Dexamethasone Suppresses Histamine Synthesis by Repressing both Transcription and Activity of HDC in Allergic Rats

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ABSTRACT
Background: Histamine synthesized by histidine decarboxylase (HDC) from L-histidine is a major chemical mediator in the development of nasal allergy which is characterized by nasal hypersensitivity. However the regulatory mechanism of histamine synthesis by HDC remains to be elucidated. The objectives of the present study were to examine the changes of histamine content, HDC activity and HDC mRNA expression in the nasal mucosa of allergy model rats sensitized by the exposure to toluene diisocyanate (TDI) and to investigate the effect of dexamethasone on the above mentioned allergic parameters.

Methods: Rats were sensitized and provocated by TDI and the nasal allergy-like behaviors were scored during a 10 minute period after provocation. Histamine content and HDC activity in the nasal mucosa were determined using fluorometric high performance liquid chromatography. The expression of HDC mRNA in nasal mucosa was determined using real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR).

Results: In TDI-sensitized rats, nasal allergy-like behaviors such as sneezing and watery rhinorrhea were induced. Histamine content, HDC activity and HDC mRNA expression in nasal mucosa were also significantly increased after TDI provocation. Pretreatment with dexamethasone significantly suppressed nasal allergy-like behaviors, up-regulation of histamine content, HDC activity and HDC mRNA induced by TDI in TDI-sensitized rats.

Conclusions: These findings indicate that increased synthesis of histamine through up-regulation of HDC gene expression and HDC activity in nasal mucosa plays an important role in the development of nasal hypersensitivity. Repression of HDC gene expression and HDC activity by dexamethasone may underlie its therapeutic effect in the treatment of allergy.

KEY WORDS
allergy, dexamethasone, HDC, histamine, rats

INTRODUCTION
Histamine the major chemical mediator in the development of nasal allergy is produced by the decarboxylation of L-histidine via the pyridoxal-requiring enzyme histidine decarboxylase (HDC).1 In eukaryotes, HDC is the primary enzyme responsible for synthesizing histamine and is consequently a crucial regulatory step for histamine biosynthesis.2

The HDC molecule is a dimer consisting of two identical 53–55 kDa subunits3,4 while the size of cDNA deduced HDC is around 74 kDa.5,6 The endogenous 74 kDa form of HDC is translated in the cytosol and then translocated to the endoplasmic reticulum, where it is post-translationally processed to the 53 kDa form.7,8 The synthesis of histamine by HDC is...
an important regulatory step in histamine signaling but the regulatory mechanism remains to be elucidated.

Steroidal anti-inflammatory drugs are known to be the most potent drugs for treatment of the inflammatory and allergic diseases. Various hypotheses on the mechanisms of anti-inflammatory effects of steroidal anti-inflammatory drugs have been presented. Recent studies suggested that one of the most important mechanisms may be the inhibition of cytokine production such as IL1, TNF and platelet-activating factor. Hirasawa et al. reported that in air pouch type allergic inflammation model in rats, histamine in the anaphylaxis phase is released from subcutaneous mast cells via an IgE-mediated mechanism and histamine in the post-anaphylaxis phase is produced from an increase in the HDC activity. They also showed that dexamethasone inhibits histamine production in allergic inflammation in rats, but the molecular mechanism remains to be elucidated.

In the present study we hypothesize that in the post-anaphylaxis phase histamine is produced through up-regulation of HDC gene expression and thereby HDC activity. Dexamethasone suppresses this up-regulation.

In our previous study we developed a nasal hypersensitivity animal model in guinea pigs sensitized by exposure to toluene diisocyanate (TDI), which expresses respiratory disorders such as asthma and nasal allergy in industrial workers. Those studies showed that repeated exposure to TDI triggered a neurogenic inflammatory reaction in the nasal mucosa correlative to the release of histamine, resulting in the development of nasal allergy-like behaviors in guinea pigs.

In the present study we used TDI-sensitized rats as an animal model of nasal hypersensitivity and investigated the changes in nasal symptoms, histamine levels, HDC activity and HDC gene expression in the nasal mucosa. The effect of dexamethasone on the above mentioned parameters in the nasal mucosa induced by TDI were also examined.

