

## Ectopic Growth Hormone-Releasing Hormone (GHRH) Syndrome in a Case with Multiple Endocrine Neoplasia Type I

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### Abstract

A 36-yr-old man with multiple endocrine neoplasia (MEN) type I had an ectopic growth hormone-releasing hormone (GHRH) syndrome due to a GHRH-secreting pancreatic tumor. The immunoreactive (IR)-GHRH concentration in his plasma ranged from 161 to 400 pg/ml ( $299 \pm 61$  pg/ml, mean  $\pm$  SD; normal,  $10.4 \pm 4.1$  pg/ml), and a significant correlation was found between his plasma IR-GHRH and GH ( $r=0.622$ ,  $p<0.02$ ). After removal of the pancreatic tumor, the high plasma GH concentration returned to nearly the normal range ( $42.2 \pm 31.3$  to  $9.6 \pm 3.8$  ng/ml). These changes paralleled the normalization of his plasma IR-GHRH ( $16.1 \pm 3.8$  pg/ml) and some of his symptoms related to acromegaly improved. However, plasma GH ( $7.7 \pm 1.3$  ng/ml) and IGF-I ( $591 \pm 22$  ng/ml) concentrations were high at 12 months after surgery, suggesting adenomatous changes in the pituitary somatotrophs.

Before surgery, exogenous GHRH induced a marked increase in plasma GH, and somatostatin and its agonist (SMS201-995) completely suppressed GH secretion, but not IR-GHRH release. No pulsatile secretion of either IR-GHRH or GH was observed during sleep. An apparent increase in the plasma GH concentration was observed in response to administration of TRH, glucose, arginine or insulin, while plasma IR-GHRH did not show any fluctuation. However, these responses of plasma GH were reduced or no longer observed one month and one year after surgery.

These results indicate that 1) a moderate increase in circulating GHRH due to ectopic secretion from a pancreatic tumor stimulated GH secretion resulting in acromegaly, and evoked GH responses to various provocative tests indistinguishable from those in patients with classical acromegaly, and 2) the ectopic secretion of GHRH may play an etiological role in the pituitary lesion of this patient with MEN type I.

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Fig. 1. B

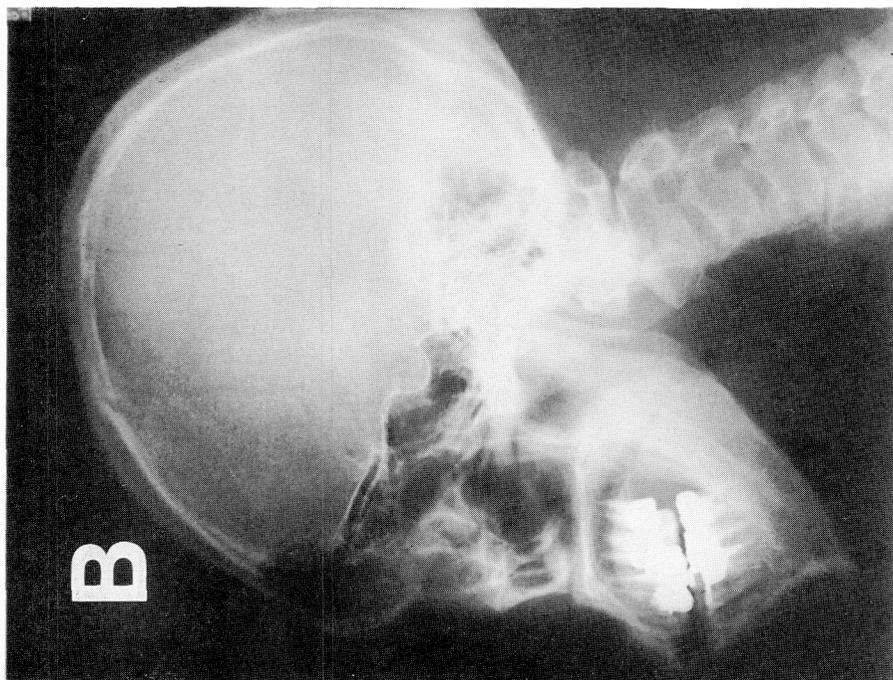
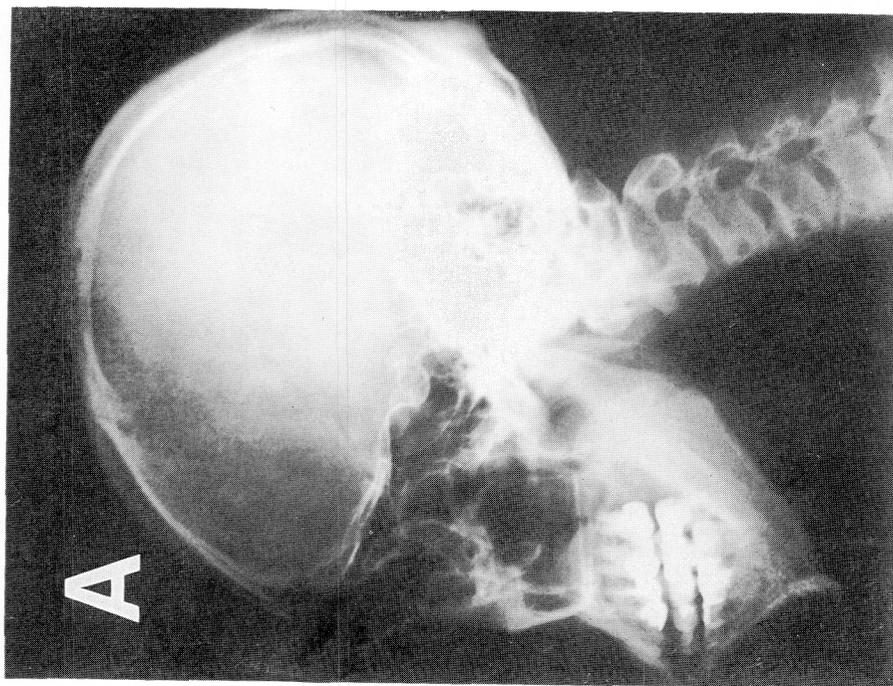
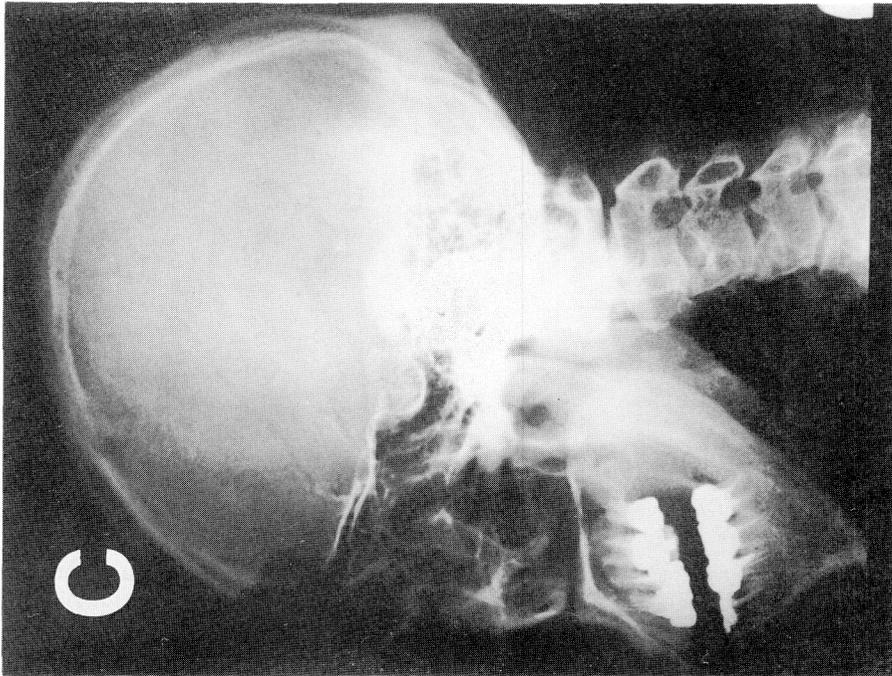


