

Respiratory pathophysiologic responses

Relationship between ultrasonically nebulized distilled water–induced bronchoconstriction and acetic acid–induced cough in asthmatic children

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Background: Although wheezing and cough are the most common complaints in asthmatic persons, the mechanisms of hyperresponsiveness to bronchoconstriction and cough are nevertheless still unclear.

Objective: To investigate the common mechanisms between them, we studied the relationship between ultrasonically nebulized distilled water (UNDW) inhalation challenge and acetic acid (AA) inhalation challenge. In addition, we evaluated the effect of inhaled furosemide on both provocation tests by means of a placebo-controlled study.

Method: In study 1 the UNDW, AA, and histamine inhalation challenges were performed in 40 asthmatic children (26 boys, aged from 7 to 16 years; mean $\pm$ SD, 11.2 ± 2.0 years). In study 2, 12 of the study 1 subjects (9 boys, 11.3 ± 2.4 years) were subjected to each challenge test after inhalation of furosemide (10 mg/body square meters), or placebo (0.9% saline solution) on separate days.

Results: There was a good correlation between the provocative dose causing a 20% fall in forced expiratory volume in 1 second in the UNDW inhalation challenge (UNDW-PD₂₀) and threshold dose causing cough in the AA inhalation challenge ($R = 0.527$; $p < 0.001$). There was no relationship either between UNDW-PD₂₀ and the provocative concentration causing a 20% fall in the histamine inhalation challenge (histamine-PC₂₀) ($R = 0.384$; $p > 0.1$), or between histamine-PC₂₀ and the cough threshold ($R = 0.308$; $p > 0.05$). In study 2 neither bronchoconstriction nor bronchodilatation after inhalation of furosemide was observed. Inhaled furosemide exerted a protective effect on UNDW-PD₂₀ and cough threshold of the AA inhalation challenge ($p < 0.01$ and $p < 0.01$, respectively), but did not attenuate histamine-PC₂₀ ($p > 0.1$).

Conclusion: These data suggest that a common mechanism may cause hyperresponsiveness against both UNDW-induced bronchoconstriction and AA-induced cough in asthmatic children. (*J ALLERGY CLIN IMMUNOL* 1995;96:193-8.)

Key words: Acetic acid–inhalation challenge, asthmatic children, bronchoconstriction, cough, furosemide, histamine inhalation challenge, ultrasonically nebulized distilled water–inhalation challenge

Cough is so common in children with respiratory tract infections and bronchial asthma that it is

important to evaluate its mechanisms. Previously, hyperresponsiveness of cough receptors in persons with asthma has been evaluated by means of inhalation challenges with chemical irritants, such as citric acid,¹ acetic acid (AA),² and capsaicin.³ Capsaicin's effects appear to occur exclusively through direct sensory nerve stimulation,^{4,5} whereas inhaled acids induce cough by ionic or osmolar changes in airway mucosa directly or indirectly.^{6,7}

Recent studies with furosemide, a diuretic

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Abbreviations used:

AA:	Acetic acid
FEV ₁ :	Forced expiratory volume in 1 second
PC ₂₀ :	Provocative concentration causing a 20% fall in FEV ₁
PD ₂₀ :	Provocative dose causing a 20% fall in FEV ₁
UNDW:	Ultrasonically nebulized distilled water

agent, have suggested one of the mechanisms of cough induced by irritants. Inhaled furosemide, which inhibited the cough induced by low chloride-content solutions, but not by capsaicin, may act by inhibiting the cough reflex indirectly, perhaps by changing local chloride ions within the vicinity of epithelial cough receptors.⁸

On the other hand, inhalation of furosemide has been reported to protect against the bronchoconstriction induced by ultrasonically nebulized distilled water (UNDW) and other stimuli in asthmatic subjects.⁹⁻¹¹ The mechanisms of the protective action of furosemide are not well known, but its lack of effect against methacholine- and histamine-induced bronchoconstriction suggests that it may not be acting directly at the airway smooth muscle level.^{12, 13} In UNDW inhalation challenge, the protective effect of furosemide is demonstrated to be dependent on its major pharmacologic action, ion channel inhibition.¹⁴

Considering these previous reports on furosemide-induced protection, we speculated that one of the common mechanisms between acid-induced cough and UNDW-induced bronchoconstriction in asthmatic patients may be ionic or osmolar changes in airway mucosa, or both. This article reports our investigation into the common mechanisms under the UNDW-induced bronchoconstriction and the AA-induced cough, in which we examined whether there is a correlation between the results of these two provocation tests and whether inhaled furosemide provides the same protection against these challenges in asthmatic children, as compared with the results of another provocation test, histamine inhalation challenge.

