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Expression and localization of histamine H_1, H_2, and H_3 receptors in rat olfactory epithelium

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ABSTRACT

Objective: Histamine is an important chemical mediator in the development of allergic rhinitis and plays a key role in eliciting the nasal symptoms of the disorder. Histamine may also affect smell as a neurotransmitter. However, whether histamine receptors are present in the mammalian olfactory epithelium has not yet been examined. The aim of this study was to investigate the expression and distribution of histamine H_1, H_2, and H_3 receptors in rat olfactory epithelium.
Methods: Real-time quantitative PCR and immunohistochemical staining were performed to examine the mRNA level and protein expression and localization of histamine receptors (H_1, H_2, and H_3) in rat olfactory epithelium.
Results: We demonstrated that mRNAs encoding histamine H_1, H_2, and H_3 receptors were detected in rat olfactory epithelium. Immunohistochemistry also showed strong positive staining for these receptors. Co-localization of histamine H_1, H_2, and H_3 receptors with olfactory mature protein revealed that these three histamine receptors were mainly localized in olfactory receptor neurons.
Conclusions: These findings indicate that histamine H_1, H_2, and H_3 receptors are present in rat olfactory epithelium and may play a physiological role in olfactory transmission.
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1. Introduction

Histamine stored in mast cells and basophils has been recognized as one of the most critical mediators in allergic reactions and plays an important role in eliciting the nasal symptoms of allergic rhinitis, such as sneezing, nasal itch, pain, rhinorrhea, and congestion [1,2]. It is now clear that histamine exerts its diverse biological effects by binding to and activating four separate G protein-coupled receptors, referred to as histamine H_1 receptor (H_1R) [3], histamine H_2 receptor (H_2R) [4], histamine H_3 receptor (H_3R) [5], and histamine H_4 receptor (H_4R) [6]. These four histamine receptor subtypes differ in their tissue distribution, agonist- and antagonist-binding affinities, structures, and functions. Stimulation of the H_1R leads to contraction of tracheal and vascular smooth muscles, elevation of vascular permeability, and stimulation of sensory nerve endings [7]. H_2R mainly mediates gastric acid secretion and airway and vascular smooth muscle relaxation [7]. H_3R can act as an autoreceptor controlling histamine synthesis and release [7] or a heteroreceptor regulating the release of neurotransmitters, such as acetylcholine, dopamine, noradrenaline, serotonin, glutamate, substance P, and gamma-aminobutyric acid [8,9]. H_4R is expressed by immunologically relevant tissues, such as spleen, thymus, mast cells, and eosinophiles and considered to exhibit immunomodulatory functions [10,11].

All four histamine receptor subtypes have been identified in human inferior turbinates [12–15].These receptors are involved in the pathogenesis of allergic rhinitis. H_1R is thought to be heavily involved with histamine-induced inflammation in the nasal mucosa. Its antagonists reduced nasal symptoms of sneezing, nasal itch, rhinorrhea, and congestion [16–18] with a decrease of the number of eosinophils in rodent models of allergic rhinitis [18]. In guinea pigs, it was reported that 20% of nasally labeled, dissociated sensory neurons responded to histamine, which was inhibited by H_1R antagonist diphenhydramine [19]. Intranasal challenge with H_2R agonists has been shown to cause nasal congestion, but failed

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to produce any other symptoms [20,21]. These responses were abolished by H_2R antagonist pretreatment. Based on the findings in other vascular systems, the H_2R-mediated nasal congestion is most likely due to the activation of H_2R on the vascular smooth muscle, causing endothelial-independent vasodilation [22]. The role of H_3R in nasal pathophysiology was reported in an experimental model of allergic nasal congestion [23]. In this study the nasal congestion caused by a mast cell degranulator, was reduced by an intravenous administration of a combination of H_1R and H_3R antagonists. Neither H_1R nor H_3R antagonist was effective when given alone. H_4R antagonist caused a significant inhibition of sneezing, nasal rubbing, and serum total IgE, with a decrease in the levels of IL-4 and an increase in the levels of INF-γ in nasal lavage [24]. Until now, investigation histamine receptors in nasal mucosa have almost completely focused on the role of inflammatory reaction in nasal respiratory mucosa.

The olfactory epithelium (OE) located at the dorsal part of the nasal cavity is segregated from the respiratory mucosa and harbors the olfactory receptor neurons (ORNs) for smell. A few studies speculated that histamine may play a role in the modulation of olfactory transmission. To identify the functions of histamine in the mammalian OE, better understanding of the distribution of each histamine receptor subtype is crucial. However, to our knowledge there is no report concerning the presence of histamine receptors in OE. The aim of the present study was to determine the expression and distribution of H_1R, H_2R, and H_3R in rat OE by real-time quantitative PCR and immunohistochemistry technique.

2. Materials and methods

2.1. Animal

Healthy 8-week-old Sprague-Dawley rats with a body weight of 250 g ± 20 g were used in this study. The care and use of the rats was approved by the Animal Care and Use Committee of the West China Medical School of Sichuan University and carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023).

