



The effect of histamine on ciliary beat frequency in the acute phase of allergic rhinitis

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Abstract

Background: There have been few studies about the effect of histamine on ciliary beat frequency (CBF) in the acute phase of allergic rhinitis.

Objective: The present study was designed to investigate CBF in the acute phase of allergic rhinitis and the effect of histamine on CBF.

Materials and methods: Nasal septal mucosae were obtained from 13 mice that had been systemically immunized and locally challenged with ovalbumin (OVA) (group A), 11 OVA immunized and phosphate-buffered saline–challenged mice (group B), and 12 nontreated negative control mice (group C). The CBFs were observed within 20 minutes of local challenge with OVA. Ciliary beat frequencies were measured before and after treatment with 10^{-5} , 10^{-3} , and 10^{-1} mol/L histamine and after administering antihistamine or Dulbecco's modified Eagle's medium to histamine-exposed mucosa.

Results: The baseline CBF in group A was higher than in groups B and C. After treatment with 10^{-1} mol/L histamine, CBF in all groups decreased to 0 within 5 to 7 minutes, whereas treatment with 10^{-5} or 10^{-3} mol/L histamine had no effect. The application of 100 μ mol/L fexofenadine or Dulbecco's modified Eagle's medium solution restored histamine-induced ciliostasis to near baseline CBF after 10 minutes.

Conclusions: Ciliary beat frequency increased in the acute phase of a mouse model of allergic rhinitis. Physiologic concentrations of histamine had no effect on CBF, and thus, it appears that other mechanisms control CBF in the acute phase of allergic rhinitis.

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1. Introduction

Mucociliary clearance is a critical aspect of host airway defense, and effective clearance requires appropriate mucus production and coordinated ciliary activity. Furthermore,

mucociliary clearance is altered by invading virulent organisms, such as viruses and bacteria, and by temperature changes, drugs, and various inflammatory mediators [1]. Decreases in the nasal ciliary beat frequency (CBF) attenuate response to external stimuli and reduce secretion in the nasal cavity and sinus. In addition, such decreases can cause upper airway diseases, such as rhinosinusitis, by obstructing the natural ostium. There are many studies of the relationship between sinusitis and CBF [2–7]. However, only a few studies have been conducted on CBF in allergic rhinitis, and those performed have produced disparate

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results. In one study, CBF in allergic rhinitis was found to decline with duration of morbidity, especially in cases of perennial allergic rhinitis [8]. In another study, an increase in CBF was observed when sensitized sheep trachea was challenged with antigen *in vivo* and *in vitro*, and this phenomenon was presumed to be caused by chemical mediators secreted from mast cells [9].

Some experiments have been performed to identify the mediators that could decrease CBF and subsequently inhibit mucociliary clearance [10–12]. In particular, histamine is known to play a major role in many processes that occur during the acute phase of allergic rhinitis. However, there has been no consensus regarding the effect of histamine on CBF. Some studies have reported stimulatory effects of histamine on CBF, whereas other studies failed to find an effect [11,13–15]. Moreover, it is difficult to compare these previous results because of the different materials and methods used. Therefore, we investigated CBF in the acute phase of allergic rhinitis and the effect of histamine on CBF.

2. Methods

2.1. Sensitization of mice and preparation of septal mucosa

2.1.1. Development of mouse model of allergic rhinitis

Thirty-six 4-week-old specific pathogen-free female BALB/c mice weighing 10 to 13 g were used in this study. All mice were treated intraperitoneally (IP) and then challenged intranasally with ovalbumin (OVA) or phosphate-buffered saline (PBS) according to the protocol depicted in Fig. 1. Briefly, group A (n = 13) mice were systemically sensitized with 25 μ g OVA IP dissolved in 300 μ L of PBS containing 2 mg alum (Pierce Biotechnology, Inc, Rockford, IL) as an adjuvant on experimental days 0, 7, and 14. One week after the third IP sensitization, mice were challenged intranasally with OVA for 7 consecutive days. Group B (n = 11) mice were systemically immunized in the same way as group A mice and were challenged

intranasally with 20 μ L of PBS for 7 consecutive days. Group C (n = 12) mice were administered PBS IP and intranasally as depicted in Fig. 1.

2.1.2. Observed nasal rubbing frequencies during the first 5 minutes after allergen challenge

Twenty-four hours after the final nasal challenge, 200 μ g OVA was instilled into nasal cavities to provoke allergic symptoms and the numbers of nasal rubbings during the 5 minutes immediately after provocation were counted.

