Virchows Arch [Pathol Anat] (1986) 409:547–554

Virchows Archiv A

Case report

Morphology of a GHRH producing pancreatic islet cell tumour causing acromegaly

W. Saeger, H.M. Schulte, and G. Klöppel

Department of Pathology of the Marienkrankenhaus Hamburg, Department of Medicine, University of Essen, and Institute of Pathology of the University of Hamburg, Federal Republic of Germany

Summary. A 54 year old woman suffered from acromegaly due to a pancreatic islet cell tumour producing GHRH. The tumour was demonstrated on CT scan. The diagnosis was established from elevated plasma levels of GHRH, GH and prolactin, and by the lack of signs of a pituitary adenoma in trans-sphenoidal surgery. Acromegaly was cured by tumour removal. Light microscopically, the tumour showed a medullary and microlobular pattern. The cells were large and often cuspidal. Small granules were found in semi-thin sections. Small aggregations of amyloid fibres were seen, mostly around capillaries. Immunocytochemistry revealed GHRH, NSE, neurotensin, serotonin, VIP and PP. S 100 was positive only in nerve fibres. Staining for GH, ACTH, calcitonin, α-HCG, β-HCG, insulin, glucagon, gastrin, substance P, bombesin and somatostatin was negative. Ultrastructure showed oval partly lobulated nuclei with small nucleoli, moderate amounts of rough endoplasmic reticulum, many free ribosomes, some large Golgi fields and small numbers of secretory granules measuring 150 nm or, in a few cells, 650 nm.

Only 4 other cases of pancreatic endocrine tumours causing acromegaly by ectopic GHRH secretion are described in the literature and these were similar to our case in many respects.

Key words: GHRH – Immunohistology – Pancreatic endocrine tumour – Acromegaly

Introduction

Acromegaly results from excess release of growth hormone (GH), usually due to a GH-secreting pituitary adenoma (Table 1). Non-pituitary endocrine tumours associated with the clinical feature of acromegaly are rare; so far 20 cases have been reported in the literature (Table 1). All of these tumours,

Offprint requests to: W. Saeger, Abteilung für Pathologie des Marienkrankenhauses, Alfredstraße 9, D-2000 Hamburg 76, Federal Republic of Germany

Table 1. Causes of acromegaly

Origin	Type of over-production	Pituitary	Other endocrine disorders	Relative frequency	References
Pituitary tumours					
Adenoma	GH	Unchanged or decreased number of paraadenomous GH cells		Very frequent	Saeger et al. (1986) (review)
Carcinoma	GH	Alterations of paraadenomous pituitary not reported		Very rare (2 cases)	Ogilvy and Jakubowski (1973)
Islet cell tumours of pancreas	GH GHRH	Changes not reported GH cell hyperplasia	1 case Zollinger-Ellison syndrome 1 case multihormonal	1 case Very rare (5 cases)	Melmed et al. (1985) see Table 3
Carcinoid of bronchus	GHRH	GH cell hyperplasia	Cushing's syndrome	Very rare (8 cases)	Scheithauer et al. (1984) (review)
duodenum	GHRH[a], ACTH, GH in metastases	GH cell hyperplasia		1 case	Leveston et al. (1981)
rectum	GHRH[a]	Clinical finding of adenoma		1 case	Buse et al. (1961)
Hypothalamic gangliocytoma	GHRH	GH cell adenoma		Very rare (6 cases)	Asa et al. (1984)

[a] Supposed, not demonstrated

which were found in the lung, the duodenum, the pancreas, the rectum and the hypothalamus proved to be neuroendocrine neoplasms.

Recently, peptides with GH releasing activity were isolated from pancreatic endocrine tumours associated with acromegaly (Guillemin et al. 1982; Rivier et al. 1982). The most potent peptide was the GH releasing hormone (GHRH-44), while two shorter fragments, GHRH-40 and GHRH-37, showed a reduced bioactivity (Böhlen et al. 1983). Further evidence that the GHRH had caused acromegaly by stimulating the GH cells of the pituitary and inducing GH cell hyperplasia (Thorner et al. 1982) was given by the disappearance of the acromegalic syndrome after tumour removal and the demonstration of GHRH immunoreactivity in the pancreatic tumours (Caplan et al. 1978; Berger et al. 1984).