Fig. 1. A



**Fig. 1.** Skull X-ray films showing enlargement of the sella turcica between 1980 and 1986. **A**, October 1980; **B**, February 1983; **C**, February 1986.

Fig. 1. C



Acromegalic symptoms are occasionally induced by ectopic secretion of growth hormone (GH)-releasing hormone (GHRH) from tumors, which has been called the "ectopic GHRH syndrome" (Frohman *et al.*, 1980). In 1982, Guillemin *et al.* (1982) and Rivier *et al.* (1982) independently isolated human pancreatic GHRH from pancreatic tumors that caused acromegaly, and subsequently the identity of this GHRH with human hypothalamic GHRH was demonstrated (Böhlen *et al.*, 1983, Ling *et al.*, 1984). The development of a radioimmunoassay (RIA) for measuring plasma immunoreactive (IR)-GHRH enabled us to differentiate the ectopic GHRH syndrome from so-called "classical acromegaly" and also clarify the mechanism of regulation of GH secretion in this pathophysiological state. Recent studies in some acromegalic patients with ectopic GHRH-producing tumor revealed that their plasma IR-GHRH concentrations were measurable in nanogram

per ml (Thorner *et al.*, 1984, Penny *et al.*, 1984, Wilson *et al.*, 1984, von Werder *et al.*, 1984, Ch'ng *et al.*, 1985, Schulte *et al.*, 1985, von Gotteschalk *et al.*, 1986, Barkan *et al.*, 1986, Roth *et al.*, 1986, Boizel *et al.*, 1987).

We report the clinical features of a patient with multiple endocrine neoplasia (MEN) type I showing the ectopic GHRH syndrome. His plasma IR-GHRH levels were considerably lower than those in the patients reported previously. We discuss here the pathophysiological role of GHRH in the regulation of GH secretion and in the formation of the acromegalic state.

## Materials and Methods

### Case Report

A 36-yr-old man was referred to our department in 1985 to clarify the cause of acromegaly. He had first been admitted to the Department of Urology of Tokushima University Hospital at 31 years of age for diagnostic evaluation of the

etiology of a renal stone. The cause was determined to be primary hyperparathyroidism. On physical examination at that time, he was found to have acromegalic features (diaphoresis, lip enlargement and low-grade frontal bossing). Abnormal laboratory values included the elevated basal GH secretion (5.5–8.4 ng/ml in 3 measurements), paradoxical plasma GH rise after thyrotropin-releasing hormone (TRH) (peak value, 34 ng/ml at 30 min) and an oral glucose load (16 ng/ml at 180 min). He underwent parathyroidectomy three times between 1980 and 1983 and chief cell hyperplasia was demonstrated histologically. In 1983, he was diagnosed as having MEN type I, since a tumor of 4.4×6.5 cm was found in the tail of the pancreas by computed tomographic (CT) scanning and his family history. His father had suffered from primary hyperparathyroidism and Cushing's disease and his younger sister had primary hyperparathyroidism.