2.1.3. Preparation of nasal septal mucosa

Mice were killed, and both nasal septal mucosae (size range, 2–3 $\times$ 3–4 mm) were harvested. Harvested nasal septal mucosae were washed in Dulbecco's modified Eagle's medium (DMEM) solution (Dulbecco's modified Eagle's medium—Ham's nutrient F12; Gibco BRL, Grand Island, NY) to remove secretions and blood then placed in the same medium during experiment. Penicillin 10 IU/mL, streptomycin 10 IU/mL, and amphotericin B 0.025 mg/mL were added to the DMEM solution, which was kept at 37°C throughout the procedure.

2.2. Measurement of CBF

We measured CBFs using a digitalized computer analysis system [16]. Harvested septal mucosae were fixed at the center of the base of 35 mm plastic culture dishes using a white gold ring and dental floss. Ciliary beat frequencies were measured using an immersion microscope at $\times$ 400 in areas in which cilia were found to be moving actively. A digital charge-coupled camera (XC-HR50; Sony Co, Tokyo, Japan) capable of operating at 60 full frames per second transferred images to a computer equipped with a frame grabber (PCVisionplus; Dalsa Co, St. Laurent, Canada). Ciliary movement was analyzed using software based on fast Fourier transform.

2.3. Design of experiment

2.3.1. Measurement of CBF *in vitro* within 20 minutes after *in vivo* nasal allergen challenge

Mucosae were harvested from the septum after euthanizing to measure CBF *in vitro*, within 20 minutes after *in vivo* nasal allergen challenge. Ciliary beat frequencies were measured at 0, 5, 10, and 15 minutes.

2.3.2. The effect of histamine on CBF

After measuring CBF for 15 minutes, solutions containing 10^{-5} , 10^{-3} , or 10^{-1} mol/L histamine in DMEM were perfused into cultures to replace the preexisting DMEM solution. Ciliary beat frequency was measured at 0, 5, 10, and 15 minutes after histamine treatment.

2.3.3. Ciliary beat frequency responses after treatment with fexofenadine and DMEM solution

To assess the effect of histamine, CBF was measured after treating histamine-exposed tissue with antihistamine or DMEM solution. Fexofenadine (HanDok Co, Seoul, South

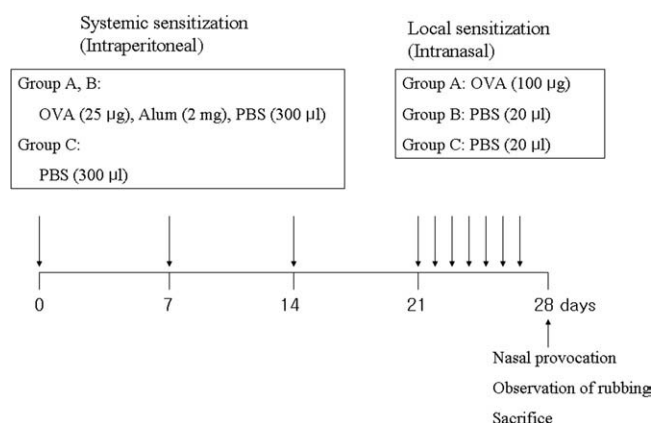


Fig. 1. Protocol for allergic model and control. Alum indicates aluminum.

Korea) was used as the antihistamine, and a concentration of 100 $\mu\text{mol/L}$ was selected based on pilot experiments.

2.4. Statistical analysis

Mann-Whitney *U* and Wilcoxon signed rank tests in SPSS version 12.0 were used to determine statistical significance. Statistical significance was accepted for *P* values of $<.05$.

The International Animal Care and Use Committee at Seoul National University approved the protocol of the animal experiments performed during this study.

3. Results

Observed nasal rubbing frequencies during the first 5 minutes postchallenge in group A were higher than those in groups B and C (7.1 ± 4.1 vs 1.2 ± 1.3 vs 0.8 ± 1.4 ; $P < .001$). Immediately after documentation of nasal rubbing, animals were killed, and CBFs were measured at 5-minute intervals in vitro for 15 minutes postchallenge. Baseline CBF in group A was higher than in group B or C (20.7 ± 3.2 Hz vs 13.2 ± 1.8 Hz vs 13.6 ± 2.7 Hz; $P < .001$). All 3 groups showed no change in CBF over the 15 minutes (Fig. 2).

After exposing mucosae to histamine, CBFs were measured for 15 minutes. Treatment with 10^{-5} or 10^{-3} mol/L histamine had no effect on CBF in any group ($P > .05$), and CBF did not change over time in any group. However, treatment with 10^{-1} mol/L histamine inhibited CBF, and complete ciliostasis was observed within 5 to 7 minutes after the application of 10^{-1} mol/L histamine ($P < .05$; Fig. 3).