To our knowledge only 4 pancreatic endocrine tumours with GHRH production and acromegaly have been described in the literature to data (Caplan et al. 1978; Berger et al. 1984; Kovacs et al. 1984; Wilson et al. 1984). We present the fifth case which is apparently, the first reported in Germany.

Case report

The patient, a 54-year old woman presented with the typical clinical features of acromegaly and showed elevated plasma levels of GH, prolactin and somatomedin-C. Tomography of the sella did not reveal any abnormalities, but CT scan seemed to demonstrate enlargement suggesting a pituitary tumour. Since subsequent bromocriptine treatment failed to reduce the patients symptoms, trans-sphenoidal surgery was performed which led to the removal of what was believed to be tumour tissue. This specimen, however, contained parts of the neurohypophysis but only very small parts of the adenohypophysis which did not permit adequate studies. By GHRH radioimmunoassay the diagnosis of an ectopic source of GHRH was established and a 10 cm mass was demonstrated by CT scan in the head of the pancreas. Removal of the tumour resulted in a complete remission of the clinical and biochemical features of acromegaly (for details on the clinical and hormonal data of this case see Schulte et al. 1985).

Licht microscopical examination of the pancreatic tumour showed carcinoid-like tissue[1] (Institute of Pathology of the University of Essen).

Methods

Immediately postoperatively, large parts of tumour tissue were frozen for further studies. For morphological investigations specimens were thawed in 3% glutaraldehyde for fixation. For light microscopy and immunohistology tissue was embedded in paraffin after post-fixation in Bouin's solution. For electron microscopy small pieces were post-fixed in osmium tetroxide and embedded in Epon 812.

Anti-GHRH was generated against GRF 1–44 amide in female New Zealand white rabbits using GRF conjugated to BSA by the carbodiimide reaction (Schulte et al. 1985). The antibody did not cross react with GH, somatostatin, GHRH, CRF, and TRH. The immunocytochemistry was performed on deparaffinised consecutive sections (5 µ) using the peroxidase-anti-peroxidase (PAP) or the avidin-biotin-complex method (ABC) with the following antibodies: anti-GHRH (dilution 1:10, PAP), anti-NSE (Dakopatts, Glostrup, Denmark, dilution 1:10, PAP), anti-neurotensin (Immunonuclear, Stillwater, USA, 1:3,000, ABC), anti-S 100 (Dakopatts, 1:20, PAP), anti-serotonin (Immunonuclear, 1:5,000, ABC), anti-VIP (Immunonuclear, 1:5,000, ABC), anti-HPP (Dr. R.F. Chance, 1:10,000, ABC), anti-GH (Kabi, Munich, F.R.G., 1:100, PAP), anti-ACTH (Dakopatts, 1:200, PAP), anti-calcitonin (Immunonuclear, 1:3,000, ABC), anti-α-HCG (own, 1:5,000, ABC), anti-β-HCG (Hybritech-Camon, Wiesbaden, F.R.G., 1:1,000, ABC), anti-insulin (Novo-Res., Copenhagen, Denmark, 1:5,000, ABC), anti-glukagon (Milab, Malmö, Sweden, 1:6,400, ABC), anti-gastrin (own, 1:7,000, ABC), anti-substanz P (Sera-Lab, Camon, Wiesbaden, F.R.G. 1:1,000, ABC), anti-somatostatin (Immunonuclear, 1:5,000, ABC).

Results

The tumour which had been removed from the head of the pancreas, weighed 300 g and measured 10 cm in maximal diameter. The mass was firm and yellowish. Metastases to the lymph nodes or the liver were not observed.

