Effects of Neuromedin B and GRP-10 on Gastrin and Insulin Release from Cultured Tumor Cells of a Malignant Gastrinoma

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Abstract

A study relating to gastrin release from gastrinoma cells by neuromedin B and C-terminal decapeptide of gastrin releasing peptide (GRP-10) has not yet been reported. Therefore, we studied the effects of neuromedin B and GRP-10 on gastrin release from cultured dispersed cells prepared from both the primary tumor in the pancreas and the metastatic tumor in the liver from a case of malignant Zollinger-Ellison syndrome. Both the primary and metastatic tumors obtained by a curative operation contained similar concentrations of gastrin and glucagon, whereas the primary tumor contained 10 times more insulin than the metastatic tumor. Gastrin release from cultured cells of both tumors was suppressed by 0.1 and 10 nM neuromedin B and tended to be suppressed by 0.1-10 nM GRP-10. However, insulin release from cultured cells of the pancreatic tumor was stimulated by GRP-10, but not by neuromedin B. These results might suggest that receptor function for the bombesin family peptides is abnormal in gastrinoma cells in both primary and metastatic tumors, and that a major source of insulin secretory cells is the contaminated normal islet cells in the primary tumor.

Zollinger-Ellison syndrome is characterized by recurrent peptic ulceration, marked gastric acid hypersecretion and islet cell tumors secreting gastrin (Zollinger and Ellison, 1955; McGuigan and Trudeau, 1969). Its incidence is low, particularly in Japan where only about 100 cases were reported prior to 1982 (Kishimoto and Miyoshi, 1983). Gastrin release from this tumor is abnormal; it is not suppressed by secretin (Lamers and Van Tongeren, 1977; McGuigan and Wolfe, 1980), and is weakly stimulated by bombesin (Basso et al., 1981; Jansen and Lamers, 1982). Neuromedin B and C-terminal decapeptide of gastrin releasing peptide (GRP-10) are mammalian bombesin-like peptides (Mina-
minato et al., 1983; Okada et al., 1984; Reeve et al., 1983) which stimulate the release of gastrin, insulin and glucagon in a similar manner to bombesin (Spindel, 1986; Kawai et al., 1988). However, no study relating to gastrin release from gastrinoma by these peptides has yet been reported.

The effects of neuromedin B and GRP-10 on gastrin release from cultured dispersed cells, from both the primary tumor in the pancreas and the metastatic tumor in the liver obtained from a case of malignant Zollinger-Ellison syndrome, are reported.

Case Report

A 43-year-old woman had suffered from epigastric pain and diarrhea for 7 years. A duodenal ulcer was diagnosed on the basis of her symptoms and antiacids prescribed by a physician. The symptoms sometimes relapsed and she received subtotal gastrectomy 9 months before her admission to the University Hospital. However, the duodenal ulcer recurred and a high plasma gastrin level was found (1090 pg/ml, normal value 40–200 pg/ml). The physician suspected Zollinger-Ellison syndrome and referred her to the University Hospital. She had no family history of peptic ulcer diseases.

The patient was 147 cm in height and 54 kg in weight, and seemed to be healthy. Her blood pressure was 120/88 mmHg. No abnormal findings were observed in the physical examination except for an operation scar in her abdomen. The blood cell count on admission showed her to be slightly anemic; RBC was $350 \times 10^4$ mm$^3$, Hb, 10.0 g/dl, and hematocrit, 39.9%. Urinalysis and blood chemistry yielded normal values; serum total protein was 7.0 g/dl, albumin, 4.0 g/dl, Ca$^{++}$ 8.9 mg/dl, and inorganic phosphate 4.5 mg/dl.

Endocrinological data on admission are shown in Table 1. The basal gastrin level was extremely high and it was not sup-

