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Full paper

Antihistamines suppress upregulation of histidine decarboxylase gene expression with potencies different from their binding affinities for histamine H_1 receptor in toluene 2,4-diisocyanate-sensitized rats

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ABSTRACT

Antihistamines inhibit histamine signaling by blocking histamine H_1 receptor (H1R) or suppressing H1R signaling as inverse agonists. The H1R gene is upregulated in patients with pollinosis, and its expression level is correlated with the severity of nasal symptoms. Here, we show that antihistamine suppressed upregulation of histidine decarboxylase (HDC) mRNA expression in patients with pollinosis, and its expression level was correlated with that of H1R mRNA. Certain antihistamines, including mepyramine and diphenhydramine, suppress toluene-2,4-diisocyanate (TDI)-induced upregulation of HDC gene expression and increase HDC activity in TDI-sensitized rats. However, *d*-chlorpheniramine did not demonstrate any effect. The potencies of antihistamine suppressive effects on HDC mRNA elevation were different from their H1R receptor binding affinities. In TDI-sensitized rats, the potencies of antihistamine inhibitory effects on sneezing in the early phase were related to H1R binding. In contrast, the potencies of their inhibitory effects on sneezing in the late phase were correlated with those of suppressive effects on HDC mRNA elevation. Data suggest that in addition to the antihistaminic and inverse agonistic activities, certain antihistamines possess additional properties unrelated to receptor binding and alleviate nasal symptoms in the late phase by inhibiting synthesis and release of histamine by suppressing HDC gene transcription.

1. Introduction

Pollinosis, a seasonal allergic rhinitis, affects approximately 30% of the Japanese population (1). Histamine plays important roles in the pathogenesis of allergic rhinitis; its action is mainly mediated through the histamine H_1 receptor (H1R). Antihistamines act as inverse agonists to inhibit histamine signaling by blocking H1R or suppressing H1R signaling (2,3). Therefore, antihistamines are employed as first-line treatment for the nasal symptoms of pollinosis.

H1R gene expression strongly correlates with the severity of allergic symptoms in toluene-2,4-diisocyanate (TDI)-sensitized rats and patients with pollinosis (4,5). Further, the suppression of upregulated H1R gene expression alleviates nasal symptoms (6–9). Allergic reactions are characterized further by disrupting the T

Abbreviations: DEPC, diethylpyrocarbonate; PCA, perchloric acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H1R, histamine H_1 receptor; HDC, histidine decarboxylase; IL, interleukin; PKCδ, protein kinase C-δ; PMA, phorbol 12-myristate 13-acetate; PUM1, pumilio RNA binding family member 1; Th1/Th2, helper T cell type 1/2; TDI, toluene-2,4-diisocyanate.

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helper cell type 1/2 (Th1/Th2) balance generating a pronounced Th2 profile (10). Th2 cytokine genes, including interleukin (IL)-4 and IL-5 genes, are upregulated in TDI-sensitized rats and patients with pollinosis; their expression levels were correlated with that of H1R gene (4,7,11), suggesting crosstalk between the H1R and Th2 cytokine signaling pathways. Intranasal application of IL-4 and histamine increases H1R gene and IL-4 gene expression, respectively (7). These findings suggest that H1R signaling is important for pollinosis development and that drugs designed to suppress H1R signaling will be effective for treating allergies.

Because histidine decarboxylase (HDC) is the only enzyme that catalyzes histamine synthesis, HDC gene expression is an important regulatory step in histamine signaling (12). Histamine is released from subcutaneous mast cells during the anaphylaxis phase via an IgE-mediated mechanism, whereas in the post-anaphylaxis phase, histamine is produced because of an increased HDC activity (13). HDC mRNA levels increase in patients with allergic rhinitis and bronchial asthma (14). Application of TDI upregulates HDC mRNA expression and HDC enzymatic activity and histamine content in the nasal mucosa (7). Moreover, suppression of HDC gene expression alleviates the nasal symptoms of TDI-sensitized rats.

Here we show that preseasonal prophylactic treatment with antihistamines suppressed the upregulation of HDC mRNA expression in patients with pollinosis and that the expression level of HDC mRNA was strongly correlated with that of H1R mRNA. Because insufficient data are available on the effect of antihistamines on HDC gene expression, we used TDI-sensitized rats to investigate the effects of antihistamines on nasal symptoms, histamine levels in nasal discharges, HDC activity, and HDC gene expression in the nasal mucosa. Our data suggest that during the late-phase response, mepyramine and diphenhydramine significantly suppressed TDI-induced upregulation of HDC mRNA, HDC activity, and histamine content in the nasal mucosa with potencies that differed from their K_i values for [^{3}H]mepyramine binding affinity to H1R.

