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PHYSIOLOGICAL STUDIES WITH SOMATOCRININ, A GROWTH HORMONE–RELEASING FACTOR

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INTRODUCTION

Our knowledge of how hormones affect normal growth and development has derived from observing the pathophysiological effects of hormonal deficiency or excess in clinical and experimental studies. These observations have led to the identification of numerous hormones that exert significant effects on somatic growth, including growth hormone (GH), insulin, thyroid hormones, glucocorticoids, and androgens. As implied by its name, GH is the fundamental factor regulating growth. Moreover, it is of particular interest since it is the hormonal link between somatic development and its regulation by the central nervous system.

GH release by the anterior pituitary is controlled by hypothalamic releasing and inhibiting factors. The recognition (1) and characterization (2) of hypothalamic growth hormone release–inhibiting factor, somatostatin, in 1973 extended our knowledge of the regulation of GH secretion. The structural elucidation of growth hormone–releasing factor (GRF) was not accomplished until almost ten years later. In November 1982, Guillemin and associates (3) reported the isolation and characterization of a 44 amino acid peptide from a pancreatic tumor causing acromegaly with potent GH-releasing activity. These investigators also described two additional GH-releasing peptides consisting of

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the first 37 and 40 amino acids of the 44 amino acid peptide. The structure of the 40 amino acid GRF was confirmed shortly thereafter by Rivier et al (4) and Esch et al (5). This information has led to the successful characterization of human hypothalamic GRF (5a) as well as murine (6), porcine (7), bovine (8), and ovine and caprine GRF (8a).

The availability of synthetic GRF has opened a new era in our investigations of the regulation of GH secretion by the anterior pituitary. The purpose of this article is to review the literature concerning the in vivo actions of growth hormone–releasing factor.

THE ONTOGENY AND DISTRIBUTION OF GRF WITHIN THE CENTRAL NERVOUS SYSTEM

Based on its hypophysiotropic function, the distribution of GRF within the central nervous system had been hypothesized as being concentrated in the hypothalamus. Immunohistochemical studies conducted to evaluate this hypothesis show that the localization of this peptidergic system within the central nervous system is highly consistent with its physiological function of releasing GH from the anterior pituitary.

GRF immunoreactivity is not observed in the hypothalami of human fetuses younger than 29 weeks of age (9). Between 29 and 31 weeks of intrauterine life, numerous immunoreactive cells are found in the infundibular (arcuate) nucleus, showing typical neuroblastic aspects such as small diameter and no processes. No immunoreactive fibers can be found in the median eminence of the hypothalamus at these ages. The hypothalamic distribution of GRF neurons is similar in older fetuses and neonates; however, cell bodies appear more immunoreactive and short, immature processes are observed. GRF immunoreactive fibers first appear in the hypothalamus after the thirty-first week of fetal life and are numerous at birth. These fibers also appear in the median eminence, with endings in contact with the hypothalamic-hypophyseal portal vessels, at about the same time.

The ontogeny of the GRF system does not parallel the development of the other hypophysiotropic factors. Neurons staining for luteinizing hormone-releasing factor (LRF) are detectable by the eleventh week of fetal life (10), for somatostatin by the fourteenth week (11), and for corticotropin-releasing factor (CRF) by the sixteenth week (12). The appearance of somatotropic cells within the pituitary occurs as early as the eighth week of gestation. Thus, it appears that the initial stages of the development and differentiation of pituitary somatotrophs are independent of hypothalamic GRF input.

Numerous cell bodies containing GRF immunoreactivity are observed in the adult human and monkey hypothalami (13, 14); the vast majority of these cell bodies are in the medial basal hypothalamus, especially in the arcuate nucleus.

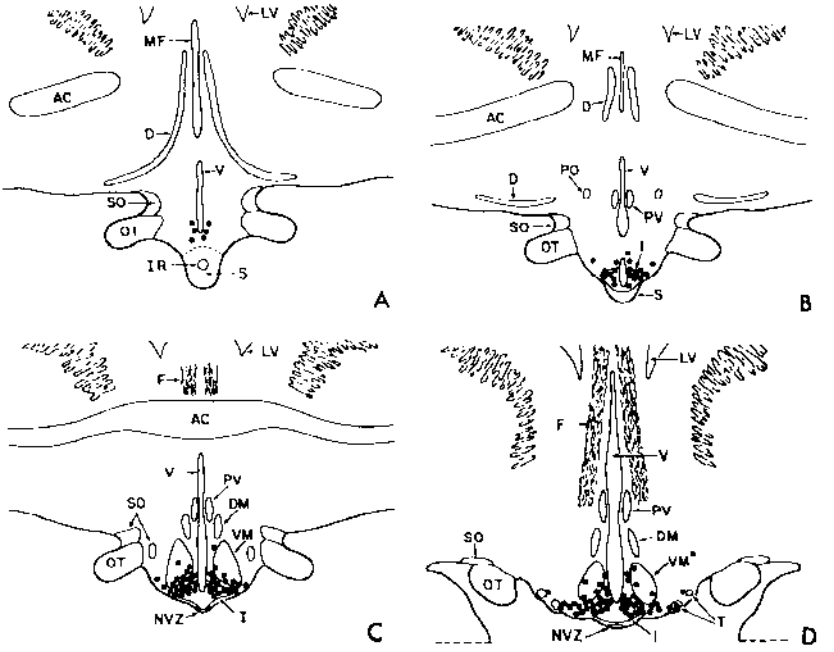

Figure 1 Diagrams of the frontal sections of the adult human hypothalamus showing the localization of GRF immunoreactive cell bodies. A–D are from anterior to posterior planes. Abbreviations: AC = anterior commissure; D = nucleus of diagonal band; DM = dorsomedial nucleus; F = fornix; I = infundibular nucleus; IR = infundibular recess; LV = lateral ventricle; MF = midline fissure; NVZ = neurovascular zone; OT = optic tract; PO = preoptic nucleus; PV = paraventricular nucleus; S = pituitary stalk; SO = supraoptic nucleus; T = lateral tuber nucleus; V = third ventricle; VM = ventromedial nucleus (taken from 9).

Some cell clusters extend into the lateral hypothalamus, and others extend dorsally near the ventromedial nucleus and along the wall of the third ventricle. Cell bodies are found as far anterior as the optic chiasm and as far posterior as the mamillary bodies (Figure 1). GRF immunoreactive fibers are present in the median eminence, arcuate nucleus, and ventromedial nuclei. Within the median eminence, the fibers appear grouped in bundles that terminate on the primary capillary plexus of the hypothalamic-hypophyseal portal system, an expected observation based on the functions of GRF.

The distribution of GRF immunoreactive structures within the rat hypothalamus is similar to that found in primate brains. The majority of cell bodies are found in the arcuate nucleus and in the medial perifornical region of the lateral hypothalamus (15–17, 22). Scattered cell bodies are also seen in the ventral and dorsal lateral hypothalamus. Of interest is the apparent absence of GRF-containing neurons in the ventromedial hypothalamus in the rat (16). Electrical stimulation (18) and lesion experiments (19–21) have strongly implicated the

ventromedial hypothalamus as an important neural locus with regard to GRF. Since GRF cell bodies are located in areas contiguous to this region, it is likely that the effects observed by destruction or stimulation of the ventromedial nucleus are due to extension of the lesions or to diffusion of the stimulation to adjacent areas. Nevertheless, neurons within the ventromedial nucleus might exert an effect on GRF neurons in the arcuate nucleus.

Dense GRF immunoreactive processes and terminals are observed in the median eminence. Bloch et al (22) have demonstrated that neonatal treatment of rats with monosodium glutamate, a procedure that selectively destroys the neurons of the arcuate nucleus, results in the almost complete and selective loss of GRF staining in the median eminence. These results demonstrate that the arcuate nucleus is the main source of GRF in the median eminence. Fibers from GRF-staining neurons in the perifornical region run perpendicular to the basal surface of the hypothalamus. They then turn medially and run parallel to it until they terminate in the median eminence.