2.2. Tissue preparation, RNA extraction and cDNA synthesis

Rats were deeply anesthetized with pentobarbital sodium (100 mg/kg, i.p.) and sacrificed by decapitation. OE was quickly removed and immediately frozen in liquid nitrogen as previously described [25]. To avoid contamination with respiratory cells, only the caudal posterior one third of the OE of the septum was peeled off from the cartilage. The tissues were then homogenized in Trizol Reagent (Invitrogen, US). Total RNA was extracted using RNeasy kit and reverse transcribed into cDNAs using Revert Aid™ First Strand cDNA Synthesis Kit (Fermentas, US) according to the manufacturer's instructions.

2.3. Real-time quantitative PCR

Primer pairs and probes used for TaqMan real-time quantitative PCR were designed using Primer-Blast (www.ncbi.nlm.nih.gov). The sequences of primers and probes for H_1R, H_2R, and H_3R genes, and for ACTB as the reference gene are shown in Table 1. Probes were labeled with the fluorescent reporter dye FAM (6-carboxyfluorescein) at the 5′ end and the fluorescent quenching group TAMRA (6-carboxy-tetramethyl-damine) at the 3′ end. The primers and the probes were synthesized and labeled by Sangon Biotech Co. Ltd. (Shanghai, China).

The PCR reaction was performed on FTC2000 Real-Time PCR system (Funglyn Biotech Inc., Canada) in a final volume of 30 μl containing 5 μl cDNA, 3 μl 10 × PCR buffer (Mg^{2+} free), 3 μl MgCl2 (25 mM), 0.36 μl dNTP mix (25 mM, Promega, US), 1 μl of each primer (10 μM), 1 μl probe (10 μM), 0.3 μl Taq DNA Polymerase (5U/μl, TAKARA, China), and 15.34 μl double-distilled H_2O. The thermal cycling conditions were 94 °C for 2 min and then amplified for 45cycles as follows: 94 °C for 20s (denaturation); different annealing temperatures for different primers and probes (see Table 1) for 20s, and 60 °C for 30s (extension), followed by measurement of fluorescence during each of the extension reaction. The specificity of the primers and probes was investigated using BLAST against public databases in NCBI (www.ncbi.nlm.nih.gov) and confirmed by sequencing of PCR products (Sangon Biotech Co. Ltd., Shanghai, China). The expression levels were analyzed as relative quantities, normalized to the endogenous control (ACTB). Values were obtained according to the equation $RQ_{sample} = 2^{-\triangle Ct}$ (where RQ_{sample} is relative quantity of sample and $\triangle Ct = Ct_{histamine\ receptor} - Ct_{ACTB}$).

2.4. Immunohistochemistry

Rats were deeply anesthetized with pentobarbital sodium (100 mg/kg, i.p.) and sacrificed by decapitation. Olfactory turbinates were quickly removed and then embedded in optimal cutting temperature mounting medium (Sakura Fine Technical Co., Ltd., Tokyo, Japan). Serial axial sections (4 μm thick) were cut with a cryostat and mounted on glass slides. Sections were treated in the following order: (1) fixation in 10% neutral formalin for 3 min at 4 °C; (2) washing in double-distilled water, 3 × 5min each; (3) washing in wash buffer (Dako, Denmark); (4) incubation with primary antibodies [rabbit anti-H_1R polyclonal antibody (1:100, Santa Cruz, US), rabbit anti-H_2R polyclonal antibody (1:100, Santa Cruz, US), or rabbit anti-H_3R polyclonal antibody (1:100, Santa Cruz, US), together with goat anti-olfactory mature protein polyclonal antibody (OMP, 1:50, Santa Cruz, US)] for 1.5 h at 37 °C; (5) washing in double-distilled water, 3 × 5min each; (6) incubation with secondary antibodies [Alexa Fluor® 488 anti-rabbit IgG (1:500; Cell signaling Technology, US) and Alexa Fluor® 594 anti-goat IgG (1:500, Life technologies, US)] for 30 min at room temperature; (7) nuclear counterstaining with 4′-6-diamidino-2-phenylindole (DAPI, 0.1 μg/ml) for 3 min at room temperature; (8) washing in double-distilled water, 3 × 5min each.

Sections of rat inferior turbinates were used for antibody specificity controls (data not shown). Additional control was performed without inclusion of primary antibodies. The fluorescent signal was visualized with a Leica fluorescence microscope (DM4000B) equipped with an appropriate filter set. Images were acquired using Leica software (at 1024 × 1024 pixel resolution) and analyzed with the Adobe Photoshop CS6 (Adobe, US).

2.5. Statistical analysis

The Statistical Package for the Social Sciences version 16.0 (SPSS Inc., Chicago, IL, US) and GraphPad Prism 5.0 (GraphPad Software, Inc., US) were used for statistical analysis. The results are presented as mean ± standard error (SE). Analysis of Variance was used to compare mRNA expression levels among H_1R, H_2R, and H_3R. P value less than 0.05 was considered to be statistically significant.

3. Results

3.1. H_1R, H_2R, and H_3R mRNA expression levels in adult rat OE

We first examined the levels of the mRNAs encoding H_1R, H_2R, and H_3R in rat OE. Our real-time quantitative PCR results, as shown in Fig. 1, revealed that H_1R, H_2R, and H_3R mRNAs were expressed in

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