An Extra-adrenal Abdominal Pheochromocytoma Causing Ectopic ACTH Syndrome

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We report a 55-year-old woman with ectopic adrenocorticotropic hormone (ACTH) secretion caused by extra-adrenal pheochromocytoma. The patient presented with a 6-month history of hypertension and diabetes mellitus. Her serum and urinary cortisol levels were extremely high and dexamethasone failed to suppress the cortisol secretion. Her plasma ACTH levels were also elevated (>300 pg/mL) and insensitive to corticotropin-releasing hormone (CRH) or metyrapone administration. Gel filtration analysis of the patient’s plasma detected the existence of large molecular weight ACTH being eluted with a major peak of authentic 1-39 ACTH. Abdominal computed tomographic scan and magnetic resonance imaging revealed a 5-cm paraganglioma located underneath the left kidney, in which 123I-MIBG tracer specifically accumulated. Bilateral adrenal glands were diffusely enlarged. After surgical removal of the paraganglioma, the patient’s clinical symptoms improved and biochemistry normalized including plasma ACTH, urinary free cortisol, and urinary catecholamines. Subsequent histologic evaluation of the transected paraganglioma tissue revealed ACTH, synaptin, and chromogranin-A histologically immunostaining. Culture of primary cells collected from the resected paraganglioma demonstrated in vitro production of ACTH, noradrenaline, and adrenaline. This is the first report of ectopic ACTH syndrome induced by an extra-adrenal abdominal paraganglioma. Am J Hypertens 2005;18:1364–1368 © 2005 American Journal of Hypertension, Ltd.

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Ectopic adrenocorticotropic (ACTH) syndrome is characterized by hypercortisolism due to the hypersecretion of ACTH outside the pituitary gland, which leads to Cushing’s syndrome. The most common causes of ectopic ACTH syndrome are malignancies including small cell type lung carcinomas, thymic carcinoids, islet cell tumors of the pancreas, medullary carcinomas of the thyroid, and bronchial adenomas or carcinoids. Adrenal pheochromocytomas involved in ectopic ACTH syndrome are very rare.1 Only six cases of ACTH-producing paragangliomas have been reported to date, in which two cases occurred in the paranasal sinus2,3 and the others were cervical4 and mediastinal/thoracic5–7 paragangliomas. There has been no report of extra-adrenal abdominal paraganglioma producing both catecholamines and ACTH.

Here we show a unique case of ectopic ACTH syndrome complicated by overproduction of catecholamines due to a paraganglioma located in the left paraortic space. We evaluated the characteristics of secreted ACTH molecules by gel filtration analysis and further proved the capability of hormonal synthesis by primary culture of cells collected from the resected paraganglioma.

