Circulating Adrenomedullin Is Increased in Patients With Corticotropin-Dependent Cushing's Syndrome Due to Pituitary Adenoma

Claudio Letizia, Romolo Di Iorio, Giorgio De Toma, Emanuela Marinoni, Sabrina Cerci, Massimiliano Celi, Stefano Subioli, and Emilio D'Erasmo

It has been demonstrated that adrenomedullin, a newly discovered peptide with structural similarity to calcitonin gene–related peptide (CGRP), is expressed in pituitary gland and affects basal and corticotropin (ACTH)-releasing factor (CRF)-stimulated ACTH release in animals, thus suggesting its potential role in regulating the hypothalamus-pituitary-adrenal axis. To evaluate whether ACTH and cortisol levels affect adrenomedullin production in humans, we studied 14 patients with Cushing's syndrome due to pituitary adenoma and 8 patients with Cushing's syndrome due to adrenal tumor, with measurement of circulating adrenomedullin by a specific radioimmunoassay (RIA). Adrenomedullin concentrations were significantly higher in patients with pituitary adenoma (37.6 ± 17.8 pg/mL) versus controls (13.7 ± 6.1 pg/mL) and patients with adrenal adenoma (17.8 ± 2.2 pg/mL). After pituitary surgical treatment, plasma adrenomedullin decreased significantly. In one patient with Cushing's syndrome due to pituitary adenoma who underwent simultaneous sampling of the inferior petrosal venous sinuses, the adrenomedullin concentration was significantly higher in plasma collected from the side with the adenoma and increased after CRF administration (Δ increase, 42.6%), according to ACTH levels. Our findings indicate that circulating adrenomedullin is increased in Cushing's disease, and the pituitary gland may represent the site of the elevated production of adrenomedullin in this condition.

ADRENOMEDULLIN is a novel peptide first isolated from human pheochromocytoma that elicits a long-lasting vasorelaxant activity[1] and shares slight homology with calcitonin gene–related peptide (CGRP).[2] Cultured endothelial cells and vascular smooth muscle cells secrete adrenomedullin and possess specific receptors for this peptide.[3,4] mRNA for adrenomedullin has been detected in various tissues including the adrenal gland, lung, heart, aorta, stomach, intestine, and kidney,[5] and it has been demonstrated that this peptide plays a role in the regulation of renal function by means of its potent natriuretic[6] and diuretic[7] properties, supporting the hypothesis that adrenomedullin may participate in the physiological regulation of blood pressure and vascular homeostasis.

Apart from the vasorelaxant effect, adrenomedullin possesses other functions, such as regulation of hormonal secretion. Adrenomedullin-immunoreactive cells have been demonstrated in the pancreatic islets, thyroid gland, gastrointestinal neuroendocrine system, anterior pituitary, thalamus and hypothalamus,[8,9] and placental and fetal membranes.[10] An in vitro study has shown that adrenomedullin inhibits basal and corticotropin (ACTH)-releasing factor (CRF)-stimulated ACTH secretion from cultured anterior pituitary cells,[11] and dexamethasone and hydrocortisone stimulate adrenomedullin production in a dose-dependent manner.[12] Furthermore, adrenomedullin, acting on CGRP-1 receptors, stimulates glucocorticoid release from cortical cells[13] and inhibits aldosterone secretion stimulated by angiotensin II in rat adrenal zona glomerulosa cells.[14] In an animal model, it has been demonstrated that adrenomedullin decreases ACTH and cortisol concentrations,[15] suggesting a potential role of this peptide in regulating the hypothalamus-pituitary-adrenal axis, but no data are available in humans in vivo.

Cushing's disease is an uncommon condition characterized by the clinical effects of increased secretion of cortisol. In ACTH-dependent Cushing's syndrome, the hypersecretion of cortisol by the adrenal gland is due to an increase of ACTH production by a pituitary gland tumor or ectopic tissue, which does not respond to the negative-feedback signal. To examine in an in vivo model the effect of a high concentration of ACTH and glucocorticoids on the adrenomedullin concentration, we studied a group of patients with Cushing's syndrome caused by pituitary ACTH tumor (Cushing's disease).

