that there are long, short, and ultrashort loops in the feedback regulation of GH secretion (see Ref. 750). Because GH lacks a distinct target endocrine gland, it was suggested that it feeds back on the hypothalamus to regulate its own secretion. The feedback regulation of GH presumably involves either activation of SS or inhibition of GHRH function, or both, not forgetting the apparent action on the pituitary through blood-borne IGF-I. In addition to GH itself, brain neurotransmitters, neuropeptides, and many factors directly or indirectly related to GH secretion, such as NEFA, glucose, glucocorticoids and thyroid and gonadal hormones, have important roles in somatotroph function (see sect. VI).

A. Growth Hormone

Early data (reviewed in Ref. 749) showed that rats with ectopic somatotrophic tumors had reduced pituitary levels of GH and their pituitary synthesize GH at a reduced rate in vitro (691). In rodents, the infusion of GH blocked the GH release induced by insulin hypoglycemia, determined using as an end point the “tibia test” bioassay (757); in humans, treatment with GH prevented insulin hypoglycemia-induced elevations of GH (4). These studies implied that elevated levels of circulating GH have an autocrine action at the level of the hypothalamus or pituitary.

With the advent of specific RIA, it was possible to show that short-term hypophysectomy was associated with a decrease in the hypothalamic content of SS and that SS levels could be restored to normal after GH administration (480, 812). Similarly, hypothalamic SS content could be reduced by selective passive immunization against GH (93). However, true assessment of the dynamics underlying these changes only became possible with molecular cloning techniques.

The knowledge that alterations of hypothalamic SS titers were due to changes in SS synthesis and release

<p>| TABLE 4. Factors or conditions that stimulate or inhibit GH secretion in primates |
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<th>Factors</th>
<th>Increase</th>
<th>Inhibition</th>
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<tr>
<td>Neurogenic</td>
<td>Sleep stages 3 and 4</td>
<td>REM sleep</td>
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<td>Stress</td>
<td>Physical</td>
<td>Psychological</td>
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<td>Monoamine</td>
<td>Epinephrine, $\alpha_2$-adrenergic stimulation</td>
<td>$\alpha_1$-Adrenergic stimulation</td>
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<td>DA agonists</td>
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<td>GABA agonists</td>
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<td>Metabolic</td>
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<td>Fasting</td>
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<td>Decreased IGF-I levels</td>
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<td>Glucocorticoids†</td>
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* For the sake of clarity, growth hormone-releasing and inhibitory neuropeptides (see sect. VI B2) have been omitted. † Under acute treatment. ‡ In acromegaly. See text for definitions.
derived from experiments in which SS mRNA was measured in individual cells of the rat PeVN. Hypophysectomy lowered the level of the messenger, per cell, an effect completely reversed by administration of GH. In the same experiments, intact GH-treated rats had higher levels of the messenger than vehicle-treated rats (897). That this effect was actually due to GH was also implied by in vitro experiments, adding GH to MBH preparations (93). Moreover, in vivo intraventricular injections of GH raised SS levels in rat portal blood after a latency of 20–40 min, suggesting action more on SS synthesis than release (235). Supporting these findings, three different dwarf mutant models of mice lacking GH, i.e., Ames dwarf df/df mice (496), Snell dwarf (dw/dw) mice (788), and transgenic dwarf mice with genetic ablation of GH-expressing cells had a selected depletion of SS mRNA in the PeVN (85).

The developmental role of GH on hypophysiotropic SS neurons has been studied in Ames dwarf df/df mice. Total SS mRNA levels were deficient in their PeVN shortly after the developmental onset of SS transcription. The absence of GH autofeedback was very likely responsible for SS mRNA deficiency in 7-day-old dwarf Ames mice compared with phenotypically normal DF+/+ controls, suggesting that GH receptors may be present on the PeVN neurons at or before 7 days of age in DF+/+ mice (496). Because the amount of SS mRNA per expressing cell in the PeVN of Ames dwarf mice was not reduced, the decrease in total SS mRNA of the whole expressing cells, also present in adults, was the result of an early failure to achieve a normal population of SS-expressing neurons (496). No studies on the developmental appearance of GHRH receptor mRNA have been reported so far in Ames dwarf mice, a topic worth further investigation to clarify the developmental role of GH on SS neurons.