Case Report

A 55-year-old Japanese woman, who presented with 6-month history of persistent headache, palpitation, hypertension, and exacerbated hyperglycemia (>500 mg/dL), was referred to our hospital. She was 155.5 cm tall and weighed 51.6 kg (body mass index, 21.5 kg/m²). Her blood pressure was 135 to 165/90 to 105 mm Hg and pulse...
rate was 110 to 130 beats/min with the medication (valsartan, 80 mg/d). She showed Cushingoid features including central distribution of body fat, round face, facial hirsutism, and severe muscle weakness of the limbs. Hyperpigmentation was not seen in the skin and the oral mucosa. Abdominal computed tomographic scan (CT) revealed a 5-cm para-aortic tumor at the level of the left kidney and remarkable bilateral enlargement of the adrenal glands (Fig. 1A). The left abdominal tumor showed high intensity by T2-weighed image unlike in the T1 image by magnetic resonance imaging (MRI) (Fig. 1B). Her pituitary did not exhibit radiologic abnormalities by cranial MRI. Laboratory examinations revealed leukocytosis (white blood cell count 11,100/μL) with eosinolymphocytopenia, hyperglycemia (252 to 390 mg/dL), hypokalemia (1.3 to 2.9 mEq/L) with metabolic alkalosis, and deranged liver function tests with hypoalbuminemia (2.9 g/dL). Endocrine profiles revealed increased levels of plasma ACTH (318.4 pg/mL; normal, 9 to 52 pg/mL) by ACTH immunoradiometric assays (IRMA) “Yuka” kit (Mitsubishi Chemical, Tokyo, Japan) and cortisol (76.5 μg/dL; normal 8 to 25 μg/dL) without normal circadian variation (Fig. 2A). Dexamethasone (1 mg) failed to suppress the endogenous cortisol secretion. Neither CRH stimulation (100 μg intravenously; Fig. 2B) nor metyrapone (1.5 g; Fig. 2C) evoked any changes in plasma ACTH levels. Urinary hormone secretion was as follows: 17-hydroxycorticosteroids, 64.9 mg/d (normal, 2.6 to 7.8 mg/d), 17-ketosteroids 32.4 mg/d (normal, 1 to 8 mg/d), free cortisol 1,000,000 μg/d (normal, 31 to 99 μg/d), adrenaline 44.1 μg/d (normal, <15 μg/d), noradrenaline 190.5 μg/d (normal, 100 to 150 μg/d), dopamine 1029.5 μg/d (normal, 100 to 700 μg/d), vanillylmandelic acid (VMA) 3.3 mg/d (normal, 1 to 7 mg/d), metanephrine 0.19 mg/d (normal, 0.05 to 0.23 mg/d), and normetanephrine 0.15 mg/d (normal, 0.07 to 0.26 mg/d). 123I-MIBG scintigraphy showed specific uptake in the left mid-abdominal region (Fig. 1C), indicating that the tumor is an extra-adrenal paraganglioma. Medication with regular insulin (~80 U/d) and supplement of potassium chloride (3.6 to 4.2 g/d) was commenced for hyperglycemia and hypokalemia, respectively. Hypertension and tachycardia were treated with a high-dose (14 mg/d) of α1-adrenergic antagonist doxazosin mesilate. In addition, metyrapone (500 mg/d) was used to suppress the hypercortisolism. The resected tumor was pathologically diagnosed as an ACTH-producing pheochromocytoma by immunohistochemical studies with synaptin, chromogranin-A, and ACTH (Fig. 3). Upon surgical removal of the paraganglioma, the patient’s blood pressure, heart rate, and potassium levels normalized gradually without medication. Moderate hyperglycemia persisted but was under control without insulin. Postoperative levels of plasma ACTH, urinary free cortisol, and urinary catecholamines normalized. Two weeks after the surgery, circulating ACTH and cortisol restored normal circadian fluctuation with a suppression response to dexamethasone (Fig. 4A) and normal response to CRH test (Fig. 4B).

**Gel Chromatography Analysis of ACTH Molecules**

Analysis of the molecular size of circulating immunoreactive (IR)-ACTH was performed using the preoperative plasma by gel chromatography as previously reported. Briefly, the plasma sample (0.4 mL) was applied to a Sephadex G-75 column (1 by 46 cm; Amersham-Pharmaacia Biotech, Piscataway, NJ) and eluted with 1% formic acid. After 2-mL fractions were collected and lyophilized, the ACTH concentration of each fraction was determined. To detect the presence of large molecular weight ACTH, ACTH levels were determined by two different IRMA methods, ACTH IRMA “Yuka” kit (using antibodies against 1-24 ACTH and 18-39 ACTH; Mitsubishi Chemical) and Nichols Allegro ACTH kit (using antibodies against 1-17 ACTH and 34-39 ACTH; Nihon Medi-Physics, Tokyo, Japan), the latter of which can detect large molecular weight ACTH molecules as we previously re-
ported. As a result, ACTH IRMA Mitsubishi “Yuka” kit detected a main peak of 1-39 ACTH with a small peak of big ACTH, whereas Nichols Allegro ACTH kit detected three different peaks of larger molecular weight ACTH molecules including pro-opiomelanocoritin (POMC) in addition to a main molecule of 1-39 ACTH (Fig. 5).

Primary Cell Culture of Resected Paraganglioma

After surgical removal, the tissues of the paraganglioma were washed, minced in ice-cold phosphate-buffered saline (PBS), and dissolved in Hanks’ balanced salt solution with trypsin (0.25%), collagenase (0.25%), and DNase (16 U) for 15 min at 37°C. The cell suspension was dispersed and replaced to a fresh Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal calf serum (FCS), penicillin, streptomycin, and L-glutamine. Cell pellets were washed and strained through 70-μm nylon mesh (BD Falcon, Bedford, MA). After counting cell numbers, 1 by 10^6 viable cells per well were plated in six-well plates with DMEM containing 1% FCS and antibiotics, and then the conditioned medium was serially collected and ACTH and catecholamine levels were determined by Mitsubishi “Yuka” IRMA kit and HPLC, respectively. Human subject protocols were approved by our institutional committee and written permission from the patient regarding the experimental use of the tissues was obtained in advance of the surgery. The results of the experiments revealed that the paraganglioma cells predominantly produce ACTH (Fig. 6A), but also secrete noradrenaline and adrenaline (Fig. 6B).

