Acromegaly due to ectopic growth hormone-releasing hormone secretion by a bronchial carcinoid tumour. Dynamic hormonal responses to various stimuli

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Abstract. Ectopic GHRH is a relatively uncommon cause of acromegaly, which should be differentiated from pituitary adenoma, in order to avoid damage to the pituitary gland from unnecessary interventions. We report here on a 66-year-old man with acromegaly due to a GHRH-secreting bronchial carcinoid tumour, who recovered completely following removal of the tumour. His hormonal status was studied before and after the operation. Basal GH, GHRH, IGF-I and PRL levels, as well as plasma GH response to glucose load and TRH administration were abnormal before the operation, and became normal thereafter. The somatostatin analogue SMS 201-995 was found to be a potent inhibitor of the ectopic GHRH and the GH secretion (>500 to 42 ng/l and 15.4 µg/l to 0.8 µg/l, respectively). The effect on GHRH proved to be due to direct effect of somatostatin on the tumour cells, as demonstrated in tissue culture studies. A mixed meal was found immediately to suppress GHRH levels without such an effect on GH secretion. We conclude that the neuroendocrine tests usually practised in acromegaly cannot differentiate between ectopic GHRH secretion and pituitary adenoma. High plasma GHRH levels may serve as a diagnostic test for excessive GHRH production, which is almost always ectopic. These high levels are suppressible by somatostatin and a mixed meal.

Ectopic secretion of growth hormone-releasing hormone is a rare cause of acromegaly, which may underlie the disease in 1-2% of all cases (1,2). The most common tumours secreting GHRH are bronchial carcinoids and pancreatic islet cell tumours (3). Differential diagnosis of pituitary adenoma is important in order to avoid unnecessary pituitary surgery or radiotherapy.

We studied the dynamic responses of GH, GHRH and PRL to various stimuli, including a somatostatin analogue and a mixed meal, in a patient with ectopic GHRH secretion as well as the in vitro response of the tumour tissue to somatostatin. This was done to establish the role of various tests in the diagnosis, as well as to understand further the pathophysiology of acromegaly due to ectopic GHRH secretion.

Patient and Methods

A 66-year-old man was admitted for investigation of acromegalic features; he had been known to have a large tumour in his right lung for more than twenty years. On examination he appeared to have typical acromegalic features. The rest of the physical examination was unremarkable.

Chest x-ray and computed tomography (CT) revealed a large well circumscribed tumour in the right lower lobe, without evidence of lymph node enlargement. Skull x-ray and CT of the head did not show sellar enlargement, but there was marked thickening of the skull bones, consistent with acromegaly. Basal hormone levels (Table 1) showed high plasma GH, IGF-I and PRL levels. Other pituitary hormones as well as thyroid hormones, cortisol and parathyroid hormone were within normal limits. The 24-h urine collection for 5-hydroxyindoleacetic acid was normal.

The patient was operated on. The right middle lobe
was resected, in which a round encapsulated mass measuring 7.5×6×6 cm was found. Histopathological examination revealed a typical picture of bronchial carcinoid and immunoperoxidase staining of frozen sections from the tumour with antibodies against GHRH and against GH showed positive cytoplasmic staining in some areas for GHRH. Staining with anti-GH was negative.

The patient had an uneventful postoperative course. Marked clinical improvement occurred, until he became totally asymptomatic after a few months. He has been followed for two years now, without any recurrence of symptoms or abnormal hormone levels, as shown later.

Basal hormone determinations and various provocation tests were performed before and after the operation. Blood samples were drawn in the morning hours in the fasting state a few times prior to the operation, and one day, two weeks and two weeks later, and again one year later (Table 2).

Stimulation tests

Oral glucose load. In solution 75 g glucose was given orally. Blood samples for GH determinations were drawn half hourly for two hours. This test was done before and two weeks after the operation.

TRH test. TRH (400 µg, Serono) was administered iv. Blood samples for GH and PRL determination were taken at 0, 20, 40, and 60 min at the pre-operative test, and at 0, 15, 30, 60 min at the second test, performed one year later.

