Multiple endocrine neoplasia with Cushing’s syndrome due to paraganglioma producing corticotropin-releasing factor and adrenocorticotropin

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Abstract. A male patient with corticotropin-releasing factor (CRF) and adrenocorticotropin (ACTH)-producing syndrome is described. Soon after being referred to us the patient developed pneumonia, anaemia, oedema and respiratory distress, and died on the 24th day after admission. Autopsy and histology revealed that he had a rare type of multiple endocrine neoplasia (type 1 + paraganglioma) with a mediastinal paraganglioma, parathyroidal hyperplasia, pancreatic islet cell adenoma, duodenal multiple carcinoid tumours and adrenocortical nodular hyperplasia. It was not possible to examine the pituitary. The paraganglioma contained a large amount of immunoreactive (IR)-CRF (606 ng/g wet weight), IR-ACTH (59.4 ng/g wet weight), IR-human proopiomelanocortin n-terminal (1–76) peptide (hNT, 156.8 ng/g wet weight) and IR-β-lipoprotein (β-LPH, 146.9 ng/g wet weight). The major IR-ACTH, β-LPH and IR-hNT were eluted at ACTH-(1–39), β-LPH and hNT marker positions, respectively. Big ACTH was not detected. IR-CRF eluted at the human CRF marker position on Sephadex G-75 chromatography and high performance liquid chromatography (HPLC). The IR-CRF fraction from the HPLC showed CRF bioactivity which paralleled that of synthetic human CRF in monolayer cultured rat anterior pituitary cells. Our results suggest that not only ACTH but CRF produced by the paraganglioma was responsible for the patient’s Cushing’s syndrome.

Upton & Amatruda (1971) first demonstrated CRF-like activity in two types of ectopic ACTH-producing tumours (pancreatic tumour and lung oat cell carcinoma). Later other cases of ectopic ACTH producing tumours which showed CRF-like activity were reported (Birkenhager et al. 1976; Yamamoto et al. 1976; Suda et al. 1977), including our case (Hashimoto et al. 1980a). In these reports tumour CRF production was detected in CRF bioassays. Vale et al. (1981) first characterized the structure of ovine CRF. The structure of human (Shibahara et al. 1983) and rat (Rivier et al. 1983) CRF were found to be the same, which permitted specific CRF radioimmunoassays and immunohistochchemical studies. Recently it has been found that several kinds of tumours contain CRF-like immunoreactivity (Kirkland & Ellison 1984; Suda et al. 1984; Wakahayashi et al. 1985). However, the CRF content was usually low, and only a few cases have been reported with ectopic CRF syndrome whose CRF production was detected by radioimmunoassay or immunohistochemistry (Carey et al. 1984; Belsky et al. 1985). We recently treated a patient with a rare type of multiple endocrine neoplasia having a CRF and ACTH-producing paraganglioma which caused Cushing’s syndrome.

Materials and Methods

Case report
A 63 year old Japanese man was admitted to an outlying hospital in June 1978 because of vertigo and short
periods of unconsciousness. He had severe anaemia, and a chest X-ray showed a tumour shadow in the left lung field. In September he was transferred to another hospital where it was found that the anaemia was due to bleeding from gastric ulcer. The abnormal lung shadow was diagnosed as a mediastinal tumour. In November an open chest surgery was conducted, but the tumour could not be removed. The tumour was biopsied and diagnosed as a paraganglioma. He then received 60Co irradiation (3000 rads) and cyclophosphamide therapy. After surgery his general condition improved, and he had been well for about 5 years until June 1983, when he experienced weakness and gait disturbances and was admitted to the Okayama Saiseikai Hospital.

His medical history indicated a partial gastrectomy in 1970 because of gastric ulcer. His mother suffered from lung tuberculosis, and his father and elder brother suffered from pleuritis.

His body weight was 57.0 kg with relative truncal obesity. The physical examination revealed a blood pressure of 170/99 mmHg with a regular pulse rate of 90 min. He was anaemic. A cervical tumour or struma was not found. He had oedema on the face, both hands and legs. His heart sound was normal, but breathing was weak and moist rales were heard in the right chest field. He had a tenderness on the left upper abdomen. The patient was alert, but both knee reflexes were weak. Muscle atrophy was noted under the limbs.