<table>
<thead>
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<th>Table 1. Endocrinological data on admission</th>
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<tbody>
<tr>
<td>Gastrin: |</td>
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<tr>
<td>Secretin injection test: |</td>
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<tr>
<td>Time (min)</td>
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<tr>
<td>Serum gastrin (pg/ml)</td>
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<td>Mixed meal loading: |</td>
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<tr>
<td>Time (min)</td>
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<tr>
<td>Serum gastrin (pg/ml) |</td>
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<td>before operation</td>
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<tr>
<td>Oral glucose tolerance test: |</td>
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<tr>
<td>Time (min)</td>
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<tr>
<td>Plasma glucose (mg/dl)</td>
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<tr>
<td>Serum insulin (µU/ml)</td>
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<td>Basal hormone levels in plasma: |</td>
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<td>Thyroxine 8.2 µg/dl, Triiodothyronine 93 ng/dl, TSH 1.7 µU/ml, Calcitonin 40 pg/ml, Parathyroid hormone 0.3 ng/ml (RIA with C-terminal-specific antibody), Growth hormone 3.6 ng/ml, ACTH 35 pg/ml, Prolactin 21 ng/ml, Luteinizing hormone 15.0 mIU/ml, Follicle-stimulating hormone 11.2 mIU/ml, Cortisol 9.1 µg/dl, Aldosterone 15.0 ng/dl.</td>
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pressed to normal by injecting secretin (2 U/kg Secrepan®, Eisai, Tokyo, Japan). The response of gastrin release to a mixed meal was low. A 75 g oral glucose loading test revealed an abnormal increase in plasma glucose level, the so called “oxyhyperglycemia” type, probably due to subtotal gastrectomy. Plasma insulin increased with a pattern of delayed response from its high fasting level (normal value 3~18 μU/ml). Basal levels of thyroid, parathyroid, pituitary and adrenal hormone were normal. Urinary excretion of epinephrine, norepinephrine, metanephrine and normetanephrine were normal at 8.1, 77.4, 40 and 100 μg/day, respectively.

Basal acid output was 35.2 mEq/hr and the maximum acid output in response to tetragastrin (4 μg/kg Gastpsin®, Nihonkayaku, Tokyo, Japan) was 39.6 mEq/hr. Abdominal ultrasound, CT scanning and angiograms revealed a tumor in the pancreatic body and multiple metastatic tumors in the liver. CT scanning of the pituitary and ultrasound of the neck were normal.

From these results, the diagnosis of Zollinger-Ellison syndrome was established with a primary tumor in the pancreas and multiple metastatic tumors in the liver. No positive data for multiple endocrine neoplasia were found. She received a resection of the body and tail of the pancreas together with the spleen and residual stomach, and a partial resection of the liver and an enucleation of hepatic metastatic tumors.

The primary tumor nodule was a solitary, solid and yellowish mass; 55 mm in size at its largest diameter and weighing 69 gr. Fig. 1 shows the microscopic findings for the primary and metastatic tumors. Histologically, the arrangement of the primary tumor cells exhibited combinations of a trabecular pattern, solid nests and a gyriform pattern. The tumor cells varied in shape, i.e. ovoid to polygonal, and the tumor cells were larger than normal islet cells. Cytoplasm of the tumor cells was

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**Fig. 1 A**

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**Fig. 1.** Histological observation of the primary tumor in the pancreas (A), capsular margin of the primary tumor (B), and the metastatic tumor in the liver (C). Hematoxylin-eosin staining (×100). Primary tumor (A): Solid
tumor growth with scanty stroma. Note the polygonal shaped cells and the round nuclei. Capsular margin of the primary tumor (B): The compressed remaining Langerhans' islets are seen at the right hand side of the photo. Liver metastasis (C): The tumor with thin fibrous capsule exhibiting a solid pattern and scanty stroma.
abundant, and the staining characteristics were granular and acidophilic. The nuclei were often giant hyperchromatic. Mitotic figures were rarely seen. In the primary nodule, capsular invasion was prominent, and the tumor cells protruded in the pancreatic duct, obstructing the lumen.

After the operation, her plasma gastrin level fell to 50 pg/ml and she was discharged.

**Materials and Methods**

**Materials**

Neuromedin B and GRP-10 used in this study were synthesized by the solid phase method (Mukai et al., 1987). They were purified by gel filtration and reverse phase high-performance liquid chromatography described previously (Mukai et al., 1987), and proved to be at least 98% pure.

**Extraction of hormone from tumors**

0.3 g each of primary pancreatic tumor and hepatic metastatic tumor were placed into 3 ml of boiling 0.1 N acetic acid for 10 min and homogenized with a Polytron (Brinkman). The homogenate was centrifuged for 30 min at 100,000×g at 4°C. The supernatant was lyophilized and dissolved in 0.2 M glycine buffer pH 8.8 containing 0.25% human serum albumin and 1% sheep serum.