On admission in January, 1986, his acromegalic symptoms seemed to progress in spite of bromocriptine therapy (7.5–15 mg/day) for 3 years, but CT scanning of the abdomen showed no enlargement and no metastasis of the pancreatic tumor. Enhanced brain CT scanning demonstrated a "hypodense area" in the enlarged sella turcica. Interestingly, skull X-ray films showed that his sella turcica had gradually enlarged between 1980 and 1986 (Fig. 1). His plasma IR-GHRH, GH and insulin-like growth factor (IGF)-I were increased (*see Results*). The concentrations of other plasma pituitary hormones, serum thyroid hormones and cortisol were normal. The concentrations of plasma insulin, glucagon, gastrin and somatostatin were 27.0  $\mu$ U/ml (normal, 2.5–16.5  $\mu$ U/ml), 160 pg/ml (33.0–256.9 pg/ml), 64 pg/ml (40–140 pg/ml) and 21 pg/ml (5.0–27.0 pg/ml), respectively. His serum Ca, plasma PTH and calcitonin and urinary 5-hydroxyindoleacetic acid were also within the normal range. In March 1986, two pancreatic tumors (large tumor, 30 g; small tumor, 2.1 g) were enucleated. After surgery, diaphoresis and soft tissue dimensions decreased. Twelve months after surgery, no changes in brain CT scanning results were observed.

On immunohistochemical examination of the large tumor, many GHRH-IR cells and a few somatostatin- and calcitonin-positive cells were observed (Sano *et al.*, 1987). In the small tumor, glucagon- and pancreatic polypeptide-IR

cells were observed, but no GHRH-IR cells were detected. No GH-IR cells were demonstrated in either tumor. The concentration of GHRH in the large tumor was 13.2  $\mu$ g/g wet weight and in both tumors that of GH was below the detection limit (1 ng/g wet weight). Northern blot analysis of RNA extracted from the large tumor using a GHRH cDNA probe provided by Dr. Gubler, Hoffmann-La Roche Inc., Nutley, USA, demonstrated a single band of mRNA consisting of about 800 nucleotides (unpublished data).

### *Clinical Studies*

Plasma IR-GHRH and GH were measured under the following conditions: 1) serial blood samplings before, during and after removal of the pancreatic tumors; 2) after a bolus intravenous injection of 100  $\mu$ g of synthetic GHRH (1–44) NH<sub>2</sub> (Sumitomo Pharmaceutical Co., Osaka, Japan) before, and one month and one year after surgery; 3) after a bolus injection of synthetic somatostatin-14 (Protein Research Foundation, Osaka, Japan) (250  $\mu$ g/5 ml saline) and then its infusion at a constant rate (10  $\mu$ g/min) for 40 min; 4) after subcutaneous injection of 50  $\mu$ g somatostatin analogue (SMS 201–995, Sandoz Pharmaceutical Co. Ltd., Tokyo, Japan); 5) during sleep, monitored by electroencephalography (EEG) and electrooculography (EOG) before surgery; and 6) after administration of TRH (500  $\mu$ g, iv), glucose (75 g, orally), arginine (0.5 g/kg, 30-min infusion) and insulin (0.1 U/kg, iv) before, and one month and one year after surgery.

All blood samples were taken from the antecubital vein, put into chilled test tubes containing 500 KIU of aprotinin and 1.2 mg of EDTA per ml of blood, and centrifuged at 3000 rpm at 4°C for 20 min. The plasma were stored at –30°C until analysis.

### *Extraction and Gel-filtration Chromatography of GHRH from plasma*

Each plasma sample was extracted with cold acetone/glacial acetic acid (100:3, vol/vol) and petroleum ether (20 ml) to eliminate substances causing non-specific interference in the RIA system, and an additional tube containing the same amount of plasma extract but no anti-GHRH serum was also set up to evaluate the rate of non-specific binding of the tracer.

The extract prepared in the same manner from 15 ml of plasma obtained before surgery

was applied to a column (1.6×85 cm) of Sephadex G-50 (fine) and eluted with 0.3 N acetic acid at a flow rate of 15 ml/hour, at 4°C. The effluent was collected in 3 ml fractions and lyophilized. The lyophilized materials were dissolved in assay buffer (0.1 M phosphate buffer, pH 7.4, containing 0.14 M NaCl, 0.05 M EDTA, 0.01% NaN<sub>3</sub>, 0.1% BSA, and 0.1% Triton X-100) and subjected to RIA for GHRH.