2. Materials and methods

2.1. Analysis of HDC mRNA expression in the nasal mucosa of patients with pollinosis

We determined HDC mRNA levels using the remaining nasal mucosa samples of patients previously analyzed for H1R mRNA levels (5). Patient information, preparations for scraping the nasal mucosa, evaluation of nasal symptoms, and other experimental conditions were previously described (5). Among 25 patients with allergic rhinitis caused by Japanese cedar pollen, 8 patients (3 males and 5 females; mean age, 47.9 years) who were treated at the Department of Otolaryngology, Yashima General Hospital before the peak pollen period (February 20–March 26, 2009) were administered prophylactic treatment with antihistamines (ebastine, 10 mg/day p.o., n = 5; and fexofenadine, 120 mg/day p.o., n = 3). The treatment lasted for 17.9 ± 11.0 days (mean ± SD) before the follow-up visit during the peak pollen period when nasal symptoms were evaluated and nasal mucosa scrapings were obtained from patients by an otolaryngologist as previously described (5).

Sneezing, watery rhinorrhea, and nasal obstruction were separately scored on a 0–5 point scale before nasal sampling. Under local anesthesia using 4% lidocaine, the surface of each patient's inferior nasal concha was scratched with a small spatula to obtain a sample of the nasal mucosa. Of the initial 25 patients, the remaining patients (8 males and 9 females; mean age, 46.3 years) who first visited the hospital during the peak pollen period but did not take antihistamines or any other treatment were designated as the no treatment group.

Total RNA isolated using the RNAqueous Micro Kit (Applied Biosystems, Foster City, CA) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The sequences of the HDC primers and TaqMan probe are listed in Table 1. The pumilio RNA binding family member 1 (PUM1) primer and probe kit (Hs 00206469-m1, Applied Biosystems) was used to generate a standard (5). The data are expressed as the ratio of HDC mRNA to PUM1 mRNA as previously described. The ethics committee of Tokushima University Hospital and Yashima General Hospital approved this study; written informed consent was obtained from each patient before the study commenced.

2.2. Animal studies

Six-week-old male Brown Norway rats (200–250 g, Japan SLC, Hamamatsu, Japan) with free access to water were kept in a room maintained at constant temperature (25 ± 2 °C) and humidity (55% ± 10%) with 12-h light/dark cycle. TDI-sensitization was performed as previously reported (12). Antihistamines were orally administered once daily for 3 weeks. The number of sneezes was measured during the 10-min period just after (early phase reaction) or 9 h (late phase reaction) after TDI provocation. The antihistamines (10 mg/kg each) were orally administered 1 h before TDI provocation, whereas the control group received water. All experimental procedures were performed in accordance with the guidelines of the Animal Research Committee of Tokushima University.

2.3. Real-time quantitative reverse transcription polymerase chain reaction

Rat nasal mucosa samples collected using RNAlater (Applied Biosystems, Foster City, CA, USA) 4 h after provocation were homogenized using a Polytron (Model PT-K; Kinematica AG, Littau/Luzern, Switzerland) in 10 volumes of ice-cold TRIzol; total RNA was prepared as previously described (7). RNA samples (8 μg) were reverse-transcribed using SuperScript II reverse transcriptase (Invitrogen); real-time PCR was conducted as previously described (7). TaqMan primers and probes were designed using Primer Express software (Applied Biosystems). The primers used to detect rat HDC mRNA are listed in Table 1. To standardize the starting material, Rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Control Reagents (VICProbe; Applied Biosystems) were used. Data are expressed as the ratio of HDC mRNA to GAPDH mRNA.

Table 1
Nucleotide sequences of primers and probes.

Primer/probe name	Sequence
Human HDC mRNA	
Sense primer	5′-CCTGAATGCAGCTCTCAATGTG-3′
Anti-sense primer	5′-CAGAGTCCCTGAAGTATATCCTCAGAC-3′
Probe	FAM-TTGCCCTCTGCAGGCCATGGTTTA-TAMRA
Rat HDC mRNA	
Sense primer	5′-GCAGCAAGGAAGAACAAAATCC-3′
Anti-sense primer	5′-CAACAAGACGAGCGTTCAGAGA-3′
Probe	FAM-AAAGCGCATGAGCCCAATGCTGAT-TAMRA
Mouse HDC mRNA	
Sense primer	5′-TCTACCTCCGACATGCCAACT-3′
Anti-sense primer	5′-CCACAGCTTAATGGAGCGAAAG-3′
Probe	FAM-CACGGACTTCATGCATTGGCAGATCC-TAMRA

