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MINIREVIEW

Growth Hormone-Releasing Factor: Chemistry and Physiology (41813)

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The hypothalamus performs a dual function in the regulation of growth hormone (GH) release. An inhibitory factor, somatostatin, was characterized in 1972, while the stimulatory factor (GRF, or somatocrinin) has eluded investigators until recently. The major problems in the characterization of this peptide have been the minute quantities of GRF present in hypothalamic tissues and the large quantities of somatostatin which interfere in the bioassay. In order to circumvent the problem of low abundance of GRF in the hypothalamus, extrahypothalamic sources of GRF were sought. The ectopic production of peptide(s) with GRF activity has been described in human carcinoids and pancreatic islet tumors as causes of acromegaly (see (1) for review). Such a tumor was used for the first successful isolation of GRF in May 1982. More recently GRF has been characterized from porcine (2), bovine (3), and murine (4) hypothalamic tissues. All these molecules share extensive common amino acid sequences and are closely related in structure (Table I).

Isolation and Characterization of Human GRF. A tumor of the pancreas was used to isolate and characterize human pancreas GRF (hpGRF) (5). The patient had acromegaly with elevated plasma GH levels and no evidence of a pituitary adenoma. An acidic extract of the pancreatic tumor contained growth hormone releasing activity with the same elution position as that of hypothalamic GRF; the tumor tissue contained 5000 times more GRF activity than that found in rat hypothalamus on a weight basis. A three-step isolation procedure including Sephadex G-75 column chromatography, high-performance liquid chromatography (HPLC) on a semipreparative reverse-phase column, and analytical reverse-phase HPLC was used to achieve final purification of the material yielding three highly purified peptides with GRF activity.

The primary structures of the three polypeptides possessing high intrinsic growth hormone-releasing activity were established by sequence analyses of the intact peptides and their cyanogen bromide cleavage fragments with a gas-phase sequenator (6). They contain 44 (hpGRF-44), 40 (hpGRF-40), and 37 (hpGRF-37) amino acids in identical sequences from their NH_2 termini. hpGRF-37 and -40 possess free carboxyl termini while hpGRF-44 is amidated. The structure of hpGRF-44 was established as: Tyr–Ala–Asp–Ala–Ile–Phe–Thr–Asn–Ser–Tyr–Arg–Lys–Val–Leu–Gly–Gln–Leu–Ser–Ala–Arg–Lys–Leu–Leu–Gln–Asp–Ile–Met–Ser–Arg–Gln–Gln–Gly–Glu–Ser–Asn–Gln–Glu–Arg–Gly–Ala–Arg–Ala–Arg–Leu–NH_2. Based on their common NH_2 terminal sequences and lower specific bioactivities *in vitro,* hpGRF-40 and -37 are most probably proteolytic degradation products of hpGRF-44. The hpGRF peptides have striking sequence homologies with various intestinal peptides, in particular with PHI-27, in which 12 out of the 23 NH_2-terminal residues are identical with those of hpGRF (Table I). Sequence homologies are also found with VIP, glucagon, secretin, GIP, and motilin (6). The trivial name *somatocrinin* has been proposed to replace the abbreviation GRF.

Subsequent to the isolation and characterization of hpGRF-44, a 40-residue peptide identical in structure to hpGRF-40 was isolated from a tumor of the pancreas from another patient who also presented with acromegaly without evidence of a pituitary adenoma (7). This finding was confirmed independently by Rivier *et al.* (8) reporting the isolation of hpGRF-40 from a sample of the same tumor.

Human Hypothalamic GRF. To compare the characteristics of tumor-derived GRF with human hypothalamic GRF the latter was purified from four fragments of human brain by

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TABLE 1. AMINO ACID SEQUENCE HOMOLOGIES AMONG THE GRFS AND VARIOUS INTESTINAL PEPTIDES

	5 10 15 20 25 30 35 40
hGRF	YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGESNQERGARARL ●
pGRF	YADAIFTNSYRKVLGQLSARKLLQDIMSRQQGERNQEQGARVRL ●
bGRF	YADAIFTNSYRKVLGQLSARKLLQDIMNRQQGERNQEQGAKVRL ●
rGRF	HADAIFTSSYRRILGQLYARKLLHEIMNRQQGERNQEQRSRFN ○
pPHI-27	HADGVFTSSYRRILGQLSAKKYLESLI ●
pVIP	HSDAVFTDNYTRLRKQMAVKKWLNSILN ●
Glucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT ○
pSecretin	HSDGTFTSELSRLRDSARLKRLLQGLV ●