#### RIAs of GHRH, GH and IGF-I

Plasma IR-GHRH was measured as reported previously (Saito *et al.*, 1984). Synthetic GHRH (1-44) NH<sub>2</sub> (Takeda Chemical Industries, LTD., Osaka, Japan) was iodinated by the chloramine T method and iodinated peptide was purified on a column (1×10 cm) of carboxymethyl cellulose (CM 23, Whatman Ltd., Maidstone, Kent, England). The anti-GHRH serum (RAS-8061, Peninsula Lab., San Carlos CA, USA; lot# 004118) used in this study did not cross-react with various neuropeptides including hypothalamic regulatory hormones and peptides of the glucagon-secretin family and was shown to recognize the N-terminus and middle portion of GHRH (1-44) NH<sub>2</sub>. The sensitivity of this assay was 4 pg/tube and the intra- and inter-assay coefficients of variation were below 15%. The mean recovery rate of GHRH added to plasma was 55%. The plasma IR-GHRH values shown are not corrected for the recovery rate.

Plasma GH was measured by double antibody RIA using a GH RIA kit provided by the

National Hormone and Pituitary Program, NIAMDD, Bethesda, MD, USA. The sensitivity of this assay was 0.2 ng/ml and the intra- and inter-assay coefficients of variation were below 10%.

Plasma IGF-I was measured by RIA for IGF-I using recombinant DNA-derived IGF-I, as reported previously (Inoue *et al.*, 1986).

All data are expressed as means±standard deviations (SD). The significance of differences between values was examined by Student's *t*-test.

## Results

The average plasma IR-GHRH level in repeated determinations before surgery (between June, 1985 and May, 1986) was 299±61 pg/ml (161–400 pg/ml, n=16), which was about 30-fold that in normal adults (10.4±4.1 pg/ml, n=72) and acromegalic subjects (8.0±3.9 pg/ml, n=15). The average plasma GH concentration in the same samples was also elevated at 42.4±31.3 ng/ml (range, 9.1–125 ng/ml). There was significant correlation between the values for plasma IR-GHRH and GH ( $r=0.622$ ;  $p<0.02$ , n=16) (Fig. 2).

The elution pattern of IR-GHRH in a plasma extract on gel-filtration chromato-

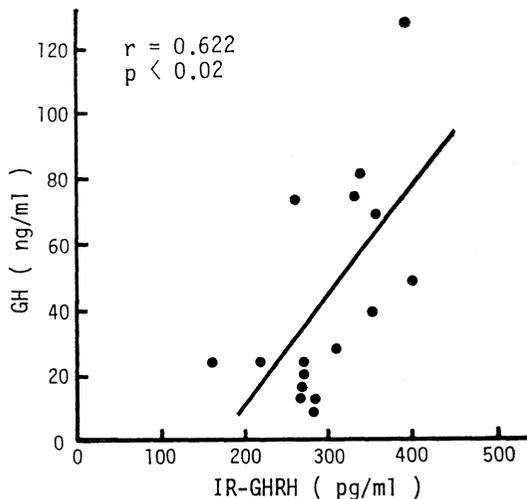
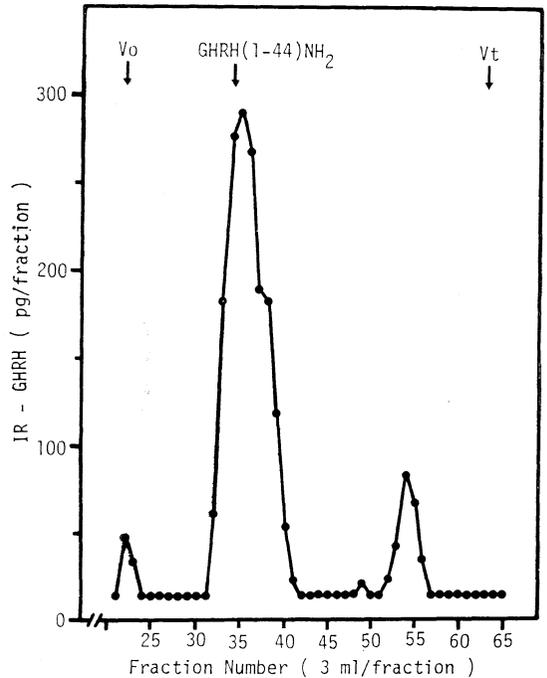


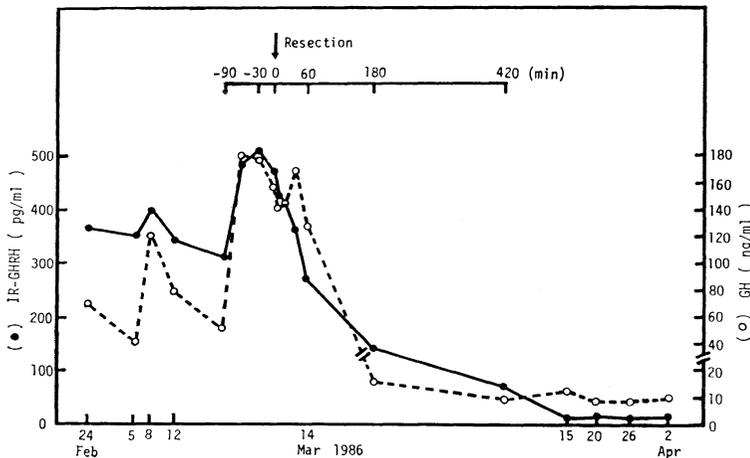
Fig. 2. Correlation between IR-GHRH and GH concentrations in 16 plasma samples obtained before surgery (between June, 1985 and May, 1986).

graphy is shown in Fig. 3. About 86% of the total GHRH-like immunoreactivity was eluted in the same position as synthetic GHRH (1-44)NH<sub>2</sub>. The remainder was distributed in two small fractions; one near the void volume and the other in positions corresponding to molecular weights of 1000-2000. With another RIA system using anti-GHRH serum (RG-107) (Sano *et al.*, 1986), which recognizes GHRH (1-44)NH<sub>2</sub> but not nonamidated GHRH fragments including GHRH (1-40)OH and GHRH (1-37)OH, GHRH-like immunoreactivity was also detected in the same position as synthetic peptide.

