Growth Hormone–Releasing Factor from a Human Pancreatic Tumor That Caused Acromegaly

Abstract. *A 44 amino acid peptide with growth hormone–releasing activity has been isolated from a human tumor of the pancreas that had caused acromegaly. The primary structure of the tumor-derived peptide is H-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. The synthetic replicate has full biological activity in vitro and in vivo specifically to stimulate the secretion of immunoreactive growth hormone. The tumor-derived peptide is identical in biological activity and similar in physicochemical properties to the still uncharacterized growth hormone–releasing factor present in extracts of hypothalamic tissues.*

The now well-established concept of a neurohumoral control of adenohypophyseal secretions by the hypothalamus indicates the existence of a hypothalamic growth hormone–releasing factor (GRF) (*1*) having somatostatin (*2*) as its inhibitory counterpart. So far, hypothalamic GRF has not been unequivocally characterized, despite earlier claims to the contrary (*3*).

Peptides with the biological activity of hypothalamic GRF have been ectopically produced (*4*), in some rare clinical cases, by human pancreatic islet cell tumors and variously located carcinoids (*5*). The tentative diagnosis of such a tumor was recently made in a patient with full-blown acromegaly; findings included nonsuppressible plasma levels of immunoreactive growth hormone > 50 ng/ml, no enlargement of the pituitary sella, and a large abdominal mass presumably in the pancreas. At surgery, two separate solid tumors were found in the pancreas (*6*); the tumor tissues were diced and collected in liquid nitrogen within 2 to 5 minutes of resection with the intent to extract them for GRF.

A portion of tissue from each tumor was extracted with 0.3*M* HCl containing the enzyme inhibitors pepstatin and phenylmethylsulfonyl fluoride; the supernatant of the extract was filtered on a column of Sephadex G-75, and the effluent was assayed for growth hormone–releasing activity (*7*). The extract of both tumors contained bioactive and immunoactive somatostatin of several molecular sizes, an observation that will not be discussed further here. The extract of both tumors also contained growth hormone–releasing activity with the same elution volume as that of hypothalamic GRF (K_{av} = 0.43, where K_{av} is the elution constant) (*8*). The amounts of GRF activity (*9*) were minute in one of the tumors [0.06 GRF unit per milligram (wet weight)], but extremely high in the other [1500 GRF units per milligram (wet weight)], 5000 times more than we had found in rat hypothalamus (*8*). The fraction containing GRF activity from the extract of the high-yield tumor was further processed by high-performance liquid chromatography (HPLC) on a semipreparative reverse-phase column using a mobile phase of *n*-propanol in pyridine formate to yield a single zone of GRF activity located by the bioassay. Final purification of this material by analytical reverse-phase HPLC yielded three highly purified peptides with GRF activity (Fig. 1). Quantitative amino acid analysis (*10*) provided estimates of the amounts of GRF-like material extracted from 7.2 g of tumor tissue of 5, 30, and 10 nmole of peptide in the fractions eluting at 60, 62, and 82 minutes, respectively. Portions of the fraction with the highest specific activity [elution time 82 minutes (see Fig. 1); 2×10^5 GRF units per nanomole or 5×10^7 GRF units per milligram] upon hydrolysis (*10*) yielded the following amino acid composition: asparagine (Asn) and aspartic acid (Asp), 4.0; threonine (Thr), 0.9; serine (Ser), 3.7; glutamine (Gln) and glutamic acid (Glu), 6.9; proline (Pro), 0; glycine (Gly), 3.4; alanine (Ala), 4.9; cysteine (Cys), 0; valine (Val), 0.9; methionine (Met), 1.0; isoleucine (Ile), 1.8; leucine (Leu), 5.0; tyrosine (Tyr), 2.1; phenylalanine (Phe), 0.9; histidine (His), 0; lysine (Lys), 2.2; tryptophan (Trp), 0; and arginine (Arg), 6.1. This material will henceforth be referred to as hpGRF, for human pancreas growth hormone–releasing factor (*11*).

We determined the primary structure of hpGRF by stepwise Edman degradation of the unmodified peptide and its cyanogen bromide cleavage fragments (1–27 and 28–44) using a gas phase sequencer (Applied Biosystems, model 470A) (*12*) and HPLC identification of the phenylthiohydantoin amino acids obtained from the sequencer (*13*). The sequence of amino acids so ascertained is H-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.