SUBJECTS AND METHODS

Patients

We measured plasma adrenomedullin levels in 14 patients with Cushing's disease, 8 patients with Cushing's syndrome due to adrenal adenoma, and 21 age-matched healthy control subjects. The diagnosis was made on the basis of the typical clinical features (asthenia, centripetal distribution of fat, pigmentation of striae, proximal myopathy, menstrual disorders, arterial hypertension, hypertrichosis and hirsutism, virilization, and impotence), laboratory results (urinary excretion of cortisol over 24 hours, circadian rhythm of plasma cortisol, dexamethasone test, plasma ACTH level, and CRF test), and the presence of tumor within the pituitary fossa or in the adrenal gland defined by computed tomographic (CT) scan or nuclear magnetic resonance (NMR).

No medications were administered to the patients in the last 2 weeks before blood sampling. All subjects received a sodium (120 to 140 mEq/d)- and potassium (40 to 60 mEq/d)-controlled diet, with evaluation of the daily uptake by means of urinary excretion. All but 3 patients with a pituitary tumor had microadenomas. In 1 patient with Cushing's disease, the diagnosis of pituitary adenoma was suspected on the basis of clinical and hormonal features, but since it was not possible to localize it using imaging techniques (CT and NMR), simultaneous sampling of the catheterized bilateral inferior petrosal venous sinuses was performed. Blood was drawn from the left and right vein separately.

From the Department of Clinical Science, Laboratory of Perinatal Medicine and Molecular Biology, Second Institute of Obstetrics and Gynecology, and Institute of Surgery, University "La Sapienza," Rome, Italy.

Submitted June 14, 1999; accepted November 1, 1999.

Address reprint requests to Claudio Letizia, MD, 2nd Institute of Internal Medicine, University "La Sapienza," Viale del Policlinico, I-00185 Rome, Italy.

0026-0495/00/4906-0006$10.00/0
doi:10.1053/meta.2000.6241

ACTH and adrenomedullin levels were measured before and 15, 30, and 60 minutes after administration of 100 μg ovine CRF. A ratio greater than 2 between the central and peripheral ACTH concentration was indicative of pituitary production. A ratio greater than 1.5 between the right and left petrosal sinus was used to localize the adenoma. Eight of 14 patients in the study group underwent surgical resection of the pituitary adenoma. In these subjects, blood was also collected after clinical and hormonal remission of the disease.

The protocol of the study was approved by the University Ethics Committee. Informed consent was obtained from all of the patients.

Blood Samples

Blood samples were collected between 8 and 9 AM from an antecubital vein after an overnight fast with the subjects in the recumbent position for at least 60 minutes. Systolic and diastolic blood pressure were measured with a mercury column sphygmomanometer, and the heart rate was registered. Blood samples (5 mL) were added to chilled tubes containing disodium EDTA (1 mg/mL) and aprotinin (500 kallikrein-inhibiting U/mL) and centrifuged at 4°C. The plasma was stored at −70°C until analysis. The plasma adrenomedullin concentration was measured after extraction and purification. Briefly, 2 mL plasma was applied to conditioned Sep-pak C18 cartridges (Millipore, Waters Chromatography, Milford, MA) and the column was sequentially washed with 5 mL isotonic saline, 5 mL 0.1% trifluoroacetic acid, and 5 mL 20% acetonitrile in 0.1% trifluoroacetic acid. The absorbed material was eluted with 4 mL 50% acetonitrile and lyophilized.

Cortisol and ACTH Assays

The cortisol level was measured in plasma and urine using a commercial radioimmunoassay (RIA) kit (Sorin, Saluggia, Italy), and the values are expressed as micrograms per deciliter. Sensitivity was 2 μg/dL, and the intraassay and interassay variation coefficients were 4.5% and 5.5%, respectively.

ACTH was determined in plasma using a commercial RIA kit (Sorin). The values are expressed as picograms per milliliter. Sensitivity was 10 pg/mL, and the intraassay and interassay variation coefficients were 6.2% and 8.3%, respectively.

Adrenomedullin Assay

After lyophilization, the samples were dissolved in 0.3 mL 50-mmol/L phosphate buffer (pH 7.4) and adrenomedullin was analyzed in duplicate in plasma extracts by RIA using a commercial kit (Phoenix Pharmaceuticals, Mountain View, CA) with rabbit polyclonal antibody raised against human adrenomedullin 1-52. The antibody cross-reacts 100% with human adrenomedullin, and no cross-reactivity was reported with rat adrenomedullin, amylin, CGRP, endothelin-1, α-atrial natriuretic peptide, brain natriuretic peptide, or ACTH. The intraassay and interassay coefficient of variation was 5.1% and 12.0%, respectively.