Discussion

Cushing’s syndrome as a consequence of ACTH-producing pheochromocytoma has been reported previously, however, the number of reported cases is very limited. Based on a recent review of the literature, approximately 3% of cases of ectopic ACTH syndrome are likely associated with pheochromocytomas. Although the mecha-
nism that evokes ectopic ACTH production in neoplasm is still poorly understood, most of the ACTH-producing tumors apparently represent undifferentiated neuroendocrine cells capable of POMC gene expression as well as post-translational processing. The POMC gene expression was previously demonstrated in pheochromocytomas unrelated to Cushing’s syndrome by de Keyzer et al., in which all the tumors contained a short splicing form of POMC mRNA in comparison with the pituitary that expresses the long form of the POMC transcripts. However, the mechanism underlying post-translational processing of ACTH peptides remains unclear in ACTH-secreting pheochromocytomas.

In the present case, aberrantly processed ACTH molecules together with authentic 1-39 ACTH molecules were clearly detected by gel chromatography of the preoperative plasma sample. Considering the remarkable increase in urinary cortisol excretion and a major peak of bioactive 1-39 authentic ACTH detected in the plasma, the capability of bioactive ACTH production by the present paraganglioma was unexpectedly high. Based on the in vitro experiments using primary paraganglioma cells, the cells secreted 1-39 ACTH, which can be selectively detected by Mitsubishi IRMA method, as well as noradrenaline and adrenaline. The levels of ACTH in the conditioned media were the highest on the first day of primary culture then gradually reduced to 100 pg/mL, which remained steady for the 10-day culture. Given that the primary cell viability was preserved for 10 days in culture leading to gradual increase in catecholamine levels in the media, this decrease of ACTH levels could be due to effects of proteolytic degradation of 1-39 ACTH during the culture.

In the therapeutic aspect, metyrapone was effective to suppress the preoperative cortisol secretion from the hypertrophic adrenals. It was notable that replacement therapy with hydrocortisone was unnecessary in our case throughout the perioperative period, suggesting that the hypothalamo-pituitary-adrenal (HPA) axis was not functionally suppressed. This condition could be associated with the following issues: 1) duration of hypercortisolism was relatively short considering the clinical onset; and 2) preoperative metyrapone treatment was helpful in stimulating the secretion of endogenous CRH and ACTH. Metyrapone is also reported to reduce ACTH and ACTH precursors in an ACTH-producing adrenal pheochromocytoma. This suggests that either metyrapone had direct effects at the level of the tumor to inhibit the production of ACTH-related peptides or that endogenous glucocorticoids could be a key regulator for inducing ACTH-related peptides. On the contrary, the paradoxic increase in ACTH precursors is also observed after hydrocortisone treatment in a subset of patients with postadrenalectomy Cushing’s disease. Thus, the regulation of ACTH precursors seems to not exactly follow the regulatory pattern for the mature ACTH molecule.

Spontaneous remission of hypercortisolism due to ectopic ACTH syndrome by adrenal pheochromocytoma was reported in three cases. The cause of the disappearance of hypercortisolism is unclear. The decrease in ACTH secretion might be explained by a change in tumor differentiation or endocrine activity accompanied by a decrease in POMC gene transcription. The synthesis of adrenaline depends on the activity of the enzyme phenylethanolamine-N-methyltransferase (PNMT). The enzymatic activity of PNMT is enhanced by increased ambient cortisol concentrations. Furthermore, some studies show
that the activity of tyrosine hydroxylase, which contributes to the synthesis of the noradrenaline and dopamine, is in turn stimulated by ACTH. However, the ACTH–cortisol axis is mutually related to catecholamine production in pheochromocytomas. In our patient, a significant increase in the secretion of adrenaline and noradrenaline was observed, whereas the levels of the urinary metabolites including metanephrine, normetanephrine, and VMA were within normal ranges. This discrepancy was also described in two other patients with adrenal ACTH-secreting pheochromocytoma. Considering the generally high sensitivity of metanephrine and VMA measurement for the diagnosis of pheochromocytomas, it is speculated that hypercortisolism or ectopic ACTH production may negatively affect the detection of urinary metanephrine excretion or the metabolic process of catecholamine including monoamine oxidase (MAO) or catechol-O-methyltransferase (COMT) activation.

In summary, we presented a case of ectopic ACTH syndrome that was developed by extra-adrenal pheochromocytoma. The existence of hormonal production of catecholamine, ACTH, and its precursors was further clarified by plasma analysis and in vitro study using resected paraganglioma. This case provides a new clinical etiology of ectopic ACTH syndrome caused by extra-adrenal abdominal paraganglioma.

References