Laboratory results. Plasma Na, 143 mEq/l; K, 3.2 mEq/l; Cl, 103 mEq/l; Ca, 6.2 mEq/l; P, 3.8 mg/dl. Blood red cell count, 310 x 10^4/mm^3; haemoglobin, 5.3 g/dl; haematocrit, 24.5%; white cell count, 10 800/mm^3 with 89% neutrophils; total protein, 4.2 g/dl; albumin, 2.27 g/dl; γ-globulin, 0.37 g/dl. Blood chemistry: serum GOT 24 KU; GPT, 30 KU; Alp-ase, 7.1 KAU; LDH, 479 WU; Fe, 47 μg/dl; blood urea nitrogen, 67.6 mg/dl; uric acid, 9.3 mg/dl; creatinine, 2.5 mg/dl; fasting blood sugar, 129 mg/dl; serum ferritin, 20.4 ng/ml; CEA, 3.23 ng/ml. Faecal occult blood was (+ +).

The baseline hormone levels are shown in Table 1. Plasma ACTH and cortisol levels were markedly elevated, and urinary excretions of 17-hydroxycorticosteroids (17-OHCS) and 17-ketosteroids (17-KS) were also quite high. Plasma noradrenaline but not epinephrine was elevated, and urinary catecholamine excretion was increased. Urinary vanillyl mandelic acid (VMA) and homo vanillic acid (HVA) excretions were also increased. Plasma c-terminal parathormone (cPTH) was greatly elevated.

The chest X-ray demonstrated cardiac enlargement and a huge abnormal shadow occupied most of the right lung field. Computerized tomography also showed a large tumour shadow in the right chest cavity which was diagnosed to be a mediastinal tumour.

This tumour shadow and the simultaneous marked elevation of plasma ACTH and cortisol suggested ectopic ACTH syndrome. Multiple endocrine neoplasia was also suspected as plasma noradrenaline and cPTH were elevated.

Clinical course after admission

He could not walk because of muscle atrophy. Hypertension persisted, and anti-hypertensive drugs were not effective. On the 5th day of admission, pneumonia developed in the left lung. On the 6th day he had a tarry stool, and his anaemia became severe. On the 8th day the oedemas increased on the face and upper-limbs. On the 12th day he had a large amount of tarry stool and became unconscious, and on the 16th day he showed respiratory disturbances. He died on the 24th day after admission.

Table 1.
Endocrine data on the patient.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Result</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma ACTH, pg/ml</td>
<td>300</td>
<td>10—100</td>
</tr>
<tr>
<td>Plasma cortisol, μg/dl</td>
<td>31</td>
<td>3.7—13.0</td>
</tr>
<tr>
<td>Urinary 17-OHCS, mg/day</td>
<td>49.3—52.1</td>
<td>2.0—8.0</td>
</tr>
<tr>
<td>Urinary 17-KS, mg/day</td>
<td>111.1—118.2</td>
<td>4.0—16.0</td>
</tr>
<tr>
<td>Plasma renin activity, ng/ml/h</td>
<td>3.5</td>
<td>0.5—2.0</td>
</tr>
<tr>
<td>Plasma aldosterone, pg/ml</td>
<td>122.4</td>
<td>&lt; 180</td>
</tr>
<tr>
<td>Serum cPTH, ng/ml</td>
<td>6.6</td>
<td>&lt; 1.3</td>
</tr>
<tr>
<td>Plasma noradrenaline, pg/ml</td>
<td>0.78</td>
<td>0.06—0.45</td>
</tr>
<tr>
<td>Plasma adrenaline, pg/ml</td>
<td>0.05</td>
<td>&lt; 0.12</td>
</tr>
<tr>
<td>Urinary catecholamine, μg/day</td>
<td>216</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>Urinary VMA, mg/day</td>
<td>14.1</td>
<td>4.7—11.4</td>
</tr>
<tr>
<td>Urinary HVA, mg/day</td>
<td>17.6</td>
<td>1.3—6.6</td>
</tr>
<tr>
<td>Urinary 5HIAA, mg/g creatinine</td>
<td>5.0</td>
<td>1.6—6.4</td>
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Autopsy and histology
At autopsy a huge tumour was found in the right anterior mediastinum, involving the pericardium, which was revealed to be the paraganglioma by the histology. The parathyroid glands were enlarged and histologically hyperplastic. An islet cell tumour was found in the body of the pancreas. Multiple carcinoid tumours were found in the duodenum. The stomach showed multiple erosions. The adrenal glands showed nodular hyperplasia. The pituitary gland could not be examined because his family would not allow an incision in the patient's head.