Somatostatin test. The slow-acting analogue (Sandostatin®, SMS 201-995) (Sandoz), 50 µg, was administered sc. Blood samples for GH and GHRH determination were drawn before and 30, 60, 90, 120, 150, 180 and 240 min following injection. The test was performed before the operation and one year later.

Mixed meal. Served as breakfast, it contained about 35 g carbohydrates, 15 g fat, and 30 g proteins. Blood samples were drawn before and half hourly for 4 h after the meal for determination of glucose, insulin, somatostatin, GH and GHRH. This test was performed before the operation and one week postoperatively.

Hormone determinations

GH was measured according to a previously described method (4), using anti-GHRH (1-44) serum and [125I]GHRH (Amersham, Buckinghamshire, UK) and GHRH(1-44) (Sigma, St. Louis, MO) as standard. The sensitivity of the assay is 7.0 ng/l and intra- and inter-assay variations at 15 ng/l are 17 and 10%, respectively.

Somatostatin determination was performed using materials provided by Incstar (Minnesota, USA) as previously described (5). The sensitivity of the assay is 8 ng/l and all the samples were determined in the same assay.

IGF-I in serum was measured by RIA using a kit (Incstar, USA) by a previously described protocol (6).

Plasma GH, PRL and insulin levels were determined by double-antibody RIA using materials provided by Sorin, D.P.C. (France) and Novo (Denmark), respectively. Blood glucose was measured by a glucose oxidase method.

Monolayer cultures of tumour tissue on extracellular matrix were prepared as previously described (7). Tumour tissue was cut into small pieces, which were vigorously shaken with collagenase-containing solution. After repeated washing, centrifugation and resuspension, tumour cells were placed on extracellular matrix-coated culture plates in a concentration of 10^6 cells/35 mm plate in RPMI-1640 medium containing antibiotics and fetal calf serum. When a monolayer was formed, secondary cultures were prepared by mild trypsin digestion and replating on new extracellular matrix-coated plates.

Three identical plates were prepared. In order to determine in vitro GHRH release, culture medium (1 ml/plate) was changed and new medium left in the plate for exactly one hour. Thereafter, it was collected for GHRH determination and replaced by 1 ml of fresh medium which contained somatostatin in a concentration of 1 ng/l. This second medium was also collected after exactly one hour for determination of GHRH levels.

Results

Basal hormone levels

Table 1 shows basal GHRH, GH, IGF-I and PRL levels at different stages before and after the operation.

Plasma GHRH levels were very high in repeated determinations before the operation. They decreased to 72 ng/l as early as at the end of the operation, and became normal one week later. Normal levels were also found one year later.

Basal GH levels were consistently high before the operation, and also decreased to near normal levels at the end of the operation and one week later. Levels at two weeks and one year after the operation were within normal limits.

IGF-I levels measured before the operation were high and returned to normal levels one year later.

Plasma PRL levels were also elevated before the operation, and slowly decreased thereafter, being still significantly elevated two weeks after the operation, but normal after a year.

Stimulation tests

Oral glucose load had no suppressive effect on the high GH levels before the operation, but it suppressed GH levels two weeks following the operation (from 3.8 to 1.9 µg/l).

TRH test (Fig. 1) revealed a marked GH stimu-
Table 1.
Basal hormone levels.

<table>
<thead>
<tr>
<th>Hormone:</th>
<th>GHRH (ng/l) normal</th>
<th>GH (μg/l) normal</th>
<th>Prolactin (μg/l) normal</th>
<th>IGF-I (nm/l) normal</th>
</tr>
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<td>&gt;500</td>
<td>20-35</td>
<td>35-40</td>
<td>135</td>
</tr>
<tr>
<td>Before operation</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Start of operation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of operation</td>
<td>72</td>
<td>7.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day after operation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After one week</td>
<td>30</td>
<td>9.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After two weeks</td>
<td>27</td>
<td>5.1</td>
<td>25</td>
<td></td>
</tr>
<tr>
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<td>7.7</td>
<td>32</td>
</tr>
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</table>

lation in the pre-operative test (from basal levels of 31 μg/l to a peak of 181 μg/l), whereas no rise occurred one year after the operation. The response of prolactin to TRH was excessive before the operation and became flat after it.