2.4. Measurement of HDC activity and histamine content in the nasal mucosa

Nasal mucosa was removed from the nasal septum 9 h after TDI-provocation and homogenized with 10 volumes of ice-cold HDC buffer containing 0.1 M potassium phosphate buffer, pH 6.8, 0.2 mM DTT, 0.01 mM pyridoxal 5′-phosphate, 1% polyethylene glycol (approximately 300 kDa), and 100 μg/ml PMSF. The homogenates were centrifuged at 10,000 × *g* for 15 min at 4 °C, and one-half of the supernatant (supernatant A) was dialyzed thrice against HDC buffer for 6 h at 4 °C (supernatant B). Histamine content in supernatant A was determined using high-performance liquid chromatography (12). The HDC activity of supernatant B was determined by incubation for 4 h at 37 °C in 0.25 mM L-histidine. HDC activities were calculated according to histamine levels (minus the blank). Protein concentrations were determined using the bicinchoninic acid protein assay reagent (Sigma) and bovine serum albumin as standard.

2.5. Determination of histamine content in nasal-cavity lavage fluid

Nasal lavage was performed 9 h after TDI provocation using a slight modification of a published method (15). Rats were anesthetized with diethyl ether and then polyethylene tubing connected to a regulated vacuum device positioned in the left nostril; slight negative pressure was then applied. Washing with 1.5 ml PBS (37 °C) was performed from the right to left nostrils and repeated in reverse order. Nasal exudates were transferred to a new tube; 10 μl perchloric acid (60% w/v) was added, The suspension was centrifuged at 10,000 × *g* for 15 min at 4 °C, transferred to another tube and stored at −20 °C. The histamine content in the supernatant was determined as described above.

2.6. Effect of antihistamines on PMA-induced upregulation of HDC gene expression in RAW 264.7 cells

RAW 264.7 cells were cultured in Eagle's minimal essential medium containing 10% fetal calf serum and 1% antibiotic–antimycotic (Invitrogen) at 37 °C in a humidified atmosphere containing 5% CO_2. RAW 264.7 cells (70% confluence) in 6-well dishes were treated with 10 μM antihistamines 1 h before the addition of 100 nM of phorbol 12-myristate 13-acetate (PMA). After 5 h, the cells were harvested using 700 μl RNAiso Plus (Takara) to isolate total RNA that was reverse transcribed (5 μg) using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Real-time PCR was performed as described above.

2.7. Statistical analysis

The results are shown as mean ± SEM, and the data acquired using nasal mucosa of patients with pollinosis are shown as mean ± SD. Statistical analyses were performed using the unpaired *t* test or one-way ANOVA using Dunnett's test with GraphPad Prism software (GraphPad Software, Inc. San Diego CA). Correlations were analyzed using the Spearman rank correlation method. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of preseasonal prophylactic treatment with antihistamine on HDC mRNA expression in the nasal mucosa of patients with pollinosis

Preseasonal prophylactic treatment with antihistamines significantly suppressed HDC mRNA expression (Fig. 1A). HDC mRNA expression levels were positively correlated with nasal symptoms (Fig. 1B) and H1R expression levels (Fig. 1C).

3.2. Effect of antihistamines on TDI-induced upregulation of HDC gene expression, HDC activity, histamine content in the nasal mucosa, and histamine levels in nasal lavage fluid of TDI-sensitized rats

Compared with controls, HDC mRNA expression in TDI-sensitized rats was significantly increased after provocation with TDI. Mepyramine and diphenhydramine strongly suppressed TDI-induced upregulation of HDC gene expression in TDI-sensitized rats (Fig. 2A). The other antihistamines, except *d*-chlorpheniramine, tended to suppress the TDI-induced increase in HDC mRNA levels. We next measured HDC activity in the nasal mucosa of TDI-sensitized rats 9 h after TDI provocation. TDI application for 3 weeks induced a significant increase in HDC activity (Fig. 2B). Pretreatment with mepyramine and diphenhydramine significantly suppressed the TDI-induced increase in HDC activity. Azelastine, epinastine, and olopatadine tended to suppress HDC activity. In contrast, *d*-chlorpheniramine, which did not suppress TDI-induced HDC upregulation, failed to suppress HDC activity. Provocation with TDI induced a significant increase in histamine content in the nasal mucosa of TDI-sensitized rats (Fig. 3). Pretreatment with mepyramine and diphenhydramine significantly decreased histamine content in the nasal mucosa. Azelastine, epinastine, and olopatadine tended to reduce the histamine level in the nasal mucosa. *d*-Chlorpheniramine had no detectable effect. Analysis of nasal lavage samples revealed a notable increase in histamine content 9 h after TDI provocation, which was significantly suppressed by mepyramine and olopatadine. Azelastine tended to decrease histamine content, whereas *d*-chlorpheniramine did not have a detectable effect (Fig. 4).