**Cell culture of tumors**

0.4 g each of primary pancreatic tumor and metastatic hepatic tumor were chopped into small pieces with scissors in Ca++-and Mg++-free Dulbecco’s modified Eagle’s medium (DMEM) (Gibco, Grand Island, NY, USA). After centrifugation at 600×g for 5 min at 4°C, the pieces were dispersed by DMEM containing 0.1% collagenase (Type IV, Worthington, Freehold, NY, USA) for 2 hr at 37°C. The dispersed cells were washed 5 times with Ca++-and Mg++-free DMEM. The washed dispersed cells were plated in tissue culture wells previously coated with a thin film of polymerized type a collagen prepared from rat tails (24 mm multiwell plates, Corning Glass works, Corning, NY, USA) and incubated in DMEM supplemented with 10% fetal calf serum and gentamicin (40 μg/ml) in humidified, 95% air and 5% CO2 at 37°C for 3 days.

**Hormone release from cultured cells**

After 3 days’ culture, the medium was aspirated and the wells were washed twice with 1 ml DMEM supplemented with 10% fetal calf serum and gentamicin (40 μg/ml). The cells were then incubated in 1 ml of the above medium at 37°C for 24 hr to determine the basal hormone release. The viability of the cells was >90% by trypan blue exclusion. After incubation, the medium was collected and washed twice with the same medium. The cells were then incubated in 1 ml of the same medium containing neuromedin B or GRP-10 at 37°C for 1 h. After the incubation, the medium was collected and the cells were again incubated in 1 ml of the medium containing the same dose of neuromedin B or GRP-10 at 37°C for 1 hr. The medium was collected and stored at −40°C until the hormone assay.

**Hormone assay**

The gastrin level was determined with a commercial kit (Gastrin-RIA kit®, Dainabot Radioisotope, Tokyo, Japan). The antiserum in this immunoassay is directed against the C-terminal residues of gastrin. Insulin and glucagon levels were measured by RIAs according to the method of Herbert et al. (1965), and Faloona and Unger (1974) with E-7 antibody (kindly donated by Dr. H. von Schenck, von Schenck, 1977), respectively. Neither neuromedin B nor GRP-10 cross-reacted with the immunoassay systems.

**Statistical analysis**

All data are presented as the mean±SEM. Statistical comparisons between groups were performed by Student’s t-test for unpaired data. P<0.05 was considered significant.

**Results**

**Hormone content of tumors**

There was 174 ng/g (tissue wet weight) gastrin in the primary tumor and 219 ng/g in the metastatic tumor. The amount of insulin in the primary tumor was 198 ng/g.
tissue wet weight and that in the metastatic tumor was estimated to be 28 ng/g because it was close to the sensitivity limit of the RIA. The amount of glucagon in the primary tumor and the metastatic tumors was 8.6 and 7.3 ng/g tissue wet weight, respectively.

**Basal hormone release from cultured tumor cells**

Table 2 shows the hormone content in the basal medium obtained after 24 hr-incubation and in the mediums of following incubation twice for 1 hr each time. Gastrin and insulin were clearly released from cultured primary tumor cells, but insulin was found not to be released from cultured metastatic tumor cells. Glucagon release was negligible in both tumor cells (data not shown).

The mean gastrin concentrations in the 24 hr incubation medium from the primary and metastatic tumors obtained from 28 tissue culture wells were $1495\pm 94\, \text{pg/ml}$ and $812\pm 41\, \text{pg/ml}$, respectively. The mean insulin concentration in the 24 hr incubation medium of the primary tumor from 28 tissue culture wells was $3657\pm 549\, \text{pg/ml}$. That of the metastatic tumor was un-

| Table 2. Hormones in the medium of control experiment (without neuromedin B or GRP-10) obtained after 24 hr incubation and following incubation twice for 1 hr each time. |
|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                   | Gastrin (pg/ml)   | Insulin (pg/ml)   |
|                   | 24 hr             | 1st 1 hr          | 2nd 1 hr          | 24 hr             | 1st 1 hr          | 2nd 1 hr          |
| Primary tumor in pancreas | $1621\pm 199$ | $646\pm 59$       | $970\pm 143$      | $3360\pm 72$      | $1239\pm 76$      | $1395\pm 112$     |
| Metastatic tumor in liver | $795\pm 18$    | $178\pm 26$       | $210\pm 22$       | Nd*               | Nd*               | Nd*               |

Mean of values from 4 tissue culture wells, * Nd; not detectable.
detectable.