On light microscopy the tumour tissue shows a medullary and microlo-

1 We thank Prof. Dr. L.-D. Leder for his kind permission to study this case in more details

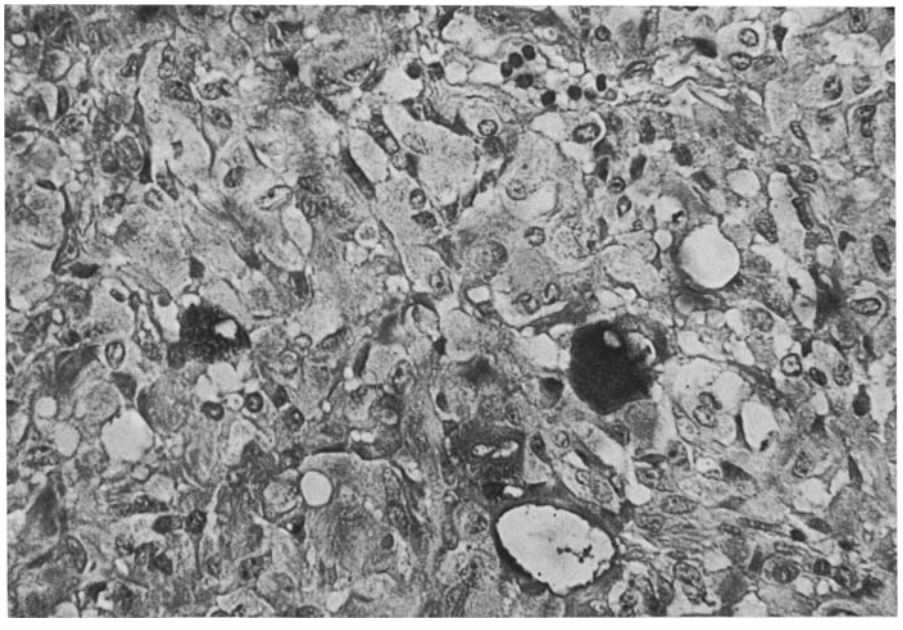

Fig. 1. GHRH producing pancreatic tumour: solid pattern, differently large cells with moderately pleomorphic nuclei. Strong reaction with anti-GHRH in some tumour cells. anti-GHRH-PAP-hematoxylin. × 440

bular pattern. The anastomosing solid tumour complexes are incompletely separated by sparse connective tissue with many capillaries. There is considerable cellular pleomorphism with many polygonal cells showing a slightly acidophilic cytoplasm and round to oval nuclei. The smaller cells have indistinct cell borders and small nuclei with condensed chromatin. Mitotic figures and cell necroses are uncommon. The cytoplasm fails to stain with PAS, but some PAS positive reaction products are found in the intercellular space. Secretory granules are identified only in semi-thin sections of epon embedded tissue. Congo red staining and subsequent polarizing microscopy revealed small starshaped or needle-shaped depositions of amyloid in the perivascular and intercellular spaces with strong birefringence.

GHRH immunoreactivity was found in about 80% of the tumour cells, approximately 5% reacting strongly (Fig. 1). Next most frequent were cells which stained positive for neurotensin and serotonin. Neuron specific enolase (NSE), vasoactive intestinal polypeptide (VIP) and pancreatic polypeptide (HPP) were found only in scattered cells. Growth hormone, corticotropin, calcitonin, α-HCG, β-HCG, insulin, glucagon, gastrin, substance P bombesin and somatostatin were negative. S 100 protein (Fig. 2) was demonstrated in small cells encasing tumour cell complexes. These cells probably correspond to Schwann' cells.

On electron microscopy (Fig. 3) the tumour cells revealed the characteristics of neuroendocrine tissue. Almost every cell contained secretory granules in varying frequencies. The granules measured 100–200 nm in diameter hav-