3.3. Effect of antihistamines on TDI-induced nasal symptoms of TDI-sensitized rats

To determine whether histamine induced an allergic reaction, we monitored sneezing just after or 9 h after provocation. Pretreatment with antihistamines reduced the number of TDI-induced sneezes at both times (Fig. 5). The potencies of inhibitory effects of the antihistamines on the number of sneezes just after provocation with TDI were similar to their respective affinities of binding to H1R (Table 2) (16–19). In contrast, 9 h after TDI provocation, these effects were similar to the inhibition of HDC mRNA expression.

3.4. Effect of antihistamines on PMA-induced upregulation of HDC gene expression in RAW 264.7 cells

PMA treatment upregulates HDC mRNA expression in RAW 264.7 cells by activating the PKC/ERK signaling pathway (20). When RAW 264.7 cells were pretreated with mepyramine and azelastine, the upregulation of HDC mRNA expression induced by PMA was not suppressed, although it was significantly suppressed by *d*-chlorpheniramine (Fig. 6).

4. Discussion

In the present study, we demonstrate that certain antihistamines, including mepyramine and diphenhydramine, alleviate allergic symptoms through their antihistaminic effects and by suppressing histamine synthesis by inhibiting HDC mRNA expression. H1R gene expression was highly correlated with the severity of allergic symptoms, and compounds that suppress H1R gene expression alleviate the symptoms of allergy of patients with

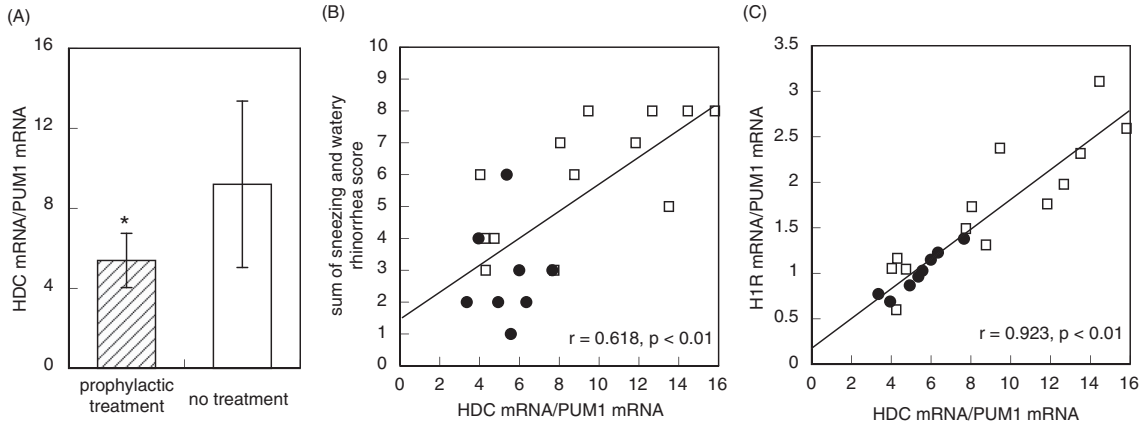

Fig. 1. Effect of preseasonal prophylactic treatment with antihistamines on the upregulation of HDC mRNA (A) and the relationship between the expression levels of HDC mRNA and the nasal symptoms (B) and the expression level of H1R mRNA (C). The scraping of the nasal mucosa from the patients with Japanese cedar pollinosis were collected and total RNA was isolated using the RNAqueous Micro Kit. HDC mRNA was determined by quantitative real-time RT-PCR. Data for the sum of sneezing and watery rhinorrhea scores and the expression of H1R mRNA were obtained from Mizuguchi *et al.* (8). In A, Data are expressed as means ± SD. For statistical analysis, unpaired t-test was used. *P < 0.05 vs. no treatment group. In B and C, filled circles and open squares represent data obtained from patients with or without preseasonal prophylactic treatment with antihistamines, respectively. Correlation was analyzed by Spearman's rank correlation test.