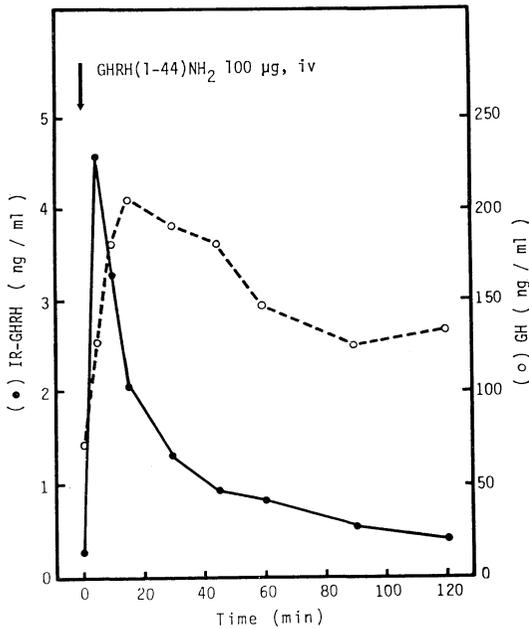
As shown in Fig. 4, after resection of the pancreatic tumors, the increased plasma IR-GHRH decreased to within the normal range ( $16.1 \pm 3.8$  pg/ml,  $n=7$ ). The half disappearance rate ( $T_{1/2}$ ) of IR-GHRH from the plasma was calculated to be about 30 min. The high plasma GH concentration decreased to nearly normal in parallel with the decrease in plasma IR-GHRH, but not to the normal range ( $9.6 \pm 3.8$  ng/ml,  $n=8$ ;  $p < 0.005$ , *vs* before surgery). The increased plasma IGF-I concentration ( $616 \pm 42$  ng/ml,  $n=6$ ; normal adults,  $147 \pm 49$  ng/ml,  $n=156$ ; acromegalic subjects,  $562 \pm 115$  ng/ml,  $n=7$ ) before surgery did not change (after



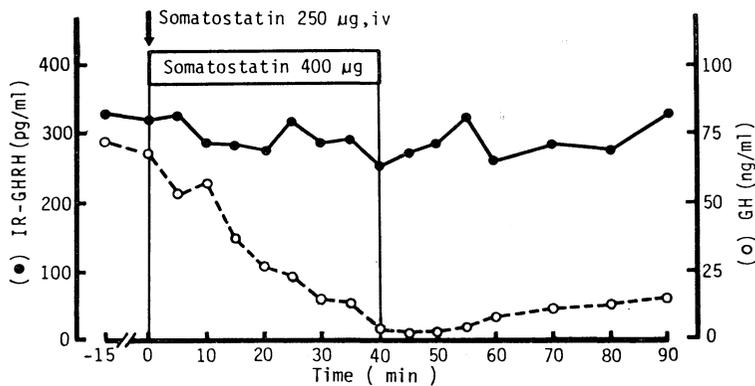
**Fig. 3.** Distribution of IR-GHRH in fractions of plasma extract from the patient on a Sephadex G-50 fine column (1.6×85 cm). The column was calibrated with blue dextran (Vo), <sup>125</sup>I-GHRH (1-44)NH<sub>2</sub> and Na<sup>125</sup>I (Vt).



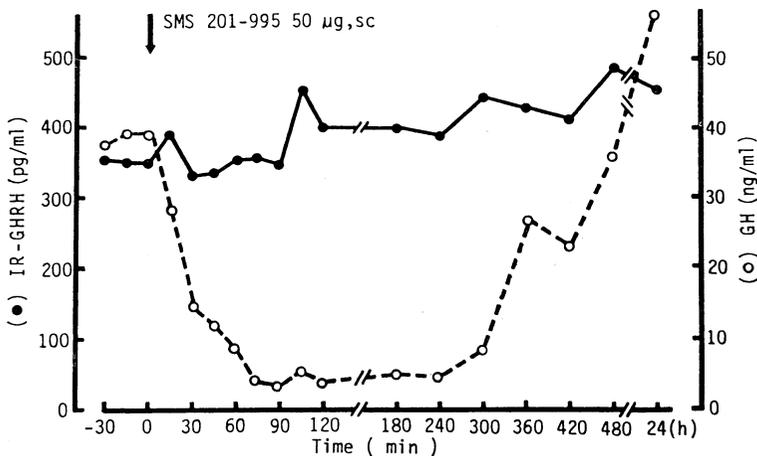
**Fig. 4.** Changes in plasma IR-GHRH and GH before, during and after resection of the pancreatic tumor. The half disappearance rate of IR-GHRH from the plasma ( $T_{1/2}$ , approximately 30 min) was calculated by direct extrapolation from the slope of the curve for decrease in plasma concentration of IR-GHRH plotted logarithmically against the time after resection of the pancreatic tumor.