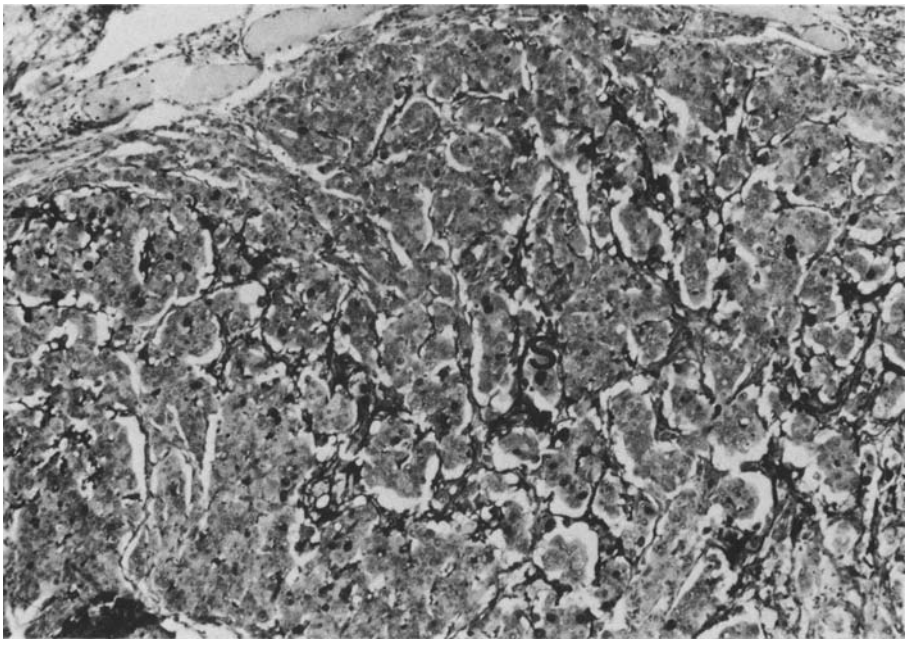

Fig. 2. GHRH producing pancreatic tumour: S 100-reactivity in fibres (S) encasing tumour cell complexes. anti-S 100-PAP. × 125

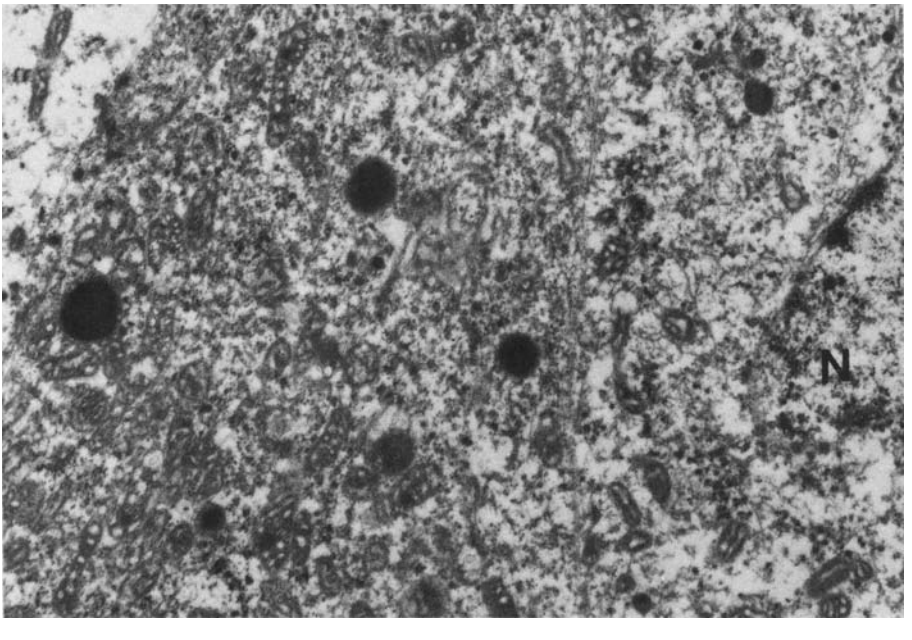

Fig. 3. GHRH producing pancreatic tumour: sparse small and large secretory granules, scattered mitochondria, many microtubules, nucleus (*N*) uranyl acetate-lead citrate. × 12,730

ing a dense core and occasionally a narrow halo. In a few cells large secretory granules measuring about 650 nm were present. Exocytoses were not observed. The Golgi fields were well developed. The membranes of rough endoplasmic reticulum were short or medium-long. Free ribosomes were partly abundant. The mitochondria varied in number showing oval structures and lamellar cristae. Lysosomes were sparse and small. Cytofilaments were sparse. The nuclei were oval or partly lobulated with finely dispersed heterochromatin and small nucleoli.

Discussion

It has been shown that pancreatic endocrine tumours are capable of producing GHRH (Bostwick et al. 1984). In three cases, the sustained hypersecretion of GHRH from pancreatic endocrine tumours had caused acromegaly (Berger et al. 1984; Kovacs et al. 1984; Wilson et al. 1984) (Table 2). In a fourth case which was observed prior to the isolation and characterisation of GHRH, it is quite likely that the pancreatic tumour had also produced GHRH because its removal completely abolished the clinical features of acromegaly in this patient (Caplan et al. 1978).

