A Case of ACTH-Independent Macronodular Adrenal Hyperplasia: Simultaneous Expression of Several Aberrant Hormone Receptors in the Adrenal Gland

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Abstract. ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a rare cause of Cushing's syndrome. Recently, aberrant expression of adrenal receptors for various hormones and/or cytokines has been identified in several cases with AIMAH, which may act as a pathogenetic factor for the disorder. We report here an AIMAH patient with a Rathke's cleft cyst. Endocrinological examinations revealed that the pituitary cyst had no hormonal secretion. Administrations of either AVP or isoproterenol provoked cortisol production in the patient, whereas DDAVP, mosapride or endogenous LH induced by GnRH did not. Reverse transcriptional-PCR analysis of total RNA obtained from the patient's adrenal tissue revealed the expression of mRNA of receptors for V1a, V1b, V2, and LH/hCG. Three of these receptors except for V1a receptor were not expressed in normal adrenal tissue. Hyperosmolar saline infusion promoted the patient's cortisol secretion through the increase in endogenous AVP (peak plasma AVP level reached 90.4 pg/ml during the test). These results suggest that endogenous AVP and catecholamines are involved in the pathophysiology of the patient. Further study will be necessary to clarify the molecular mechanisms that regulate tissue-specific expression of these receptors and their role in the overgrowth of adrenal in AIMAH.

Key words: ACTH-independent macronodular adrenal hyperplasia, AVP, Catecholamines, Aberrant receptors, Rathke's cleft cyst

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ACTH-INDEPENDENT macronodular bilateral adrenal hyperplasia (AIMAH), an unusual cause (less than 10%) of Cushing's syndrome, might consist of several subtypes with different pathogenetic mechanisms [1–3]. Recently, aberrant expression of adrenal receptors for various hormones and/or cytokines has been identified in several cases with AIMAH, which might act as a pathogenetic factor for the disorder [4–22]. The hypothesis that these abnormally expressed

receptors promote the adrenal overproduction of cortisol and the overgrowth of the adrenal gland has been partly proven by successful therapies for patients with AIMAH such as the suppression of possible endogenous ligands for the abnormal receptors, or the administration of specific antagonists for them [7, 12, 13, 18]. In the present study, we report a male patient affected with overt Cushing's syndrome due to AIMAH and complicated with pituitary tumor that was suggested to be non-functional. He underwent bilateral adrenalectomy. The aim of this study is to demonstrate his unique endocrinological features with provocative tests, and to analyze his adrenal tissue of the adrenalectomized samples for expression of ectopic or abnormal hormone receptors by reverse transcriptional (RT)-PCR method.

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Patient and Methods

Case report

A 49-yr-old man was referred and admitted to our hospital with suspicion of Cushing's syndrome. He had been treated for chronic type B hepatitis and hypertension for seven years. The patient had noticed rounding of his face and edema of both legs for two years. Diabetes mellitus was diagnosed at another hospital three months prior to this hospitalization.

Physical findings revealed facial plethora, so-called moon face, mild trunkal obesity (weight, 62 kg; height, 162 cm; body mass index, 24), multiple bruises, and high blood pressure (170/96 mm Hg). On laboratory examination, serum potassium was 2.4 mEq/l, sodium 147 mEq/l, albumin 3.3 g/dl, AST 82 U/l, and ALT 176 U/l. Metabolic alkalosis (pH: 7.50, BE: 10.6 mEq/l) was shown on arterial blood gas analysis. Hemoglobin A1c was 8.3% and urinary C-peptide excretion was 190 µg/day. Abdominal CT scan and magnetic resonance imaging (MRI) revealed bilateral large macro-

Fig. 1. (A) Abdominal computed tomography scan of the patient. (B) An adrenal scintigram imaging with ^{131}I -adosterol. (C) A T1-weighted cranial MRI imaging enhanced with Gd-DTPA. (D) A T2-weighted cranial MRI imaging. The pituitary tumor was $14 \times 15 \times 19$ mm, isointense on the T1-weighted and rather hyperintense on T2-weighted images (indicated by arrowheads). The normal pituitary gland seems to be pressed away by the mass. On the dynamic enhancing MRI scan using Gd-DTPA, neither the substance nor the capsule of the tumor was enhanced, while the supposed normal pituitary gland was enhanced (C, indicated by an arrow).