Transgenic mice expressing heterologous and ectopic GH have been used as models for studying the feedback effects of elevated nonregulated GH on hypophysiotropic neurons and peripheral functions. It would seem that feedback effects extend beyond dynamic regulation to influence hypophysiotropic neuron differentiation and/or survival, when the deficiency (see above), or excess of GH is genetic and lifelong, such as in spontaneous mutant or transgenic models (837). Somatostatin expression was markedly increased in mice bearing either bovine or human transgenes. Human, but not bovine, GH reportedly has lactogenic properties in mice and appears to stimulate prolactin-inhibiting TIDA neurons. The results in the murine transgene models suggested that although even extremely high levels of circulating GH of bovine origin did not stimulate TIDA neurons, lifelong high levels of human GH had a stimulatory and graded effect on the developmental differentiation of TH and DA production, supporting the concept of prolactin as a trophic factor for TIDA neurons.

Although extensive studies have been done in animal models to elucidate the various components of this regulatory system, studies in humans have been limited because of the inaccessibility of the hypothalamus and pituitary and their vascular connections. However, in healthy subjects given an acute dose of GH, total suppression of the subsequent GH response to GHRH (906), before any rise of circulating IGF-I (907), was evident. Moreover, similar to what is observed with SS antisera in the rat (592, 1039) or with muscarinic cholinergic agonists inhibiting hypothalamic SS release (1039; and see sect. vB3), in humans too this class of compounds (671, 907) completely restored the GH response to GHRH, inhibited by a previous GHRH bolus (410, 672), suggesting SS is involved in the short-loop negative autofeedback. However, these experiments, which imply the intervention of the GH-SS axis, cannot exclude a mechanism mediated by blood-borne or locally produced IGF-I (see also below).

However, the GH-mediated inhibitory feedback has more experimental than clinical relevance, as shown in GH-deficient patients who, despite chronic treatment with GHRH, have persistently elevated GH and IGF-I plasma levels (1035), or patients with ectopic GHRH secretion who develop acromegaly (376).

In addition to SS, GHRH is also implicated in GH feedback mechanisms. In rats, intracerebroventricular infusion of GH reduced the amplitude of spontaneous GH secretory bursts, an effect that was counteracted not by SS antisera but by the combination of supramaximal systemic doses of GHRH and SS antibodies (592). A more direct approach had relied on direct measurement of GHRH-IR in the hypothalamus of hypophysectomized rats. Hypothalamic GHRH-IR concentrations were reduced in these rats and were partially restored after GH treatment (386). However, in this instance, simple evaluation of GHRH content was misleading and contrasted the reported ability of GH to lower hypothalamic GHRH content in intact rats (386) or rats with a GH-secreting tumor (799). In a sequel to these studies, it was shown that GHRH mRNA was significantly increased in the hypothalamus of hypophysectomized rats and was partially reduced by GH (237, 290).

The negative-feedback action of GH on GHRH mRNA was also demonstrated in infant rats, in which a dose of GH reduced pituitary GH content and abolished the GH rise in response to acute GHRH; this was restored to normal when GH treatment was combined with GHRH (207).

These findings might be relevant to the GH therapy of nonclassical GH-deficient short children, whose GHRH-secreting neurons are functionally preserved (399, 1055). However, a recent study in children with severe short stature and impaired GH responses to suprapituitary stimuli but normal responsiveness to GHRH negates this
mechanism. During GH therapy, the GH response to a GHRH bolus was only slightly reduced, and a second bolus of GHRH elicited a GH response similar to that in the pretreatment test (927). This indicates a significant resilience of GHRH neurons to the feedback mechanisms triggered by elevated circulating GH concentrations.

Feedback regulation of GHRH function by GH has been studied in animal models of spontaneous GH deficiency (933). In Ames dwarf mice not only was total GHRH mRNA expression enhanced, but the number of GHRH-IR neurons was increased (838). Conversely, transgenic mice bearing a TH-human GH transgene had targeted expression of GH in the PeVN, ARC, and adrenal medulla and were growth retarded (68). They also had low pituitary GH and GH mRNA and low hepatic IGF-I mRNA and serum IGF-I levels. In these rats, feedback effects occurred in the PeVN and ARC, leading to increases of SS and its messenger and decreases in GHRH and its messenger (996).

