NOTE

In vivo and in vitro ACTH Response to Ovine Corticotropin-Releasing Factor in a Bronchial Carcinoid from a Patient with Ectopic ACTH Syndrome

MITSUMASA KUBO, KOJI NAKAGAWA, KAZUMASA AKIKAWA, TATSUYA ISHIZUKA AND MIYAO MATSUBARA

Second Department of Medicine, Hokkaido University
School of Medicine, Sapporo 060

Abstract

ACTH response in vivo and in vitro to synthetic ovine corticotropin-releasing factor (o-CRF) was examined in a bronchial carcinoid from a patient with ectopic ACTH syndrome. o-CRF, 1 µg/kg iv bolus, scarcely increased plasma ACTH or cortisol on one occasion, but they showed a low response on retesting. On the other hand, 10⁻⁸ and 10⁻⁷ M of o-CRF significantly stimulated ACTH release in cultured bronchial carcinoid cells.

Differential diagnosis between Cushing’s disease and ectopic ACTH syndrome often runs into difficulties at present. Since ovine corticotropin-releasing factor (o-CRF) was isolated by Vale et al. (1981), the o-CRF test has been performed in normal subjects and patients with hypothalamo-pituitary-adrenal disorders. Plasma ACTH response to o-CRF in Cushing’s disease was normal or exaggerated in vivo (Orth et al., 1982; Müller et al., 1983; Pieters et al., 1983; Lytras et al., 1984; Kubo, 1985) and o-CRF stimulated ACTH release in the pituitary adenoma from the patients with Cushing’s disease in vitro (Suda et al., 1984; Oosterom et al., 1984). On the other hand, plasma ACTH responses to o-CRF in ectopic ACTH syndrome in vivo were reported to be less remarkable (Lytras et al., 1984) or absent (Müller et al., 1983; Chrousos et al., 1984; Lytras et al., 1984), but there has been no report about in vitro ACTH response to o-CRF in the ACTH-producing tumor from the patients with ectopic ACTH syndrome. In this study, we examined in vivo and in vitro ACTH release by o-CRF in the ACTH-producing bronchial carcinoid from a patient with ectopic ACTH syndrome.

Materials and Methods

Synthetic o-CRF (Peptide Institute, Minoh, Osaka), 1 µg/kg body weight, was injected as a bolus intravenously at 9:30 a.m. after overnight fasting in two occasions. Blood samples were collected at -30, 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min via a catheter previously inserted into an antecubital vein and the blood was immediately mixed with 1 mg EDTA/ml blood and centrifuged. The plasma was stored at -20°C until assayed. Plasma ACTH was measured by RIA with the kit purchased from...
CEA-IRON-SORIN, France, according to the modified method reported previously (Kubo, 1985). Plasma cortisol was measured by RIA with the kit from Eiken ICL, Tokyo.

The carcinoid tumor was washed with Ca, Mg-free phosphate-buffered saline (PBS), minced and dispersed in PBS containing 2.5 mg/ml trypsin, 200 μg/ml collagenase, and 500 μg/ml glucose at 37°C for 15 min. The cells were collected by centrifugation at 1,000 rpm for 10 min and were incubated in plastic dishes (60×15 mm, Falcon) at a concentration of approximately 10^5 cells/4 ml Dulbecco modified Eagle’s medium (DMEM) containing 10% fetal calf serum in 95% air-5% CO₂ at 37°C. Three, 7, 12 and 29 days thereafter, the culture dishes were washed twice with DMEM and were incubated with DMEM only or DMEM with o-CRF, Dex, or LVP for four hours. The medium was removed and stored at -20°C until assayed. ACTH in the medium was measured by RIA with an antibody supplied by NIAMDD which recognizes the N-terminal of human ACTH. Statistical analysis of data was performed using Student's t-test.

Case Report

A 54-year-old woman with obvious Cushingoid features was admitted to our hospital. An X-ray film of the chest showed a well demarcated nodule in the left lower lung field behind the heart shadow. The morning plasma ACTH levels were 248 ± 23 (mean ± SD) pg/ml (n = 8) and the morning plasma cortisol levels were 43.5 ± 17.0 μg/dl (n = 8). The 24-hour urinary 17-hydroxycorticosteroids (u-17-OH-CS) and 17-ketosteroids were 29.9 ± 8.7 mg (n = 19) and 17.1 ± 4.2 mg (n = 19), respectively. After the oral administration of dexamethasone (Dex), 0.5 mg every six hours for 48 hours, u-17-OH-CS fell only from 24.6 to 21.0 mg/day and from 19.3 to 9.7 mg/day after 2 mg every six hours for 48 hours. A metyrapone test (750 mg every four hours for six doses) caused a rise in u-17-0H-CS from 24.6 to 98.0 mg/day. A lysine-vasopressin (LVP) test (10 μl.im) showed a low response of plasma ACTH (152 to 171 pg/ml) but a definite response of plasma cortisol (33.2 to 51.2 μg/ml). CT scan revealed a partial empty sella. There was no difference between the ACTH levels for bilateral inferior petrosal veins (370–380 pg/ml) and between them and that for an antecubital vein (356 pg/ml). A left lower lobectomy of the lung was performed and a tumor removed (3.0×2.5 cm). The ACTH levels in the left lower pulmonary vein and antecubital artery ob-