METHODS

Subjects

Forty asthmatic children (26 boys, 14 girls; mean age $\pm$ SD 11.2 ± 2.0 years) were enrolled in study 1. Twelve of the study 1 children (9 boys, 3 girls; 11.3 ± 2.4 years old) were also enrolled in study 2 (Table I). The clinical

diagnosis of bronchial asthma was based on a characteristic history of recurrent attacks of dyspnea with perceptible wheezing, with the diagnosis having been established after more than 2 years of follow-up. All subjects had been free of upper respiratory tract infections for at least 2 weeks, had no spontaneous coughing or other symptoms at the time of the study, and had not received any medication for at least 16 hours before the tests. No child was receiving long-term antihistamine drugs.

Informed consent for this study was obtained from all patients or their parents before the examination.

Study protocol

In study 1 40 atopic asthmatic children underwent AA inhalation challenge, UNDW inhalation challenge, and histamine inhalation challenge. Each subject attended on three different occasions, at least 1 week apart, at the same time of the day. Twelve attended on more than three occasions for study 2. On each study day the subjects rested for 5 minutes before undergoing measurement of forced expiratory volume in 1 second (FEV₁) to check lung function, after which AA, UNDW, or histamine was administered. On day 1 the AA inhalation test was performed, and FEV₁ was again measured after the challenge. On day 2 (second trial) and day 3 (third trial), UNDW inhalation challenge and histamine inhalation challenge were performed.

In study 2 the 12 subjects who agreed to undergo evaluation of the furosemide effect were entered into a single blind, placebo-controlled study. Each subject attended on six different occasions, at least 1 week apart, at the same time in the same season. On each day, the subjects rested for 5 minutes before measurement of FEV₁; then AA, UNDW, or histamine was administered. Either furosemide or placebo was inhaled 15 minutes before the inhalation challenges. FEV₁ was measured after furosemide or placebo inhalation. On day 1 or day 2 (second trial), AA inhalation challenge after pretreatment with furosemide or placebo was performed in 12 subjects. On day 3 (third trial) or day 4 (fourth trial), UNDW inhalation challenge after pretreatment with furosemide or placebo was performed in 12 subjects. On day 5 (fifth trial) or day 6 (sixth trial), histamine inhalation challenge was performed after pretreatment with furosemide or placebo in eight subjects, because we had only one opportunity to administer histamine inhalation challenge to patients 1, 3, 7, and 10, who underwent the histamine inhalation challenge with placebo pretreatment.

Acetic acid inhalation challenge test

The procedure of AA inhalation test, which has been previously described,¹⁵ was slightly modified. Briefly, the AA solution was prepared on the same day and diluted twofold with 0.9% saline solution (from 0.04% to 20%; pH from 3.13 to 1.62; osmolarity from 292 to 1113 mOsm/kg) and administered by means of a De Vilbiss 646 nebulizer (De Vilbiss Co., Somerset, Pa.). The output of the De Vilbiss 646 nebulizer was 0.15 ± 0.01

TABLE I. Characteristics of subjects in study 2

No.	Sex	Age (years)	Height (cm)	Weight (kg)	Duration* (year)	Total IgE (IU/ml)	Allergens	Treatment
1	M	8	120	21	6	35	—	D, T
2	M	8	120	23	6	1970	Mite	D, T
3	F	9	130	25	8	687	Mite	D, T
4	M	9	131	28	4	956	Mite; cat	D, T
5	M	10	141	30	9	75	—	D, T, B
6	M	12	146	35	11	1936	Mite	D, T, B
7	M	12	135	30	9	2000	Mite; cat	D, T
8	M	12	148	35	10	480	Mite; mold	D, T
9	M	12	156	48	3	282	Mite; cat	D, T
10	M	14	168	57	9	2000	Mite	D
11	F	14	154	54	12	15	—	D, T, B
12	F	16	145	48	9	534	Mite	D, T

D, Disodium cromoglycate inhalation; *T*, theophylline preparation; *B*, β -adrenergic agonist preparation.