● = amidated carboxy-terminus ○ = free-acid form

gel filtration and reverse-phase HPLC (9). Bioassay with the pituitary monolayer culture system and radioimmunoassay using two specific antisera, one directed against the amidated COOH-terminal sequence and the other against the central portion of hpGRF-44 peptide, revealed the presence of two major forms of GRF activity. These coeluted with hpGRF-44-NH_2 and hpGRF-40, thereby providing strong evidence for the identity of the two major hypothalamic peptides with hpGRF-40-OH and hpGRF-44-NH_2. Ultimate proof of the similarity of the human hypothalamic GRF and the tumor-derived GRF would require only a supply of human hypothalami larger than that which we could obtain to establish a complete primary sequence of the hypothalamic material.

Porcine, Bovine, and Murine Hypothalmic GRF. More recently our laboratory has isolated and characterized porcine and bovine hypothalamic GRF. Both these peptides have 44 amino acid residues and are C-terminally amidated. Both pGRF and bGRF share a very extensive structural homology with hpGRF-44-NH_2 (Fig. 1). The isolation and structural characterization of rat GRF was reported as that of a 43-residue polypeptide (6). The reported sequence shows 14 amino acid differences between rGRF and hpGRF-44-NH_2 and most of these differences reside in the COOH-terminal end.

Synthesis of GRF and Structure Activity Relationships. Total synthesis of all these molecules with GRF activity (hpGRF-44, hpGRF-40, hpGRF-37, pGRF, bGRF, and rGRF) and fragments thereof was achieved by solid-phase techniques (5).

The synthetic peptides were tested in a normal pituitary cell monolayer culture system to assess quantitatively their GH-releasing potency (10). Of the GRF peptides tested, hpGRF-44-NH_2 was shown to be the most potent (ED_{50} = 12 pM; E_{max} = 100 pM). Based upon a potency ranking of 100 for hpGRF-44-NH_2, GRF(1-44)OH = 61; (1-40)NH_2 = 49; (1-40)OH = 30; (1-37)NH_2 = 28; (1-37)OH = 12; (1-34)OH = 17; (1-31)OH = 9; (1-28)OH = 6; (1-24)OH = 0.014; (1-21)OH = <0.002 and (1-19)OH = <0.001. Thus it appears that amidation of the carboxyl terminal residue increases the potency of a GRF peptide approximately 1.5-fold over that of the free-acid form. These studies also indicate that the biologically active core of the molecule probably resides in the amino terminal part of the molecule between residues 2 and 24. Met 27 is not exclusively required for biological activity and can be replaced by structurally related amino acids. Moreover, Tyr-1 can be replaced by His-1, yielding a molecule with

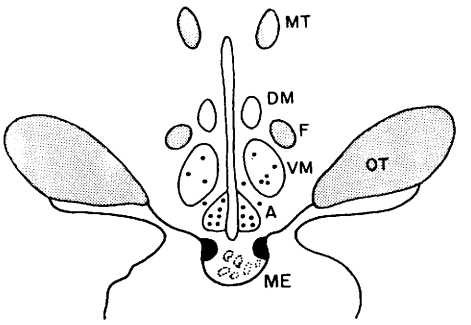

FIG. 1. Topographical representation of neurons containing hpGRF immunoreactivity in monkey hypothalamus; coronal Section. Small black circles represent cell bodies. The two black areas in median eminence indicate the fiber bundles and the small dots represent the endings in contact with portal vessels. OT, optic tract; ME, median eminence; A, arcuate nucleus; VM, ventromedial nucleus; DM, dorsomedial nucleus; F, fornix; MT, mamillothalamic tract.

equal potency. These studies strongly indicate that the amino-terminal portion of the molecule is most probably involved in the binding of the molecule to the receptor while the C-terminal portion is most likely involved in regulating the potency of the GRF peptides. Human and murine GRF are equipotent when tested, *in vitro;* pGRF and bGRF have a somewhat lower potency than hpGRF when tested in rat pituitary cells *in vitro.*

Structure of Human Pancreas PreproGRF. The primary structure of two precursors for human pancreas growth hormone releasing factor, hpGRF-44, was established by molecular cloning (11). The two precursor polypeptides contain the sequence of hpGRF-44 flanked by basic processing sites. The precursors include a putative signal sequence and a carboxyl terminal amidation signal for hpGRF-44. The two forms of mRNA code for preproGRF-107 and -108. The latter differs from preproGRF-107 by the insertion of serine in the carboxyl terminal portion of the precursor. The molecular weight of preproGRF as ascertained by *in vitro* translation of tumor poly(A^+)-RNA, followed by immunoprecipitation with GRF-specific antiserum was shown to be approximately 13,000.

