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Immunocytochemical localization of adrenomedullin 2/intermedin-like immunoreactivity in human hypothalamus, heart and kidney

Kazuhiro Takahashi[a,e,*], *Kumi Kikuchi*[b], *Yutaka Maruyama*[e], *Tomoko Urabe*[f], *Kiichiro Nakajima*[f], *Hironobu Sasano*[c], *Yutaka Imai*[d,e], *Osamu Murakami*[b], *Kazuhito Totsune*[d,e]

[a] *Department of Analytical Medical Technology, Tohoku University School of Health Sciences, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan*
[b] *Department of Medicine, Tohoku University Hospital, Aoba-ku, Sendai 980-8574, Japan*
[c] *Department of Pathology, Tohoku University School of Medicine, Aoba-ku, Sendai 980-8575, Japan*
[d] *Department of Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Pharmaceutical Sciences and Medicine, Aoba-ku, Sendai 980-8578, Japan*
[e] *Tohoku University 21st COE Program Comprehensive Research and Education Center for Planning of Drug Development and Clinical Evaluation (CRESCENDO), Aoba-ku, Sendai 980-8578, Japan*
[f] *Peptide Institute Inc., Minoh-shi, Osaka 562-8686, Japan*

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ABSTRACT

Adrenomedullin 2/intermedin (AM2/IMD) is a novel member of the calcitonin/calcitonin gene-related peptide (CGRP) peptide family. AM2/IMD has a vasodilator action, and anti-diuretic and antinatriuretic effects in mice. The aim of the present study is to clarify immunolocalization of AM2/IMD in human hypothalamus, heart and kidney obtained at autopsy. Immunocytochemistry showed AM2/IMD-immunoreactive cell bodies in the paraventricular and supraoptic nuclei of human hypothalamus. Both parvocellular and magnocellular cells in the paravetricular nucleus are immunostained with AM2/IMD. Immunostaining of serial sections showed co-localization of AM2/IMD-like immunoreactivity and vasopressin in the paraventricular nucleus. Myocardial cells of the heart and renal tubular cells were positively immunostained with AM2/IMD, whereas neither renal glomeruli nor vasculature in the heart and kidney were immunostained. Reverse-transcriptase polymerase chain reaction confirmed expression of AM2/IMD mRNA in the brain, pituitary, heart and kidney. The present study has shown the wide expression of AM2/IMD in human hypothalamus, heart and kidney, raising the possibility that this novel peptide may be related to the central and peripheral regulation of the circulation and water–electrolyte metabolism.

* *Corresponding author*. Tel.: +81 22 717 7482; fax: +81 22 717 7482.
E-mail address: ktaka-md@mail.tains.tohoku.ac.jp (K. Takahashi).
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1. Introduction

Adrenomedullin 2/intermedin (AM2/IMD) is a novel member of the calcitonin/calcitonin gene-related peptide (CGRP) peptide family, which consists of adrenomedullin (AM), CGRP, calcitonin and amylin [1,10,14,15]. Two research groups discovered this peptide almost simultaneously by searching the genome database, and named intermedin [10] and adrenomedullin 2 [15], respectively. The complex of calcitonin receptor-like receptor (CL) and one of the three receptor activity-modifying proteins (RAMPs) forms receptors for AM and CGRP [7]. The complex of CL and RAMP1 generates receptors for CGRP, whereas the complex of CL and RAMP2 or 3 generates receptors for AM. AM2/IMD can bind to the complex of CL and one of three RAMPs non-selectively.

AM2/IMD stimulates cAMP production and has a potent vasodilator action like AM and CGRP [10,15]. Intravenous injection of AM2/IMD decreased arterial pressure and induced antidiuresis and antinatriuresis in mice [15]. Furthermore, AM2/IMD suppressed gastric emptying activity and food intake in mice, when administered intraperitoneally [10]. AM2/IMD is highly expressed in the intermediate lobe of the rat pituitary and human gastrointestinal tract, particularly the muscularis mucosae layer of the stomach [10]. Reverse-transcriptase polymerase chain reaction (RT-PCR) has shown that AM2/IMD mRNA is widely distributed in various tissues of mice, particularly, submaxillary gland, kidney, stomach, ovary, lymphoid tissues and pancreas [15]. Immunocytochemistry of the mouse heart and kidney showed that AM2/IMD immunoreactivity was detected in the endothelial cells of the coronary arteries and veins, the endothelial cells of glomerular capillaries and vasa recta that run in parallel with renal tubules [14].