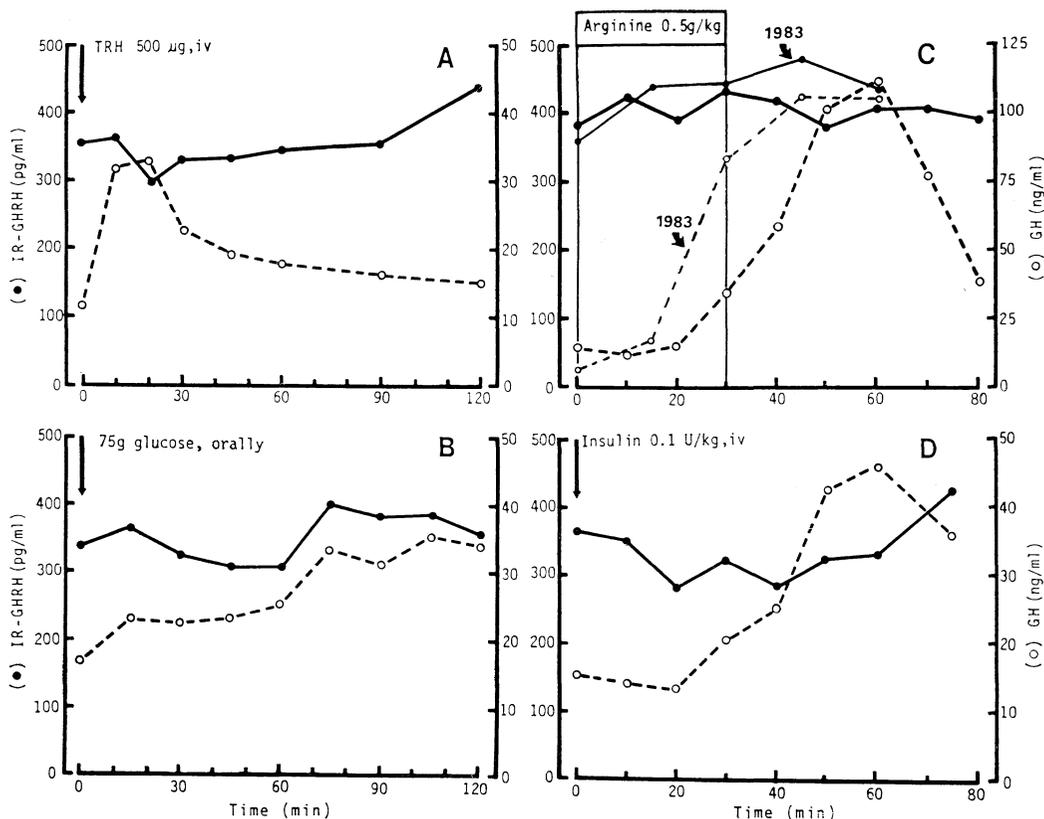


**Fig. 5.** Plasma IR-GHRH and GH after iv administration of exogenous GHRH (1-44) NH<sub>2</sub> (100 µg) before surgery.



**Fig. 6.** Effects of somatostatin-14 (250 µg iv bolus and 10 µg/min over 40 min) and SMS-201-995 (50 µg sc) on plasma IR-GHRH and GH before surgery.





**Fig. 7.** Effects of administrations of TRH (500  $\mu$ g, iv; A), glucose (75 g, orally; B), arginine (0.5 g/kg, 30 min infusion; C) and insulin (0.1 U/kg, iv; D) on plasma IR-GHRH and GH before surgery.

surgery,  $595 \pm 49$  ng/ml,  $n=5$ ). At 12 months after surgery, plasma GH ( $7.7 \pm 1.3$  ng/ml,  $n=6$ ) and IGF-I ( $591 \pm 22$  ng/ml,  $n=6$ ) concentrations were still high.

Before surgery, plasma GH increased rapidly after GHRH (1-44)  $\text{NH}_2$  administration, reaching a maximum of 205 ng/ml after 15 min (Fig. 5). Plasma IR-GHRH increased from the basal value of 260 pg/ml to a maximum of 4590 pg/ml after 5 min. The effects of synthetic somatostatin-14 and its long-acting agonist (SMS201-995) on plasma IR-GHRH and GH before surgery are shown in Fig. 6. Both somatostatin and SMS201-995 completely suppressed GH secretion, but failed to inhibit GHRH

release. The rebound surge of GH was not observed when somatostatin infusion was discontinued.

Neither IR-GHRH nor GH in the plasma showed any remarkable changes even in the slow wave stage during sleep.