Immunocytochemical Localization of GRF. GRF-like immunoreactivity was detected in human and primate brain (12, 13). These studies indicate the presence of immunoreactive cell bodies in the arcuate nucleus and the ventromedial nucleus. Both in man and monkey the majority of immunoreactive cells are found in the arcuate nucleus indicating that this area may be the primary site for the production of GRF. The cell bodies found in the arcuate nucleus have positive-staining fibers projecting to various areas of the hypothalamus including the median eminence, where they end in contact with portal vessels. Hypothalamic fibers which were found outside the median eminence appear to originate from the arcuate and ventromedial nuclei and project in areas located in close proximity to the third ventricle (14). In immunocytochemical studies using human hypothalami from fetuses, neonates, infants and adults (using two antisera with recognition sites in the midportion 28–39 residues of hpGRF and in the amidated carboxyl terminal of the molecule, respectively) GRF-staining neurons were found primarily in the arcuate nucleus. The topographical representation of neurons containing hpGRF in primate hypothalamus is shown in Fig. 1.

Effect of GRF *in vitro*. All the experimental data indicate that GRF stimulates secretion of GH *in vitro* and *in vivo.* In pituitary cells in monolayer culture or in a pituitary cell perifusion system (15) GRF stimulates release of GH, in a dose- and time-dependent manner and exclusively, i.e., without affecting secretion of other pituitary hormones (15). In the perifusion system using dispersed rat anterior pituitary cells, the release of GH is observed within 30 sec following exposure to GRF. Somatostatin-28 and somatostatin-14 (SS-28; SS-14) inhibit the response of normal pituitary cells to GRF in a noncompetitive manner (16) (Fig. 2). Additional studies using the pituitary monolayer system show that GRF-dependent GH release is antagonized by the calcium channel blocker, cobalt. GH release is enhanced by the cAMP analog, 8-bromocyclic AMP, by the phosphodiesterase inhibitor, IBMX, and by agents such as forskolin and cholera toxin which activate the adenylate cyclase system (16). GRF stimulates the efflux

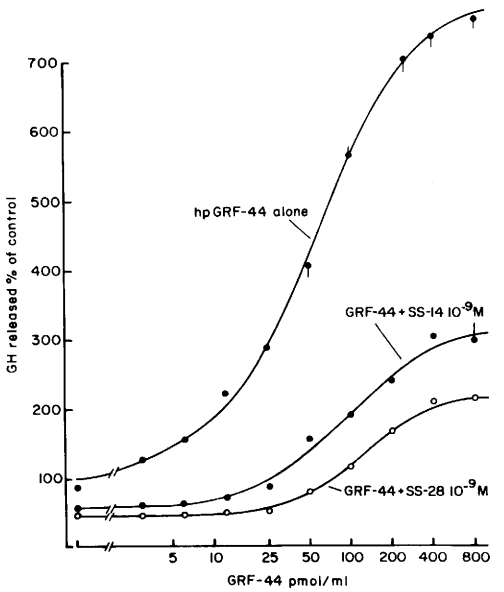

FIG. 2. Effect of GRF and somatostatin 14 (SS-14) or somatostatin 28 (SS-28) on GH release by rat pituitary cells in monolayer culture. There is a dose-dependent release of GH by GRF. SS-14 and SS-28 inhibit the response to GRF in a noncompetitive manner.

of cAMP by pituitary cells. In short-term studies (<15 min) with GRF, release of GH, efflux of cAMP and accumulation of intracellular cAMP occur in parallel (14). Additional studies indicate an additive effect of prostaglandin E_2 on GRF-mediated GH-release in normal pituitary cells (16). Thyroxine, triiodothyronine and synthetic glucocorticoids have been shown to enhance response of normal pituitary cells to synthetic GRF (17).