**Effects of neuromedin B and GRP-10 on hormone release from cultured tumor cells**

Gastrin release from both primary and metastatic tumor cells decreased significantly with 0.1 nM neuromedin B. 1 nM neuromedin B and 10 nM neuromedin B also inhibited gastrin release from metastatic tumor cells and from primary tumor cells, respectively. 0.1–10 nM GRP-10 tended to inhibit gastrin release from both cultured primary and metastatic tumor cells (Fig. 2). Insulin release from cultured primary tumor cells was stimulated by 0.1 and 1 nM GRP-10 and was not affected by 0.1–10 nM neuromedin B. (Fig. 3). Glucagon release tended to be suppressed by neuromedin B and GRP-10, but the values were too small to make a statistical analysis when the glucagon concentration in the incubation medium itself was subtracted (data not shown).

**Discussion**

The hormonal aspects of a case of malignant gastrinoma with a primary tumor in the pancreas body and multiple metastatic tumors in the liver has been investigated. Both the primary and metastatic tumor cells contained gastrin and glucagon to the same extent, whereas the insulin concentration in the primary tumor cells was almost 10 times higher than that in the metastatic tumor. Gastrin release from both tumor cells was suppressed by neuromedin B and tended to be suppressed by GRP-10. However, insulin release from pancreatic tumor cells was stimulated by GRP-10 and

![Fig. 3. Effects of neuromedin B (NMB) and GRP-10 on insulin release from cultured primary tumor cells. The results are shown as percent changes in insulin content in the first 1 hr-incubation medium plus that in the second 1 hr-incubation medium against insulin content in the 24 hr basal incubation medium (without NMB or GRP-10). N=4, mean±SEM. *P<0.05 vs. control (vehicle of NMB or GRP-10).](image-url)
not by neuromedin B.

Neuromedin B and GRP-10 are mammalian bombesin-like peptides which stimulate the release of gastrin, insulin and glucagon. The potency of GRP-10 was found to be over 10 times greater than that of neuromedin B in our previous study (Kawai et al., 1988). These effects of bombesin-like peptides are considered to be result of their binding to receptor sites. However, in this study gastrin release was suppressed by neuromedin B, which might be ascribed to an abnormality in the function of the receptor for bombesin-like peptides in tumor cells. The absence of the suppression of gastrin release by secretion in this patient and its small increase after the ingestion of a mixed meal (Table 1) suggest a similar abnormality. The abnormal regulation of hormone release in endocrine tumors is sometimes observed, i.e., no stimulation of insulin release by glucose in insulinoma, no suppression of growth hormone release by glucose and an abnormal stimulation of it by TRH in acromegaly, although the precise mechanisms of these abnormalities has not yet been understood.

However, the response of insulin release from cultured cells to GRP-10 was normal. The amount of insulin in tumors was about 100 times lower than that in the normal pancreas, and the amount in the metastatic tumor in the liver was about 15% that in the primary tumor in the pancreas. These results suggest that the majority of insulin from the tumor in the pancreas is derived from contaminated islet cells as shown in Fig. 1-B, although the tumor was well defined from a normal pancreas. The amount of glucagon in the primary tumor was also slightly higher than that in the metastatic tumor.

Previous in vivo studies of gastrinoma demonstrated that the plasma gastrin level of patients increased little in response to an intravenous infusion of bombesin, compared with patients with hypergastrinemia of antral origin (Basso et al., 1981 and Jansen and Lamers 1982). Gastrin release was not clearly stimulated by the infusion of bombesin in 4 patients out of 8 with a non-operated gastrinoma (Jansen and Lamers, 1982), or in 2 patients out of 16 with Zollinger-Ellison syndrome (Basso et al., 1981). In addition, the response seems to be weak in patients with liver metastases (Basso et al., 1981). Since the effects of bombesin-like peptides on gastrointestinal and pancreatic hormone release are thought to be similar, the suppression of gastrin release by neuromedin B in this in vitro study is somewhat different from previous in vivo studies, suggesting poor response of gastrinoma cells to bombesin. Although the data presented are these for only one malignant gastrinoma, it is suggested that neuromedin B might suppress gastrin release from a malignant gastrinoma taking into account of the abnormal response to bombesin in patients with malignant gastrinoma (Basso et al., 1981). Therefore, it is possible that the infusion of neuromedin B may be useful in distinguishing between malignant and benign gastrinoma. In this patient, we have not examined the response of the plasma gastrin level to neuromedin B or GRP-10, because the use of these peptides was limited to animals in this study.

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