To confirm the effect of 10^{-1} mol/L histamine, 100 $\mu\text{mol/L}$ fexofenadine was added to bath media 10 minutes after complete ciliostasis. Ciliary beat frequency increased significantly within 10 to 15 minutes in all groups (group

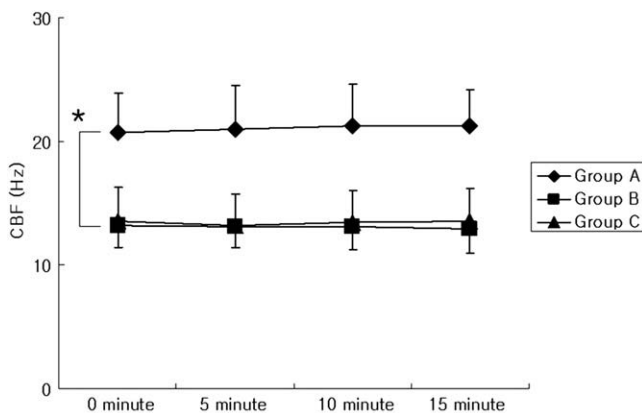


Fig. 2. Ciliary beat frequency over time after nasal provocation. Ciliary beat frequencies in group A (allergic rhinitis model, $n = 13$) were higher than in groups B ($n = 11$) and C ($n = 12$). Increases were significant at 0, 5, 10, and 15 minutes postchallenge ($*P < .001$).

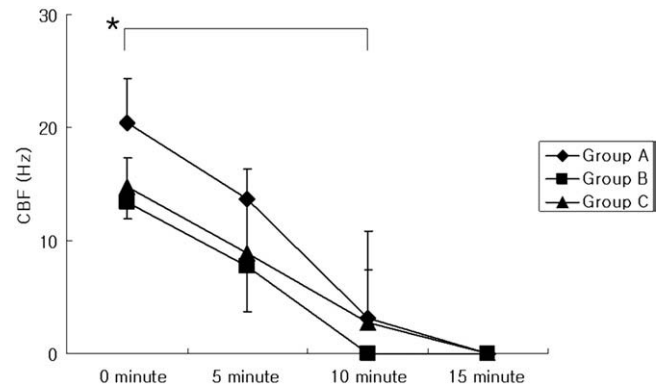


Fig. 3. Changes in CBF after the application of 10^{-1} mol/L histamine. Ciliary beat frequencies in all 3 study groups (group A, $n = 13$; group B, $n = 11$; group C, $n = 12$) decreased to 0 at 5 or 7 minutes postchallenge ($*P < .05$).

A, 16.0 ± 1.1 Hz; group B, 12.5 ± 1.7 Hz; group C, 12.8 ± 1.1 Hz at 15 minutes; $P < .05$). Furthermore, this increase in CBF was greater in group A than in group B or C ($P < .05$; Fig. 4).

In addition, we replaced 10^{-1} mol/L histamine with fresh DMEM solution 10 minutes after complete ciliostasis to remove the histamine. Recovery of CBF was observed after 10 to 15 minutes, but recovered CBF values were not significantly different in the 3 groups (group A, 18.1 ± 2.2 Hz; group B, 11.6 ± 0.4 Hz; group C, 13.0 ± 1.4 Hz at 15 minutes, $P > .05$).

4. Discussion

In this study, CBFs were measured in vitro within 20 minutes after in vivo nasal allergen challenge. Nasal mucosae that had been challenged with allergen after sensitization to OVA (group A) showed higher baseline

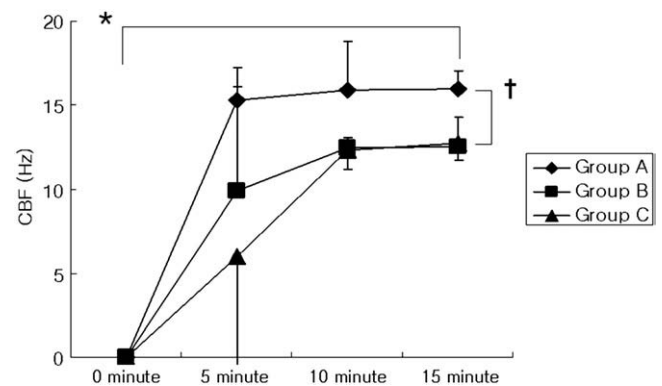


Fig. 4. Changes in CBF after the application of 100 $\mu\text{mol/L}$ fexofenadine to tissue exhibiting histamine-induced ciliostasis. Ciliary beat frequencies increased in all groups ($*P < .05$) and CBFs in group A (allergic rhinitis model, $n = 13$) were higher than in group B ($n = 11$) or C ($n = 12$) at 15 minutes postchallenge ($†P < .05$).