Fig. 2. Microscopic view of nodular lesion of the adrenal gland. (A) Original magnification $\times 5$. (B) Original magnification $\times 50$ (hematoxylin-eosin staining). Nodules were composed predominantly of markedly hyperplastic large clear cells interspersed with colonies of acidophilic small compact cells.

nodular adrenal tumors (Fig. 1A). Adrenal scintigram imaging with ^{131}I -adosterol disclosed uptake of the isotope in the area corresponding to both adrenals (Fig. 1B). A pituitary tumor was detected on a cranial MRI scan. The tumor size was $14 \times 15 \times 19$ mm, which was isointense relative to the cerebral matter on the T1-weighted images, and was rather hyperintense on T2-weighted ones (Fig. 1C, D, indicated by arrowheads). The normal pituitary gland was being pressed away in the cranial and frontal direction by the mass, which was detected in a line-shaped or belt-shaped image. On the dynamic enhancing MRI scan using Gd-DTPA, neither the substance nor the capsule of the tumor was enhanced, while the supposed normal pituitary gland was positively enhanced (Fig. 1C, indicated by arrow). According to these radiological findings, the tumor was diagnosed as a Rathke's cleft cyst.

Basal endocrinological examinations

The basal levels of anterior pituitary hormones, the circadian variations of plasma cortisol and ACTH, urine levels of free cortisol, 17OHCS, and 17KS were examined. Overnight dexamethasone suppression test was performed.

Provocative tests

Anterior pituitary hormones: Three agents including human CRH (100 μg , i.v.), TRH (500 μg , i.v.) and LHRH (100 μg , i.v.) were administered simultaneously.

Cortisol: Various provocative tests including ACTH (250 μg , i.v.), human CRH (100 μg , i.v.), insulin (0.1 U/kg, i.v.) induced hypoglycemic stress, AVP (10 U, i.m.), desmopressin acetate (dDAVP 2.5 μg , s.c.), isoproterenol (20 ng/kg/min, d.i.v. for 30 min), and mosapride citrate (15 mg, p.o.) were performed. A hyperosmolar stress test by continuous intravenous infusion of 5 % NaCl solution for 120 min (3.6 mL/min) was also performed. Every test was done on separate day after an overnight fast.

In vitro study (RT-PCR analysis)

Primer sets for amplification of V1a, V1b, V2, LH/hCG, ACTH, β -adrenergic, 5-HT-4, and glucose-dependent insulinotropic hormone (GIP) receptors were used as described elsewhere (19). RNA preparation, reverse transcription method, and PCR conditions were also as described elsewhere (19). Normal adrenal cortex tissue was obtained from a normal gland (kidney removal; kindly provided by Yoshihiro Wada, M.D.).

These studies were approved by the local ethics committee and informed consent was obtained from the patients involved.

Clinical course

After investigation, bilateral adrenalectomy was performed. Both adrenals were diffusely enlarged with multinodules; the left adrenal weighed 106 g, and the

right adrenal 63 g. On microscopic examination, those nodules were composed predominantly of markedly hyperplastic large clear cells interspersed with colonies of acidophilic small compact cells (Fig. 2A, B). The diagnosis of ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) was confirmed by these pathological findings.

Results

Basal endocrinological examinations

The basal levels of anterior pituitary hormones were normal except for ACTH and TSH (Table 1). The circadian rhythm of plasma cortisol variation was lost (25.6, 22, and 21.4 $\mu\text{g/dL}$ at 0900, 1700, and 2100 h, respectively), and morning levels were not suppressed by 1 mg or 8 mg overnight dexamethasone (33.2 or 21.3 $\mu\text{g/dL}$, respectively). Plasma ACTH was undetectable throughout the day. Urine levels of free cortisol, 17OHCS, and 17KS were high. Other hormone levels are summarized in Table 1.

Provocative tests

Anterior pituitary hormones: Simultaneous loading of CRH, TRH, and LHRH induced normal responses of TSH, PRL, and LH, a blunted response of FSH, and no response of ACTH (Fig. 3). These results, combined with the radiological analysis of cranial MRI scan, indicated that the pituitary tumor was a Rathke's cleft cyst with no hormonal secretion.