GRF does not stimulate release of GH from a line of rat pituitary tumor cells (GH_3) which have been hitherto widely used to study the regulation of the GH and prolactin (PRL) genes (18). The mechanism of spontaneous release of GH by this cell line thus appears to be different from those underlying the secretion of GH by normal pituitary cells.

At micromolar concentrations, hpGRF-44-NH_2 stimulates release of neurotensin (NT) in a clonal line of rat medullary thyroid carcinoma cells (of C-cell origin) (19). This is the first evidence of an extrapituitary target for GRF-mediated peptide release. In these cells as in pituitary cells there is concomitant release of peptide and cAMP in response to GRF. An additive effect of norepinephrine (NE) is found on GRF-stimulated NT release.

Although the mechanism(s) whereby GRF stimulates release of GH is not known, there is evidence indicating that hpGRF-44-NH_2 stimulates cAMP-induced histone phosphorylation in isolated hog pituitary granules in a dose-dependent manner (20). This effect of GRF is seen at concentrations as low as 0.3 pM. On the basis of these findings (20) it is possible that GRF stimulates GH release by activating exocytosis through a phosphorylation mechanism mediated by a granular "receptor" most probably coupled to a cAMP-dependent protein kinase.

An effect of hpGRF-44-NH_2 on GH-mRNA levels was studied in normal pituitary cells in culture and in GH_3 cells. The cytoplasmic dot hybridization technique (21) was employed to examine the effect of GRF. Pituitary cells incubated for 24 hr with 25 to 400 fmole GRF had a significant increase in GH-mRNA levels over the controls. Maximal GH-mRNA levels were noted following 72 hr incubation of normal pituitary cells with 10^{-9} *M* GRF. In a similar experimental design, GRF did not stimulate PRL release or relative PRL-mRNA levels. GRF does not stimulate GH- or PRL-mRNA levels in GH_3 cells (we have reported above that GRF does not affect GH secretion by GH_3 cells).

***In vivo* Studies with GRF.** *In vivo* effects of GRF have been studied in rats anesthetized with pentobarbital (22), and in conscious freely moving rats outfitted with a chronic indwelling catheter (23). In the anesthetized rat, GRF produces a dose-dependent release of plasma GH and does not affect plasma prolactin (PRL), thyroid stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and corticosterone. In conscious rats, administration of GRF results in release of GH approximately 30% of the time. The inconsistent response to the peptide is attributed to stress-induced endogenous release of somatostatin, which counteracts the effect of exogenous GRF. When a somatostatin antiserum is administered to the animals there is now a consistent response to GRF administration, and plasma GH levels increase 5- to 10-fold. (Figs. 3a, b).

Effects of anatomical and functional lesions of the CNS upon GRF-stimulated GH release have been studied in the rat. Electrolytic lesions of the ventromedial hypothalamus (VMH) result in impaired growth and a suppression of spontaneous GH pulses. In rats bearing VMH lesions, iv administration of GRF causes a marked surge in plasma GH levels within 5 min. This effect of GRF can be reversed by the concomitant administration of somatostatin-14 (24, 25). GRF has also been shown to reestablish GH secretion in rats with a chemical lesion of the arcuate nucleus induced by neonatal treatment with monosodium glutamate, and in rats treated with α-methyl-*p*-tyrosine and reserpine, agents causing functional lesions of normal catecholamine metabolism. Thus, as shown in a variety of animal models, GRF stimulates GH release by acting directly on the pituitary gland and not at some proximal locus within the central nervous system. GRF is the only substance known to reestablish GH secretion in animals with these anatomical or functional lesions.

Clinical Studies with GRF. Clinical interest in a "true" GRF, i.e., a substance that would directly stimulate secretion of GH at pituitary level, has always been considerable, since all