CBF values than those of animals' nasal mucosae sensitized with allergen but challenged with PBS (group B) or those not sensitized (group C). This result demonstrates that our in vitro preparations reflected previous in vivo sensitization and allergen challenge. Furthermore, the increases in CBF were associated only with allergen challenge after in vivo sensitization (group A) and not by sensitization alone (group B). We speculate that the mediators present in the early phase of allergic rhinitis were responsible for increasing CBFs. One previous study in which CBF showed no changes after antigen provocation in pediatric patients with allergic rhinitis is in disagreement with these results [17]. However, this earlier study differed substantially from the present study and had several limitations. More specifically, the subjects were human, the authors did not objectively define allergic rhinitis, and a photometric method was used to measure CBF. On the other hand, the findings of other studies concur with our findings. In particular, CBF in acute allergic reaction was found to increase in experiments with sheep trachea sensitized with *Ascaris suum*. Furthermore, these authors concluded that chemical mediators from mast cells were responsible for this phenomenon because this effect was blocked by cromolyn sodium [9]. However, they did not identify the chemical mediators responsible for increasing CBF.

Based on the findings of this earlier study, various chemicals have been investigated with respect to their effect on CBF. Histamine was the main target because it is the main mediator in the acute allergic reaction. Mechanistically, the binding of histamine to histamine receptor results in an increase in intracellular calcium [18], which can induce increase of CBF [19]. In one in vitro human study using a photoelectric method, histamine at concentrations ranging from 10^{-6} to 10^{-3} mol/L was found to have no effect on CBF [10]. In the present study, we observed no change in CBF at histamine concentrations of 10^{-5} to 10^{-3} mol/L but found a significant decrease at 10^{-1} mol/L. These results are partially in accord with previous reports, but the observation that 10^{-1} mol/L histamine decreases CBF is novel. The concentration of histamine in the nasal secretions of patients after nasal allergen challenge is known to be approximately 10^{-7} to 10^{-5} mol/L [20]. Therefore, we speculate that physiologic concentrations of histamine have no effect on CBF in the acute phase of allergic rhinitis, although high concentrations of histamine can reduce and even stop ciliary beating.

The increase in CBF in the acute phase of allergic rhinitis is explained by the presence of many chemical mediators such as leukotriene C₄, prostaglandin E₂, and substance P. Leukotriene C₄ was found to increase CBF in an in vitro animal study [11], and prostaglandin E₂ has been demonstrated to increase CBF in vitro in both animal and human studies [11,12]. Ciliary beat frequency has also been reported to be enhanced by substance P [21], which is a neuropeptide released from sensory nerve C fibers in response to chemical and thermal irritation [22]. In addition,

nasal endogenous nitric oxide, which is elevated in allergic rhinitis has been shown to increase CBF via the nitric oxide (NO)–cyclic guanosine monophosphate (cGMP)–protein kinase G pathway [23,24].

Neural reflex is another possible mechanism underlying CBF modulation. Allergic reactions stimulate sensory nerves, and these signals are transmitted to the central nervous system and further travel through parasympathetic efferent nerves. Then nitric oxide production is enhanced and increased nitric oxide activates guanyl cyclase, thus cGMP is accumulated in the cells. This cGMP induces ciliostimulation [25–27].

In the present study, high concentrations of histamine were observed to have an inhibitory effect on CBF irrespective of allergic status. This suggests that these high concentrations of histamine may lead to adverse effects on epithelial cells irrespective of allergic state. Alternatively, at high concentrations, histamine increases nasal mucosal permeability and induces secretions [28], and we surmise that, under the immersion microscope, ciliary movement is impeded by secretions. However, no previous study has mentioned the effect of high concentrations of histamine (above 10^{-1} mol/L) on CBF or the mechanism involved, and thus, further study is required on this topic.

In the present study, no statistical difference was observed between fexofenadine and DMEM in terms of magnitude of recovery. So we believe that our results regarding the effect of antihistamine and DMEM challenge after ciliostasis are probably caused by a washout effect.

We believe that the results of this study will be helpful for determining the mechanism underlying the effect of histamine on CBF.

5. Conclusion

In summary, the present study shows that the increase in CBF observed during the acute phase of allergic rhinitis is mainly caused by chemical mediators other than histamine. We surmise that increase in ciliary motion induced by acute allergic inflammatory mediators is an aspect of innate immune response.

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