Data Analysis

All data are presented as the mean ± SD. Statistical calculations were performed using the PRIMER software (Primer of Biostatistics, S.A. Glantz; McGraw-Hill, San Francisco, CA, 1987). The individual values were inserted by group on the spreadsheet and evaluated by 1-way ANOVA and Bonferroni's test where appropriate. The correlation between variables was evaluated by Spearman's correlation coefficient. The percent increment in hormones with respect to basal levels is indicated by Δ% ([value post-stimulus/basal value × 100] − 100). Statistical significance was set at a P level less than .05.

RESULTS

Table 1 shows the demographic characteristics and clinical and hormonal parameters of the study groups. As expected, the mean values for arterial pressure, heart rate, plasma and urinary cortisol, and ACTH were significantly higher ($P < .01$) in patients with Cushing's disease than in controls. No differences were present in the diastolic or systolic blood pressure, heart rate, and cortisol level between patients with pituitary adenoma and those with adrenal tumor, although ACTH was significantly lower in the latter group. There were no significant differences in ACTH and plasma and urinary cortisol concentrations in patients with pituitary macroadenoma or microadenoma (data not shown).

Table 1. Demographic, Clinical, and Hormonal Features of the Study Groups (mean ± SD)

Variable	Control (n = 21)	Adrenal Adenoma (n = 8)	Pituitary Adenoma (n = 14)
Age (yr)	31.1 ± 12	37.5 ± 14.2	47.5 ± 13.7
Diastolic blood pressure (mm Hg)	76.4 ± 6.5	98.7 ± 6.9*	98.9 ± 4.5*
Systolic arterial pressure (mm Hg)	120.1 ± 6.6	152.5 ± 7.1*	151.1 ± 8.8*
Heart rate (bpm)	62.1 ± 7.3	74.1 ± 3.1*	72.9 ± 2.8*
Plasma ACTH (pg/mL)	25.2 ± 18.6	11.0 ± 5.2*†	224.7 ± 11.7*
Plasma cortisol (μg/dL)	9.8 ± 4.7	38.4 ± 10.9*	32.8 ± 8.4*
Urinary cortisol excretion (μg/24 h)	45.2 ± 7.1	479.9 ± 282.7*	254.2 ± 91.8*

*$P < .01$ *v* control.
†$P < .01$ *v* pituitary adenoma.

In patients with Cushing's disease who received surgical treatment, cortisol and ACTH decreased significantly after surgery and were similar to the levels found in the control group (data not shown). Circulating adrenomedullin concentrations were significantly ($P < .01$) higher in patients with Cushing's disease (37.6 ± 17.8 pg/mL) compared with the controls (13.7 ± 6.1 pg/mL) or those with adrenal adenoma (17.8 ± 2.2 pg/mL; Fig 1). Adrenomedullin levels in patients with microadenoma or macroadenoma were similar (34.7 ± 17.7 and 48.1 ± 16.8 pg/mL, respectively). Adrenomedullin decreased significantly ($P < .01$) after surgical treatment (16.7 ± 9.2 pg/mL). No correlation was found between adrenomedullin and ACTH or cortisol levels in affected and control subjects. ACTH and adrenomedullin concentrations of the patient undergoing simultaneous bilateral inferior petrosal venous sinus sampling are shown in Table 2. The adenoma was localized on the right side, as confirmed by the basal and CRF-stimulated ACTH ratio. Adrenomedullin levels were higher in blood collected from the right vein and increased significantly after CRF administration up to +42.6% at 30 minutes in the right petrosal sinus compared with the basal values. In peripheral blood, no significant increase was found in adrenomedullin after CRF infusion.

DISCUSSION

In the present study, we examined plasma levels of adrenomedullin in patients before and after pituitary surgery for Cushing's disease. Adrenomedullin concentrations were elevated in untreated Cushing's disease and normalized after surgery. In one patient who underwent petrosal sinus sampling, adrenomedullin levels were about double on the side where the adenoma was localized.