Cortisol: Plasma cortisol sharply increased in response to ACTH and AVP, and mildly increased in response to isoproterenol, but did not respond to dDAVP, hCRH, mosapride, or insulin induced hypoglycemia (Fig. 4). After continuous intravenous infusion of 5% NaCl solution for 120 min (3.6 mL/min), plasma cortisol increased by 2.5-fold with a sharp increase in plasma AVP level (Fig. 5). Basal AVP was 4.4 pg/ml and reached the peak value of 90.2 pg/ml at 120 min. Plasma ACTH remained in the undetectable range during all of these tests.

Table 1. Basal hormone levels

	Patient's basal level	Normal range
ACTH (pg/ml)	< 4	4.4 – 48
Cortisol ($\mu\text{g/dl}$)	25.6	5.6 – 21.3
GH (ng/ml)	0.26	< 1.46
PRL (ng/ml)	5.6	1.5 – 9.7
LH (mIU/ml)	3.5	1.1 – 8.8
FSH (mIU/ml)	4.4	1.8 – 13.6
TSH ($\mu\text{IU/ml}$)	0.32	0.4 – 4.1
Testosterone (ng/dl)	198	320 – 1030
U-17OHCS (mg/day)	24.8	3.4 – 12.0
U-17KS (mg/day)	14.9	4.6 – 18.0
Free urinary cortisol ($\mu\text{g/day}$)	1896	35 – 160
Aldosterone (pg/ml)	56	30 – 160
Plasma renin activity (ng/ml/h)	0.7	0.3 – 2.9
DHEA-S (ng/ml)	30	400 – 3500
Free T3 (pg/ml)	2.0	2.3 – 4.2
Free T4 (ng/dl)	0.63	0.72 – 1.52
AVP (pg/ml)	5.0	0.3 – 3.5

Fig. 3. A provocative test for anterior pituitary hormones secretion. CRH, TRH and LHRH were administered simultaneously. Left panel; responses of ACTH, TSH, and prolactin. Right panel; responses of LH and FSH.

Fig. 4. Various provocative tests for cortisol secretion. Plasma cortisol sharply increased in response to ACTH and AVP, and mildly increased in response to isoproterenol, but did not respond to dDAVP, hCRH, mosapride, or insulin induced hypoglycemia.

Fig. 5. Hypertonic saline infusion test. Plasma cortisol increased by 2.5-fold with a sharp increase in plasma AVP level. Basal AVP was 4.4 pg/ml and reached the peak value of 90.2 pg/ml at 120 min. Plasma ACTH remained in the undetectable level during the test.

In vitro study (RT-PCR analysis)

Amplification of RNA from resected adrenal tumor of the patient revealed distinct expression of receptors for vasopressin (V1a, V1b and V2), LH/hCG and ACTH (Fig. 6; upper panel). In the normal control, the expression of V1a and ACTH receptors was detected, whereas no band for V1b, V2, or LH/hCG receptors

was detected (Fig. 6; lower panel). No band for β -adrenergic, 5-HT-4 or GIP receptor was detected either in the patient or normal control.

Discussion

The present study described a patient affected with Cushing's syndrome with both bilateral adrenal tumor and a pituitary tumor. Pathophysiological and endocrinological examinations revealed that the Cushing's syndrome was resulted from AIMAH, whereas the pituitary tumor was diagnosed as Rathke's cleft cyst with no hormonal activity. These two disorders should be regarded as having developed coincidentally, because plasma ACTH level was very low (less than 4 pg/ml) at all times throughout our observation.

There are two distinct Cushing's syndromes caused by ACTH independent hyperplasia of the adrenal glands; primary pigmented nodular adrenocortical dysplasia (PPNAD) and AIMAH. Both of them have been demonstrated to be directly or indirectly associated with protein kinase A signaling. In most cases PPNAD is caused by mutations of the PRKAR1A gene [23], and AIMAH has been associated with aberrant and/or ectopic expression, and presumably regulation of various G protein-coupled receptors including those

Fig. 6. RT-PCR of various receptors mRNA. Lane 1, PCR products for V1a receptor (718 bp); lane 2, V1b (492 bp); lane 3, V2 (657 bp); lane 4, ACTH (513 bp); lane 5, LH/hCG (518 bp); lane 6, β -adrenalin; lane 7, GIP; lane 8, 5-HT₄; lane 9, β -actin (positive control). Expression of the receptors for vasopressin (V1a, V1b and V2), LH/hCG and ACTH was observed, whereas no band for β -adrenergic, 5-HT-4 or GIP receptor was detected in the adrenal tumor (upper panel). In a normal control, expression of the receptor for V1a and that for ACTH was observed, whereas no band for any other receptor was detected (lower panel).

for AVP [5, 8, 19, 21], GIP [9-11, 20], LH [12, 13, 19, 20], catecholamines [7, 19], 5HT-4 [13, 22], and angiotensin II [17]. Thus, the study of ectopic receptor expression in AIMAH may have wider implications for adrenal tumorigenesis, although the molecular mechanisms by which the abnormal receptor expression leads to adrenal hyperplasia are still unknown.