*Duration: Duration of asthmatic symptoms.

ml/min. Subjects used a mouthpiece for inhalation. Saline solution (0.9%) was used for a control. After local anesthesia (procaine hydrochloride) was applied to the nasopharynx by swabbing, the subjects first inhaled the saline solution as a control for 10 seconds, and then inhaled successive concentrations of the AA solution in a seated position for 10 seconds each, using a nose clip, after which they were instructed to breathe normally. The concentration inducing the first cough that was recognized by more than two independent observers was considered to be the threshold of the responsiveness of individual cough receptors.

UNDW inhalation challenge test

The challenge of UNDW was performed according to previous methods,^{16,17} slightly modified. Spirometry was performed correctly. After their inhalation of 0.9% saline solution, subjects inhaled distilled water (pH 6.9 to 7.1) for 2 minutes. The aerosols were generated by an Omron NE-U 10B (Omron Co., Tokyo, Japan), and were inhaled through a two-way valve, using the nose clip, by tidal breathing. The nebulizer was equipped with a fan that produced a continuous airflow. We adjusted the volume output of the nebulizer by adjusting its amplitude of oscillation and set the output as 1.0 ml/min. The response was measured by the change in FEV₁ after each challenge until a 20% fall in FEV₁ from the baseline was observed. The provocative dose of the distilled water required to produce a 20% fall in FEV₁ (UNDW-PD₂₀) was obtained by linear interpolation of the last two points on a stimulus-response curve, with UNDW-PD₂₀ considered to represent the bronchial hyperresponsiveness to distilled water.¹⁸

Histamine inhalation challenge test

The histamine inhalation challenge was performed as previously reported.^{19,20} Histamine (Daichi Kagaku

Yakuin Co., Tokyo, Japan) was prepared on the same day, serially diluted twofold with 0.9% saline solution (from 20 mg/ml to approximately 40 μ g/ml), and administered by means of a De Vilbiss 646 nebulizer. Spirometry was performed correctly in each subject by using an electronic spirometer (Nihon Koden Co., Tokyo, Japan) before and after inhalation of the 0.9% saline solution for 2 minutes as a control. Then each subject inhaled the lowest concentration of histamine for 2 minutes by using a nose clip and mouthpiece, and spirometry was repeated after each inhalation. Subsequent doses were then doubled until a 20% fall in FEV₁ from the baseline occurred. The provocative concentration of histamine required to produce a 20% fall in FEV₁ (histamine-PC₂₀) was considered to represent the bronchial hyperresponsiveness to histamine.

Pretreatment with furosemide

Furosemide (10 mg/ml; Hoechst Japan Ltd., Tokyo, Japan) was diluted with 0.9% saline solution to 5.0 mg/ml, and subjects inhaled this for 5 to 8 minutes by using a De Vilbiss 646 nebulizer.¹⁴ The total amount of inhaled furosemide was calculated from body square (10 mg/m²), and 0.9% saline solution was used as a placebo. The pH and osmolarity of furosemide and placebo were 8.16 and 7.64, 288 mOsm and 286 mOsm, respectively. Fifteen minutes before these inhalation challenges, the subjects were treated with furosemide or placebo inhalation.

Statistical analysis

Analysis of variance of the paired data was performed by means of Wilcoxon's rank test. Spearman's rank correlation coefficient was used to analyze the relationship between two data. Data except age are reported as mean $\pm$ SEM.

