Growth hormone-releasing factor

A tale of two islets

from George Fink

THE two recent reports^{1,2} of the isolation and sequencing of human pancreatic growth hormone-releasing factor (hpGRF) from the laboratories of Roger Guillemin and Wylie Vale have rapidly been followed by immunohistochemical evidence from Guillemin's group that hpGRF is the same as the hypothalamic factor that normally stimulates growth hormone release. In this issue of *Nature* (p.607), Bloch and colleagues³ show that an antiserum against the hpGRF peptide specifically stains neural cell bodies in areas of the primate hypothalamus associated with the hypothalamic peptide (GRF).

The isolation and sequencing of hpGRF was only made possible by the existence of two islet cell tumours, one in a patient at Charlottesville, Virginia and another in a patient at Lyon. Carcinomas of the pancreatic islets (the islets of Langerhans) are relatively infrequent (less than 1/100,000 of the population⁴) and because they often secrete large amounts of different types of peptide hormones they are extremely difficult to diagnose. The tumours most commonly secrete the hormones indigenous to the pancreas — insulin (symptoms of hypoglycaemia), gastrin (gastric or duodenal ulceration and diarrhoea; Zollinger–Ellison syndrome), glucagon (mild diabetes mellitus and dermatitis) and somatostatin (hypochlorhydria, steatorrhoea and diabetes mellitus)^{4,5}. Less frequently they secrete hormones ectopic to the pancreas such as secretin (leading to 'pancreatic cholera'), adrenocorticotropin (presenting with features of Cushing's syndrome), serotonin (associated with watery diarrhoea, flushes and tachycardia)⁴ and in the present case, hpGRF (associated with acromegaly).

The isolation of hpGRF was carried out quite separately by two teams at the same institute — the Salk Institute — and headed respectively by Wylie Vale (Peptide Biology Laboratory) and his former mentor Roger Guillemin (Laboratories for Neuroendocrinology). Peptide isolation, like the Americas Cup, offers no second place, but in this case the race appears to have been a dead heat.

Vale's tumour was removed from a 21-year-old woman who presented at Mike Thorner's (formerly of Bart's) clinic in Charlottesville with Turner's syndrome and the signs and symptoms of acromegaly⁶. Guillemin obtained his tumour by way of the 'French connection'; it was removed from a man who presented with acromegaly at the clinic of G. Sassolas at Lyon¹. Both teams used essentially the same methods — gel filtration, HPLC and Edman degradation. Guillemin¹ isolated three peptides, the largest of which was 44

residues long and had the sequence 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-NH₂. The peptide isolated by Vale² was 40 amino acid residues long and had a sequence identical to the first 40 residues of Guillemin's peptide. The two other factors found by Guillemin to release growth hormone comprised the 1–37 and 1–40 sequences of the 44-residue hpGRF.

Both Vale and Guillemin agree that the N-terminus is essential for biological activity [deletion of -Tyr (ref. 4), or Tyr-Ala or Tyr-Ala-Asp (ref. 1) leads to almost total loss of activity] but whereas Guillemin found that hpGRF_{1–37} had only 12 per cent of the activity of hpGRF_{1–44}, Vale² reported that the first 29 residues were sufficient for full expression of activity *in vitro*. The peptide has several sequence homologies with peptides of the glucagon-secretin family, but none of these peptides has growth hormone-releasing activity. The greatest homology is with a 27-residue peptide recently isolated from porcine gut, PHI-27 (ref. 7), which has 12 residues that are also present in equivalent positions in hpGRF_{1–27}.

The isolation of hypothalamic GRF, like the other hypothalamic regulatory peptides, has been a will-o-the-wisp. Andrew Schally caused a flurry of interest when in 1971 he published the structure of GRF (from porcine hypothalamus)⁸ as H-Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala-OH, but although his peptide appeared to release growth hormone as assessed by bioassays (which used, in the main, the capricious pituitary growth hormone depletion–tibia assay), it was never found to release growth hormone as measured by radioimmunoassay.

Guillemin attributes the difficulty of isolating GRF from hypothalamus to the incredibly small tissue concentration in comparison with that of other hypothalamic neuropeptides (femtomoles compared with pico- and nanomoles). Guillemin and Vale both argued that hpGRF is likely to be identical to the hypothalamic GRF by analogy with other ectopic hormones, the structure of which has always been similar or identical to the structure of the same hormone produced by the tissue to which it is indigenous. Further, both groups had shown that partially purified rat hypothalamic GRF (rGRF) co-eluted with hpGRF on chromatography and that in terms of growth hormone released, the dose–response curves for rGRF were parallel to those for hpGRF.

The work reported from Guillemin's group in this issue³ (p.607) has come close to clinching the deal by showing that in the monkey and human, nerve fibres around the primary capillary plexus of the hypophyseal portal vessels stain immunohistochemically with an antiserum raised in rabbits against hpGRF_{1–40}. Cell bodies in the tuberal nuclei were also immunoreactive. The antiserum did not stain fibres or cell bodies in the hypothalamus of either the rat or the ox, perhaps not surprising since the antiserum was raised against a human peptide. Bloch *et al.*³ speculate that this may be due to species differences in the 28–40 region of the molecule which is devoid of GRF bioactivity. Species differences may also explain some of the differences in the GRF activity of extracts of porcine stalk median eminence compared with that of GRF partially purified from islet tumour tissue⁹.

Peptide isolation aside, there is much fundamental biology to be learned from the studies carried out on Thorner's patient⁶ as well as from the elegant case reports of Lawrence Frohman¹⁰ whose work on extrapituitary GRF activity must be considered as the stimulus and model for the studies by Guillemin and Vale. Thorner's patient showed the hallmarks of acromegaly due to a pituitary adenoma: plasma growth hormone concentrations were increased in response to glucose (instead of suppressed), increased after TRH administration (instead of no change) and decreased with dopamine infusion (instead of increased). Were these abnormal responses due to intrinsic changes in the hypertrophic pituitary somatotrophs or changes in ligand–receptor interactions brought about by excessive exposure to GRF, or did these factors (glucose, TRH and dopamine) act on the tumour directly to affect hpGRF release? What happens to somatostatin (in brain and pancreas) in these situations in view of the fact that somatostatin inhibits the growth hormone response to GRF release in a non-competitive manner^{5,9}? Whatever the answers to these and other questions regarding GRF biosynthesis and its role in gut and pancreas, the diagnosis and removal of two islet cell carcinomas has made two patients, two peptide chemists and several biological firms very happy. □

George Fink is Director of the MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ.

- Guillemin, R. *et al.* *Science* **218**, 585 (1982).
- Rivier, J., Spiess, J., Thorner, M. & Vale, W. *Nature* **300**, 276 (1982).
- Bloch, B. *et al.* *Biochem. biophys. Res. Commun.* **45**, 235 (1971).
- Schein, P.S. *et al.* *Ann. int. Med.* **79**, 239 (1973).
- Larsson, L.-I. *et al.* *Lancet* **i**, 666 (1977).
- Thorner, M.O. *et al.* *J. clin. Invest.* **70**, 965 (1982).
- Tatemoto, K. & Mutt, V. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6603 (1981).
- Schally, A.V., Baba, Y., Nair, R.M.G. & Bennett, C.A. *J. biol. Chem.* **246**, 6647 (1971).
- Szabo, M., Chu, L. & Frohman, L. *Endocrinology* **111**, 1235 (1982).
- Frohman, L.A., Szabo, M., Berelowitz, M. & Stachura, M.E. *J. clin. Invest.* **65**, 43 (1980).