Lowering GHRH function during fetal life in these mice affected the density of pituitary GHRH receptors and impaired somatotroph development (996). Thus this type of transgenic mouse serves as a model of idiopathic GH deficiency in humans and a means for studying factors affecting somatotroph proliferation (374).

Flavell et al. (362) reported that in transgenic rats targeting the expression of human GH (hGH) to GHRH neurons can induce dominant dwarfism. A line of these rats bearing a single copy of a GHRH-hGH transgene had low levels of production of hypothalamic GHRH and mRNA, in contrast to the increased GHRH expression accompanying GH deficiency in other models of dwarfism. These rats also had a sexually dimorphic pattern of GH secretion and a lower GH response to GHRH or GHRP-6 challenge than their nontransgenic littermates; nevertheless, despite the pituitary GH deficiency and dwarfism, repeated dosing of GHRH or GHRP-6 did stimulate their growth. To our knowledge, these rats are the first genetic animal model of GH deficiency in which dwarfism could be corrected by exogenous GH secretagogues (1093).

In contrast to the feedback data in young rats, the feedback effects of GH are disrupted in senescent rats, which are spontaneously deficient in GH. In 20-mo-old male rats, 4 days of treatment with GH increased plasma IGF-I levels but did not significantly change hypothalamic GHRH and SS mRNA levels (291). However, old rats apparently losing their ability to sense the feedback action of GH in the hypothalamus responded normally to GH with increases in SS and IGF-I gene expression in the cerebral hemispheres (637). This may explain the improvement of some psychological symptoms after GH treatment in GH-deficient patients (see also below).

The direct feedback action of GH on the brain involves GH binding to its own receptor. Specific GH receptors have been found in the choroid plexus, hippocampus, hypothalamus, and pituitary gland (583, 1084). Receptors in the choroid plexus are involved in transporting the hormone across the BBB (583). Growth hormone is reportedly present in low concentrations in the CSF in humans (616), but direct evidence that it can cross the BBB has been recently provided in GH-deficient adults where CSF GH levels rose significantly after 1 or 21 mo of GH treatment (154, 528). This was accompanied by parallel increases in IGF-I, IGFBP-3, and β-endorphin concentrations and by decreases in homovanillic acid, VIP, and free thyroxine concentrations. These changes in CSF composition might explain the gain in psychological well-being usually reported in GH-deficient patients after they start GH treatment.

The mRNA for GH receptors was widely expressed in the PeVN, PVN, and ARC of the hypothalamus (155). In the PeVN and PVN, the majority of SS neurons coexpressed GH receptor mRNA, suggesting that GH acts directly on SS at these sites. Intriguing was the observation that GH receptor mRNA was also expressed in the ARC, although the vast majority of GHRH and SS cells of the ARC did not appear to express it, implying that GH effects in ARC were transduced by other neurons (155). However, single- and double-label in situ hybridization showed that GH induced c-fos expression in the ARC of intact rats (156) and in the ARC and PeVN of hypophysectomized rats (728), indicating that immediate early gene expression plays a role in the feedback actions of GH into the brain. However, although c-fos-expressing neurons in the medial ARC were abundant, few if any were GHRH or SS neurons.

The pattern of distribution of c-fos expression in the ARC was strikingly similar to that of GH-receptor mRNA, suggesting that receptor binding and the induction of c-fos gene expression in this nucleus occurred within the same population of unidentified cells.

The precise identity of these cells remained elusive until Kamigai et al. (538) demonstrated that GH induces the expression of c-fos mRNA in NPY neurons of the ARC. Although this suggested that most NPY neurons were responsive to GH, it remained to be established whether GH acts directly on NPY neurons or whether there was some other population of cells receiving the GH signal. Studies of coexpression of GH receptor mRNA and NPY mRNA in the ARC indicated that the majority of NPY neurons in the ARC coexpressed GH receptor mRNA (217). Granted that NPY neurons may serve as a receiving population for the GH signal, it remains to be seen how, or even if, the signal is relayed to GHRH or SS neurons in the ARC (see also sect. vB6).