The ontogeny and distribution of GRF immunoreactivity has also been studied in the cat (23). As in other species, cell bodies are most abundant in the arcuate nucleus, with additional staining observed in the paraventricular, supraoptic, and dorsomedial nuclei and anterior periventricular areas. Again, little or no immunoreactivity is observed in the ventromedial nucleus. Consistent with what is observed in humans, the development of the GRF pathways in the cat is much later than that of the other hypothalamic releasing and inhibiting factors. Although the GRF cell bodies are developed by 15 days of age, only scarce GRF immunoreactive fibers in the median eminence can be observed. By 30 days of age nerve fibers are abundant and well developed, and terminals can be seen in close proximity to the primary capillary bed of the hypothalamic-hypophyseal portal system. GRF perikarya have also been observed outside the hypothalamus in the cat; however, questions concerning the specificity of the GRF antiserum in relation to feline GRF leave these observations open to interpretation.

GRF REGULATION OF GH RELEASE

Extensive studies on the in vivo action of GRF were initiated as soon as the synthetic replicate of GRF became available. Initial experiments were designed to establish the dose-response relationship and the specificity of GRF in stimulating GH release in a variety of animal models. Likewise, studies were conducted to evaluate whether the various GRFs characterized possess different biological activity. Since the 44 amino–acid peptide isolated from human tissue has in vivo biological activity similar to the 40 and 37 amino–acid peptides (Figure 2) and since human and murine GRF are equipotent in vivo

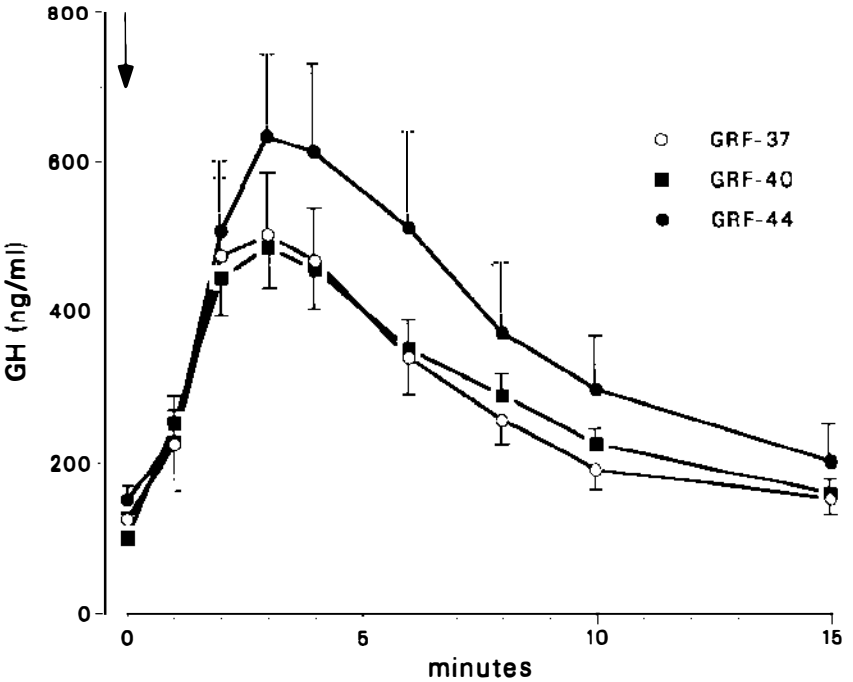

Figure 2 The relative potency of 0.02 nmole human GRF (1-44), (1-40), and (1-37) (see 3) in anesthetized male rats. Animals were treated with 60 mg/kg sodium pentobarbital administered intraperitoneally 15 minutes prior to the initiation of blood sampling. The peptides were administered intravenously immediately after time 0 in 0.5 ml saline. Data points represent the mean of results obtained in 19 rats; vertical bars represent the SEM (W. Wehrenberg, N. Ling, unpublished observations).

(23a), little distinction will be given here to which specific molecule of GRF was used in a given study.

Doses

HUMAN AND SUBHUMAN PRIMATES The identification of a substance with direct and potent GH releasing activity has stimulated substantial clinical interest. Intravenous administration of GRF into normal adults in doses of 0.1–10 μg/kg body weight results in a significant increase in plasma GH concentrations (24–29). Peak concentrations of GH are reached 15–30 minutes post-injection and concentrations return to baseline values in between one and two hours (Figure 3). The pituitary GH response to GRF is specific; the secretion of no other anterior pituitary hormone is altered by GRF administration (24). Thorner et al (30) have reported that GRF is also effective in stimulating GH release following subcutaneous and intranasal administration.

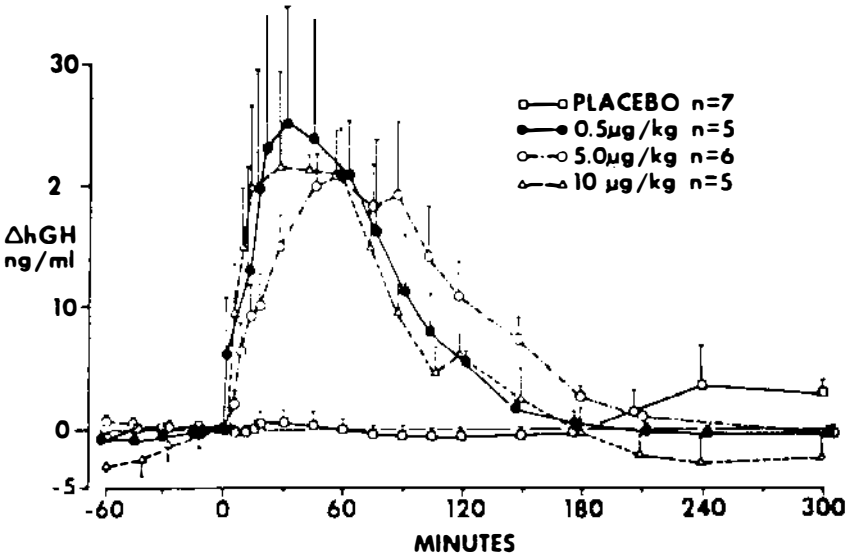

Figure 3 Elevation of plasma GH concentrations in response to GRF in normal adult human volunteers. Response to the 0.5, 5, and 10 μg/kg doses of GRF were all significantly higher than the placebo. GH concentrations rose within five minutes and reached a maximum at 30–45 minutes (0.5 μg/kg), 45–90 minutes (5 μg/kg), and 30–120 minutes (10 μg/kg). Results are expressed in ng hGH/ml (mean ± SEM) (taken from 25).

The minimum effective doses of GRF required for subcutaneous administration are approximately 30 times greater than those required for intravenous administration (30). Intranasal administration of GRF requires doses about 100 times greater than those required for intravenous administration (30, 31).

The specific pituitary GH response to GRF and its dose-response relationship has also been confirmed in subhuman primates (32). Increases in GH are observed in normal female monkeys following the intravenous administration of 5–100 μg GRF/kg. Maximum GH concentrations are measured approximately 30 minutes following injection; concentrations return to baseline within one hour following lower doses of GRF, but they remain elevated for up to two hours following higher doses.

The reports published thus far on the effects of GRF on GH secretion in humans and monkeys demonstrate a marked heterogeneity of the GH response to a given dose of GRF. This applies to the response observed within an individual given repeated doses of GRF and to the response observed between individuals. It is unclear what factors are involved in modulating the GH response to GRF. It is possible that somatostatin is actively involved, since this neuropeptide plays a major role in regulating GH secretion and it has already been shown to modulate the GH response to GRF in laboratory animals (33,

43). Regardless of the variability in the GH response to GRF, this peptide is already of immense importance in the clinical diagnosis of GH disorders.