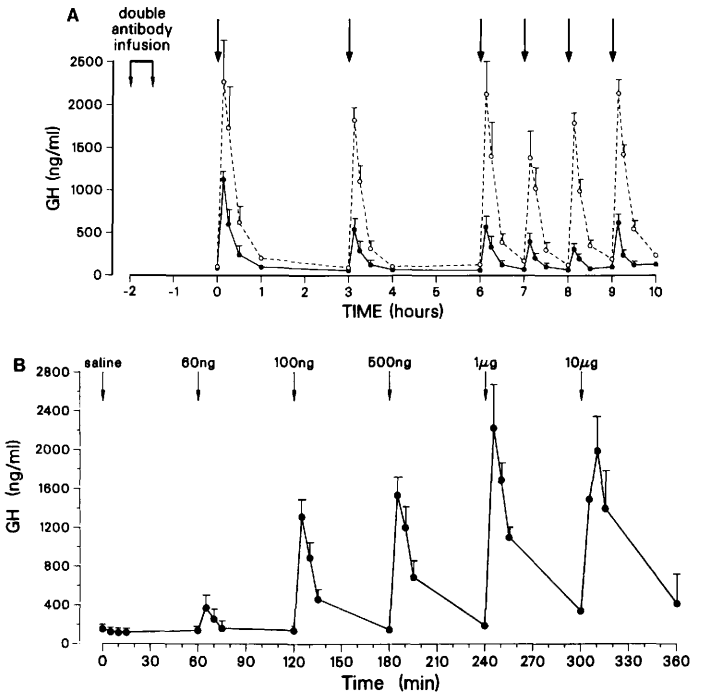

FIG. 3. (A) The capacity of the pituitary in conscious, freely moving male rats (N = 6) to secrete GH in response to repeated intravenous injections of a moderate (0.25 μg; ●) and maximal (5 μg; ○) dose of hpGRF-40. Two hours before the first injection rats were treated with antiserum against somatostatin and monoclonal antibody against rat hypothalamic growth hormone-releasing factor. Arrows indicate the injection of synthetic hpGRF-40. Data points represent the mean GH concentration, and the vertical bars represent the SEM. (B) The dose-dependent response of the pituitary in conscious, freely moving male rats (N = 6) to secrete GH in response to hpGRF-40 (iv). Two hours before the saline injection rats were treated with antiserum against somatostatin and monoclonal antibody against rat hypothalamic growth hormone-releasing factor. The dose and time of injection of synthetic hpGRF-40 are indicated. Data points represent the mean GH concentration, and the vertical bars represent the SEM.

current methods to stimulate secretion of GH in human subjects (i.e., hypoglycemia, L-Dopa, infusion of amino acids, etc.) are indirect, i.e., mediated by some brain center. Many clinical studies with synthetic GRF were promptly started and are currently expanding (26–28). The results have not been disappointing. Intravenous injection into normal young adults, male or female, of synthetic GRF or of the fragment GRF(1-40) in doses ranging from 0.1 to 10 μg/kg body wt regularly produces elevation of plasma immunoreactive GH, with a peak response at 15–30 min and lasting for 1–2 hr (Fig. 4). The response to GRF is highly specific for the secretion of GH, with no effect on plasma levels of all the other pituitary hormones or gut peptides (26). Obviously synthetic GRF should and will replace the indirect methods (see above) currently used to assess GH secretion. Since all the current GH-secretion tests work indirectly, i.e., through the central nervous system, none of them being truly hypophysiotropic, they do not permit a diagnosis of GH pituitary insufficiency as truly pituitary or hypothalamic in origin. Indeed, injection of GRF to "hypopituitary dwarfs" has already led to the recognition of GH secretory deficiencies truly pituitary in origin (no response to GRF) and of a second category which is best qualified as suprapituitary (most likely hypothalamic) in origin (positive response to GRF). Chronic administration of GRF should be the treatment of choice for these cases of "hypothalamic dwarfism." Early studies indicate that the acute pituitary response to GRF in human

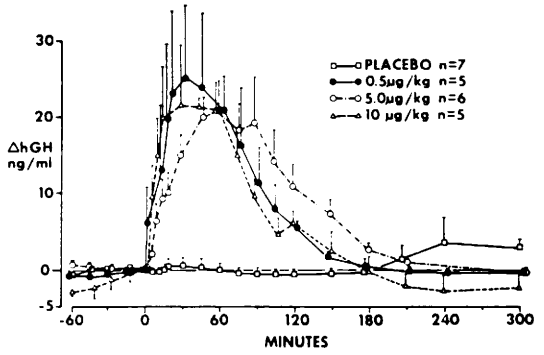

FIG. 4. Elevation of plasma GH levels in response to hpGRF in normal adult human volunteers. The response to 0.5-, 5-, and 10-μg/kg doses of hpGRF-44 were all significantly higher than the placebo. GH levels rose within 5 min and reached peak concentrations at 30–45 min (0.5 μg/kg); 45–90 min (5 μg/kg), and 30–120 min (10 μg/kg). Results are expressed in ng hGH/ml (mean ± SEM). (From Ref. 28.)

subjects may be highly age dependent, the response being quasi-abolished after age 40. This remarkable observation remains to be confirmed and explained.

