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Immunohistochemical localization of alpha and beta adrenergic receptors in the human nasal turbinate

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ABSTRACT

Objective: Adrenergic receptors (ARs) include four general types (α1, α2, β1 and β2), which are found in different target tissues. α-AR agonists are commonly used for decongestant therapy of upper airway diseases. In order to clarify the roles of AR subtypes in the upper airways, we investigated the localization of these receptors by immunohistochemistry.

Methods: Human turbinates were obtained after turbinectomy from 12 patients with nasal obstruction refractory to medical therapy. The specific cells expressing α- and β-AR proteins were identified by immunostaining using an anti-human AR subtype-specific antibodies (α1A-, α1D-, α2C- and β2-ARs) antibody.

Results: Immunohistochemical analysis revealed that immunoreactivities for α1D- and β2-ARs were densely distributed in submucosal glands. In contrast, immunoreactivities for α1A- and 2C-ARs were densely distributed in vascular smooth muscle.

Conclusion: Our results suggested that adrenergic receptor (AR) subtypes had different roles in upper airway diseases, such as allergic rhinitis and nonallergic rhinitis.

1. Introduction

Adrenergic receptors (ARs) are cell membrane receptors belonging to the seven-transmembrane-spanning G-protein coupled receptor superfamily. ARs respond to the sympathetic neurotransmitter noradrenaline and to the hormone adrenaline (in addition to various exogenous agonists) by producing a response within the cell that involves second messengers or ion channels. Molecular cloning has definitively identified the existence of three α1-AR subtypes: α1A, α1B and α1D; three α2-AR subtypes: α2A, α2B and α2 C; and three β-AR subtypes: β1, β2 and β3 [1]. α-AR agonists are sympathomimetic agents that function through receptor excitatory functions and decrease resistance to airflow by decreasing the volume of nasal mucosa. Such decongestants are commonly used in the treatment of rhinitis. Additionally, β-AR agonists (particularly β2-AR agonists) have been widely used as bronchodilators for the treatment of asthma, and few studies have examined the role of β-AR in the nose. However, some reports have suggested the clinical benefits of β2-AR agonists in the treatment of nasal allergies [2–4].

To date, only two reports have described the distribution of human AR subtypes at mRNA level in nasal structures using in situ hybridization [5,6]. No reports have described the distribution of AR subtypes in nasal structures at the protein level. Therefore, because of the recent availability of subtype-specific antibodies for α-AR and β-AR, we carried out the present study to examine the distribution of AR subtypes using immunohistochemical analysis in human nasal turbinate tissue.

2. Materials and methods

2.1. Tissue preparation

Human inferior turbinates were obtained after turbinectomy from 12 patients with nasal obstruction refractory to medical therapy as previously reported [7]. Written informed consent was obtained from all patients and this study was approved by the ethics committee of Sapporo Medical University (approval

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number: 24–14). All patients were nonsmokers. Six patients had a perennial allergy against mites as defined by questionnaire and CAP test (Phadia K.K., Tokyo, Japan). All medications, including antibiotics, were prohibited for at least 3 weeks prior to the study. The demographic and clinical characteristics of the patients are summarized in Table 1. After collection, nasal mucosal specimens were immediately fixed in 10% formalin for immunohistochemistry.

2.2. Immunohistochemistry

For immunohistochemistry of the ARs, we used commercial antibodies available for paraffin-embedded sections obtained by the catalog data. Deparaffinized sections were initially incubated with 3% H_2O_2 in methanol for 30 min to quench endogenous peroxidase activity. After microwave treatment (10 min at 500 W) in Target Retrieval Solution (Dako, Glostrup, Denmark), the sections were incubated in peroxidase-blocking solution (Dako) for 10 min followed by overnight incubation with the primary antibody at 4 °C. For immunohistochemistry of α1A-AR and β2-AR, mouse monoclonal anti-human α1-AR antibodies (diluted 1:100, sc-100291: Santa Cruz Biotechnology, Santa Cruz, CA, USA) ormouse monoclonal anti-human β2-AR antibodies (diluted 1:200, sc-81577: Santa Cruz Biotechnology) were used as the primary antibody. For immunohistochemistry of α1D-AR and α2C-AR, rabbit polyclonal anti-human α1D-AR antibodies (diluted 1:100, sc-10721: Santa Cruz Biotechnology) or rabbit polyclonal anti-human α2C-AR antibodies (diluted 1:50, sc-1480-R: Santa Cruz Biotechnology) was used as the primary antibody. The sections were washed and incubated for 30 min with MAX-PO (M) or MAX-PO (R) (Nichirei Bioscience, Tokyo, Japan). After additional washing with phosphate-buffered saline (PBS), the sections were developed with DAB (Dako) chromogen for signal visualization. The slides were counterstained with Mayer's hematoxylin and mounted on cover slips in mounting medium. Negative controls were obtained by replacing primary antibodies with mouse IgG1 and rabbit immunoglobulin fraction (Dako).