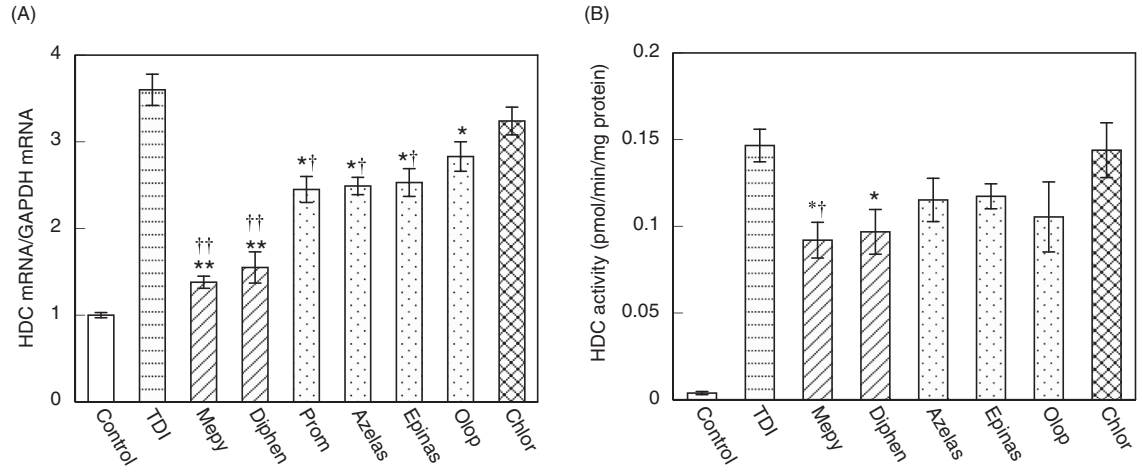

Fig. 2. Antihistamines block TDI-induced upregulation of HDC mRNA (A) and suppress HDC activity (B). In A, HDC mRNA was determined by quantitative real-time RT-PCR 4 h after provocation. In B, HDC activity in the nasal mucosa was measured by HPLC 9 h after provocation. Data are expressed as means ± SEM. Control, vehicle control; TDI, TDI-provoked rats without administration of antihistamines (TDI-control); Diphen, diphenhydramine; Mepy, mepyramine; Diphen, diphenhydramine; Prom, promethazine; Azlas, azelastine; Epinas; epinastine; Olop, olopatadine; Chlor, *d*-chlorpheniramine. $^{**}P < 0.01$, and $^{*}P < 0.05$ vs. TDI; $^{\dagger\dagger}P < 0.01$ and $^{\dagger}P < 0.05$ vs. *d*-chlorpheniramine (n = 4).

pollinosis (4–9). Th2 cytokines also play important roles in the pathogenesis of allergic inflammation (10). For example, in patients with pollinosis, histamine influences the expression and actions of Th2 cytokines through crosstalk between the H1R and Th2 cytokine signaling pathways; and Th2 cytokines, in turn, modulate the production and release of histamine (21,22).

We demonstrated that prophylactic treatment with antihistamines suppressed TDI-induced upregulation of H1R and IL-4 mRNA expressions in TDI-sensitized rats (4). We also showed that direct administration of IL-4 into the nasal cavities of normal non-TDI-treated rats upregulated H1R mRNA expression (7). Intranasal histamine application increased IL-4 mRNA levels in normal rats (7), and H1R expression level was strongly correlated with that of IL-5 in patients with pollinosis (11). These findings suggest that H1R gene expression is correlated with that of Th2-cytokine genes and that suppression of H1R gene expression affects the expression levels of Th2 cytokines. Therefore, we consider that the suppression of H1R signaling is important for treating allergies.

In the present study, we used TDI-sensitized rats as a model of allergic rhinitis. Allergic rhinitis is defined as an IgE-mediated disease (1). In contrast, rhinitis caused by TDI is a non-IgE mediated disease (23). For example, TDI-specific IgE is not detected in mice exposed to TDI, although total IgE and TDI-specific IgG titers are elevated (24). Therefore, nasal symptoms and pathogenesis observed in TDI-sensitized rats may differ from those of antigen-induced IgE-dependent rhinitis. However, intranasal application of TDI induces nasal symptoms, including sneezing, and watery rhinorrhea in TDI-sensitized guinea pigs (25), which are similar to those observed in patients with allergic rhinitis. TDI-sensitized rats display many characteristic features of human allergic rhinitis (e.g., nasal symptoms), including infiltration of eosinophils and mast cells (26), increased levels of cytokines (27–30), elevation of H1R mRNA and protein expression (31), increased levels of HDC mRNA, HDC activity, and histamine content (12). Accordingly, distinguishing between immunological sensitization and irritation by TDI is difficult, probably because that the underlying mechanisms driving TDI-induced rhinitis share similarities with allergic rhinitis caused by ubiquitous airborne protein allergens. Therefore, we consider that TDI-sensitized rats can serve as a model for human allergic rhinitis.