Total synthesis of hpGRF was achieved by solid-phase techniques (*14*). This was conducted on a *p*-methylbenzhydrylamine resin with a peptide synthesizer (Beckman 990). The major product of the synthesis was purified by gel permeation, ion exchange, and partition chromatography (*14*). Amino acid analysis and microsequencing confirmed the correct synthesis of hpGRF. Native and synthetic hpGRF migrate together and are well separated by reverse-phase and cation exchange HPLC from synthetic hpGRF-OH, confirming that native hpGRF was isolated in the amide form, with the Met residue not oxidized.

The synthetic peptide has the full biological activity of native hpGRF in the in vitro assay (*7*) (Fig. 2). The median effective dose (ED_{50}) of the native material or of the synthetic replicate is about 15

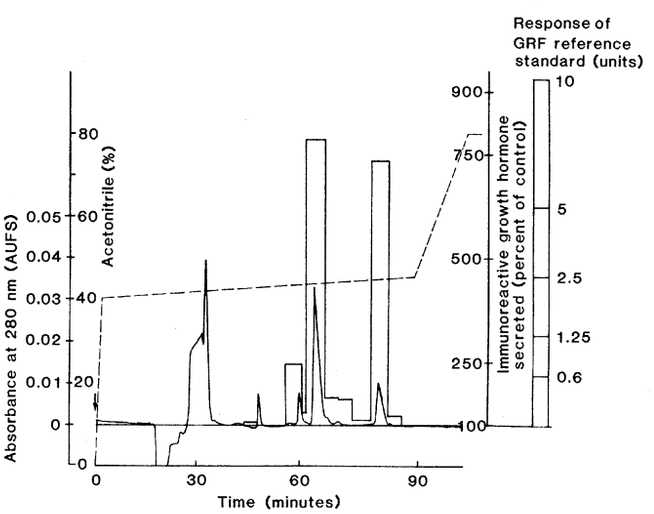

Fig. 1. Final purification of hpGRF by reverse-phase HPLC. The column (Ultrasphere C18), 25 by 0.4 cm, 5-μm particle size, was eluted with a gradient of acetonitrile (---) in 0.5 percent (by volume) heptafluorobutyric acid at a flow rate of 0.6 ml/min. Fractions (2.4 ml) were collected as indicated on the abscissa, and portions were used for bioassay (*7*). The vertical bars represent the amount of growth hormone secreted in the assay of each fraction of the effluent, expressed as percentage of the amount of growth hormone secreted by control pituitary cells receiving no treatment. *AUFS*, absorbance units full scale.

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fmole/ml; the high potency and high intrinsic activity of this molecule as a growth hormone–releasing factor are characteristics in favor of a true physiological significance to the results presented here. Regression analysis of the dose response data for hpGRF, either native or synthetic, shows the same slope as that calculated for rat hypothalamic GRF (Fig. 2a). This implies that the two GRF peptides have the same mechanism of action on the same pituitary receptors. Synthetic hpGRF is also highly active in vivo; it stimulates secretion of growth hormone in rats anesthetized with Nembutal (Fig. 2b), in conscious rats or dogs fitted with a permanent venous catheter (not shown), and in rats with a stereotaxic lesion of the ventromedial nucleus of the hypothalamus (*15*), a lesion known to abolish endogenous secretion of growth hormone. Both in vitro and in vivo, hpGRF or its synthetic replicate stimulates the pituitary to secrete only growth hormone. Like purified murine or porcine hypothalamic GRF (*8*), it has no effect on the secretion of β-endorphin, prolactin, lutropin, follitropin, or thyrotropin (data not shown) when tested in vitro.

We have established that the biological activity of hpGRF resides in the amino terminal part of the molecule; the carboxyl terminal fragment (28–44) generated by allowing hpGRF to react with cyanogen bromide has no biological activity. Moreover, the tyrosine amino terminus is essential for biological activity. Deleting the amino terminal Tyr, Tyr-Ala, or Tyr-Ala-Asp in synthetic replicates yields compounds that have less than 0.1 percent of the specific activity of hpGRF (their respective potencies are 0.2×10^{-3}, 0.2×10^{-4}, and 0.3×10^{-5}, compared with 1.0 for hpGRF).

The other two components with GRF activity in the extract of the tumor tissue (Fig. 1) have also been characterized. The material eluting at 60 minutes has the sequence (1–37)-OH of hpGRF; its specific activity is 12 percent that of hpGRF (with confidence limits of 9 and 16 percent) on the release of growth hormone in vitro. The peptide eluting at 62 minutes has the sequence (1–40)-OH of hpGRF; its specific activity is 30 percent that of hpGRF (with confidence limits of 25 and 37 percent) on the release of growth hormone in vitro. All three compounds have full intrinsic activity; that is, they produce statistically identical maximal responses (E_{max}).