Another neuropeptide, galanin, which is coexpressed in GHRH neurons (699, 778; see sect. mA2), seems to be involved in the feedback control of GH. Galanin mRNA is reduced in GHRH neurons of Lewis dwarf rats and in
hypophysectomized rats and is restored by exogenous GH (218). A subset of SS neurons in the PeVN also expresses the galanin receptor mRNA, whereas few GHRH neurons, if any, appeared to do so (218). Galanin, like its co-transmitter GHRH, is the target for GH action, and galanin may play a role in the feedback control of GH secretion, through a direct effect on SS neurons. These, in turn, would inhibit GHRH ARC neurons, although the underlying mechanism remains to be elucidated.

High levels of expression of the mRNA for two prototypic receptors of the SS family, sstr1 and sstr2, have been found (81), and 2 wk after hypophysectomy, sstr1 and sstr2 mRNA labeling density was reduced in the ARC. Seven days of treatment with hGH raised the labeling density of sstr1 mRNA selectively in the ARC of hypophysectomized rats (448). Thus the expression of sstr1 and sstr2 receptor subtypes must be under the regulatory influence of pituitary hormones, and GH may participate in its own secretion by modulating hypothalamic SS and, secondarily, by up- or down-regulating sstr1 receptor mRNA on GHRH-containing neurons. However, in view of the long-term exposure to GH reported in this study, it is still questionable whether this regulatory feedback mechanism would operate under physiological conditions and participate in the genesis of episodic GH secretion or is only manifested under pharmacological conditions.

Growth hormone, in addition to its direct short-loop effects on the hypothalamic neurons, directly affects lipolysis and impairs glucose metabolism and indirectly affects growth by stimulating the production of IGF-I. Therefore, IGF-I, NEFA, and glucose might all have roles in the long-loop feedback regulation of GH secretion (see sect. vi, B1 and B3).

B. Insulin-Like Growth Factor I

Insulin-like growth factor I participates in the growth and function of almost every organ in the body (283) and is synthesized primarily in the liver, although it is produced everywhere in the body. Insulin-like growth factor I shows structural similarities with IGF-II and insulin, with which it has in common 60% of amino acids. However, unlike insulin, which circulates in picomolar concentrations and has a short half-life, IGF-I and -II circulate in nanomolar concentrations and have a much longer half-life because they are largely bound to a variety of BP, six of which have been characterized (530). These BP, like IGF-I and IGF-II, are synthesized by most tissues, where they act in an autocrine or paracrine manner to regulate different functions (530). Both IGF-I and IGF-II are essential for fetal development (67), and nanomolar concentrations persist in the circulation into adult life.

The main role of IGF-I after birth is to regulate growth, whereas that of IGF-II is still unknown (606). The circulating BP modulate the activity of these growth factors by limiting their access to specific tissues and receptors. Binding protein 3 binds >95% of circulating IGF-I and -II. This complex binds an acid-labile protein subunit, forming a ternary complex that has a serum half-life of many hours. Once released from this complex, the IGF can enter target tissues with the aid of other BP. Growth hormone raises the serum concentrations of BP-3 and the acid-soluble subunit (530). Some IGF-I BP have a greater affinity for IGF than receptors. This can prevent activation of intracellular signaling pathways (606).

The local production of IGF-I is under different control in different tissues. For example, GH, parathyroid hormone, and sex steroids regulate the production of IGF-I in bone, whereas sex steroids are the main regulators of locally produced IGF-I in the reproductive system. The functions of circulating IGF-I are becoming clearer, but the actions of locally produced IGF have yet to be defined. Age, sex, nutritional status, and GH affect serum IGF-I concentrations. Plasma levels of IGF-I are low at birth, rise substantially during childhood and puberty, and begin to decline in the third decade (59, 607), after the changes in GH secretion.