To identify ARs expression in nasal vascular endothelial cells, the double immunofluorescence staining using anti-AR monoclonal or polyclonal antibodies and anti-von Willebrand factor polyclonal or monoclonal antibody was performed. For the staining of α1A-AR and β2-AR, the sections were incubated for 3 h at 4 °C with a combination of a rabbit polyclonal anti-von Willebrand factor antibody (diluted 1:150, ab6994, Abcam, Cambridge, UK) and one of the mouse monoclonal anti-α1A-AR antibodies (diluted 1:50, sc-100291: Santa Cruz Biotechnology) or β2-AR antibodies (diluted 1:100, sc-81577: Santa Cruz Biotechnology) after microwave treatment (5 min at 500 W) in Target Retrieval Solution, pH9 (Dako). The sections were washed in PBS and incubated for 30 min with both Alexa Fluor 594-conjugated goat anti-mouse IgG and Alexa Fluor 488-conjugated goat anti-rabbit IgG (diluted 1:50; Molecular Probes, Eugene, OR, USA). For the staining of α1D-AR and α2C-AR, the sections were incubated for 3 h at 4 °C with a combination of a mouse monoclonal anti-von Willebrand factor antibody (diluted 1:50, F8/86 clone, Dako) and one of the rabbit polyclonal anti-α1D-AR antibodies (diluted 1:50, sc-10721: Santa Cruz Biotechnology) or α2C-AR antibodies (diluted 1:30, sc-1480-R: Santa Cruz Biotechnology) after microwave treatment (5 min at 500 W) in Target Retrieval Solution, pH9 (Dako). The sections were washed in PBS and incubated for 30 min with both Alexa Fluor 594-conjugated goat anti-rabbit IgG and Alexa Fluor 488-conjugated goat anti-mouse IgG (1:50; Molecular Probes). Then, the sections were mounted using Vectashield® mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, CA, USA) and examined under an Olympus BX51 microscope fitted with a DP70 CCD camera (Olympus Optical, Tokyo, Japan).

Using the similar methods, the double immunofluorescence staining using anti-AR monoclonal or polyclonal antibodies and anti-alpha smooth muscle actin polyclonal or monoclonal antibody was performed to identify ARs expression in the vascular smooth muscles. For the staining of α1A-AR and β2-AR, the sections were incubated for 3 h at 4 °C with a combination of a rabbit polyclonal anti-alpha smooth muscle actin antibody (diluted 1:200, ab139370, Abcam) and one of the mouse monoclonal anti-α1A-AR antibodies (diluted 1:50, sc-100291: Santa Cruz Biotechnology) or β2-AR antibodies (diluted 1:100, sc-81577: Santa Cruz Biotechnology) after microwave treatment (5 min at 500 W) in Target Retrieval Solution, pH9 (Dako). For the staining of α1D-AR and α2C-AR, the sections were incubated for 3 h at 4 °C with a combination of a mouse monoclonal anti-alpha smooth muscle actin antibody (diluted 1:50, 1A4 clone, Dako) and one of the rabbit polyclonal anti-α1D-AR antibodies (diluted 1:50, sc-10721: Santa Cruz Biotechnology) or α2C-AR antibodies (diluted 1:30, sc-1480-R: Santa Cruz Biotechnology) after microwave treatment (5 min at 500 W) in Target Retrieval Solution, pH9 (Dako). All image analysis was performed with the DP Controller and DP Manager Software (Olympus Optical). Using this method, the ARs-expressing cells were visualized in red, vascular endothelial cells in red, and the merged signal in yellow.

Table 1
Demographic characteristics of allergic and non-allergic patients.

	Allergic rhinitis $N=6$	Non-allergic rhinitis $N=6$
Sex (male/female)	2/4	3/3
Age	31 (19–58)	39 (28–55)
Specific IgE to house dust mite (d1) (kU/L)	2.7 (1.0–13)	<0.35
Total IgE (kU/L)	210 (10–387)	110 (10–185)
Blood eosinophils (cells/μL)	370 (70–690)	135 (55–240)
Current nasal symptoms (number of patients)		
Nasal obstruction	6 (all patients)	6 (all patients)
Sneezing	4	0
Rhinorrhea	3	2

Data expressed as median values and range (in brackets).

3. Results

As shown in Fig. 1A, the immunoreactivity of α1A-AR was densely distributed in vascular smooth muscle. Epithelial cells, endothelial cells and submucosal glands were sparsely labeled. The basal cell layers were moderately labeled. In contrast, immunoreactivity for β2-AR was densely distributed in submucosal glands, while epithelial cells, endothelial cells and vascular smooth muscle were moderately labeled (Fig. 1B). As shown in Fig. 2A, immunoreactivity for α1D-AR was densely distributed in epithelial cells and submucosal glands, while endothelial cells were sparsely labeled. In contrast, immunoreactivity for α2C-AR was densely distributed in vascular smooth muscle, while epithelial cells and endothelial cells were sparsely labeled. There was no specific labeling in submucosal glands (Fig. 2B). The high power field images showed both cytoplasmic and membrane staining of these adrenergic receptors (Figs. 1D,E, and 2D,E). The specificity of the staining was also confirmed by the absence of labeling with normal mouse IgG1 (Fig. 1C) and the rabbit immunoglobulin fraction (Fig. 2C).

To further clarify the vascular cell types expressing AR subtypes, double immunofluorescence staining for ARs and vascular endothelial cells or vascular smooth muscles was performed. As shown in Figs. 3 and 4, overlay images show the

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