METHODS

TDI SENSTITIZATION AND PROVOCATION

Six-week-old male Brown Norway rats (Nihon Charles River, Kanagawa, Japan) were used for the present study. The rats were kept in a room maintained at a constant temperature (25 ± 2°C) and humidity (55 ± 10%) with a 12 hour light/dark cycle. Sensitization to TDI was performed by the method described by Tanaka et al. with slight modifications. Briefly, 10 μl of a 10% solution of TDI (Wako Chemical Co., Tokyo, Japan) in ethyl acetate (Wako Chemical Co.) was painted bilaterally on the nasal vestibule once a day for five consecutive days (sensitization). This sensitization procedure was then repeated after a 2-day interval. Seven days after the second sensitization, 10 μl of 10% TDI solution was again applied to the nasal vestibule to provoke nasal allergy-like behaviors in the sensitized rats (provocation) (Fig. 1). Control rats were treated with ethyl acetate, the vehicle by the same procedure. Nasal allergy-like behaviors were measured by the number of sneezes and the extent of watery rhinorrhea on a scale ranging from zero to three (Table 1) during a 10-minute period just after provocation by the method of Abe et al.

All animal experiments were approved by the Ethical Committee for Animal Studies of School of Medicine, the University of Tokushima.

PRETREATMENT WITH DEXAMETHASONE

Dexamethasone (Wako Chemical Co.) was administered intraperitoneally 24 hours before TDI provocation at the dose of 1 mg/kg. In the saline-pretreated group, saline was administered instead of the drug.

COLLECTION OF NASAL MUCOSA AND ISOLATION OF TOTAL RNA

At different indicated periods of time after provocation, rats were sacrificed and nasal mucosa was removed from the nasal septum. Samples were frozen in RNA later (Takara Biochemicals, Tokyo, Japan) and stored in a tube at −84°C until assayed. Total RNA was isolated using TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer’s instructions. In short, nasal mucosa was homogenized using Polytron (Model PT-K; Kinematica AG, Littau/Luzern, Switzerland) in 10 volumes of ice-cold TRIzol Reagent until complete disruption. The homogenates were mixed with chloroform and centrifuged at 12,000 × g for 15 minutes at 4°C. The aqueous phase containing RNA was transferred to a new tube and the RNA was precipitated by the addition of isopropanol. Samples were incubated at room temperature for 5 minutes and centrifuged at 12,000 × g for 15 minutes at 4°C. The RNA pellet was washed with 70% ice-cold ethanol, air-dried and dissolved in 20 μl of diethylpyrocarbonate-treated water. The purity and yield of total RNA were determined spectrophotometrically at 260 and 280 nm. The ratio of absorption (260 : 280) of all preparations was >1.8.
REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (REAL-TIME RT-PCR)

RNA samples were reverse-transcribed to cDNA in a 40 μl reaction volume in the presence of first-strand buffer (375 mM KCl, 250 mM Tris-HCl, pH 8.3, at room temperature), 15 mM MgCl₂, 0.8 mM concentrations of each deoxyribonucleoside triphosphate (dNTP), 40 μM of oligo (dT) primers, 0.004 units of RNase inhibitor, 8 units of reverse transcriptase, and 8 units of reverse transcriptase and were incubated at 37°C for 60 minutes. Then 2 μl of 2N NaOH was added before they were incubated again at 65°C for 30 minutes. Subsequently, 12.8 μl of 1 M Tris-HCl, pH 8.0, was added before samples were heated at 95°C for 10 minutes and chilled to 4°C for 5 minutes. TaqMan primers and probe were designed using Primer Express software (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The sequences of the primers were as follows: sense primer 5'-GCA GCA AGG AAG AAC AAA ATC C-3'; antisense primer 5'-CAA CAA CAA GAC GAG GTG TCA GAG A-3'. The sequence of the probe was as follows: FAM-AAA GCG CAT GAG CCC AAT GCT GAT-TAMRA. To account for differences in starting material, rodent glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers and probe from Applied Biosystems were used as recommended by the manufacturer. The transcripts were utilized for a 40-cycle 3-step PCR using the GeneAmp 5700 Sequence Detection System (Perkin Elmer Applied Biosystems) in 20 mM Tris, pH 8.4, 50 mM KCl, 3 mM MgCl₂, 200 μM dNTPs, 900 nM concentrations of each primer and 0.25 units of Platinum Taq. Amplicon size and reaction specificity were confirmed by agarose gel electrophoresis. The identity of the PCR products was verified by sequencing using a DNA sequencer (4200 L-1; Aloka, Tokyo, Japan).