We now add a fifth case of a pancreatic endocrine tumour with acromegaly, which by immunocytochemistry and radioimmunoassay disclosed intensive production and secretion of GHRH. Due to the uncontrolled release of GHRH from the tumour the patient had high levels of GH and somatomedin C. Presumably, he had also a GH cell hyperplasia of the pituitary, as could be demonstrated in the case of Thorner et al. (1982), but the surgical specimen removed from the pituitary was too small for establishing the diagnosis of a GH cell hyperplasia. Like other endocrine tumours of pancreatic islets our tumour was immunocytochemically positive for NSE. In addition to GHRH, it contained neurotensin, serotonin, VIP and HPP. S 100 reactivity of our case is unusual for a pancreatic endocrine tumour. It could be explained by the existence fo scattered Schwann' cells. Berger et al. (1984) found positivity also for somatostatin, glucagon and hormones of the proopiomelanocortin (Table 2). The patient of Wilson et al. (1984) suffered from an additional Zollinger-Ellison syndrome and thus in this tumour gastrin was also demonstrable (Table 2).

Some tumours of the pancreatic islets or carcinoids of the gut although immunohistologically positive for GHRH do not induce acromegaly (Bostwick et al. 1984), probably because the hormone is biologically inactive or the quantity secreted is too small. The ultrastructure of GHRH tumours in acromegaly described by Caplan et al. (1978), Thorner et al. (1982), Kovacs et al. (1984) and Berger et al. (1984) was similar to our tumour (Table 2). Nevertheless, Caplan et al. (1978) noted more densely developed microtubules, microfilaments and Nebenkerns which were also found by Thorner et al. (1982). In the tumour studied by Thorner et al. (1982) calcification was demonstrable. Some larger secretory granules, found by us, were also seen in the tumour of Caplan et al. (1978). Exocytoses were lacking in all these tumours.

Table 2. GHRH secreting pancreatic islet tumours with acromegaly (literature review of reported cases)

Authors	Age (years) sex	Tumour size	Dignity and specialities	Immunohistology	Electron microscopy
Caplan et al. (1978)	30, ♀	7.5 cm		not performed	Secretory granules 100–250 nm and 300–450 nm, Nebenkerns, many micro-filaments and microtubules
Thorner et al. (1982) Kovacs et al. (1984)	21, ♀	5 cm	Turner's syndrome	GHRH[a] NSE[b]	Secretory granules 150 nm Nebenkerns, calcifications
Berger et al. (1984)	55, ♂	25 cm two connecting masses, whole gland involved	Metastases MEA type 1 multi-hormonal activities	GHRH[b], HPP[b], Somatostatin[a] NSE[b], Glucagon[b], β-Endorphin[b], ACTH[b], α-Endorphin[b], Lipotropin[b]	Secretory granules 100–150 nm
Wilson et al. (1984)	25, ♀	?	Metastases Zollinger-Ellison syndrome	GHRH[b], Gastrin[b] Gastrin-releasing peptide[b]	
This report	54, ♀	10 cm		GHRH[b], NSE[a], (S 100[b]), Neurotensin[b], Serotonin[b], VIP[a], HPP[b]	Secretory granules 150 nm and a few 650 nm

[a] scattered cells positive; [b] more cells positive

The GHRH producing pancreatic endocrine tumours were identified by immunocytochemistry and by electron microscopy as neuroendocrine tumours. According to their structure and hormone content, they may be called "carcinoids". However, it seems to be better to confine the term "carcinoid" to the tumours of the disseminated neuroendocrine cell system (Klöppel 1981; Heitz et al. 1982).

Since metastases were found in two cases (Berger et al. 1984; Wilson et al. 1984), the tumour has a malignant potential. It should therefore be designated as a GHRH producing islet cell tumour. Although it is a very rare lesion GHRH producing tumours of the bronchus or the pancreatic islets have to be suspected if a pituitary adenoma cannot be demonstrated in cases with typical acromegaly in future. For a clear morphological diagnosis GHRH-immunocytochemistry is essential.