OTHER SPECIES Extensive research has been conducted on the biological relationships between GRF and GH in the laboratory rat. Initial studies were conducted in rats anesthetized with sodium pentobarbital. This animal model has proven very useful for the study of GRF, since pentobarbital anesthesia appears to inhibit the release of both endogenous somatostatin and GRF (34). Studies with GRF have demonstrated that the maximum GH response occurs within 3–5 minutes post-intravenous injection; concentrations begin to decline within 15 minutes and return to baseline by 30 minutes. The dose-response relationship for GRF has been clearly demonstrated in the rat, in contrast to results obtained in the human. The minimum effective dose of GRF to elicit a GH response is approximately 100 ng/kg and the maximum dose is in the range of 5 μg/kg (Figure 4). The subcutaneous administration of GRF is also effective, the minimum effective dose being approximately 25 μg/kg (35). Rats anesthetized with urethane also appear to be a useful model (35, 36). However, urethane anesthesia is known to increase somatostatin concentrations in hypothalamic-hypophyseal portal blood compared to sodium pentobarbital anesthesia (37), and thus it is not unexpected that the amount of GRF needed to stimulate GH secretion is greater in the urethane-anesthetized rat.

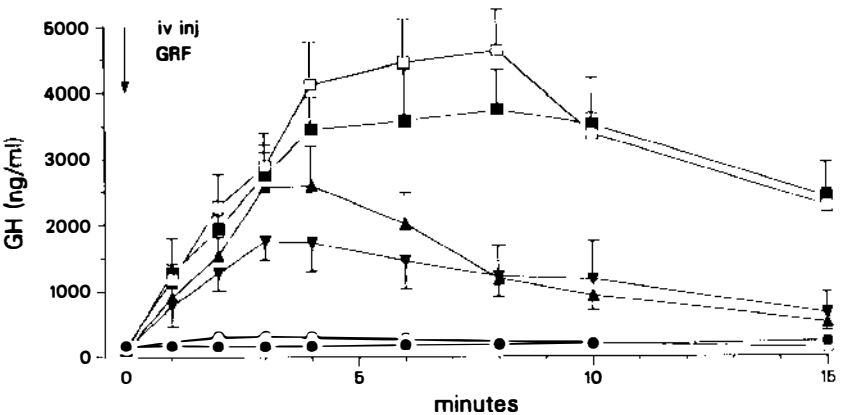

Figure 4 The increase in plasma GH concentrations following the intravenous administration of saline (●), 0.15 (○), 0.5 (◀), 1.5 (▲), 15 (□), and 25 μg/kg (■) of GRF in anesthetized male rats three months of age. Animals were treated immediately after the time 0 sample, data points represent the mean response in six rats, and vertical bars represent the SEM. Note that the increase in GH concentrations following the 0.15 μg/kg dose is significant ($p < 0.05$), although it is difficult to illustrate this increase here because of the tremendous responses observed at the higher doses (taken from 90).

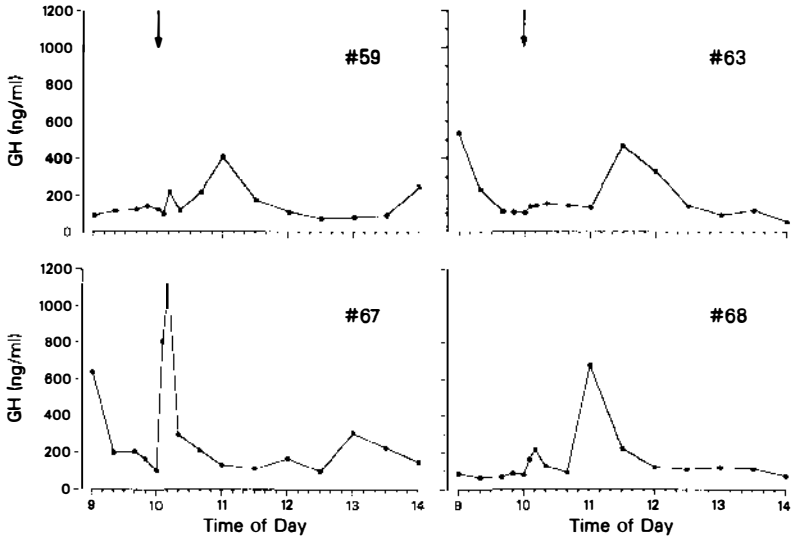

Figure 5 The effect of 10 μg GRF administered intravenously on GH secretion in four conscious, freely moving male rats. Injections (indicated by arrows) were made at a time known to be between spontaneous GH pulses. Note the absence of response in rat #63 and the partial response in rats #59 and #68 compared to the response in rat #67 (taken from 33).

While anesthetized animals are a very useful model for numerous studies, it is apparent that they are not the best model for studies designed to investigate some of the physiological interactions between GRF and GH. Ideally, such studies should be carried out in conscious, freely moving animals. Initial studies using this animal model yielded very perplexing results (33). The intravenous administration of GRF, which is unequivocally bioactive in vitro (3, 4) and in vivo in anesthetized rats (3, 4), only induces an increase in plasma GH concentrations in 30% of the rats tested (Figure 5). This inconsistency in response, which to some degree appears similar to the heterogeneity of responses observed in humans, can be completely eliminated by pretreating the rats with antibodies raised against somatostatin (Figure 6). An additional study was conducted to further establish the role of somatostatin in modulating the pituitary GH response to GRF. Following a 72-hour fast, a treatment reported to increase endogenous somatostatin release (38), rats were injected with GRF. The administration of GRF did not elicit a significant GH response in fasted rats, a result that can be reversed by pretreatment of the animals with somatostatin antibodies (33). These results demonstrate the dynamic and opposite roles exerted by GRF and somatostatin in regulating GH secretion (see further discussion below).

An additional fact that can complicate the interpretation of results obtained from studies involving the administration of GRF to conscious, freely moving

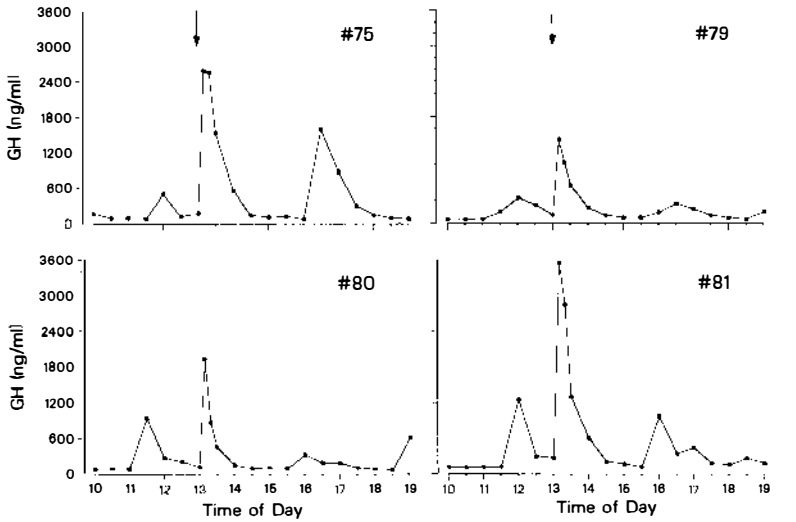

Figure 6 The effect of 1 μg GRF administered intravenously on GH secretion in four conscious, freely moving male rats pretreated with antiserum against somatostatin. Injections (indicated by arrows) were made at a time known to be between spontaneous GH pulses. Note the change in dose of GRF administered and scale of GH concentrations compared to Figure 5 (taken from 33).

animals is that the endogenous release of GH by the pituitary is pulsatile in nature (39). Using a monoclonal antibody raised against rat GRF (40), Wehrenberg et al (41) have shown that these spontaneous GH pulses are GRF dependent. The fact that plasma GH concentrations during spontaneous pulses can approach those observed in response to exogenously administered GRF make it difficult to delineate spontaneous and induced changes in plasma GH concentrations. To circumvent the problems created by endogenous somatostatin and GRF, Wehrenberg et al (42) treated conscious, freely moving animals with antiserum against somatostatin and with a monoclonal antibody against rat GRF that does not recognize GRF isolated from human tissue. This animal model is ideal in that it represents an immediate, noninvasive, yet reversible, functional lesion of the hypothalamo-pituitary axis that is specific only for endogenous somatostatin and rat GRF. Using this model, these investigators have shown that the pituitary GH response to repeated doses of human GRF is virtually unchanged over time (Figure 7). In addition, dose-response studies conducted in this animal model confirm studies performed in anesthetized rats. The minimum effective dose of GRF required to elicit a GH response is 100–200 ng/kg and the maximum GH response is observed with approximately 5 μg/kg (Figure 8).