Fig. 1. Plasma ACTH and cortisol responses to iv injection of 1 μg/kg of o-CRF. Open circles: first test; closed circles: second test.
tained simultaneously at the time of the operation were 2,145 and 476 pg/ml, respectively. A pathohistological examination revealed an argyrophil type of bronchial carcinoid. The carcinoid was positive for ACTH in the immunohistochemical staining (DAKO PAP Kit; anti ACTH1-24). The ACTH content of the tumor was 36.7 μg/g wet tissue and the CRF content assayed with a human CRF RIA system was less than 10 ng/g wet tissue. Two days after the operation, the morning plasma ACTH level was less than 10 pg/ml.

Results

In vivo o-CRF stimulating test (Fig. 1)
In the first trial, o-CRF caused little response of plasma ACTH and cortisol (Fig. 1, open circle), but, on retesting, plasma ACTH showed about a 1.4 fold increase (against the mean of two basal samples) 15 and 180 min after o-CRF administration, and plasma cortisol about 1.2 fold at 120 min.

In vitro ACTH release (Figs. 2 and 3)
Basal ACTH release in 4 hours measured on the 3rd, 7th, 12th and 29th day of culture declined rapidly (Fig. 2).

On day 3, o-CRF at a concentration of 10^{-8} M stimulated ACTH release by 33% (p<0.02) (Fig. 3a). Dex, 10^{-7} M, did not suppress the basal ACTH release but suppressed ACTH release by o-CRF (Fig. 3a).

On day 7, o-CRF, 10^{-7} M, again increased ACTH release by 65% and Dex, 10^{-6} M, had no effect (Fig. 3b). LVP, 1 mU/ml, caused a 3.7 fold increase in ACTH release.

Discussion

This patient had some remarkable features of Cushing's syndrome and u-17-OH-CS were suppressed by 50% with high-dose Dex and were hyperresponsive to metyrapone. Plasma ACTH and cortisol were increased with LVP. These results had supported the diagnosis of Cushing's disease. However, o-CRF caused no response or only a slight response of plasma ACTH or cortisol and there was no difference between the ACTH levels for bilateral inferior petrosal veins and an antecubital vein. The diagnosis of ectopic ACTH syndrome was finally confirmed by the facts as follow: (1) After the removal of a bronchial carcinoid tumor, the plasma ACTH level fell to the normal range and her clinical symptoms were lessened. (2) The ACTH concentration in the tumor was very high. (3) Immunohistochemical staining showed cells positive for ACTH. (4) ACTH release was found in cultured carcinoid cells.

Some patients with ectopic ACTH syndrome were reported to have some laboratory findings as in our patient that were identical to those seen in patients with Cushing's disease (Strott et al., 1968; Mason
et al.; 1972). Recently, plasma ACTH and cortisol responses to o-CRF in Cushing’s disease have been reported as normal or exaggerated, but less remarkable or absent in ectopic ACTH syndrome. Therefore, the o-CRF stimulating test is thought to be useful in differentiating Cushing’s disease from ectopic ACTH syndrome (Chrousos et al., 1984). In our case, plasma ACTH and cortisol showed very little response to o-CRF in one occasion, but a low response on re-testing. In cultured carcinoid cells, 10^{-8} and 10^{-7} M of o-CRF increased ACTH release significantly. However, the increase was relatively small in spite of much higher concentrations of CRF than in plasma in the in vivo CRF test (Kubo, 1985), and this may explain the difference between in vivo and in vitro responses to o-CRF in this patient. Hirata et al. (1979) reported that rat median eminence (ME) extract stimulated in vitro ACTH secretion in medullary carci-
noma of the thyroid and malignant epithelial thymoma from each of two patients with ectopic ACTH syndrome though rat ME extract contained possible nonspecific ACTH secretagogues other than CRF.

Dex did not suppress the basal ACTH release in cultured carcinoid cells. The difference between in vivo and in vitro responses is inexplicable. However, Dex, 10^{-7} M significantly suppressed in vitro ACTH release by 10^{-8} M of o-CRF.

The in vivo and in vitro data of the present patient were incompatible with the presumption of Orth (1984) that ectopic ACTH-secreting carcinoid adenomas that responded to Dex would respond to o-CRF. Further analysis in many cases would be necessary to arrive at a definite conclusion concerning this problem.

Acknowledgements

We are grateful to Dr. N. Yanaihara of Shizuoka Pharmaceutical College, for his generous gift of synthetic human CRF, Dr. T. Suda of Tokyo Women's Medical College for his generous gift of anti-human CRF antiserum, and Dr. T. Nojima of Hokkaido University Hospital for the immunohistochemical staining. We also thank NIAMDD, U.S.A. for supplying anti-human ACTH antiserum, and Shionogi Laboratory for supplying synthetic human ACTH.

References