References

Asa SL, Scheithauer BW, Bilbao JM, Horvath E, Ryan N, Kovacs K, Randall RV, Laws ER, Singer W, Linfoot JA, Thorner MO, Vale W (1984) A case of hypothalamic acromegaly: a clinicopathological study of six patients with hypothalamic gangliocytomas producing growth-hormone-releasing factor. J Clin Endocr Metab 58:796–803

Berger G, Trouillas J, Bloch B, Sassolas G, Berger F, Partensky C, Chayvialle JA, Brazeau P, Claustrat B, Lesbros F, Girod C (1984) Multihormonal carcinoid tumor of the pancreas secreting growth hormone-releasing factor as a cause of acromegaly. Cancer 54:2097–2108

Böhlen P, Brazeau P, Block B, Ling N, Gaillard R, Guillemin R (1983) Human hypothalamic growth hormone releasing factor (GRF): evidence for two forms identical to tumour derived GRF-44-NH_2 and GRF-40. Biochem Biophys Res Commun 114:930–936

Bostwick DG, Quan R, Hoffman AR, Webber RJ, Chang J-K, Bensch KG (1984) Growth-hormone-releasing factor immunoreactivity in human endocrine tumors. Am J Pathol 117:167–170

Buse J, Buse MG, Roberts WJ (1961) Eosinophilic adenoma of the pituitary and carcinoid tumor of the rectosigmoid area (letter). J Clin Endocr Metab 21:735–738

Caplan RH, Koop L, Abellera RM, Pagliara AS, Kovacs K, Randall RV (1978) Cure of acromegaly by operative removal of an islet cell tumor of the pancreas. Am J Med 64:874–882

Guillemin R, Brazeau P, Böhlen P, Esch F, Ling N, Wehrenberg WB (1982) Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science 218:585–587

Heitz PhU, Kasper M, Polak JM, Klöppel G (1982) Pancreatic endocrine tumors: immunocytochemical analysis of 125 tumors. Hum Pathol 13:263–271

Klöppel G (1981) Endokrines Pankreas und Diabetes mellitus. In: Doerr W, Seifert G (Hrsg) Spezielle pathologische Anatomie, Band 14: Pathologie der endokrinen Organe. Springer, Berlin Heidelberg New York, p 523–728

Kovacs K, Ryan N, Horvath E, Asa SL, Thorner MO, Leong DA, Vale V, Rivier J, Scheithauer BW, Randall RV, Carpenter PC, Caplan RH (1984) Somatoliberinoma: morphologic characteristics. Arch Pathol Lab Med 108:355–356

Leveston SA, MacKeel DW jr, Buckley PJ, Deschryver K, Greider MH, Jaffe BM, Daughaday WH (1981) Acromegaly and Cushing's syndrome associated with a foregut carcinoid tumor. J Clin Endocr Metab 53:682–689

Melmed S, Ezrin C, Kovacs K, Goodman RS, Frohman LA (1985) Acromegaly due to secretion of growth hormone by an ectopic pancreatic islet-cell tumor. New Engl J Med 312:9–17

Ogilvy KM, Jakubowski J (1973) Intracranial dissemination of pituitary adenomas. J Neurol Neurosurg Psychiatr 36:199–205

Rivier J, Spiess J, Thorner M, Vale V (1982) Characterization of a growth hormone-releasing factor from a human pancreatic islet tumour. Nature 300:276–278

Saeger W, Riedel M, Lüdecke DK (1986) Pituitary morphology in acromegaly. International Symposium. Challenge of Hypersecretion: 6H. New York: Raven Press (in press)

Scheithauer BW, Carpenter PC, Bloch B, Brazeau P (1984) Ectopic secretion of a growth hormone-releasing factor. Report of a case of acromegaly with bronchial carcinoid tumor. Am J Med 76:605–616

Schulte HM, Benker G, Windeck R, Olbricht T, Reinwein D (1985) Failure to respond to growth hormone releasing hormone (GHRH) in acromegaly due to a GHRH secreting pancreatic tumor: dynamics of multiple endocrine testing. J Clin Endocr Metab 61:585–587

Thorner MO, Perryman RL, Cronin MJ, Rogol AD, Draznin M, Johanson A, Vale W, Horvath E, Kovacs K (1982) Somatotroph hyperplasia – successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. J Clin Invest 70:965–977

Wilson DM, Ceda GP, Bostwick DG, Webber RJ, Minkoff JR, Pont A, Hintz RL, Bensch KG, Kraemer FB, Rosenfeld RG, Hoffman AR (1984) Acromegaly and Zollinger-Ellison syndrome secondary to an islet cell tumor: characterization and quantification of plasma and tumor human growth hormone-releasing factor. J Clin Endocrinol Metab 59:1002–1005

Accepted March 13, 1986