As depicted in Fig. 7, intravenous administration of TRH or an oral glucose load evoked a paradoxical increase in plasma GH and no apparent fluctuation in the plasma IR-GHRH concentration was observed in either test. On the other hand, a marked increase in plasma GH in response to arginine infusion or insulin hypoglycemia (blood glucose level; 109 to 60 mg/dl) was observed without any significant change in

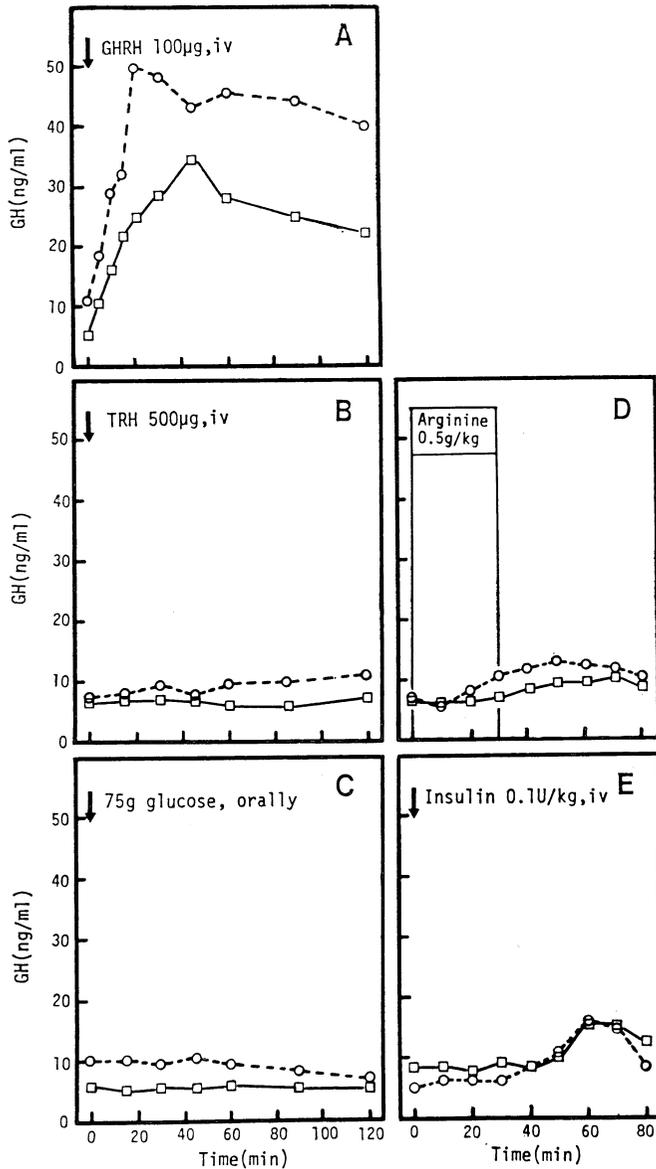


Fig. 8. Changes in plasma GH concentration in response to exogenous GHRH (1-44) NH<sub>2</sub> (A), TRH(B), glucose (C), arginine (D) and insulin (E) one month (○---○) and one year (□—□) after surgery.

the plasma IR-GHRH concentration. A similar result was obtained in an arginine infusion test in 1983.

The effects of GHRH (1-44) NH<sub>2</sub>, TRH, glucose, arginine or insulin on plasma GH were examined one month and one year after removal of the pancreatic tumors, and

results are shown in Fig. 8. At one month, an apparent increase in plasma GH was observed in response to exogenous GHRH (1-44) NH<sub>2</sub>, but the rate of increase ( $\Delta$ GH at 15 min, 46.5 ng/ml) was considerably lower than that before surgery (114 ng/ml). In addition, the paradoxical rise in plasma

GH in response to TRH or glucose load was abolished after removal of the pancreatic tumors. As in the GHRH test, the responses of GH release to arginine infusion and insulin hypoglycemia (blood glucose concentration: 116 to 49 mg/dl) were less than before surgery. At twelve months, the responses of GH release in these endocrine tests (insulin hypoglycemia: 120 to 48 mg/dl) were similar to those one month after removal of the pancreatic tumors.

### Discussion

In this paper we examined the clinical features and changes in plasma IR-GHRH and GH in a patient with MEN type I secondary to ectopic GHRH syndrome before and after resection of the pancreatic tumor. We also demonstrated GHRH mRNA and GHRH-IR cells in the excised tumor tissue.

The plasma IR-GHRH concentration was about 30-fold higher than normal, but considerably lower than in the cases of ectopic GHRH-producing tumors reported previously. However, GHRH released from the tumor in the present case seemed to play a pathophysiological role in hypersecretion of GH resulting in acromegaly. The reasons for this conclusion are as follows: 1) A significant correlation was found between the concentrations of IR-GHRH and GH in the plasma before surgery. 2) After removal of the GHRH-producing pancreatic tumor, plasma GH decreased significantly to nearly the normal range with slight remission of acromegalic symptoms. 3) Plasma IR-GHRH was about 350 pg/ml, which is enough to stimulate GH secretion as reported by Frohman *et al.* (1984). In addition, most of the GHRH-like immunoreactivity in the plasma was the same molecular size as GHRH (1-44)NH<sub>2</sub>, as shown by gel-filtration chromatography, and the T<sub>1/2</sub> of about 30 min was similar to that of exogenous GHRH administered

to humans (T<sub>1/2</sub>; 41.3±3.0 min) (Frohman *et al.*, 1984).

The absence of a GH response to exogenous GHRH in patients with the ectopic GHRH syndrome was reported by Ch'ng *et al.* (1985), Schulte *et al.* (1985) and Boizel *et al.* (1987), suggesting that the somatotrophs in their patients were fully sensitized by ectopic GHRH. In acromegalic patients this phenomenon indicates the presence of an ectopic GHRH-producing tumor. However, in our case, a marked rise in plasma GH was evoked by exogenous GHRH administration as observed by Barkan *et al.* (1986), possibly because somatotrophs had not been stimulated maximally by ectopic GHRH secretion. Therefore, patients with the ectopic GHRH syndrome may not show a consistent pattern of GH responsiveness to exogenous GHRH, and the determination of circulating GHRH should be necessary to confirm this syndrome.