It is therefore likely that AM2/IMD acts as a regulator in the circulation. The information on the presence of AM2/IMD peptide in human tissues is very limited: only its expression in human gastrointestinal tract is reported [10]. We therefore studied localization of AM2/IMD-like immunoreactivity in human hypothalamus, heart and kidney by immunocytochemistry. Moreover, we studied expression of AM2/IMD mRNA in human brain, pituitary, heart and kidney by RT-PCR.

2. Methods

2.1. Materials

This study has been approved by the Ethics Committee of Tohoku University School of Medicine. Human hypothalamus, heart and kidney were obtained at autopsy performed at the Department of Pathology, Tohoku University Hospital within 4 h postmortem for immunocytochemistry. Human hypothalamic tissues were obtained from five male patients (52–74 years old). These patients had neither neurological nor endocrinological diseases. Heart tissues were obtained from five subjects without cardiac diseases (one male and four female, 48–79 years old). Kidney tissues were obtained from five subjects without renal diseases (three male and two female, 43–54 years old). The tissues were fixed in 4% formalin and embedded into paraffin. Informed consent was obtained from the family of the subjects. Human brain tissues, pituitary, left ventricles of hearts and kidneys were also obtained at autopsy for the RT-PCR analysis, and stored at −80 °C until the RNA extraction.

2.2. Antiserum

The antiserum against human AM2/IMD (Cat-010-B01, Peptide Institute, Minoh-Shi, Japan) was raised in a rabbit by injecting human AM2/IMD (1–7) conjugated with bovine thyroglobulin.

The antiserum was characterized by enzyme-linked immunosorbent assay (ELISA). The peptides (AM2/IMD, adrenomedullin, calcitonin and CGRP; Peptide Institute) were dissolved in phosphate buffered saline, added to 96-well plates (Iwaki, Chiba, Japan) at concentrations of 0.1 μg/well (about 20 pmol/well), and incubated overnight at room temperature. The plates were washed by water and blocked by Block Ace (Dainippon Pharmaceutical Co., Osaka, Japan) for 6 h. After washing by water, the antiserum was added to each well at dilutions of 1000–16,000, and incubated overnight at room temperature. After washing by water, the immune-complex was reacted with secondary antibody (horseradish peroxidase-conjugated anti-rabbit IgG antibody, Bio-Rad, Tokyo, Japan) for 90 min. ABTS (Roche Diagnostics, Mannheim, Germany) was used as chromogenic substrate for peroxidase. OD_{415} was measured by the microplate reader.

2.3. Immunocytochemistry

Immunocytochemistry was performed by the ABC method using the Vector ABC Kit (Vector Laboratories, Burlingame, CA, USA), as previously reported [11,13]. Briefly, 4 μm sections were deparaffinized, and incubated in methanol containing 0.3% H_2O_2 for 30 min and then with normal goat serum (1:20) to block non-specific staining. Sections were intensely washed in 0.01 mol/L phosphate-buffered saline (pH 7.4) between the procedures. Sections were incubated in antiserum against human AM2/IMD (1:1000) for 20 h at 4 °C. Sections were incubated in biotinylated secondary antibody to rabbit IgG (1:200) (Vector Laboratories) for 30 min at room temperature, and subsequently incubated with peroxidase-conjugated avidin for 30 min using the Vector ABC Kit (Vector Laboratories). These sections were visualized by immersion in 3,3′-diaminobenzidine solution (0.01 mol/L 3,3′-diaminobenzidine in 0.05 mol/L Tris–HCl buffer, pH 7.6 and 0.01% H_2O_2).

Immunocytochemistry of AM2/IMD and vasopressin was performed using serial sections of human hypothalami in order to clarify co-localization of AM2/IMD and vasopressin in the hypothalamus. The antiserum against arginine vasopressin (no. #S701–730) was raised in a rabbit and the characteristics of this antiserum were previously reported in details [8]. The vasopressin antiserum was used at a dilution of 1:3000 in immunocytochemistry.

As negative controls, AM2/IMD antiserum preabsorbed with synthetic AM2/IMD (Peptide Institute) or normal rabbit serum (at a dilution of 1:1000) was used instead of the IMD/AM2 antiserum. An absorption test for AM2/IMD was performed using the antiserum incubated with an excess amount of peptide (10 nmol peptide/1 ml of the diluted antiserum) for 20 h at 4 °C prior to the use.