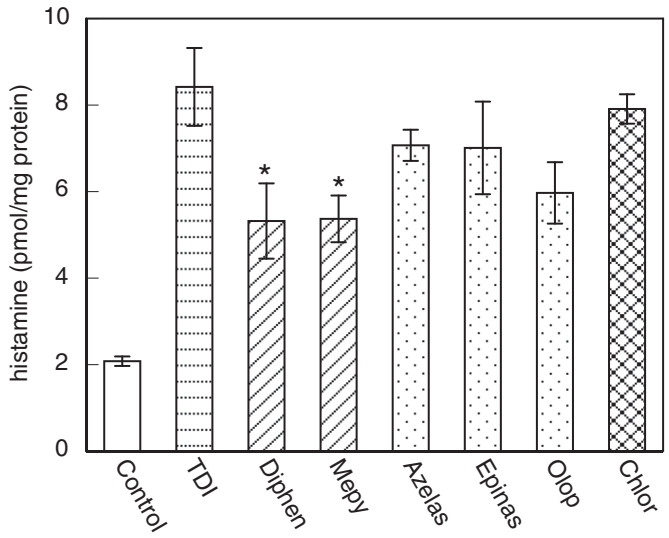

Fig. 3. Antihistamines suppress histamine synthesis. Histamine content in the nasal mucosa was measured by HPLC 9 h after provocation. Data are expressed as means ± SEM. Control, vehicle control; TDI, TDI-control; Diphen, diphenhydramine; Mepy, mepyramine; Azlas, azelastine; Epinas, epinastine; Olop, olopatadine; Chlor, *d*-chlorpheniramine. $^{*}P < 0.05$ vs. TDI (n = 4).

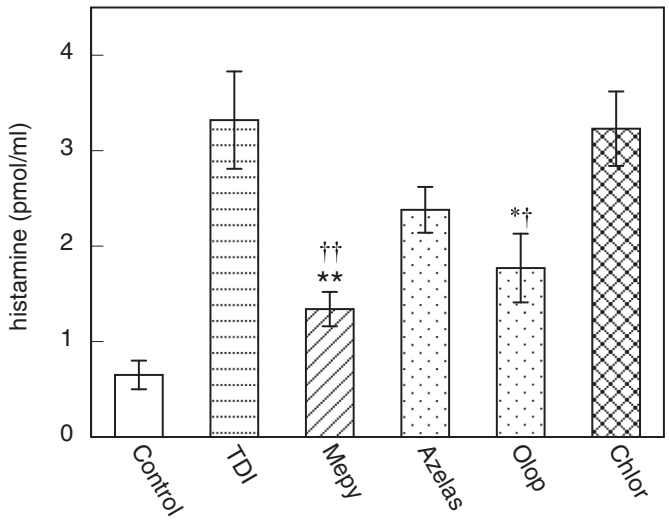

Fig. 4. Antihistamines restrain histamine release in nasal discharge in nasal allergy model rats. Nasal discharge from both vestibules was collected by polyethylene tubing connected to a regulated vacuum 9 h after provocation. Histamine content was measured by HPLC. Data are expressed as means ± SEM. Control, vehicle control; TDI, TDI-control; Mepy, mepyramine; Azlas, azelastine; Olop, olopatadine; Chlor, *d*-chlorpheniramine. $^{**}P < 0.01$ and $^{*}P < 0.05$ vs. TDI; $^{\dagger}P < 0.01$ and $^{\dagger\dagger}P < 0.05$ vs. *d*-chlorpheniramine (n = 4).

Because histamine upregulates H1R gene expression (32), the regulation of HDC expression is considered to be important. Preseasonal prophylactic treatment with antihistamine suppressed the upregulation of HDC mRNA expression in patients with pollinosis (Fig. 1). Further, HDC mRNA expression level was strongly correlated with that of H1R mRNA. These findings suggest that the suppression of HDC expression decreases histamine-induced H1R mRNA expression level and alleviates nasal symptoms, supporting our hypothesis that the regulation of HDC expression is important for treating allergies. The importance of histamine in allergies is also demonstrated by the results of studies using HDC-deficient mice (33,34).

Sensitization and provocation with TDI elevates HDC mRNA levels in the nasal mucosa, followed by increases in HDC activity and histamine content (12). Here pretreatment with antihistamines, except *d*-chlorpheniramine, suppressed TDI-induced HDC mRNA upregulation at different levels, which indicates the existence of novel targets and mechanisms of antihistaminic action as well as inverse agonistic activities. The potencies of the inhibitory effects of antihistamines on the increase in HDC mRNA levels were different from their binding affinities for the H1R (16–19), suggesting that this effect is unrelated to H1R binding. Moreover, human nasal mucosal epithelial and vascular endothelial cells express H1R (35). The results are consistent with our unpublished immunohistochemical analysis of TDI-sensitized rats. To our knowledge, there are no reports of HDC expression in epithelial and endothelial cells; thus, it is unlikely that these cells produce histamine in an autocrine manner. These findings support our hypothesis that the suppressive effect of antihistamines on the HDC-induced elevation of mRNA expression is not related to H1R-binding.