Complete disruption of the IGF-I gene in a 15-yr-old boy who presented with severe intrauterine growth failure, deafness, and mild mental retardation indicates that the absence of IGF-I is compatible with life. However, it suggests that IGF-I plays a major role in human fetal growth and CNS development (1104) (see also below). In addition, low IGF-I levels may result from GH deficiency or from mutations in the gene encoding the GH receptor, such as in Laron dwarfism (593).

The nutritional status also markedly affects plasma IGF-I concentrations. Starvation causes complete resistance to GH, and restriction of protein or calories causes a lesser degree of resistance with a consequent reduction of hepatic IGF-I production (517). Low IGF-I levels with GH resistance are also seen in other catabolic states, such as severe trauma and sepsis. In IDDM, hepatic resistance to GH, with elevated serum GH and low IGF-I levels in plasma, result probably from an inadequate insufficient action of insulin on the liver (450). During puberty, these changes may limit somatic growth. The high levels of GH may worsen the hyperglycemia by opposing the peripheral actions of insulin in poorly controlled IDDM or NIDDM. Treatment with IGF-I improves glycemic control in IDDM and reduces insulin resistance by lowering serum GH and glucagon (61, 327), and in NIDDM it prevents hyperinsulinemia (742, 1127).

Over recent years, there has been increasing interest in the roles of IGF-I within the CNS. In addition to its activity on proliferation and differentiation during embryonic and postnatal development (67, 623, 876), IGF-I also promotes survival and stimulates neurite outgrowth from central and peripheral neurons in culture. Insulin-like
growth factor I increases oligodendrocyte-induced myelin synthesis and survival (1116). Targeted disruption of the IGF-I gene results in reduced brain size, hypomyelination, and neuronal loss (82).

Insulin-like growth factor I mRNA is transiently expressed in CNS areas related to long projection neurons (123, 387). The transient expression of IGF-I by projection neurons during axon growth and synaptogenesis suggests it has an important role in these processes (123). This contention is borne out by the permanent expression of IGF-I and its receptors in the olfactory bulb, where neuroend synaptogenesis persist during adult life (123). Insulin-like growth factor I therefore has a pleiotropic role in the CNS essential for proliferation, differentiation, and survival in the developing brain, and in several neuropathological conditions (319), gene expression reaching its highest levels in the early phase of neuronal growth, myelination, or nerve regeneration.

Concerning the factors and mechanisms involved in the regulation of CNS IGF-I gene expression, Wood et al. (1104) reported that in adult hypophysectomized rats GH given centrally or systemically could restore normal IGF-I mRNA levels. Ye et al. (1116) found that GH played a role in the regulation of brain IGF-I gene expression during development too. Growth hormone, which regulates IGF-I gene expression, may be produced in the brain (482) or from the pituitary, since GH levels in brain are reduced after hypophysectomy (483), and systemically administered GH increases brain IGF-I mRNA (1104).

In addition, IGF-I might reach the brain from the periphery, since IGF-I transport across the BBB into specific thalamic and hypothalamic nuclei has been reported (881).

1. Feedback actions of IGF-I

It has long been known that a long-loop feedback regulation of GH secretion is operated by IGF-I. Berelowitz et al. (90) were the first to demonstrate that a purified IGF-I preparation inhibited GH release from pituitary cells in culture and stimulated dose-related release of SS from hypothalamic explants. The IGF long-loop feedback was further strengthened by the finding (1011) that intraventricular injection of a preparation containing both IGF-I and -II markedly suppressed spontaneous GH secretion in the rat. Insulin-like growth factor I receptors have been found in normal rat pituitary cells and in human GH-secreting adenomas (196). Insulin-like growth factor I has a potent and persistent inhibitory action on cultured human pituitary adenoma cells, resulting in the reduction of GH release, under basal and stimulated conditions (197); it dose-dependently lowers the levels of GH mRNA (1115). It also has inhibitory action, not only on GH gene expression but also on the pituitary specific transcriptional factor GHRF-I/Pit-I (973).

Although in vitro data clearly demonstrated the inhibitory role of IGF-I in the pituitary, in vivo experiments in rats and humans yielded contrasting results. As mentioned above, experiments in rats indicated that IGF participate in GH regulation through a hypothalamic action. However, those experiments suffered the limitation of having used a partially purified IGF preparation (1011), a point that was reexamined with the advent of recombinant IGF preparations. Separate intraventricular injection of IGF-I and -II, even at high concentrations, did not modify the pulsatile pattern of GH secretion in the rat. Only combined IGF-I and -II significantly inhibited GH secretion (454, 455). The mechanism of this interaction is still not clear.