GRF has been shown to be biologically active in all of the animal species tested thus far. Intravenous injection of 1–10 μg GRF in rabbits causes a

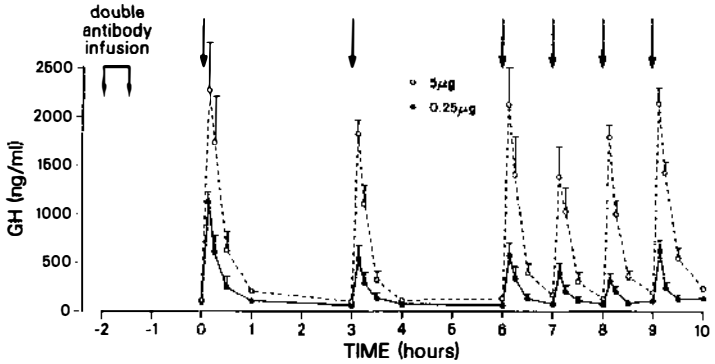

Figure 7 The capacity of the pituitary in conscious, freely moving male rats (n = 6) to secrete GH in response to repeated intravenous injections of a moderate (0.25 μg; ●) and maximal (5 μg; ○) dose of human GRF. Two hours before the first injection, the rats were treated with an antiserum against somatostatin and a monoclonal antibody against rat GRF that does not recognize human GRF. Arrows indicate the injection of human GRF. Data points represent the mean GH concentration and the vertical bars represent the SEM (taken from 42).

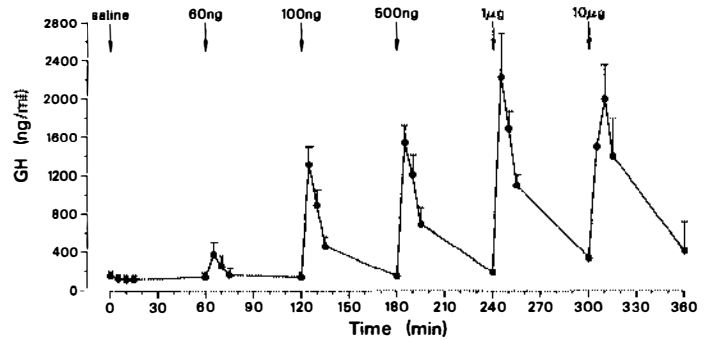

Figure 8 The dose-dependent response of the pituitary in conscious, freely moving male rats (n = 6) to secrete GH in response to human GRF administered intravenously. Two hours before the saline injection, the rats were treated with an antiserum against somatostatin and a monoclonal antibody against rat GRF that does not recognize human GRF. The dose and time of injection of human GRF are indicated, data points represent the mean GH concentration, and the vertical bars represent the SEM (taken from 42).

significant and dose-related increase in plasma GH concentrations (43). As in the rat, the GH response is significantly greater in rabbits pretreated with somatostatin antiserum than in rabbits pretreated with control serum. The GH response to repeated doses of GRF is also unaltered over time in rabbits, again illustrating the capacity of the pituitary to secrete GH. GRF is effective in releasing GH in dogs (3). In domestic animals, GRF stimulates GH secretion in ovine (44, 45), porcine (46), and bovine species (47). It is also active in chickens (48, 49) and turkeys (50). GRF releases GH in at least one lower

vertebrate species, the goldfish (51). It is our opinion that, as further research is conducted, GRF will continue to be shown as a potent and specific releaser of GH across all species.

Animal Models for the Study of GRF Regulation of GH Release

As already discussed, the stimulation of GH secretion by GRF can be clearly illustrated in normal animals following various routes of administration. However, the GH response to GRF is quite variable due to the interaction of endogenous somatostatin and the pulsatile nature of spontaneous GH secretion. For this reason, various animal models have been investigated for their potential use in studying the GH response to GRF. Rats with electrolytic lesions of the ventromedial-arcuate region of the hypothalamus have a marked suppression of the GH release (52) that is not due to increased somatostatin release (21). Using such lesioned animals, Tannenbaum et al (54) and Wehrenberg et al (55) have reported a potent effect of GRF on GH secretion. Pretreatment of these animals with somatostatin antibodies results in an enhancement of the GH response to GRF. Other animal models in which spontaneous GH secretion can be modified have also been evaluated. Chemical lesion of the arcuate nucleus by neonatal treatment with monosodium glutamate and the pharmacological interruption of catecholamine synthesis and storage by α-methyl-p-tyrosine and reserpine respectively are all known to inhibit spontaneous GH release. In each of these animal models, the GH response to GRF is enhanced by prior treatment of the animals with somatostatin antiserum (55).

CENTRAL NERVOUS SYSTEM PATHWAYS INVOLVED IN GRF SECRETION

Numerous stimuli are known to release GH in vivo. Their actions are indirect, since they are unable to stimulate GH release in vitro. Initial reports are now appearing in the literature that indicate that these indirect pathways for releasing GH ultimately involve GRF. Opiates and opioid peptides are potent releasers of pituitary GH in vivo, yet are devoid of any GH releasing activity in vitro. Wehrenberg et al (42) reported that the GH response to morphine sulfate administration in rats can be completely blocked by the prior administration of GRF antibodies. These results have been expanded by the recent report by Miki et al (58) that shows that passive immunization of rats with an antibody raised against human GRF that recognizes rat GRF completely abolishes the GH response to FK 33–824, an enkephalin analogue. We have also observed that such passive immunization inhibits the GH-releasing activity of β-endorphin when administered into the lateral cerebral ventricles (57). Miki et al (58) have also shown that the α–adrenergic stimulating pathways involved in GH secre-

tion require the active involvement of GRF. These initial observations indicate that GRF is the final common pathway for the ultimate regulation of GH secretion.

FACTORS MODULATING GRF REGULATION OF GH RELEASE

Other Hypothalamic Releasing Factors

The specificity of previously characterized hypothalamic releasing factors in releasing their respective pituitary target hormones is well established. Thyrotropin-releasing factor (TRF) stimulates the release of thyrotropin (TSH) and prolactin; luteinizing hormone–releasing factor (LRF) stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH); corticotropin-releasing factor (CRF) stimulates the release of adrenocorticotropin (ACTH) and β-endorphin. Likewise, GRF is specific for the release of GH (3, 4, 24, 34). Under numerous pathophysiological conditions, the secretion of anterior pituitary hormones is altered. For example, stress increases the secretion of ACTH and decreases the secretion of gonadotropins and TSH (60, 61). This change in pituitary secretion may be due to a variety of mechanisms, one of which might be changes in the pituitary response to the releasing factors in the presence or absence of other releasing factors (i.e. interactions). To investigate the possibility of interactions between the four hypothalamic releasing factors on anterior pituitary secretion, Wehrenberg et al (62) conducted in vitro and in vivo experiments using a $2 \times 2 \times 2 \times 2$ factorial design. This design allows for the evaluation of both main effects of the releasing factors as well as of all possible interactions between them. The results obtained confirm the specificity of each of the releasing factors on their respective target cells in the anterior pituitary under normal physiological conditions. There were no significant interactions between any of the releasing factors on anterior pituitary hormone secretion. These results suggest that changes in the pituitary secretion observed under pathophysiological conditions are not due to interactions of the releasing factors at the level of the pituitary, but rather to other secondary interactions that modify the hypothalamic release of the releasing factors or the pituitary response to the stimuli. An important implication of these results is that the clinical pituitary reserve test conducted in humans to evaluate pituitary response to the hypothalamic releasing factors can be expanded to include all four releasing factors, since any lack of response will reflect pituitary dysfunction and not an interaction of the releasing factors.