The number of target copies in each sample was interpolated from its detection threshold (C_T) value using a purified PCR product standard curve included

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### Table 1 Criteria for grading the severity of TDI-induced nasal allergy-like behavior in rats.

<table>
<thead>
<tr>
<th>Score</th>
<th>Number of sneezes</th>
<th>Watery rhinorrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>1</td>
<td>1−4</td>
<td>at the nostril</td>
</tr>
<tr>
<td>2</td>
<td>5−11</td>
<td>between 1 and 3</td>
</tr>
<tr>
<td>3</td>
<td>12&lt;</td>
<td>drops of discharge from the nose</td>
</tr>
</tbody>
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### Table 2 Effect of Dexamethasone on TDI-induced nasal symptoms

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TDI treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pretreated with saline</td>
<td>pretreated with Dexamethasone</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>3.3±0.4*</td>
</tr>
</tbody>
</table>

* P < 0.01 compared with saline pretreated group.
on each plate. Each PCR run included the four points of the standard curve (four fold serially diluted cDNA), a no template control, the calibrator cDNA and the unknown cDNAs. The measurements were calibrated using the calibrator included on each PCR plate. For the quantification of gene expression, we used GAPDH mRNA as the endogenous control. To determine whether the amplification products came exclusively from the RNA, a reverse-transcriptase negative reaction was run in which the enzyme was replaced by RNase-free water for each sample.

**HDC ACTIVITY AND HISTAMINE CONTENT**

Nasal mucosa was homogenized with 10 volumes of ice-cold HDC buffer solution, which consisted of 0.1 M potassium phosphate buffer (pH 6–8), 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate, 1% polyethylene glycol (average molecular weight 300) and 100 μg/ml phenylmethylsulfonyl fluoride. Histamines were centrifuged at 10,000 g for 15 minutes at 4°C and the supernatant (supernatant A) was collected. Half of the supernatant A was dialyzed three times against adequate volume of HDC buffer solution for 6 hours at 4°C (supernatant B).

Histamine content in supernatant A was determined using a high-performance liquid chromatography (HPLC) with a cation exchanger (Tosoh, Tokyo, Japan) and an automated o-phthalaldehyde fluorometric detection system (Hitachi, Tokyo, Japan) in accordance with the method of Yamatodani et al. Histamine activity was determined by incubating supernatant B for 4 hours at 37°C with 0.25 mM L-histidine. HDC activities were calculated based on the formiation of histamine after the subtraction of blank value. The protein concentrations of these preparations were determined by the method of Lowry et al. in triplicate with bovine serum albumin as a standard.

**STATISTICAL ANALYSIS**

The results are presented as mean±standard error (SE). All P values were determined by using one-way ANOVA and Fisher’s paired least-significant difference test. P values less than 0.05 were considered significant.

**RESULTS**

**NASAL ALLERGY-LIKE BEHAVIORS**

Intranasal application of TDI induced nasal allergy-like behaviors such as sneezing and watery rhinorrhea in sensitized rats. The total score of nasal allergy-like behaviors was 5.2. Pretreatment with dexamethasone (1 mg/kg i.p.) significantly reduced nasal allergy-like behaviors, of which total score was reduced to 3.3 (Table 2). However, control rats sensitized by exposure to vehicle showed no nasal allergy-like behaviors after provocation.

**HISTAMINE CONTENT**

As histamine is the prime chemical mediator in the development of nasal allergy, we measured the histamine content in nasal mucosa at different time after TDI provocation and observed the effect of dexamethasone on it. The histamine content in nasal mucosa was significantly increased after TDI provocation in sensitized rats (Fig. 2A). The maximum level was achieved after 9 hours of provocation. Dexamethasone (1 mg/kg, i.p.) significantly reduced nasal allergy-like behaviors after provocation.