Clinical interest in GRF extends over its use as a diagnostic tool and a treatment of hypothalamic dwarfism: use of GRF to promote anabolism in chronic debilitating diseases, so long as the dietary intake is adequate, and to promote the healing of wounds and bone fractures. The availability of GRF with its highly specific effect in stimulating GH secretion should permit once and for all investigation of the proposed role of GH in diabetic retinopathy. Structural analogs of GRF acting as competitive antagonists (as we have already reported in an early series (10)) may be of major clinical significance in the treatment of these accidents of juvenile diabetes, something that somatostatin eventually could not offer in view of its too numerous sites of action as they came to be recognized.

Conclusions. In conclusion, the long-sought growth hormone-releasing factor has been characterized and sequenced in several species (human, porcine, bovine, and murine). The noteworthy achievement with GRF has been the rapidity with which the sum of information has been gathered. In the space of 12 months most of the pioneering studies on the mechanism of action of the peptide *in vivo* and *in vitro* were described. Immunocytochemical mapping of GRF neurons was carried out. Structure–function studies were initiated. Clinical trials were started and confirmed the potent GH-releasing activity of GRF in man. The effect of the peptide on specific GH mRNA levels was described and molecular cloning was used to establish the structures of human (pancreas) preproGRF. All the hypothalamic releasing factors which had been postulated in the early fifties as humoral regulators of the secretion of each pituitary hormone have now been characterized.

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1. Frohman L, Szabo M. Ectopic production of growth hormone-releasing factor by carcinoid and pancreatic islet tumors associated with acromegaly. In: Physiopathology of Endocrine Diseases and Mechanisms of Hormone Action. New York, Liss, pp259–271, 1981.
2. Böhlen P, Esch F, Brazeau P, Ling N, Guillemin R. Isolation and characterization of the porcine hypothalamic growth hormone releasing factor. Biochem Biophys Res Commun **116**:726–734, 1983
3. Esch F, Böhlen P, Ling N, Brazeau P and Guillemin R. Isolation and structural characterization of the bovine hypothalamic growth hormone releasing factor. Biochem Biophys Res Commun **117**:772–779, 1983.
4. Spiess J, Rivier J, Vale W. Characterization of rat hypothalamic growth hormone-releasing factor. Nature (London) **301**:532–535, 1983.
5. Guillemin R, Brazeau P, Böhlen P, Esch F, Ling N, Wehrenberg W. Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science **218**:585–587, 1982.
6. Esch F, Böhlen P, Ling L, Brazeau P, Wehrenberg W, Guillemin R. Primary structures of three human pancreas peptides with growth hormone-releasing activity. J Biol Chem **258**:1806–1810, 1982.
7. Esch F, Böhlen P, Ling N, Brazeau P, Wehrenberg W, Thorner M, Cronin M, Guillemin R. Characterization of a 40 residue peptide from a human pancreatic tumor with growth hormone releasing activity. Biochem Biophys Res Commun **109**:152–158, 1982.
8. Rivier J, Spiess J, Thorner M, Vale W. Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. Nature (London) **300**:276–278, 1982.
9. Böhlen P, Brazeau P, Bloch B, Ling N, Gaillard R, Guillemin R. Human hypothalamic growth hormone releasing factor (GRF): Evidence for two forms identical to tumor-derived GRF-44-NH_2 and GRF-40. Biochem Biophys Res Commun **114**:930–936, 1983.