The multiplicity of these GRF peptides, all biologically active, all related to a common core and precursor, is reminiscent of the current status regarding multiple forms of, for instance, somatostatin, gastrin, cholecystokinin, or the enkephalins (*16*). Similarly, we have reported that multiple fractions with GRF activity exist in extracts of porcine or murine hypothalamus (*8*).

There are sequence homologies between hpGRF and several peptides of gut origin, all of the secretin-glucagon family. The largest homology is with the recently described intestinal peptide PHI-27 (*17*) (Fig. 3). Probably because of the discrepancy at the amino terminus (histidine for PHI-27 and tyrosine for hpGRF, tyrosine being essential for GRF activity), native or synthetic PHI-27 has no GRF activity when tested at 30,000 times the minimal active dose of hpGRF.

Is this tumor-derived GRF identical to hypothalamic GRF? The question will not be answered until a GRF of hypothalamic origin has been chemically characterized. This is not a simple proposal; we know now that one hypothalamic fragment of fresh rat brain contains only femtomoles of GRF; its occurrence in such minute amounts—even when compared to those of the other hypothalamic releasing factors—is probably one of the major problems in isolating hypothalamic GRF. However, biologically active peptides, produced ectopically by tumors, have always been shown to be identical to the products of the physiological source of such peptides or to corresponding fragments of the precursor molecule [reviewed in (*18*)]. Although differences in the regulation of gene expression may be involved, the gene products are identical in the physiological and the ectopic tissues. Indeed, in all studies conducted so far, hpGRF and hypothalamic GRF have been indistinguishable except by the monoclonal antibodies (*19*) to rat hypothalamic GRF. What can certainly be said is that the molecule we have now characterized has all the attributes expected from the long-sought hypothalamic releasing factor for growth hormone.

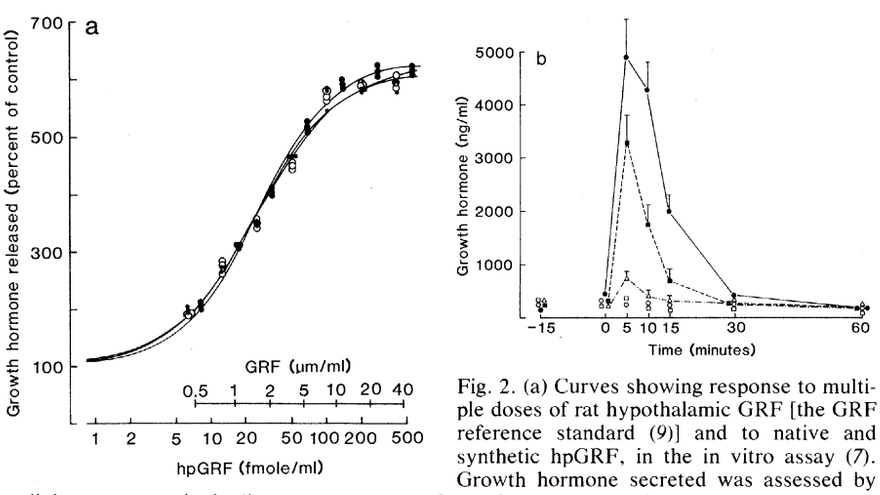

Fig. 2. (a) Curves showing response to multiple doses of rat hypothalamic GRF [the GRF reference standard (*9*)] and to native and synthetic hpGRF, in the in vitro assay (*7*). Growth hormone secreted was assessed by radioimmunoassay in duplicate measurements for each treatment, and treatments were done in triplicate. Actual experimental data for native hpGRF (●), synthetic hpGRF (○), and GRF reference standard (●) are expressed as percentage of control values. Lines are the theoretical curves, computer-calculated and drawn, from the four-parameter logistic equations for each set of data. Curves shown here are drawn without constraints [program ALLFIT (*20*)]. The mathematical analysis shows the slopes of the three sets of curves to be identical as are values for E_{max}. Potency of synthetic hpGRF versus native hpGRF is 0.99 with 95 percent confidence limits of 0.79 and 1.24 [program BIOPROG (*20*)]. (b) Plasma growth hormone levels in response to the intravenous administration of saline (□) and of synthetic hpGRF at 0.01 μg (○), 0.1 μg (△), 1.0 μg (■), and 10.0 μg (●). Male Sprague-Dawley rats weighing 280 to 320 g were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) at time −30 minutes. Samples (0.2 ml) were drawn by venipuncture at times indicated; saline or hpGRF was administered immediately after time 0. Four animals were used for each treatment. Results are shown as the mean of responses, with the vertical line representing the standard error of the mean.