Antihistamines have pharmacological properties unrelated to receptor binding (36). Azatadine, *d*-chlorpheniramine, mepyramine, and promethazine reduce the release of proinflammatory mediators from mast cells and basophils, chemotaxis and activation of inflammatory cells, particularly eosinophils, and expression of adhesion molecules in epithelial cell lines induced by immunological or nonimmunological stimuli (37). Desloratadine inhibits IgE-mediated IL-4 and IL-13 secretion from human basophils (38), and antihistamines suppress Th2 response (3,7–9,12,39). However, no reports demonstrate that antihistamines suppress HDC expressions. To the best of our knowledge, the present study reports the first evidence demonstrating that antihistamines suppress the upregulation of HDC mRNA expression.

Although several studies demonstrate the upregulation of HDC mRNA expression, the mechanism is unknown. Because PMA upregulates HDC gene expression (40), PKC signaling may induce the upregulation of HDC gene transcription. For example, the PKC/ERK/CEBPβ pathway mediates PMA-induced upregulation of HDC mRNA expression in RAW 264.7 cells (20). Our present data demonstrated that mepyramine and azelastine did not inhibit PMA-induced upregulation of HDC gene expression in RAW 264.7 cells, although they suppressed HDC gene expression in TDI-sensitized rats. In contrast, *d*-chlorpheniramine did not suppress HDC expression in TDI-sensitized rats, although it inhibited PMA-induced HDC upregulation in RAW 264.7 cells. Therefore, it is unlikely that PKC signaling upregulates HDC mRNA expression induced by TDI. NF-κB represents another candidate because its expression is elevated in patients with allergic rhinitis (41). Dexamethasone significantly reduces the upregulation of HDC mRNA expression in TDI-sensitized rats (12), and the glucocorticoid receptor–dexamethasone complex binds to AP-1 or NF-κB and suppresses their transcriptional activities. Therefore, the suppressive effect of dexamethasone on TDI-induced HDC mRNA upregulation may be explained by the inhibition of NF-κB signaling. However, we have no data to prove this, and there are no reports of NF-κB-induced HDC gene expression.

It was reported that the expression of HDC gene was transcriptionally regulated by DNA methylation at the region including the GC box sequence in the promoter (42). Demethylation of this GC box by the treatment with 5-azacytidine induced a high level of HDC mRNA expression, suggesting that HDC expression levels were positively correlated with hypomethylation of the HDC promoter region (43). It was also reported that Sp-1 binds to the GC box in the HDC promoter and protects from *de novo* methylation (42). Since long-term effect was observed in TDI-sensitized rats, DNA methylation status or Sp-1 dependent transcription may be affected by antihistamines such as mepyramine and diphenhydramine. Investigation of the effect of

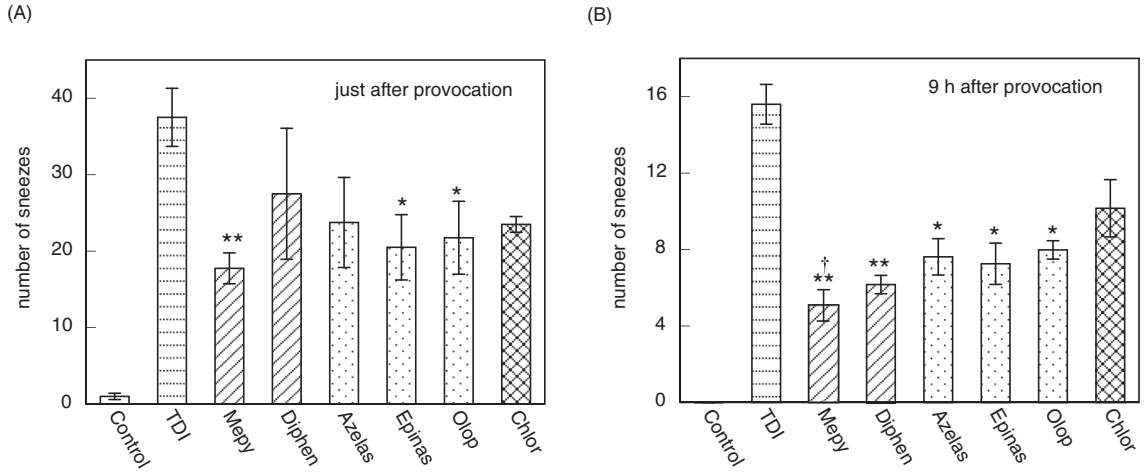

Fig. 5. Pretreatment with antihistamines reduced the number of sneezes in nasal allergy model rats. The number of sneezes was counted for 10 min just after provocation (A) and 9 h after provocation (B). Data are expressed as means ± SEM. Control, vehicle control; TDI, TDI-control; Diphen, diphenhydramine; Mepy, mepyramine; Azlas, azelastine; Epinas, epinastine; Olop, olopatadine; Chlor, *d*-chlorpheniramine. $^{**}P < 0.01$ and $^{*}P < 0.05$ vs. TDI; $^{\dagger}P < 0.05$ vs. *d*-chlorpheniramine (n = 4).

these antihistamines on the DNA methylation of the HDC promoter or Sp-1-dependent HDC gene transcription is under way in our laboratory.