Hypothalamic Inhibiting Factor: Somatostatin

As discussed earlier, somatostatin plays an active role in modulating the GH response to GRF. One of the most perplexing observations made thus far is the

apparent randomness of the GH response to GRF in conscious, freely moving animals (33, 43). When normal animals are pretreated with somatostatin antiserum, the GH response is normalized. These observations suggest that somatostatin is released in a pulsatile or episodic fashion. Arancibia et al (63) and Kasting et al (64) have reported that the concentrations of somatostatin released from the median eminence is pulsatile, with a period interval of approximately 60–120 minutes. Evidence thus suggests that both GRF and somatostatin are released in a pulsatile fashion. Apparently, somatostatin predominates in this system, since administration of somatostatin always reduces plasma GH concentrations (65) and the administration of GRF does not always increase GH (33). For the exogenous administration of GRF to be effective, hypothalamic somatostatin secretion must be at a nadir. Much work is needed in this area to further delineate the relationship between GRF and somatostatin in regulating GH secretion.

Pituitary Hormones

To date, only the effects of reduced or excess GH on the pituitary response to GRF have been reported. These reports have centered primarily on clinical studies in patients suffering from acromegaly or hypopituitarisms. Shibasaki et al (66) have observed that the time of peak GH concentrations, as well as the magnitude of the pituitary response to GRF, was highly variable in ten acromegalic patients. In light of the clinical history of these individuals, the significance of the results is unclear. A preliminary report has appeared that suggests that the exogenous administration of GH to normal subjects reduces the GH response to GRF (67).

The effects of GRF administration under conditions of GH deficiency can be summarized by stating that GRF is very useful in distinguishing pituitary dysfunction from hypothalamic dysfunction (68–71). The significance of this as a clinical tool is apparent. Pintor et al (72) have also observed that GRF has a prolactin-lowering effect in GH-deficient children. This observation will need further confirmation.

Other Endocrine Effects

Glucocorticoids enhance GH production by somatotrophs in in vitro systems (73–75). Yet in vivo, the exogenous administration of these steroids results in an inhibition of somatic growth. To study this apparent dichotomy in the actions of glucocorticoids, the pituitary response to GRF has been evaluated in intact and adrenalectomized rats receiving glucocorticoid replacement therapy. Administration of the glucocorticoid dexamethasone significantly enhanced the GH response to GRF in intact as well as in adrenalectomized rats (76). These observations demonstrate that these steroids enhance the pituitary GH response to GRF in vivo. This fact, coupled with the fact that glucocorticoids

inhibit somatic growth, leads to the hypothesis that adrenocortical steroids are positive modulators of the GH response at the pituitary level but negative modulators at peripheral sites. Thyroid hormone also enhances GH production by somatotrophs in vitro (74, 75). Preliminary observations in vivo are consistent with these observations in that thyroid hormone appears to enhance the GH response to GRF (77). These results are in agreement with in vitro data (78).

Males of most vertebrate species, including humans and laboratory rodents, are much larger than their female counterparts. Sex differences in weight are known to be due to direct and potent anabolic effects of androgens on metabolic processes in target tissues. In addition, some evidence suggests that gonadal steroids mediate their effects on growth by regulating GH synthesis and release (79–84). Wehrenberg et al (85) have reported that testosterone replacement therapy in gonadectomized male rats causes a significant enhancement of the GH response to GRF, but estradiol replacement therapy in gonadectomized female rats does not alter the response. Other results in pre- and post-pubescent rats suggest that the enhanced response observed in male rats is not observed until after puberty.

Other Factors

The amino acid sequence of GRF shows considerable homology with peptides in the secretin-glucagon family of gut peptides and suggests that peptides within this family might interact. Glucagon, gastric inhibitory peptide (GIP), and secretin cause a slight inhibition of plasma GH concentrations when administered to anesthetized rats (86). When GIP is administered in combination with GRF, the pituitary GH response is significantly augmented. In contrast, when secretin is administered in combination with GRF, the pituitary response is significantly reduced. It is unclear whether these results reflect a direct interaction of these peptides with GRF receptors on the somatotrophs or are due to extrapituitary mechanisms.

One of the standard clinical tests to elicit pituitary GH secretion in man is insulin-induced hypoglycemia. The fact that hyperglycemia suppresses GH secretion is well known. In light of this relationship, the effects of blood glucose on the response of GH to GRF have been evaluated in humans (87, 88). Under conditions of hyperglycemia, the GH response to GRF is significantly suppressed.

Significant age-related variations in the spontaneous secretion of GH exist in man and experimental animals. At or near the time of puberty, GH secretion is near a maximum for both the frequency of spontaneous GH pulses and the magnitude of plasma GH concentrations. With advancing age, both of these parameters are significantly reduced. The cause of these age-related changes is unknown; however, a possible mechanism could be changes in pituitary response to GRF. The data reported in humans are unequivocal: the pituitary GH

response to GRF in men in their twenties and thirties is significantly greater than the response observed in men in their forties or older (89). It is not known whether this change in response reflects decreased pituitary sensitivity to GRF or increased involvement of somatostatin.

Studies conducted in rats show an absence of any age-related change in GH response to GRF. Wehrenberg et al (90) have reported no age-related change in the pituitary response to either submaximal or maximal doses of GRF when administered to anesthetized male rats. Using a similar experimental design, Sonntag et al (91) have reported a decreased response to GRF in old rats as compared to young rats. In vitro data published by these investigators show that there is no change in the somatotrophs' response to GRF when these cells are isolated from hypothalamic influences. In light of these in vitro results, Sonntag et al (91) have suggested that the reduced response to GRF in vivo may be due to increased release of or enhanced sensitivity to somatostatin rather than to a decrease in the pituitary response to GRF.

There is no doubt that the somatomedins play a significant role in mediating the actions of GH. To date, we have little information on what role somatomedins have in the feedback regulation of GRF or how they might modify the GH response to GRF. Motilin is another peptide that might be involved, since it has been reported to have a direct effect on pituitary GH secretion in vitro (92), and passive immunization of animals with antiserum raised against motilin suppress plasma GH concentrations (93). An additional factor that may be involved is an enkephalin analog with growth hormone–releasing activity in vitro and in vivo (94–96). How these factors integrate into the regulation of GH secretion remains to be determined.

CHRONIC ADMINISTRATION OF GRF AND ITS EFFECTS ON SOMATIC GROWTH

The GH content of the anterior pituitary is very high compared to that of other anterior pituitary hormones. This suggests that the pituitary may have a large capacity to secrete GH after repeated or chronic administration of GRF. As previously discussed, the pituitary GH response to GRF does not change after repeated doses of GRF in the rat (Figure 7) (42). In contrast, earlier observations indicated that the pituitary becomes refractory to the continuous administration of GRF in less than one hour (34). In a more extensive study, Wehrenberg et al (97) have shown that the capacity of the pituitary to respond to GRF can be exhausted after a 12–24 hour administration of a relatively high dose of GRF in rats pretreated with somatostatin antiserum. The loss of response is due at least in part to depletion of pituitary GH content. The possible involvement of GRF receptor desensitization or down-regulation has not been evaluated and therefore can not be ruled out.

One of the most obvious applications for the chronic use of GRF is to enhance somatic growth. There is no doubt that GRF is critical in this process. Indeed, passively immunizing rats with GRF antibodies for as short a time as eight days causes a significant inhibition of somatic growth (98). The absence of any published reports indicating the successful use of GRF to enhance growth undoubtedly reflects the complex nature of the hypothalamic regulation of GH secretion by GRF and somatostatin.

TOXICOLOGICAL AND PHARMACOLOGICAL EFFECTS

The potential toxic effects of GRF have been evaluated in various animal models (28; W. Wehrenberg, N. Ling, P. Brazeau, R. Guillemin, unpublished observations). The gross and microscopic pathology of tissues obtained from animals receiving over 1,000 times the maximum effect dose of GRF are normal. No morbidity or mortality of animals was noted in studies in which large doses of GRF were administered for extended periods (more than seven days). Administration of GRF to humans does cause a transient flushing of the face and upper torso (24–29). These effects occur during the first few minutes following GRF administration, and no long-term effects have been observed.