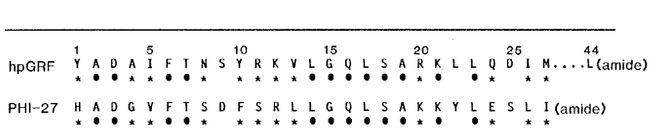

Fig. 3. Sequence homologies between hpGRF and PHI-27. Identical residues are underscored by ●. Residues differing in the genetic code by only one base are underscored by ∗. A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; and Y, tyrosine.

The physiological and clinical significance of this peptide will now be established in consequence of what has been expected of GRF in the many years of the search for it. Beyond that, and in keeping with other past experience, probably the most interesting role, effect, or use of GRF or of some of its future structural analogs is currently totally unsuspected.

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21. We thank G. Sassolas and C. Partensky for their active interest and collaboration, M. Brown and V. Webb for making available and handling the trained dogs used in some of the results reported here, and S. Ying and D. Fuller for performing the radioimmunoassays for lutropin, follitropin, and thyrotropin. Native PHI-27 was received from V. Mutt, Karolinska Institute, Stockholm; synthetic PHI-27 was obtained from Peninsula Laboratories, San Carlos, California. We also acknowledge the excellent and devoted technical assistance of D. Martineau, M. K. Culkin, F. Castillo, R. Schroeder, G. Kleeman, M. Mercado, D. Lappi, M. Regno, D. Olshefsky, and G. Textor. Supported by NIH grants HD-09690 and AM-18811 and by grants from the Robert J. and Helen C. Kleberg Foundation.

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A New Perceptual Context-Superiority Effect: Line Segments Are More Visible Against a Figure than Against a Ground

Abstract. *Context, specifically the perceived figure or ground of an ambiguous form that surrounds a diagonal line segment, can influence the discrimination of that line segment even though the physical attributes of the context remain the same during figure-ground reversals. When the line segment was flashed on a region of the form seen as figure, discrimination was twice as accurate as when the line segment was flashed in isolation, and it was at least three times as accurate as when the line segment was flashed on that same region seen as ground.*

A barely visible, briefly flashed line segment is discriminated with greater accuracy when it is part of a pattern that looks like an object than when it is flashed alone or when it is part of a pattern that appears to be a random collection of lines (*1*). A letter is typically identified better when it is presented as part of a pronounceable word than when it is flashed among an unpronounceable string of letters or alone (*2*). And an object is better recognized when it is part of a coherent scene than when it is flashed in a scene whose parts have been jumbled (*3*). These object, word, and scene superiority effects can all be classified more generally as "context effects" in perception. Such context effects show that perceptual variables influence task performance quite apart from the physical aspects of the stimuli.

We now report effects of context that are entirely perceptual. Visual discrimination is dramatically enhanced when line segments are flashed in a region that is perceived as figure. Discrimination is substantially degraded when the same region is seen as ground even though the physical stimulus remains identical throughout figure-ground reversals.

In our experiment, we chose Rubin's face-vase reversible figure as the context stimulus (Fig. 1) (*4*). If one fixates at *A*, the perception of two identical faces, one on each side of the central region, alternates with the perception of a vase in the middle of the figure. When the central region is perceived as a vase (or figure) the surrounding regions become a background (ground) with no definite shape. Conversely, when the surrounding regions are seen as two faces, the central region loses its figural identity and assumes the characteristic of a formless background. The common boundary contour shared by the central and flanking regions seems to belong to the region seen as figure (*4*). In this stimulus, local and global environments, spatial frequency and phase, in fact, all the physical aspects of the stimulus are identical whether a region is seen as figure or as ground. Only the perception varies.

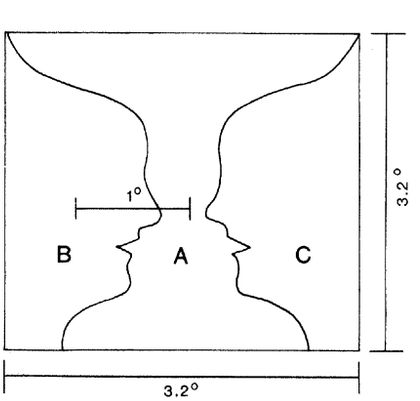

Fig. 1. Reversible face-vase figure (*4*).

Our experiment compared observers' ability to identify the direction of tilt of a test line that was flashed within a given region of Fig. 1 when that region was perceived as figure or when seen as ground. The context pattern occupied a region 3.2° by 3.2° with a dim fixation point located at the center. The target was a line 0.9° long and 0.06° wide. On a

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