We have examined the involvement of those illegitimate receptors with *in vivo* challenge tests and *in vitro* analysis using RT-PCR. In the challenge tests, AVP and catecholamine (isoproterenol) revealed positive effect on cortisol secretion in the patient. It is noteworthy that a hyperosmolar saline infusion also promoted the patient's cortisol secretion through the increase in endogenous AVP (the peak plasma AVP level during the test reached 90.4 pg/ml). Since plasma ACTH level stayed below the detection limit of assay during these tests, it seems certain that the ACTH-cortisol axis had little effect on these results. RT-PCR analysis of the extirpated adrenal tissues of the patient revealed expression of receptors for V1a, V1b, V2, LH/hCG, and ACTH. Comparing these data with those of normal control, it was suggested that the expression of V1b, V2, and LH/hCG in the patient's adrenal glands were ectopic, while that of V1a and ACTH were eutopic.

Neither LHRH nor DDAVP administration stimulated cortisol secretion in the patient under the aberrant expression of receptor for LH/hCG or V2, respective-

ly. Meanwhile, isoproterenol stimulated cortisol secretion although the expression of β -adrenergic receptor was not confirmed by the RT-PCR analysis. These discrepancies remain unexplained in the present study. However, our results described above suggested the possibility that adrenal stimulation via the aberrant receptor(s) for AVP, and/or LH/hCG, and/or catecholamine is involved in the pathogenesis of AIMAH in the patient. Several reports have demonstrated aberrant cortisol stimulation by a couple of hormones in each case with AIMAH; in one case by LH/hCG or CRH and cisaprid [13], and in another case by GIP and LH/hCG [20]. Bourdeau *et al.* have suggested that the synchronous expression of a number of receptors was frequently observed in AIMAH [16]. Our study also emphasizes the importance of screening for these abnormal hormone receptors, which may be expressed solely, or may be expressed together with other receptors in adrenal glands of AIMAH.

Surgical removal of bilateral adrenal gland has been the most common treatment for AIMAH, which needs subsequent cortisol replacement therapy throughout life. However, subtotal adrenal resection and the metyrapone treatment have been described as alternative choices for AIMAH patients [24, 25]. In addition, successful therapeutic trials for AIMAH using each antagonist for β -adrenaline or LH/hCG receptor have been reported in view of the treatment for hypercortisolism [7, 12, 13]. Therefore, our patient could also have

been treated pharmacologically with such antagonists for aberrant hormone receptors instead of bilateral adrenalectomy.

Recently, we have described a family in which a mother and her son were affected with AIMAH [19]. They are not related to the present case. Exogenous AVP stimulated cortisol secretion in the two patients of the familial AIMAH, and so did exogenous isoproterenol in one of them. To our surprise, the aberrant expression of V1b, V2, and LH/hCG receptors was also detected by RT-PCR in one family member who underwent adrenalectomy. The simultaneous adrenal expression of these aberrant receptors commonly observed in the present case and another unrelated familial case implies the possibility that some specific factors underlie the pathogenesis of some of these patients. The unknown factors may include abnormal transcriptional regulations that are involved in expression of several G-protein coupled receptors such as those for V1b, V2, LH/hCG, and β -adrenaline simul-

taneously. Analyses of the promoter regions or their cis/trans acting factors of the genes encoding these receptors may clarify the molecular mechanisms that regulate tissue-specific expression and lead to increased adrenal expression of them.

In conclusion, we reported a rare case of AIMAH patient with a Rathke's cleft cyst. *In vivo* and *in vitro* examinations suggested that altered regulation of a group of aberrant adrenal receptors for V1b, V2, LH/hCG, and possibly β -adrenaline was involved in the pathogenesis of AIMAH in the patient.

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