The novel mechanism of the regulation of HDC gene expression described here is not uniform among antihistamines. For example, we showed that mepyramine and diphenhydramine significantly suppressed HDC activity and histamine content in the nasal mucosa of TDI-sensitized rats, whereas they were also suppressed by azelastine, epinastine, and olopatadine, although the effects of the latter were not statistically significant. In contrast, *d*-chlorpheniramine had no detectable effect. The potencies of the inhibitory effects of antihistamines were different from their binding affinities for the H1R. Because mepyramine, diphenhydramine, epinastine, and *d*-chlorpheniramine are inverse agonists and olopatadine is a neutral antagonist (3), it is unlikely that the ability to suppress HDC mRNA expression was correlated with inverse agonistic activity.

The diversity among antihistamines in the suppression of HDC mRNA expression is of interest because uncovering their structure–activity relationships may facilitate the discovery of new drugs for treating allergies. When we investigated the levels of histamine in the nasal discharges, similar results were obtained. For example, mepyramine and olopatadine significantly suppressed the TDI-induced release of histamine. The ability of olopatadine to inhibit histamine release was not related to the suppression of HDC mRNA expression but related to its function as a membrane stabilizer (44). Here the inhibitory effect of azelastine was not statistically significant, and *d*-chlorpheniramine did not affect histamine release.

Table 2
K_i values of antihistamines used in this study determined by [^{3}H]mepyramine displacement assay.

Antihistamines	K_i (nM)
Mepyramine	1.7[a], 4.5[b]
d-Chlorpheniramine	7.5[a], 8.0[b]
Diphenhydramine	17[b]
Promethazine	2.9[b]
Azelastine	7.0[c]
Epinastine	3.8[c]
Olopatadine	41[d]

Data were taken from:
[a] Ref. (16).
[b] Ref. (17).
[c] Ref. (18).
[d] Ref. (19).

To assess the clinical significance of these results, we measured the number of sneezes for 10 min just after and 9 h after provocation. Each antihistamine significantly suppressed sneezing at both times, and the potency of suppression in the early phase was similar to receptor-binding affinity. In contrast, in the late phase, the suppressive potency might be correlated with histamine synthesis, suggesting that certain antihistamines suppressed the nasal symptom by inhibiting histamine synthesis through the suppression of HDC mRNA expression.

In conclusion, we demonstrate here that the long-term effects of certain antihistamines are mediated through inhibition of HDC gene expression and down-regulation of the H1R signaling pathway. These findings provide a new perspective on the long-term effects of antihistamines on allergies. Specifically, mepyramine and diphenhydramine inhibited allergic responses during the late phase. Therefore, detailed knowledge on the mechanism that regulates HDC gene expression may be helpful for the development of new drugs for treating allergies.

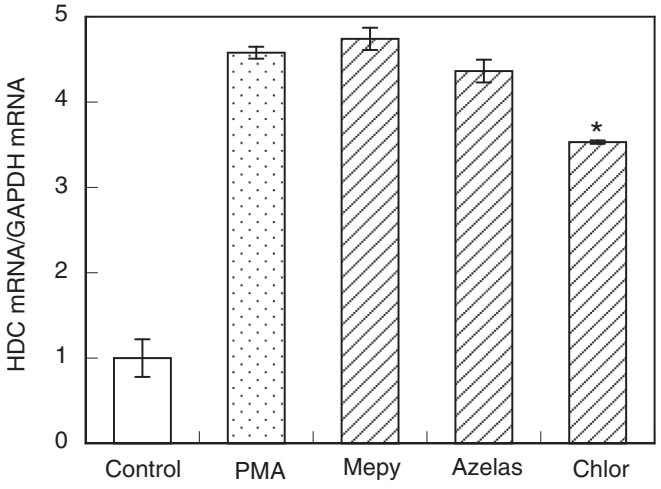

Fig. 6. Effect of antihistamines on PMA-induced upregulation of HDC gene expression in RAW 264.7 cells. RAW 264.7 cells were treated with 10 μM of antihistamine 1 h before stimulation with 100 nM PMA. After a 5-h treatment with PMA, total RNA was isolated, and the HDC mRNA levels were determined by real-time quantitative RT-PCR. Data are presented as the means ± SEM. (n = 4). $^{*}P < 0.05$ vs. PMA.

Conflicts of interest

The authors declare no financial conflicts of interest.

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