SHORT COMMUNICATION

Expression of adrenomedullin and adrenomedullin mRNA in ectopic ACTH-secreting tumors

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Abstract

Objective: To study the expression of adrenomedullin, a potent vasodilator peptide originally isolated from a pheochromocytoma, in ectopic ACTH-secreting tumors.

Methods: Tumor tissue concentrations of adrenomedullin, calcitonin gene-related peptide, neuropeptide Y, endothelin-1, corticotropin-releasing hormone and ACTH were measured in three ectopic ACTH-secreting tumors by RIA. The expression of adrenomedullin mRNA was examined by northern blot analysis of tissue from one of the tumors.

Results: Immunoreactive adrenomedullin was detected in tumor tissues of three ectopic ACTH-secreting tumors (0.60–18.5 pmol/g wet weight). Calcitonin gene-related peptide, neuropeptide Y, endothelin-1 and corticotropin-releasing hormone were also detected in the tumor tissues. The tumor tissue concentrations of immunoreactive adrenomedullin were comparable to those of these four peptides, but much lower than those of ACTH. Northern blot analysis showed the expression of adrenomedullin mRNA in one tumor from which sufficient tissue was available for such study. The plasma concentration of immunoreactive adrenomedullin was increased in one patient (41.3 pmol/l, control 13.5 ± 3.6 pmol/l, mean $\pm$ s.d., $n = 12$).

Conclusions: These results suggest that adrenomedullin is produced by ectopic ACTH-secreting tumors, together with other neuropeptides, and raise the possibility that adrenomedullin is related to the pathophysiology of these tumors.

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Introduction

Adrenomedullin (ADM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma (1). The C-terminal portion (16–52) of ADM has about 27% homology with calcitonin gene-related peptide (CGRP). ADM has been shown to regulate the production and secretion of some hormones, such as ACTH (2) and aldosterone (3, 4), and to modulate the proliferation and differentiation of cells (5).

ADM mRNA and ADM receptor mRNA are expressed in various kinds of tumors, including pulmonary tumors (6). ADM is produced and secreted by cultured choroid plexus carcinoma cells (7) and by cultured glioblastoma cells (8). It is known that ectopic ACTH-secreting tumors produce and secrete not only ACTH but also other peptides, such as calcitonin. We reported the presence of immunoreactive (IR)-endothelin, IR-neuropeptide Y (NPY), and IR-corticotropin-releasing

hormone (CRH) in the tumor tissue of an ectopic ACTH-secreting bronchial carcinoid tumor (9). There is no information on ADM in ectopic ACTH-secreting tumors. We therefore studied the expression of ADM and ADM mRNA in tumor tissue of ectopic ACTH-secreting tumors.

Materials and methods

Samples

Tumor tissues were obtained at surgery from three patients with ectopic ACTH-secreting tumors (Table 1a). Tumor tissues were stored at -80°C until required for the extraction of peptides and RNA. All the patients had increased plasma concentrations of ACTH and cortisol (Table 1a). After surgery, plasma concentrations of ACTH and cortisol were decreased and patients 1 and 3

Table 1 Clinical characteristics and results of analyses of plasma IR-ADM concentrations and tumor tissue concentrations of IR-ADM, ACTH and four other peptides in three patients with ectopic ACTH-secreting tumors.*a Clinical characteristics*

Pt. No.	Age (years)	Sex	Plasma ACTH (pmol/l)	Plasma cortisol (nmol/l)	Plasma aldosterone (pmol/l)	Serum K ⁺ (pmol/l)	Major clinical symptoms and signs	Histology of tumors
1	58	F	18.1	847	<42	4.2	Edema in the legs, hypertension, moon face, central obesity	Bronchial carcinoid
2	58	M	39.9	861	<42	2.5	Metastasis of the tumor to the iliac bone, diabetes mellitus, hypertension, muscle atrophy, moon face	Neuroendocrine tumor of thymus
3	42	M	56.8	905	114	2.2	Muscle weakness, gait disturbance, hypertension, edema in the legs, central obesity, moon face	Neuroendocrine tumor of thymus

b Results of analyses

Pt. No.	Plasma ADM (pmol/l)	Tumor tissue concentrations (pmol/g wet weight)					
		ADM	CGRP	NPY	ET	CRH	ACTH
1	n.e.	0.60	45.7	8.4	3.37	1.76	3702
2	17.5	2.32	0.8	24.6	0.19	0.62	22
3	41.3	18.5	0.1	37.8	0.11	0.11	309

Pt, patient; n.e., not examined; ADM, adrenomedulin; CGRP, calcitonin gene-related peptide; NPY, neuropeptide Y; ET, endothelin; CRH, corticotropin-releasing hormone.

received dexamethasone (0.5 mg/day). In patient 2, plasma concentrations of ACTH and cortisol were decreased after surgery, but only to approximately 50% of the preoperative values, possibly because of a metastasis of the tumor in the iliac bone. Plasma samples for the measurement of IR-ADM were available from patients 2 and 3. Control plasma samples were obtained from 12 healthy subjects (seven men and five women, 21–40 years old). The study was approved by the Ethics Committee of Tohoku University School of Medicine.