Tumour extraction
A part of the paraganglioma was extracted for CRF detection by an acid acetone-petroleum ether method (Hashimoto et al. 1985). For ACTH, hNT and β-LPH detection, the tumour was extracted with 0.05 N HCl (Hashimoto et al. 1980a).

Sephadex gel filtration
The tumour extract was applied to a 0.9 x 70 cm column of Sephadex G-75 and eluted with 0.1 N HCl. The eluate was fractionated, and each 1 ml fraction was lyophilized and stored at -40°C for CRF, ACTH, hNT and β-LPH assays.

HPLC
HPLC was carried out as described previously (Hashimoto et al. 1983). Briefly, samples were freshly dissolved in the primary aqueous solvent (0.045 M NaCl-0.01 N HCl) and centrifuged. Then, the supernatant was injected onto a stainless steel column (4 x 250 mm) packed with Hitachi gel 3053 (octadecylsilane). The column was eluted using a gradient elution of increasing acetonitrile concentration (0–89%) in a mixture of 0.045 M NaCl-0.01 N HCl, at a flow rate of 1.0 ml/min, monitoring the column effluent at 220 nm with a UV detector. Each 2 ml fraction was divided into 0.1 and 1.9 ml, and they were lyophilized for subsequent CRF radioimmunoassay and bioassay, respectively.

Radioimmunoassays (RIAs)
The amount of CRF in the tumour extract was assayed employing synthetic CRF (Peptide Institute Inc, Osaka) and anti-human CRF serum prepared in our laboratory (Hashimoto et al. 1985). (Tyr) human CRF was used for tracer preparation. ACTH was assayed employing ACTH-(1–39) and its antiserum (Hashimoto et al. 1976) which did not react with β-LPH or hNT. β-LPH assay was carried out employing β-LPH and its antiserum (Hashimoto et al. 1980b) (donated by Dr C. H. Li) which did not react with ACTH-(1–39) or hNT. Human NT assay was performed employing hNT and its antiserum which did not react with ACTH-(1–39) and β-LPH (Chan et al. 1983; Motomatsu et al. 1984).

CRF bioassay
CRF bioactivity was measured using a previously reported method (Hashimoto et al. 1979), with monolayer cultured rat anterior pituitary cells.

Statistical analysis was conducted using Student t-test.

![Fig. 1](image)

Displacement of [125I](Tyr) human CRF from anti-CRF serum by serially diluted human CRF and paraganglioma extract.
Immunohistochemical study

The presence of insulin, gastrin, glucagon, vasoactive intestinal polypeptide (VIP) or somatostatin containing cells were investigated in the paraganglioma, islet cell tumour and duodenal carcinoid by the peroxidase-anti-peroxidase technique using specific antisera to these peptides. Antiserum to insulin, gastrin, glucagon, VIP and somatostatin were obtained from Ortho Diagnostic System Inc. (HISTOSET, USA).

Results

CRF, ACTH-(1–39), hNT and β-LPH immunoreactivity in the paraganglioma

The displacement of {[I²⁵³]Tyr}human CRF by the serially diluted tumour extract paralleled that of synthetic human CRF (Fig. 1). The tumour contained a large amount of CRF immunoreactivity (approximately 606 ng/g wet weight (wt) tissue). When the extract was chromatographed on a Sephadex G-75 column, most IR-CRF co-eluted with human CRF marker (Fig. 2). A small peak of CRF-like immunoreactivity also appeared on the void volume. ACTH, hNT and β-LPH immunoreactivities were 59.4, 156.8 and 146.9 ng/g wet weight tissue, respectively. A major IR-ACTH co-eluted with ACTH-(1–39). A major

Fig. 2.

Gel filtration of CRF-like immunoreactivity in paraganglioma extract (315 mg wet tissue weight equivalent) on a Sephadex G-75 column (0.9 x 70 cm) with 0.1 n HCl.

Fig. 3.