Both somatostatin and SMS201-995 completely suppressed GH secretion, but unexpectedly, did not inhibit IR-GHRH release in our patient. There are reports (von Werder *et al.*, 1984, Ch'ng *et al.*, 1985, Barkan *et al.*, 1986) that the release of GHRH as well as GH in cases of metastatic GHRH-producing tumors was significantly suppressed by SMS201-995 and somatostatin when used as maintenance therapy. However, we demonstrated that the release of GHRH from monolayer cultures of pancreatic tumor cells from the patient was inhibited by incubation for 48 h with 10 nM somatostatin (unpublished observation). This discrepancy may be explained at least in part by the fact that the concentrations of somatostatin and SMS201-995 were not great enough to inhibit GHRH release from the tumor *in vivo*.

No apparent fluctuations in the plasma IR-GHRH and GH were observed during sleep, in spite of the GH response to exogenous GHRH and somatostatin. This sug-

gests that inhibition of hypothalamic GHRH release may occur through a negative feedback mechanism by excessive secretions of plasma GHRH and/or GH. On the other hand, recent studies by Hizuka *et al.* (1985) and Rochiccioli *et al.* (1986) demonstrated that continuous infusion of GHRH accelerated pulsatile GH secretion during sleep throughout the night in children with GH deficiency. Their studies suggest that continuous GHRH infusion may induce a periodic surge of hypothalamic somatostatin. If this is the case, patients with the ectopic GHRH syndrome may not show a periodic surge of hypothalamic somatostatin release.

The paradoxical rise in the plasma GH concentration in response to TRH before surgery was abolished after removal of the GHRH-producing pancreatic tumor, as in the two cases reported by Thorner *et al.* (1982) and Schulte *et al.* (1985), while only one reported case showed a diminished GH response to TRH after surgery (Boizel *et al.*, 1987). In an *in vitro* experiment by Borges *et al.* (1983), the addition of TRH to the perfusate during GHRH perfusion of rat anterior pituitary cells resulted in further stimulation of GH release, suggesting that TRH can stimulate the pituitary after sensitization with a maximal concentration of GHRH. However, the paradoxical GH response after TRH could occur without maximal exposure of the somatotroph to GHRH, since such GH release was observed in our patient, who showed a further GH rise in response to exogenous GHRH. Simultaneously, abolishment of a paradoxical increase in plasma GH in response to an oral glucose load was also observed after surgery. These findings indicate that the paradoxical GH rise is modulated by ectopic GHRH or unidentified factors induced by excess GHRH, which stimulate the somatotroph chronically but not maximally. On the other hand, after surgery the responses of GH release to arginine infusion and insulin hypoglycemia

were diminished. Therefore, the effects of arginine and insulin on GH release were enhanced by ectopic secretion of GHRH.

In our patient, a paradoxical rise in plasma GH in response to TRH and an oral glucose load was observed more than 6 years before surgery, GHRH may have been released from his pancreatic tumor from that time. In fact, a moderate increase in circulating GHRH and a pancreatic tumor were detected 3 years previously. Interestingly, skull X-ray films showed that his sella turcica became gradually enlarged, and an enhanced brain CT scanning showed a "hypodense area" in the pituitary, which may have been due to bromocriptine therapy or infarction. Therefore, ectopic secretion of GHRH from the pancreatic tumor stimulated his pituitary chronically (for more than 6 years), and resulted in development of an acromegalic state. His plasma GH and IGF-I did not return to normal until at least 12 months after surgery despite the fact that the plasma GHRH concentration became normal. The co-existence of an ectopic GH-producing tumor (Melmed *et al.*, 1985) was excluded in this patient by immunohistochemistry and a biochemical study of his pancreatic tumors. Delay in recovery of the hypothalamo-pituitary axis seems to be unlikely, because all previous cases without pituitary tumors showed normalization of the plasma GH concentration soon after resection of the GHRH-producing tumor. Therefore, it is most probable that adenomatous changes occurred in his pituitary somatotrophs as in a case reported by Roth *et al.* (1986). However, the possibility that pituitary adenoma may arise primarily independent from chronic stimulation by ectopic GHRH cannot be excluded, since the pituitary lesion is considered to be one of the phenotypes in MEN type I.

An association of pituitary and pancreatic tumors is frequently seen in MEN type I. The pathogenesis of the pancreatic

tumor is unknown, but pituitary hyperplasia or adenoma could be induced by ectopic over-production and secretion of releasing hormone with biological activity, such as GHRH which acts on the pituitary. Indeed, in our case and others reported previously (Wilson *et al.*, 1984, Ch'ng *et al.*, 1985, Schulte *et al.*, 1985, Thorner *et al.*, 1982, Aida *et al.*, 1977, Caplan *et al.*, 1978, Sassolas *et al.*, 1983), ectopic secretion of GHRH released from the pancreatic tumor may have been involved in pituitary lesions. Recently, Roth *et al.* (1986) reported a case of somatotroph hyperplasia and acromegaly secondary to a pheochromocytoma secreting catecholamines and GHRH. Thus, multiple endocrine syndromes including MEN may be caused at least in part by a single neoplasm, but further studies are required to establish their etiology.

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