10. Ling N, Brazeau P. Structure activity relationship in the molecule of hpGRF, growth hormone releasing factor. Endocrinology **112**:A295, 1983.
11. Gubler U, Monahan J, Lomedico P, Bhatt R, Collier K, Hoffman B, Böhlen P, Esch F, Ling N, Zeytin F, Brazeau P, Poonian M, Gage L. Cloning and sequence analysis of cDNA for the precursor of human growth hormone releasing factor, somatocrinin. Proc Natl Acad Sci USA **80**:4311–4314, 1983.
12. Bloch B, Brazeau P, Ling N, Böhlen P, Esch F, Wehrenberg W, Benoit B, Bloom F, Guillemin R. Immunohistochemical detection of growth hormone-releasing factor in brain. Nature (London) **301**:607–608, 1983.
13. Bloch B, Brazeau P, Bloom F, Ling N. Topographical study of the neurons containing hpGRF immunoreactivity in monkey hypothalamus. Neurosci Lett **37**:23–28, 1983.
14. Bloch B, Gaillard R, Brazeau P, Lin H, Ling N, Reichlin S, Guillemin R. Topographical and ontogenetical study of the neurons producing growth hormone-releasing factor in human hypothalamus. Reg Peptides, in press.
15. Brazeau P, Ling N, Böhlen, Esch F, Ying S-Y, Guillemin R. Growth hormone releasing factor, somatocrinin, releases pituitary growth hormone in vitro. Proc Natl Acad Sci USA **79**:7909–7913, 1982.
16. Brazeau P, Ling, Esch F, Böhlen P, Mougin C, Guillemin R. Somatocrinin (growth hormone-releasing factor) *in vitro* bioactivity; Ca^{++} involvement, cAMP mediated action and additivity of effect with PGE_2. Biochem Biophys Res Commun **109**:588–594, 1982.
17. Vale W, Vaughan J, Yamamoto G, Spiess J, Rivier J. Effects of synthetic human pancreatic (tumor) GH releasing factor and somatostatin, triiodothyronine and dexamethasone on GH secretion in vitro. Endocrinology **112**:1553–1555, 1983.
18. Gick G, Zeytin F, Brazeau P, Ling N, Esch F, Bancroft F. Growth hormone-releasing factor regulates growth hormone mRNA in primary cultures of rat pituitary cells. Proc Natl Acad Sci USA, in press.
19. Zeytinoglu F, Ling N, Mougin C, Esch F. Growth hormone releasing factor stimulates release of neurotensin and efflux of cAMP by a rat C-cell line. Endocrinology **112**:A293, 1983.
20. Lewin M, Reyl-Desmars F, Ling N. A somatocrinin receptor coupled with cAMP-dependent protein kinase on anterior pituitary granules. Proc Natl Acad Sci USA **80**:6538–6541, 1983.
21. White B, Bancroft F. Epidermal growth factor and thyrotropin-releasing hormone interact synergistically with calcium to regulate prolactin mRNA levels. J Biol Chem **258**:4618–4622, 1983.
22. Wehrenberg W, Ling N, Brazeau P, Esch F, Böhlen P, Baird A, Ying S-Y, Guillemin R. Somatocrinin, growth hormone releasing factor, stimulates secretion of growth hormone in anesthetized rats. Biochem Biophys Res Commun **109**:382–387, 1982.
23. Wehrenberg W, Ling N, Böhlen P, Esch F, Brazeau P, Guillemin R. Physiological roles of somatocrinin and somatostatin in the regulation of growth hormone secretion. Biochem Biophys Res Commun **109**:562–567, 1982.
24. Tannenbaum G, Eikelboom R, Ling N. Human pancreas GH-releasing factor analog restores high-amplitude GH pulses in CNS lesion-induced GH deficiency. Endocrinology **113**:1173–1175, 1983.
25. Wehrenberg WB, Bloch B, Zhang, C-L, Brazeau P, Ling N, Guillemin R. 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, in press.
26. Thorner M, Spiess J, Vance M, Rogol A, Kaiser D, Webster J, Rivier J, Borges J, Bloom S, Cronin M, Evans W, Macleod R, Vale W. Human pancreatic growth-hormone-releasing factor selectively stimulates growth hormone secretion in man. Lancet I:24–28, 1983.
27. Sassolas G, Rousset H, Cohen R, Chatelain P, Claustrat B, Laporte S, Laferrère B, Charfi A, Ferry S. La somatocrinine provoque la libération de somathormone dans un cas de déficit en somathormone d'origine hypothalamique chez l'enfant. C R Acad Sci, Paris **296**:527–530, 1983.
28. Rosenthal S, Schriock E, Kaplan S, Guillemin R, Grumbach M. Synthetic human pancreas growth hormone-releasing factor (hpGRF 1-44-NH_2) stimulates growth hormone secretion in normal men. J Clin Endocrinol Metab **57**:677–679, 1983.