How IGF-I affects SS and GHRH mRNA levels was studied in GHRH-deprived rats. No effect was seen after systemic administration of IGF-I, whereas intraventricular IGF-I lowered GHRH mRNA and raised SS mRNA levels (924). The difference between rats and humans was not explained. In the rat, GH secretion was inhibited only after intraventricular injection, whereas in humans, it was also inhibited after peripheral administration (196). It was initially shown that an intravenous bolus injection of IGF-I immediately inhibited GH secretion, in normal and Laron dwarf patients, also reducing blood glucose (596). In normal subjects, a low-dose euglycemic infusion of IGF-I rapidly suppressed the GH secretion enhanced by fasting (459) or by GHRH or hexarelin (M. Rolla, unpublished results), indicating an action most likely through IGF-I receptors and independent of its insulin-like metabolic actions.

The observation that in ewes intraventricular infusions of IGF-I and IGF-II, alone or in combination, had no effect on GH secretion whereas intrapituitary infusion inhibited it (363) strongly suggests the pituitary is the main site of IGF feedback on GH. In the rat, continuous infusion of IGF-I for 3 days significantly raised SS mRNA levels in the hypothalamus of control and starved rats. In the same model, repeated doses of rhGH stimulated SS mRNA levels in control but not starved rats, indicating that IGF-I generation was necessary in the GH autofeedback mechanisms operated through SS neurons (416).

The physiological relevance of the feedback effect of IGF-I on the CNS depends on its access in vivo to those areas that participate in SS and GHRH secretion. Specific binding sites for IGF-I have been found in the pituitary, in the external palisade zone of the ME, where SS nerve terminals project (122), and in other brain areas (435); IGF-I is also present in the CSF (80).

By all these mechanisms IGF-I inhibits the posttranslational processing of GH and reduces GH exocytosis, antagonizing the effect of GHRH and blunting the GH-releasing actions of forskolin, cAMP, and TPA (741, 1112). The relevant IGFBP role in the effect of the peptide is now understood to be important. It is thus possible that spe-
cies differences of IGF-I’s effects on GH secretion after different routes of administration are related to different expression of IGFBP in the CNS (196).

All in all, IGF-I can play an important role in the feedback control of GH by acting acutely on SS secretion which, in turn, inhibits pituitary GH secretion and in the long run by acting directly on the pituitary.

VIII. CONCLUDING REMARKS

The neural control of GH secretion in mammals is thought to be exerted primarily through the subtle interplay of GHRH, the physiological stimulator of GH synthesis and release and of somatotroph proliferation, and SS, which through its inhibitory influences is the principal modulator of GH release and regulator of GH pulsatility. The identification, characterization, and cloning of animal and human GHRH receptors should extend our understanding of GH regulation under different physiological and pathological conditions, leading us to use GHRH better for the therapy of pituitary dwarfism, GH deficiency of adults, and other GH deficiency states.

A variety of recently cloned sstr subtypes mediate the various effects of SS and will help us correlate their regional distribution within the CNS with the peptides’ functional properties. Development of subtype-selective SS agonists and antagonists may result in new therapies for the treatment of CNS and peripheral tumors and neurological disorders in which the SS system is reportedly altered. A new family of extremely effective GH releasers, the peptidyl and nonpeptidyl GH-releasing peptides, whose mechanism of action and endogenous receptor ligands have yet to be identified, nevertheless offer therapeutic potential in a variety of hyposomatotropic states, when given alone or in combination with GHRH. They are also posing questions about some traditional beliefs of GH regulation, adding new complexity to the understanding of how the classic brain neurotransmitters, GHRH and SS, but also an increasing cohort of GH-releasing factors (growth hormone-releasing hormone and gamma-aminobutyric acid) in the regulation of growth hormone secretion in the neonatal and adult rat. "A superfusion study. Endocrinology 129: 1790–1798, 1987.

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