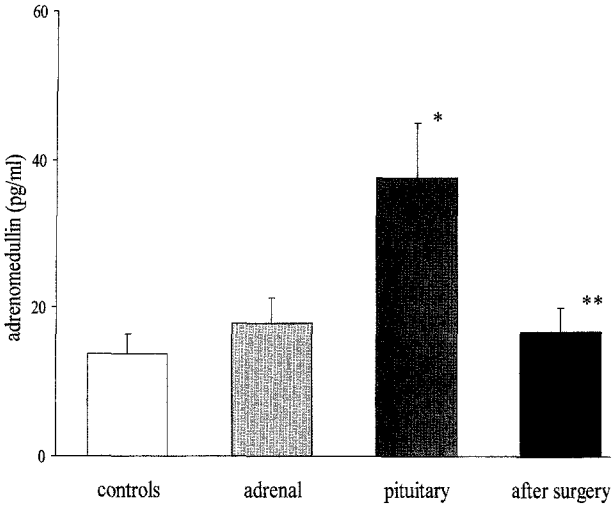

Fig 1. Mean peripheral plasma adrenomedullin concentration in control subjects and in patients with adrenal adenoma (adrenal) and pituitary ACTH-dependent Cushing's disease before (pituitary) and after surgical resection (n = 8). *$P < .01$ *v* controls and adrenal; **$P < .01$ *v* Cushing's disease before surgery. There were no differences in adrenomedullin levels in patients with Cushing's disease after surgery and control or adrenal adenoma groups.

The mechanism of the circulating adrenomedullin elevation in patients with Cushing's disease is not clear. An elevation of plasma adrenomedullin in these patients may be determined by excessive endogenous secretion of cortisol, which may stimulate adrenomedullin synthesis and secretion in plasma. In fact, it has been reported that steroids, in particular glucocorticoids, elicit a stimulation of adrenomedullin production in vascular smooth muscle cells, very likely through activation of the glucocorticoid receptor pathway.[12] However, this hypothesis is not supported by the findings of normal adrenomedullin levels in patients with Cushing's syndrome due to adrenal tumor, which instead suggest that it is produced concomitantly with ACTH. Studies in vitro have shown that rat adrenomedullin inhibits basal and CRF-stimulated ACTH release from cultured anterior pituitary cells in a dose-dependent manner, while no effects have been shown on luteinizing hormone and growth hormone release.[11] Moreover, an in vivo study has shown that intravenous adrenomedullin infusion in conscious sheep decreases ACTH and cortisol concentrations in peripheral blood.[15] In patients with Cushing's syndrome and pituitary adenoma producing ACTH, high ACTH levels induce an increase in circulating cortisol levels, which in turn may upregulate adrenomedullin production. Since ACTH secretion is not downregulated, it is also possible that ACTH itself may directly stimulate adrenomedullin secretion by positive feedback within the pituitary gland. After surgery, adrenomedullin decreased significantly to the levels found in the normal subjects. In accordance with these data, we reported high levels of adrenomedullin in patients with primary adrenal failure (Addison's disease) characterized by elevated ACTH which decreased concomitantly after therapy.[16]

Although we failed to show a direct correlation between adrenomedullin and ACTH or cortisol, our findings indicate that the increase of adrenomedullin in Cushing's disease may be induced by inappropriate ACTH or cortisol production as a compensatory mechanism. Hormonal findings in the patient with Cushing's disease who underwent catheterization of the inferior petrosal venous sinuses clearly indicate that the pituitary gland represents the site of production of the high levels of adrenomedullin found in this condition. In fact, the adrenomedullin concentration was significantly higher in the right versus the left vein, in accordance with ACTH levels. Moreover, CRF administration determined a concomitant increase in ACTH and adrenomedullin that was greater in the right petrosal sinus where the adenoma was localized, and was maintained up to 30 minutes according to the increase in ACTH. The prompt increase of adrenomedullin in response to CRF infusion is in accordance with the effects of actinomycin D and cycloheximide on adrenomedullin production reported by Minamino et al[12] indicating that the biosynthesis of the peptide is designed for a quick response to physiological stimulation. Finally, we cannot exclude that adrenomedullin is increased in association with the clinical complications that characterize Cushing's disease such as arterial hypertension and hyperglycemia. It has been extensively reported that adrenomedullin is increased in patients with hypertension compared with normotensive controls[17-19] and that it plays a role in the regulation of insulin secretion.[20] However, systolic and diastolic blood pressure were similar in the 2 groups with Cushing's syndrome, suggesting that blood pressure should not represent a confounding variable.

In conclusion, the present investigation demonstrates that in Cushing's syndrome, patients with a pituitary adenoma producing ACTH have a remarkable increase in circulating adrenomedullin as compared with normal subjects, and this increase seems related to ACTH production. However, further studies are needed to verify the role of adrenomedullin in Cushing's disease.