CONCLUSIONS

Since the isolation and characterization of GRF, initially from tumor tissue but subsequently from normal hypothalamic tissue, most of the fundamental studies of the in vivo actions of GRF have been performed. Immunohistochemical mapping of GRF neurons in the central nervous system has been completed, the dose-response relationships between GRF and GH have been established, and the clinical use of the peptide has been initiated. Our present knowledge of the actions of GRF will serve as a solid foundation for the numerous additional studies that must be conducted before we will be able to completely understand the physiology of GRF. It is easiest to illustrate the immense amount of research that is currently being conducted and that remains to be performed by stating that in the week prior to the submission of this manuscript, over 100 abstracts on the actions of GRF were presented at the seventh international congress on endocrinology held in Quebec City, Canada.

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Literature Cited

1. Krulich, L., Dhariwal, A. P. S., McCann, S. M. 1968. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. *Endocrinology* 83:783–90
2. Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., et al. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 179:77–79
3. Guillemin, R., Brazeau, P., Bohlen, P., Esch, F., Ling, N., Wehrenberg, W. B. 1982. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* 218:585–87
4. Rivier, J., Spiess, J., Thorner, M., Vale, W. 1982. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. *Nature* 300:276–78
5. Esch, F. S., Bohlen, P., Ling, N. C., Brazeau, P. E., Wehrenberg, W. B., et al. 1982. Characterization of a 40 residue peptide from a human pancreatic tumor with growth hormone releasing activity. *Biochem. Biophys. Res. Commun.* 109: 152–58

5a. Ling, N., Esch, F., Bohlen, P., Brazeau, P., Wehrenberg, W. B., et al. 1984. Isolation, primary structure and synthesis of human hypothalamic somatocrinin: Growth hormone-releasing factor. *Proc. Natl. Acad. Sci. USA*. 81: 4302–6

6. Spiess, J., Rivier, J., Vale, W. 1983. Characterization of rat hypothalamic growth hormone-releasing factor. *Nature* 303:532–35
7. Bohlen, P., Esch, F., Brazeau, P., Ling, N., Guillemin, R. 1983. Isolation and characterization of the porcine hypothalamic growth hormone releasing factor. *Biochem. Biophys. Res. Commun.* 116: 726–34
8. Esch, F., Bohlen, P., Ling, N., Brazeau, P., Guillemin, R. 1983. Isolation and characterization of the bovine hypothalamic growth hormone releasing factor. *Biochem. Biophys. Res. Commun.* 117: 772–79

8a. Brazeau, P., Böhlen, P., Esch, F., Ling, N., Wehrenberg, W. B., Guillemin, R. 1984. Growth hormone–releasing factor from ovine and caprine hypothalamus: Isolation, sequence analysis and total synthesis. *Biochem. Biophys. Res. Commun.* In press

9. Bloch, B., Gaillard, R. C., Brazeau, P., Lin, H. D., Ling, N. 1984. Topographical and ontogenetic study of the neurons producing growth hormone-releasing factor in human hypothalamus. *Reg. Peptides* 8:21–31
10. Bugnon, C., Bloch, B., Lenys, D., Fellmann, D. 1978. Cytoimmunological study of the LH-RH neurons in humans during fetal life. In *Brain Endocrine Interaction. 3rd Int. Symp. Wurzburg, 1977. III. Neural Hormones and Reproduction*, ed. D. E. Scott, G. P. Kozlowski, A. Weindl, pp. 183–96. Basel: Karger
11. Bugnon, C., Fellmann, D., Bloch, B. 1977. Immunocytochemical study of the ontogenesis of the hypothalamic somatostatin-containing neurons in the human fetus. *Cell Tissue Res.* 183:319–28
12. Bugnon, C., Fellmann, D., Bresson, J. L., Clavequin, M. C. 1982. Etude immunocytochimique de l'ontogenese du systeme neuroglandulaire a CRF chez l'homme. *C.R. Acad. Sci.* 294:491–94
13. Bloch, B., Brazeau, P., Ling, N., Bohlen, P., Esch, F., et al. 1983. Immunohistochemical detection of growth hormone-releasing factor in brain. *Nature* 301:607–8
14. Bloch, B., Brazeau, P., Bloom, F., Ling, N. 1983. Topographical study of the neurons containing hpGRF immunoreactivity in monkey hypothalamus. *Neurosci. Lett.* 37:23–28
15. Jacobowitz, D. M., Schulte, H., Chrousos, G. P., Loriaux, D. L. 1983. Localization of GRF-like immunoreactive neurons in the rat brain. *Peptides* 4:521–24
16. Sawchenko, P. E., Swanson, L. W., Rivier, J., Vale, W. W. 1984. The distribution of growth hormone-releasing factor (GRF)-immunoreactivity in the central nervous system of the rat: An immunohistochemical study using antisera directed against rat hypothalamic GRF. *J. Comp. Neurol.* In press
17. Merchenthaler, I., Vigh, S., Schally, A. V., Petrusz, P. 1984. Immunocytochemical localization of growth hormone-releasing factor in the rat hypothalamus. *Endocrinology* 114:1082–85
18. Martin, J. B. 1972. Plasma growth hormone (GH) response to hypothalamic or extrahypothalamic electrical stimulation. *Endocrinology* 91:107–15
19. Frohman, L. A., Bernardis, L. L. 1968. Growth hormone and insulin levels in weanling rats with ventromedial hypothalamic lesions. *Endocrinology* 82: 1125–32

20. Frohman, L. A., Bernardis, L. L., Kant, K. J. 1968. Hypothalamic stimulation of growth hormone secretion. *Science* 162: 580–82
21. Eikelboom, R., Tannenbaum, G. S. 1983. Effects of obesity-inducing ventromedial hypothalamic lesions on pulsatile growth hormone and insulin secretion: Evidence for the existence of a growth hormone-releasing factor. *Endocrinology* 112:212–19
22. Bloch, B., Ling, N., Benoit, R., Wehrenberg, W. B., Guillemin, R. 1984. Specific depletion of immunoreactive growth hormone–releasing factor by monosodium glutamate in rat median eminence. *Nature* 307:272–73
23. Bugnon, C., Gouget, A., Fellmann, D., Clavequin, M. C. 1983. Immunocytochemical demonstration of a novel peptidergic neurone system in the cat brain with an anti-growth hormone-releasing factor serum. *Neurosci. Lett.* 38:131–37

23a. Wehrenberg, W. B., Ling, N. 1983. *In vivo* biological potency of rat and human growth hormone–releasing factor and fragments of human growth hormone-releasing factor. *Biochem. Biophys. Res. Commun.* 115:525–53