Subcutaneous somatostatin administration (Fig. 2) in the pre-operative state induced a marked decrease in plasma GHRH levels from basal levels of >500 to a trough of 42 ng/l, and in the plasma GH levels from 15.4 to a trough of 0.8 μg/l. This effect persisted for at least 4 h, until termination of the test. One year later, while basal levels of both hormones were much lower, no significant effect of somatostatin could be detected.

Mixed meal administration before the operation induced a rapid and marked decrease in plasma GHRH from >500 to a minimum level of 110 ng/l at 30 min. This was followed by a slow recovery toward basal levels. This decrease of GHRH was not accompanied by any decrease in plasma GH levels, which, on the contrary, rose toward 210 min (from 9.8 to 23 μg/l). Plasma somatostatin increased continuously from a basal level of 5.2 to a peak of 19.6 ng/l at 150 min. Glucose and insulin levels showed a slight increase following meal with a peak at 60 min.

One week later, when basal GH and GHRH levels were much lower, and no particular response of GH and GHRH to a similar meal could be detected.

**In vitro somatostatin effect**
In vitro GHRH secretion into tissue culture supernatant (Table 2) ranged between 100 and 310 ng/h. Following somatostatin administration into medium GHRH secretion decreased by 58-76%.

**Discussion**
Ectopic GHRH secretion is considered a rare cause of acromegaly. It is reported in 1-2% of patients in large series of acromegaly (1,2).

Having diagnosed a man with a bronchial carcinoid causing acromegaly, we investigated several parameters of the pathophysiology of excessive GHRH secretion and tried to determine which tests can differentiate between ectopic GHRH and pituitary adenoma.

Our findings confirm previous observations that
most hormonal responses in patients with ectopic GHRH secretion are indistinguishable from those of patients with GH-secreting pituitary adenomata. These include high basal GH levels, abnormal GH response to glucose load, abnormal GH response to TRH, and high basal as well as TRH-stimulated PRL levels (3,8-18).

All these tests indicate a marked similarity between responses of adenomatous and chronically stimulated but essentially normal somatotropes. They all normalized shortly after the removal of the tumour. The only reliable test to distinguish between ectopic GHRH and pituitary adenoma was plasma GHRH levels. This test can differentiate between the two etiologies, with the exception of very rare cases of excessive GHRH of hypothalamic origin (1,19).

The finding that acute administration of somatostatin to our patient induced a dramatic long-lasting suppression of both GH and GHRH is consistent with other reported cases of ectopic GHRH, where somatostatin had a dual effect, both GHRH suppression and a direct effect on GH secretion (8-10,13,14,20,21). By studying the tumour in a monolayer tissue culture, we proved for the first time that the effect of somatostatin on plasma GHRH is mediated by a direct immediate action on
the GHRH-secreting cells of the tumour. The mechanism underlying this direct action is yet to be determined.

Whereas plasma immunoreactive GHRH was found to increase slightly following meals in healthy normal subjects (22,23), in our patient, there was a dramatic suppression of GHRH following a mixed meal, which was not accompanied by a parallel decrease in GH. The mechanism whereby the decrease in GHRH occurs is not known, but the finding that plasma somatostatin increased after a mixed meal in our patient, as was also reported in healthy and diabetic subjects (23,24), suggests a role for peripheral somatostatin in the decreased plasma GHRH level. The lack of a parallel decrease in plasma GH is consistent with other reports showing dissociation between plasma GHRH and pituitary GH secretion (25), as well as with the paradoxical response of GH to glucose elevation in these cases (8,9).

We conclude that most hormonal responses of patients with ectopic GHRH secretion are indistinguishable from those of patients with pituitary adenomas. The one useful test in the differential diagnosis is plasma GHRH levels which are high in patients with ectopic GHRH. These high levels are suppressible by somatostatin and a mixed meal.

Acknowledgment

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