**HDC ACTIVITY**

To confirm whether increased synthesis of histamine is mediated through HDC activity, a time course
study was also done to investigate the HDC activity at different time intervals after TDI provocation. HDC activity in the nasal mucosa was significantly increased after provocation in TDI sensitized rats (Fig. 3A). The HDC activity reached a maximum over the initial level after 9 hours of provocation. Pretreatment with dexamethasone (1 mg/kg, i.p.) almost completely suppressed HDC activity both 9 hours and 15 hours after provocation (Fig. 3B).

HDC mRNA EXPRESSION
In an attempt to confirm the molecular mechanism of HDC activity up-regulation, the effect of TDI on HDC gene expression in the nasal mucosa was performed using real-time PCR. The HDC mRNA expression was significantly increased after provocation in sensitized rats compared to control (Fig. 4A).

The increase of HDC mRNA in the nasal mucosa was observed 1 hour after provocation and reached a maximum (6 times over the initial level) after 4 hours. The level of HDC mRNA returns close to its baseline within 24 hours. Pretreatment with dexamethasone (1 mg/kg, i.p.) significantly suppressed HDC transcription in both 4 hours and 12 hours after provocation (Fig. 4B).

DISCUSSION
In the present study, intranasal application of TDI induced nasal allergy-like behaviors, such as sneezing and watery rhinorrhea in TDI-sensitized rats. This finding corroborates the results of our previous studies that showed the trigger of such behaviors after TDI stimulation. We also have reported that TDI-sensitization enhances the synthesis of substance P and calcitonin-gene related peptide in the trigeminal ganglion neurons as well as their axonal transport and antidromic release towards the peripheral endings. Moreover, substance P and calcitonin-gene related peptide has been reported to induce the release of histamine from mast cells in the nasal mucosa. This observation has been confirmed by reports showing that both the histamine content and number of mast cells in nasal mucosa were higher in TDI-sensitized guinea pigs as compared to controls.

Nasal allergy-like behaviors during 10 minutes just after provocation was studied to check the effect of dexamethasone on the anaphylaxis phase which is due to the release of preformed histamine during the sensitization process. Dexamethasone improves nasal allergy-like behaviors by suppressing the release of inflammatory mediators from mast cells and other types of cells. It also suppresses proliferation of activated lymphocytes, mast cell development and maturation and expression of FcεRI. Although this data is not relevant for the present study it does support the previous studies.

In addition, the expression of HDC mRNA in the nasal mucosa was significantly increased after TDI provocation in sensitized rats (Fig. 4A). This finding is in line with studies in which the HDC mRNA level was increased in patients with allergic rhinitis and bronchial asthma. HDC activity and histamine content in the nasal mucosa were also significantly increased after TDI provocation (Figs. 2A, 3A). This finding suggests that HDC gene expression is an important step in histamine signaling pathways in allergic responses.

We also examined the effect of dexamethasone on the up-regulation of histamine content, HDC activity and gene expression after intranasal application of TDI in TDI-sensitized rats. Our results in this study demonstrate that pretreatment with dexamethasone significantly suppressed the increase of histamine.
content, HDC activity and HDC mRNA expression in the nasal mucosa after provocation (Figs. 2B–4B). Since histamine is the major chemical mediator for allergy, it is suggested that pretreatment with dexamethasone improves allergic diseases by its inhibitory effect on histamine synthesis. Histamine synthesis in vivo is regulated through either the translation or transcription stage or through both. HDC mRNA was suppressed approximately by half the level whereas HDC activity was almost completely suppressed by dexamethasone. These results indicate that dexamethasone exhibit it’s inhibitory effect both at the transcription and translation level. At present we have no data to describe precisely the step between mRNA transcription and matured protein with enzymatic activity inhibited by dexamethasone. Suppression of HDC mRNA may be due to a decrease in RNA transcription rate or RNA stability. We have yet to determine which of these mechanisms might account for the decrease of HDC mRNA, but we suspect that a reduction in transcription rate may be involved. Concordantly HDC enzymatic activity suppression may be mediated by low rates of translation or rapid degradation of HDC protein. Due to lack of sufficient data, we are currently unable to explain the exact reason behind this. Sequence analysis of rat HDC gene has identified several putative regulatory abilities to activate the transcription rate.29