24. Thorner, M. O., Spiess, J., Vance, M. L., Rogol, A. D., Kaiser, D. L., et al. 1983. Human pancreatic growth-hormone-releasing factor selectively stimulates growth-hormone secretion in man. *Lancet* 1:24–28
25. Rosenthal, S. M., Schriock, E. A., Kaplan, S. L., Guillemin, R., Grumbach, M. M. 1983. Synthetic human pancreas growth hormone–releasing factor (hpGRF 1–44–NH2) stimulates growth hormone secretion in normal men. *J. Clin. Endocrinol. Metab.* 57:677–79
26. Chatelain, P., Cohen, H., Sassolas, G., Exclerc, J. L., Ruitton, A., et al. 1983. Somathormone plasmatique induite par la somatocrinine: Formes circulantes et active biologique. *Ann. Endocrinol.* 44: 159
27. Wood, S. M., Ch'ng, J. L. C., Adams, E. F., Webster, J. D., Joplin, G. F., et al. 1983. Abnormalities of growth hormone release in response to human pancreatic growth hormone releasing factor [GRF (1-44)] in acromegaly and hypopituitarism. *Br. Med. J.* 286:1687–91
28. Gelato, M. C., Pescovitz, O., Cassorla, F., Loriaux, D. L., Merriam, G. R. 1983. Effects of a growth hormone releasing factor in man. *J. Clin. Endocrinol. Metab.* 57:674–76
29. Losa, M., Stalla, G. K., Muller, O. A., von Werder, K. 1983. Human pancreatic growth hormone-releasing factor (hpGRF): Dose-response of GRF- and GH- levels. *Klin. Wochenschr.* 61:1249–53
30. Thorner, M. O., Vance, M. L., Kaiser, D. L., Chitwood, J., Evans, W. S. 1984. Intravenous, subcutaneous, and intranasal administration of hpGRF-40 in normal men. *7th Int. Congress of Endocrinology, Quebec City*, p. 1415. Amsterdam: Excerpta Medica
31. Evans, W. S., Borges, J. L. C., Kaiser, D. L., Vance, M. L., Sellers, R. P., et al. 1983. Intranasal administration of human pancreatic tumor GH-releasing factor-40 stimulates GH release in normal men. *J. Clin. Endocrinol. Metab.* 57:1081–83
32. Almeida, O. F. X., Schulte, H. M., Rittmaster, R. S., Chrousos, G. P., Loriaux, D. L., et al. 1984. Potency and specificity of a growth hormone-releasing factor in a primate and *in vitro*. *J. Clin. Endocrinol. Metab.* 58:309–12
33. Wehrenberg, W. B., Ling, N., Bohlen, P., Esch, F., Brazeau, P., et al. 1982. Physiological roles of somatocrinin and somatostatin in the regulation of growth hormone secretion. *Biochem. Biophys. Res. Commun.* 109:562–67
34. Wehrenberg, W. B., Ling, N., Brazeau, P., Esch, F., Bohlen, P., et al. 1982. Somatocrinin, growth hormone releasing factor, stimulates secretion of growth hormone in anesthetized rats. *Biochem. Biophys, Res. Commun.* 109:382–87
35. Murphy, W. A., Lance, V. A., Sueiras-Diaz, J., Coy, G. H. 1983. Effects of secretin and gastric inhibitory polypeptide on human pancreatic growth hormone-releasing factor(1–40)–stimulated growth hormone levels in the rat. *Biochem. Biophys. Res. Commun.* 112: 469–74
36. Szabo, M., Dudlak, D., Thominet, J. L., Frohman, L. A. 1983. Ectopic growth hormone-releasing factor stimulates growth hormone secretion in the urethane-anesthetized rat in vivo. *Neuroendocrinology* 37:328–31
37. Chihara, K., Arimura, A., Schally, A. V. 1979. Immunoreactive somatostatin in rat hypophyseal portal blood: Effects of anesthetics. *Endocrinology* 104:1434–41
38. Tannenbaum, G. S., Epelbaum, J., Colle, E., Brazeau, P., Martin, J. B. 1978. Antiserum to somatostatin reverses starvation-induced inhibition of growth hormone but not insulin secretion. *Endocrinology* 102:1909–14
39. Tannenbaum, G. S., Martin, J. B. 1976. Evidence for an endogenous ultradian

rhythm governing growth hormone secretion in the rat. *Endocrinology* 98:562–70
40. Luben, R. A., Brazeau, P., Bohlen, P., Guillemin, R. 1982. Monoclonal antibodies to hypothalamic growth hormone–releasing factor with picomoles of antigen. *Science* 218:887–89
41. Wehrenberg, W. B., Brazeau, P., Luben, R., Bohlen, P., Guillemin, R. 1982. Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth releasing factor (GRF). *Endocrinology* 111:2147–48
42. Wehrenberg, W. B., Brazeau, P., Luben, R., Ling, N., Guillemin, R. 1983. A noninvasive functional lesion of the hypothalamo-pituitary axis for the study of growth hormone-releasing factor. *Neuroendocrinology* 36:489–91
43. Chihara, K., Minamitani, N., Kaji, H., Kodama, H., Kita, T., et al. 1983. Human pancreatic growth hormone-releasing factor stimulates release of growth hormone in conscious unrestrained male rabbits. *Endocrinology* 113:2081–85
44. Ohmura, E., Jansen, A., Chernick, V., Winter, J., Friesen, H. G., et al. 1984. Human pancreatic growth hormone releasing factor (hpGRF-1-40) stimulates GH release in the ovine fetus. *Endocrinology* 114:299–301
45. Baile, C. A., Della-Fera, M. A. 1984. An update on the roles for brain peptides in controlling behavior and metabolism. *Monsanto Techn. Symp. Fresno, Calif.*, pp. 13–34. St. Louis: Nutr. Chem. Div., Monsanto
46. Lance, V. A., Murphy, W. A., Sueiras-Diaz, J., Coy, D. H. 1984. Super-active analogs of growth hormone-releasing factor (1-29)-amide. *Biochem. Biophys. Res. Commun.* 119:265–72
47. Moseley, W. M., Krabill, L. F., Friedman, A. R., Olsen, R. F. 1984. Growth hormone response of steers injected with synthetic human pancreatic growth hormone-releasing factors. *J. Anim. Sci.* 58:430–35
48. Scanes, C. G., Carsia, R. V., Lauterio, T. J., Huybrechts, L., Rivier, J., et al. 1984. Synthetic human pancreatic growth hormone releasing factor (GRF) stimulates growth hormone secretion in the domestic fowl (*Gallus domesticus*). *Life Sci.* 34:1127–34
49. Leung, F. C., Taylor, J. E. 1983. In vivo and in vitro stimulation of growth hormone release in chickens by synthetic human pancreatic growth hormone releasing factor (hpGRFs). *Endocrinology* 113:1913–15
50. Proudman, J. A. 1984. Growth hormone and prolactin response to thyrotropin releasing hormone and growth hormone releasing factor in the immature turkey (41770). *Proc. Soc. Exp. Biol. Med.* 175:79–83
51. Peter, R. E., Nahorniak, C. S., Vale, W., Rivier, J. 1984. Human pancreatic growth hormone-releasing factor stimulates growth hormone release in goldfish. *J. Exp. Zool.* In press
52. Martin, J. B., Renaud, L. P., Brazeau, P. 1974. Pulsatile growth hormone secretion: Suppression by hypothalamic ventromedial lesions and by long-acting somatostatin. *Science* 186:538–40
53. Deleted in proof
54. Tannenbaum, G. S., Eikelboom, R., Ling, N. 1983. Human pancreas GH-releasing factor analog restores high-amplitude GH pulses in CNS lesion-induced GH deficiency. *Endocrinology* 113:1173–75
55. Wehrenberg, W. B., Bloch, B., Chong-Li, Z., Brazeau, P., Ling, N., et al. 1984. Pituitary response to growth hormone-releasing factor in rats with functional or anatomical lesions of the central nervous system that inhibit endogenous growth hormone secretion. *Reg. Peptides* 8:1–8
56. Deleted in proof
57. Wehrenberg, W. B., Bloch, B., Ling, N. 1985. Pituitary secretion of growth hormone in response to opioid peptides and opiates is mediated through growth hormone-releasing factor. *Neuroendocrinology* In press
58. Miki, N., Ono, M., Shizume, K. 1984. Evidence that opiatergic and α-adrenergic mechanisms stimulate rat growth hormone release via growth hormone-releasing factor (GRF). *Endocrinology* 114:1950–52
59. Deleted in proof
60. Sachar, E. J. 1975. Neuroendocrine abnormalities in depressive illness. In *Topics in Neuroendocrinology*, ed. E. J. Sachar, pp. 135–58. New York: Grune & Stratton.
61. Ducommun, P., Vale, W., Sakiz, E., Guillemin, R. 1967. Reversal of the inhibition of TSH secretion due to acute stress. *Endocrinology* 80:953–56
62. Wehrenberg, W. B., Baird, A., Ying, S. Y., Rivier, C., Ling, N., et al. 1984. Multiple stimulation of the adenohypophysis by combinations of hypothalamic releasing factors. *Endocrinology* 114:1995–2001