Gel filtration of IR-ACTH, IR-hNT and IR-β-LPH in paraganglioma extract (315 mg wet tissue weight equivalent) on a Sephadex G-75 column (0.9 x 70 cm) with 0.1 n HCl.
IR-hNT eluted at the hNT marker position. A big peak of IR-β-LPH eluted at the β-LPH marker position, and other peaks also appeared after the β-LPH marker (Fig. 3).

When fractions with CRF immunoreactivity on Sephadex G-75 gel filtration (Fig. 2, 27–32 ml) were chromatographed by HPLC, one peak of IR-CRF appeared at the human CRF marker position (Fig. 4).

Noradrenaline and adrenaline contents in the tumour were 18.7 and 3.3 µg/g wet weight tissue, respectively, but dopamine was not detected.

**CRF bioactivity of the paraganglioma**

When the peak IR-CRF fraction from HPLC (39–40 ml) was lyophilized and tested for CRF bioactivity in monolayer cultured rat anterior pituitary cells, it showed significant CRF bioactivity which paralleled that of synthetic human CRF (Fig. 5).

**Immunohistochemical study**

The peroxidase-antiperoxidase staining method showed the presence of insulin, glucagon and somatostatin containing cell in the islet cell tumour, and gastrin and somatostatin containing cells in the duodenal carcinoid tumour. The paraganglioma, parathyroidal and adrenal tissues showed no insulin, gastrin, VIP, glucagon or somatostatin containing cells. Immunohistochemical staining for CRF and ACTH has not been done in these tissues.

**Discussion**

This patient apparently had ectopic ACTH syndrome due to the paraganglioma. The elevated cPTH level, high serum Ca level and parathyroidal hyperplasia suggested that he also had primary hyperthyroidism. Elevated serum noradrenaline and urinary catecholamine levels were ascribed to the paraganglioma. These results suggest that the patient had a multiple endocrine neoplasia which was confirmed by the autopsy data. The autopsy revealed that he had a pan-
creatic islet cell adenoma and multiple duodenal carcinoid tumours which contained gastrin producing cells, suggesting that he had Zollinger-Ellison's syndrome.

Pituitary adenoma, parathyroidal adenoma or hyperplasia, pancreatic islet cell adenoma and duodenal carcinoid tumour are usually involved in multiple endocrine neoplasia type 1 syndrome, but paraganglioma is not included. Adrenomedullary pheochromocytoma and paraganglioma are included in multiple endocrine neoplasia type 2. Although the pituitary was not examined in this patient, our results indicate that he had a rare type (type 1 + paraganglioma) of multiple endocrine neoplasia.

Most of the ACTH, hNT and β-LPH immunoreactivity co-eluted respectively with synthetic ACTH-(1–39), hNT and β-LPH, and the big form of ACTH was not detected. These results and the high plasma ACTH levels suggest that these ACTH related peptides were well processed from their precursor (proopiomelanocortin) in the tumour tissue and were secreted into systemic circulation. Gamma-LPH and other β-LPH like-immunoreactive fragments might be responsible for multiple peaks of IR-β-LPH, although some smaller peaks might be due to laboratory artifacts. Thus, ectopic ACTH production in the paraganglioma was probably responsible for the Cushing's syndrome in this patient.

Since synthetic CRF became available, it has been shown that CRF-like immunoreactivity is widely present in normal and tumour tissues outside the central nervous system (Suda et al. 1984; Kirkland et al. 1984; Wakabayashi et al. 1985). But only a few cases of Cushing's syndrome have been reported due to ectopic production of CRF. Recently, Asa et al. (1984) reported a case of Cushing's disease associated with an intrasellar gangliocytoma producing corticotropin-releasing factor. Ann Int Med 101: 789–792.


because of the patient's severe condition, and we were not able to examine the patient's pituitary. Therefore, we can not definitively conclude that the CRF produced in the tumour stimulated the secretions of ACTH and related peptides from the tumour and the pituitary. However, the paraganglioma had a great amount of CRF immunoreactivity which was identical to human hypothalamic CRF and had significant ACTH-releasing activity. Therefore, it is possible that tumour CRF secreted into systemic circulation stimulated pituitary ACTH secretion, which might have contributed to the ACTH pool that affected the ultimate tissue, the adrenal glands. IR-CRF at the void volume of Sephadex G-75 chromatography might be ascribed to a precursor of CRF, although it was not confirmed in the present investigation.

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References


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