Table 2. ACTH and Adrenomedullin Concentrations in One Patient With ACTH-Dependent Cushing's Syndrome Before and After CRF Administration in Blood Samples Collected From the Catheterized Inferior Petrosal Sinuses and the Peripheral Antecubital Vein

	Left Petrosal Sinus				Right Petrosal Sinus				Antecubital Vein			
Variable	0	+15 min	+30 min	+60 min	0	+15 min	+30 min	+60 min	0	+15 min	+30 min	60 min
ACTH (pg/mL)	275	1,113*	373	268	385	5,429†	2,253	371	141	424‡	392	232
Adrenomedullin (pg/mL)	10.9	14.4§	13.7	13.9	22.3	28.1	31.8‖	15.7	16.9	18.4¶	17.2	15.3

*Δ = 304%.
†Δ = 1,310%.
‡Δ = 199%.
§Δ = 32%.
‖Δ = 42.6%.
¶Δ = 8.8%.

REFERENCES

1. Kitamura K, Kawamoto M, Ichiki Y, et al: Adrenomedullin: A novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun 192:553-560, 1993

2. Nuki C, Kawasaki H, Kitamura K, et al: Vasodilator effect of adrenomedullin and calcitonin-gene related peptide receptors in rat mesenteric vascular beds. Biochem Biophys Res Commun 196:245-251, 1993

3. Sugo S, Minamino N, Kangawa K, et al: Endothelial cells actively synthesize and secrete adrenomedullin. Biochem Biophys Res Commun 201:1160-1166, 1994

4. Kato J, Kitamura K, Kangawa K, et al: Receptors for adrenomedullin in human vascular endothelial cells. Eur J Pharmacol 289:383-385, 1995

5. Kitamura K, Sakata J, Kangawa K, et al: Cloning and characterization of cDNA encoding a precursor of human adrenomedullin. Biochem Biophys Res Commun 194:720-725, 1993

6. Ebara T, Miura K, Okamura M, et al: Effect of adrenomedullin on renal hemodynamics and functions in dogs. Eur J Pharmacol 263:69-73, 1994

7. Hirata Y, Hayakawa H, Suzuki Y, et al: Mechanism of adrenomedullin-induced vasodilatation in the rat kidney. Hypertension 25:790-795, 1995

8. Ichiki Y, Kitamura K, Kangawa K, et al: Distribution and characterization of immunoreactive adrenomedullin in human tissue and plasma. FEBS Lett 338:6-10, 1994

9. Washimine H, Asada Y, Kitamura K, et al: Immunohistochemical identification of adrenomedullin in human, rat, and porcine tissue. Histochem Cell Biol 103:251-254, 1995

10. Marinoni E, Di Iorio R, Letizia C, et al: Immunoreactive adrenomedullin in human fetoplacental tissues. Am J Obstet Gynecol 179:784-787, 1998

11. Samson WK, Murphy T, Schell DA: A novel vasoactive peptide, adrenomedullin, inhibits pituitary adrenocorticotropin release. Endocrinology 136:2349-2352, 1995

12. Minamino N, Shoji H, Sugo S, et al: Adrenocortical steroids, thyroid hormones and retinoic acid augment the production of adrenomedullin in vascular smooth muscle cells. Biochem Biophys Res Commun 211:686-693, 1995

13. Mazzocchi G, Musajo FG, Neri G, et al: Adrenomedullin stimulates steroid secretion by the isolated perfused rat adrenal gland in situ: Comparison with calcitonin gene–related peptide effects. Peptides 17:853-857, 1996

14. Yamaguchi T, Baba K, Doi Y, et al: Effect of adrenomedullin on aldosterone secretion by dispersed rat adrenal zona glomerulosa cells. Life Sci 56:379-387, 1995

15. Parkes DG, May CN: ACTH-suppressive and vasodilatator actions of adrenomedullin in conscious sheep. J Neuroendocrinol 7:923-929, 1995

16. Letizia C, Cerci S, Centanni M, et al: Circulating levels of adrenomedullin in patients with Addison's disease before and after corticosteroid treatment. Clin Endocrinol (Oxf) 48:20-23, 1998

17. Kohno N, Hanehira T, Kano H, et al: Plasma adrenomedullin concentrations in essential hypertension. Hypertension 27:102-107, 1996

18. Tanaka M, Kitamura K, Ishizaka Y, et al: Plasma adrenomedullin in various diseases and exercise-induced change in adrenomedullin in healthy subjects. Intern Med 34:728-733, 1995

19. Di Iorio R, Marinoni E, Letizia C, et al: Adrenomedullin, a new vasoactive peptide, is increased in preeclampsia. Hypertension 32:758-763, 1998

20. Martínez A, Weaver C, López J, et al: Regulation of insulin secretion and blood glucose metabolism by adrenomedullin. Endocrinology 137:26-32, 1996