63. Arancibia, S., Epelbaum, J., Alonso, G., Assenmacher, I. 1983. Pulsatile secretion of somatostatin (SRIF) into the 3rd ventricle of unanesthetized rats. *C.R. Acad. Sci.* 296:47–52
64. Kasting, N. W., Martin, J. B., Arnold, M. A. 1981. Pulsatile somatostatin release from the median eminence of the unanesthetized rat and its relationship to plasma growth hormone levels. *Endocrinology* 109:1739–45
65. Brazeau, P., Rivier, W., Vale, W., Guillemin, R. 1974. Inhibition of growth hormone secretion in the rat by synthetic somatostatin. *Endocrinology* 94:184–87
66. Shibasaki, T., Shizume, K., Masuda, A., Nakahara, M., Hizuka, N., et al. 1984. Plasma growth hormone response to growth hormone-releasing factor in acromegalic patients. *J. Clin. Endocrinol. Metab.* 58:215–17
67. Rosenthal, S. M., Hulse, J. A., Kaplan, S. L., Grumbach, M. M. 1984. Exogenous growth hormone (GH) inhibits growth hormone releasing factor (GRF-44-NH)-induced GH secretion in normal men: Further evidence for GH autoregulation. See Ref. 30, p. 1144
68. Borges, J. L. C., Gelato, M. C., Rogol, A. D., Vance, M. L., MacLeod, R. M., et al. 1983. Effects of human pancreatic tumour growth hormone releasing factor on growth hormone and somatomedin C levels in patients with idiopathic growth hormone deficiency. *Lancet* 1:119–24
69. Grossman, A., Wass, J. A. H., Sueiras-Diaz, J., Savage, M. O., Lytras, N., et al. 1983. Growth-hormone-releasing factor in growth hormone deficiency: Demonstration of a hypothalamic defect in growth hormone release. *Lancet* 1:137–38
70. Takano, K., Hizuka, N., Shizume, K., Asakawa, K., Miyakawa, M., et al. 1984. Plasma growth hormone (GH) response to GH-releasing factor in normal children with short stature and patients with pituitary dwarfism. *J. Clin. Endocrinol. Metab.* 58:236–41
71. Pintor, C., Fanni, V., Loche, S., Locatelli, V., Cella, S. G., et al. 1983. Synthetic hpGRF 1-40 stimulates growth hormone and inhibits prolactin secretion in normal children and children with isolated growth hormone deficiency. *Peptides* 4:929–33
72. Pintor, C., Corda, R., Puggioni, R., Locatelli, V., Cella, S. G., et al. 1983. Prolactin-lowering effect of growth-hormone-releasing factor in children with growth-hormone deficiency. *Lancet* 1: 1088–89
73. Bancroft, F. C., Levine, L., Tashjian, A. H. 1969. Control of growth hormone production by a clonal strain of rat pituitary cells: Stimulation by hydrocortisone. *J. Cell Biol.* 43:432
74. Shapiro, L., Samuels, H., Yaffee, B. 1979. Modulation of thyroid hormone nuclear receptor levels by 3'5' triiodo-L-thyronine in GH cell. *J. Biol. Chem.* 252:6052
75. Spindler, S., Mellon, S., Baxter, J. 1982. Growth hormone gene transcription is regulated by thyroid and glucocorticoid hormones in cultured rat pituitary tumor cells. *J. Biol. Chem.* 257:11627–32
76. Wehrenberg, W. B., Baird, A., Ling, N. 1983. Potent interaction between glucocorticoids and growth hormone–releasing factor in vivo. *Science* 221:556–58
77. Ruestow, P. C., Kramer, D. E., Szabo, M. 1984. Growth hormone (GH) response to systemic thyrotropin-releasing hormone (TRH) and GH-releasing factor (GRF) administration in rats: Effect of hypothyroidism and thyroid hormone replacement. See Ref. 30, p. 1145
78. Vale, W., Vaughan, J., Yamamoto, G., Spiess, J., Rivier, J. 1983. Effects of synthetic human pancreatic (tumor) GH releasing factor and somatostatin, triiodothyronine and dexamethasone on GH secretion *in vitro*. *Endocrinology* 112:1553–55
79. Somana, R., Visessuwan, S., Samridtong, A., Holland, R. C. 1978. Effect of neonatal androgen treatment and orchidectomy on pituitary levels of growth hormone in the rat. *J. Endocrinol.* 79:399–400
80. Eastman, C. J., Lazarus, L., Stuart, M. C., Casey, J. H. 1971. The effect of puberty on growth hormone secretion in boys with short stature and delayed adolescence. *Austr. NZ J. Med.* 1:154–59
81. Illig, R., Prader, A. 1970. Effect of testosterone on growth hormone secretion in patients with anorchia and delayed puberty. *J. Clin. Endocrinol. Metab.* 30:615–18
82. Jansson, J. O., Eriksson, E., Eden, S., Modigh, K. 1982. Effects of gonadectomy and testosterone replacement on growth hormone response to alpha-2 adrenergic stimulation in the male rat. *Psychoneuroendocrinology* 7:245–48
83. Shupnik, M. A., Baxter, L. A., French, L. R., Gorski, J. 1979. In vivo effects of estrogen on ovine pituitaries: Prolactin and growth hormone biosynthesis and

messenger ribonucleic acid translation. *Endocrinology* 104:729–35
84. Negro-Vilar, A., Ojeda, S. R., Advis, J. P., McCann, S. M. 1979. Evidence for noradrenergic involvement in episodic prolactin and growth hormone release in ovariectomized rats. *Endocrinology* 105:86–91
85. Wehrenberg, W. B., Baird, A., Ying, S. Y., Ling, N. 1985. The effects of testosterone and estrogen on the pituitary growth hormone response to growth hormone-releasing factor in rats. *Biol. Reprod.* In press
86. Murphy, W. A., Lance, V. A., Sueiras-Diaz, J., Coy, D. H. 1983. Effects of secretin and gastric inhibitory polypeptide on human pancreatic growth hormone-releasing factor (1-40)-stimulated growth hormone levels in the rat. *Biochem. Biophys. Res. Commun.* 112:469–74
87. Sharp, P. S., Foley, K., Chahal, P., Kohner, E. M. 1984. The effect of plasma glucose on the growth hormone response to human pancreatic growth hormone releasing factor in normal subjects. *Clin. Endocrinol.* 20:497–501
88. Masuda, A., Shibasaki, T., Shizume, K., Nakahara, M., Imaki, T., etal. 1985. The effect of glucose on growth hormone releasing factor-mediated GH secretion in man. *J. Clin. Endocrinol. Metab.* In press
89. Shibasa i, T., Shizume, K., Na ahara, M., Masuda, A., Jibiki, K., et al. 1984. Age-related changes in plasma growth hormone response to growth hormone-releasing factor in man. *J. Clin. Endocrinol. Metab.* 58:212–14
90. Wehrenberg, W. B., Ling, N. 1983. The absence of an age-related change in the pituitary response to growth hormone-releasing factor in rats. *Neuroendocrinology* 37:463–66
91. Sonntag, W. E., Hylka, V. W., Meites, J. 1983. Impaired ability of old male rats to secrete growth hormone *in vivo* but not *in vitro* in response to hpGRF (1-44). *Endocrinology* 113:2305–7
92. Samson, W. K., Lumpkin, M. D., McCann, S. M. 1982. Motilin stimulates growth hormone release in vitro. *Brain Res. Bull.* 8:117–21
93. Samson, W. K., [redacted] M. D., Nilaver, G., McCann, [redacted] 84. Motilin: A novel growth hormone releasing agent. *Brain Res. Bull.* 12:57–62
94. Momany, F. A., Bowers, C. Y., Reynolds, G. A., Chang, D., Hong, A., etal. 1981. Design, synthesis and biological activity of peptides which release growth hormone *in vitro*. *Endocrinology* 108:31–39
95. Momany, F. A., Bowers, C. Y., Reynolds, G. A., Hong, A., Newlander, K. 1984. Conformational energy studies and *in vitro* and *in vivo* activity data on growth hormone-releasing peptides. *Endocrinology* 114:1531–36
96. Bowers, C. Y., Momany, F. A., Reynolds, G. A., Hong, A. 1984. On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology* 114:1537–45
97. Wehrenberg, W. B., Brazeau, P., Ling, N., Textor, G., Guillemin, R. 1984. Pituitary growth hormone response in rats during a 24-hour infusion of growth hormone-releasing factor. *Endocrinology* 114:1613–16
98. Wehrenberg, W. B., Bloch, B., Phillips, B. J. 1984. Antibodies to growth hormone-releasing factor inhibit somatic growth. *Endocrinology* 115:1218–20