RIA and chromatography

Tumor tissues were extracted as previously reported (10). Plasma samples were extracted by Sep-Pak C18 cartridges (Waters, Milford, MA, USA). Plasma (2 ml) was acidified with 2 ml 0.75 mol/l acetic acid containing 0.5% (w/v) casein (Wako Pure Chemical Industries, Ltd, Osaka, Japan) and loaded onto the cartridge, which was pretreated with 10 ml acetonitrile, 10 ml methanol and then 10 ml 0.75 mol/l acetic acid. The peptide was eluted with 2 ml 60% (v/v) acetonitrile–water containing 0.1% (v/v) trifluoroacetic acid from the cartridge. The eluate was air-dried, reconstituted with assay buffer and assayed. The recovery of this extraction method ranged from 55% to 61%.

The ADM RIA was performed using the antiserum against human ADM(1–52) (No. 102), as previously reported (7, 11). The assay showed less than 0.001%

cross reactivity with other peptides such as α CGRP, IR-CGRP, IR-NPY, IR-endothelin, IR-CRH and IR-ACTH in the tumor tissues were measured by RIA as described previously (9, 12–14). The CGRP RIA showed less than 0.01% crossreaction with human ADM(1–52). The endothelin RIA was performed using an antiserum against endothelin-1 (BP6), which was kindly supplied by Prof. S R Bloom and Dr M A Ghatei (Royal Postgraduate Medical School, London, UK). The endothelin assay showed 60% crossreactivity with endothelin-2 and 70% with endothelin-3 (14).

IR-ADM in the tissue extracts was characterized by reverse-phase HPLC using a μ Bondapak C18 column (3.9 mm $\times$ 300 mm, Waters) as reported previously (7).

RNA extraction and northern blot analysis

Tumor tissue for RNA extraction was available only from patient 3. Total RNA was extracted by the guanidine thiocyanate–cesium chloride method and subjected to northern blot analysis as reported previously (7). The tumor tissue of a pheochromocytoma obtained from a 63-year-old female patient was included as a positive control. The HindIII/EcoRI fragment of pBS-hAM2 (7) was labeled with [α -³²P]dCTP by the random priming method and was used as a hybridization probe. Expression of β -actin mRNA was examined as an internal control.

Results

IR-ADM was detected in all three tumor tissues of ectopic ACTH-secreting tumors (0.60–18.5 pmol/g wet weight) (Table 1b). Reverse phase HPLC of the tumor tissue extracts of these three patients showed a single immunoreactive peak eluting in the position of human ADM(1–52) (data not shown). CGRP, NPY, endothelin and CRH were also detected in the tumor tissues of these ectopic ACTH-secreting tumors (Table 1b). The concentrations of IR-ADM in the tumor tissues were comparable to those of these four peptides, but much lower than those of ACTH.

The plasma concentration of IR-ADM was increased in patient 3 (41.3 pmol/l; control, 13.5 ± 3.6 pmol/l, mean $\pm$ s.d., $n = 12$), but not in patient 2 (17.5 pmol/l) (Table 1b).

Northern blot analysis of the total RNA prepared from the tumor tissue of patient 3 showed the expression of ADM mRNA (Fig. 1) at a level comparable to that in a pheochromocytoma.

Discussion

The present study has shown the expression of ADM and ADM mRNA in the tumor tissues of ectopic ACTH-secreting tumors. The tumor tissue concentrations of IR-ADM were comparable to those in pheochromocytomas in our previous study (11). The tumor tissue

concentration of IR-ADM in patient 3 (18.5 pmol/g wet weight) was greater than the concentrations in normal adrenal glands (12.6 ± 1.0 pmol/g wet weight) (11). The tumor tissue concentrations of IR-ADM in patients 1 and 2 were comparable to those in human brain (0.26–1.4 pmol/g wet weight) (11). In addition, an increased plasma concentration of IR-ADM was found in one patient, suggesting that ADM secreted from the tumor may contribute to plasma IR-ADM concentrations in some patients. Our findings concerning the presence of IR-NPY, IR-endothelin and IR-CRH in the tumor tissues are compatible with those of our previous study (9). The present study has also shown the presence of IR-CGRP in these tumor tissues.

It has been reported that ADM has a direct inhibitory effect on the secretion of aldosterone (3, 4) and an indirect stimulatory effect on it, possibly through catecholamine release (4). Rat ADM(11–50) inhibited ACTH release from dispersed rat anterior pituitary cells (2). ADM secreted from ectopic ACTH-secreting tumors may modulate signs and symptoms in patients with these tumors. Low plasma concentrations of aldosterone were found in all three of our patients. An increased plasma concentration of IR-ADM was observed only in one of them (patient 3), however, and further studies are required to clarify the effects of ADM on signs and symptoms in these patients. Another possible pathophysiological role of ADM in ectopic ACTH-secreting tumors may be through local actions on the differentiation and proliferation of tumor cells. It has been reported that ADM stimulated DNA synthesis and cell proliferation via increases in cAMP in Swiss 3T3 cells (4). In this regard, endothelin-1 is known to have a mitogenic action (15). ADM produced by the tumor may act locally on the growth and differentiation of the tumor, together with endothelin-1. The findings of the present study suggest possible pathophysiological roles of ADM, in addition to CGRP, NPY, endothelin-1 and CRH, in ectopic ACTH-secreting tumors.

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Figure 1 Northern blot analysis of adrenomedullin mRNA in the tumor tissue of an ectopic ACTH-secreting tumor (patient 3). The tumor tissue of a pheochromocytoma (Pheo) was included as a positive control. The lower panel shows the expression of β -actin mRNA as an internal control.

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