In agreement with our results, several studies in rat and mouse lung have shown that glucocorticoid hormones may be involved in the regulation of HDC enzyme activity.30,32 Cynthia A et al. showed that glucocorticoid hormones down-regulate HDC mRNA and enzyme activity in lung but failed to show any down-regulation of histamine content in normal rats.2 It may be due to short time treatment of dexamethasone. In order to clarify this phenomenon, in the present study we treated rats with dexamethasone for a long time period (24 hours) and found that dexamethasone improves allergic diseases through suppression of histamine synthesis. Maeda et al. showed that HDC activity in human mast cell line HMC-1 induced by PMA and Inomycin is regulated at the translation level as this activity was inhibited by cyclohexamide but not by actinomycin D.30 In human basophilic leukemia KU-812-F cells, induction of HDC activity by PMA is regulated at the translation level as well.34 Whereas in murine macrophage-like cell line RAW 264.7, induction of histamine production by thapsigargin was due to the increased expression of HDC protein and dexamethasone inhibited thapsigargin-induced HDC protein expression and histamine production via inhibition of MAP kinase activation.35

In contrast to our studies, glucocorticoids have been shown to activate HDC expression in mastocytoma and basophilic leukemia cell lines.36 Experiments with cell lines provide valuable information but must be interpreted with caution because they do not precisely duplicate the conditions and the multiple variables that exist in the whole animal. On the other hand according to the article, the transcription starting site was tentatively identified, but the possibility that the transcription starts from multiple sites was excluded. This may be one of the reasons for the discrepancy in the effects of dexamethasone between in vivo and in-vitro studies. Dexamethasone increased both histamine content and the de novo synthesis of HDC from cultured mouse mastocytoma p 815 cells and cultures of rat hepatic cells.31,37 This regulation was further studied by examining the synergistic effects of glucocorticoids and the protein kinase C (PKC) activator 12-O-tetradecanoylphorbol 13 acetate (TPA) on HDC synthesis from mouse p815 cells.38 Previously, it had been shown that activation of PKC may be involved in the glucocorticoid hormone induced synthesis of HDC in mouse mastocytoma cells.38 Taken together, these results suggest that the effects of glucocorticoid hormones on HDC activity may be tissue or cell specific.

Several studies have been done previously for investigating the sources of histamine in vivo. Histamine is produced by mast cells, basophilic cells, macrophages, neutrophils and lymphocytes.39-41 Mast cells and basophilic cells constitutively express HDC of 74 and 53 kDa molecular masses and store the produced histamine in their granules.42 Other types of cells produce histamine in response to various stimulants and release it piecemeal.35 In the present study we did not check the sources of histamine produced in TDI-sensitized allergic rats but speculate that all of the above mentioned cells were the sources of histamine.

Clinically, histamine plays a key role in producing nasal symptoms and glucocorticoids constitute the most potent treatment in nasal allergy. In fact, glucocorticoids have a strong anti-inflammatory capacity in reducing cytokine and chemokine release and are able to decrease the cellular infiltration of antigen presenting cells, T cells and eosinophils in the nasal mucosa.43 Current pharmacotherapy for nasal allergy also includes H1-antihistamines. However, in our preliminary study, d-chlorpheniramine did not suppress the TDI-induced up-regulation of HDC mRNA. Therefore, the suppression of up-regulation of HDC mRNA and HDC activity may be the mechanisms responsible for the superiority of dexamethasone to H1 antihistamines in the treatment of nasal allergy.

In conclusion, we used TDI-sensitized rats as an animal model of nasal hypersensitivity induced by
neurogenic inflammation and showed that the up-regulation of HDC mRNA in nasal mucosa plays an important role in the development of nasal hypersensitivity. Dexamethasone significantly reduced nasal allergy-like behaviors, up-regulation of HDC mRNA, HDC activity and histamine content induced by TDI. These results suggest that the suppression of the up-regulation of HDC genes and HDC enzymatic activity by dexamethasone may underlie its therapeutic effect in the treatment of allergies.

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