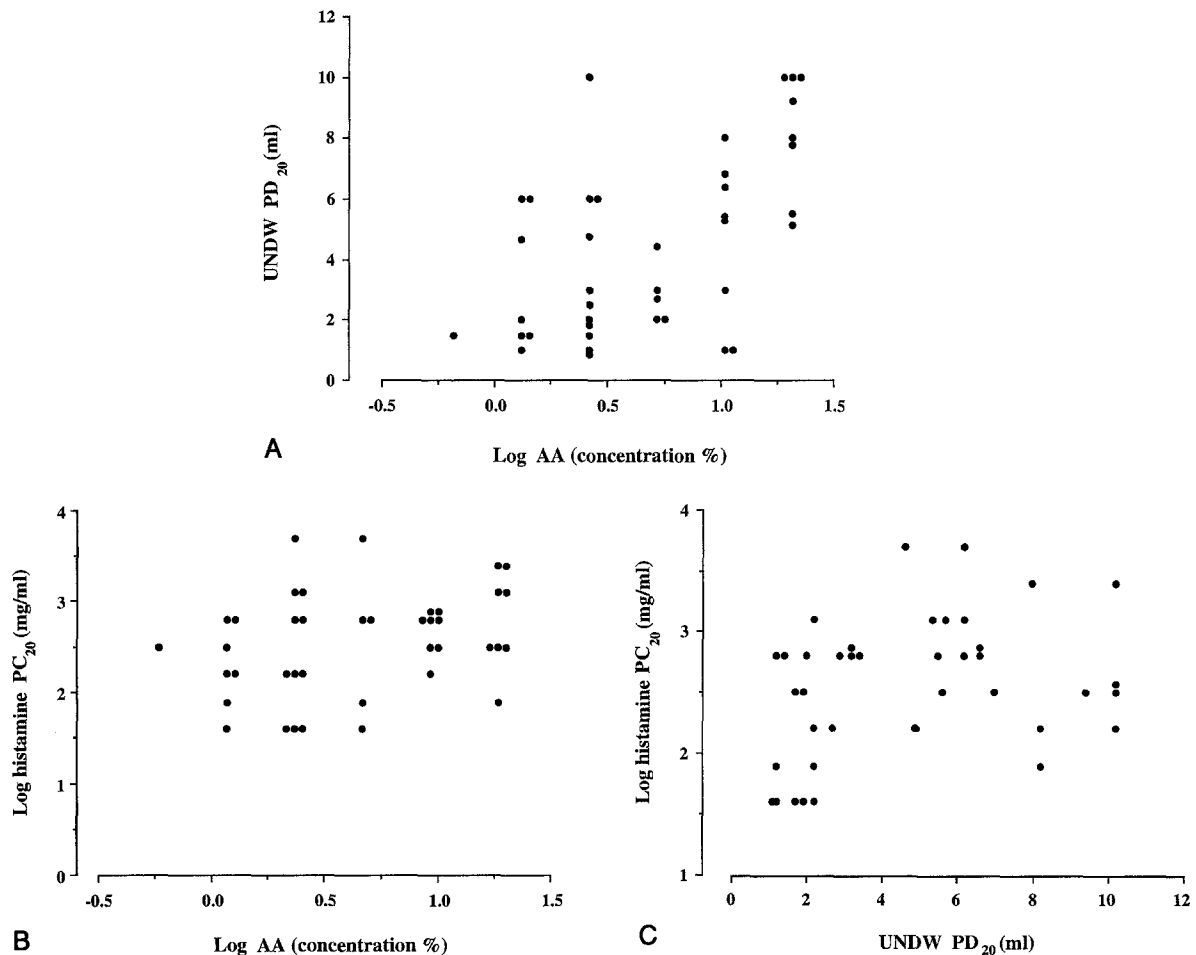


FIG. 1. **A**, Relationships between UND W-PD₂₀ and threshold dose causing cough in AA inhalation challenge. There was a good correlation between them ($n = 40$; $r_s = 0.527$; $p < 0.001$). **B**, Relationships between histamine-PC₂₀ and threshold dose causing cough in AA inhalation challenge. There was no relationship between them ($n = 40$; $r_s = 0.384$; $p > 0.1$). **C**, Relationships between UND W-PD₂₀ and histamine-PC₂₀. There was no relationship between them ($n = 40$; $r_s = 0.308$; $p > 0.05$).

RESULTS

Relationships between acetic acid inhalation challenge, UND W inhalation challenge, and histamine inhalation challenge

Fig. 1 shows the results of study 1 ($n = 40$). The mean value and standard error of the mean of UND W-PD₂₀, histamine-PC₂₀, and threshold of cough in AA inhalation challenge were 4.50 ± 0.47 (ml), 2.54 ± 0.09 log(mg/ml), and 0.67 ± 0.07 log(concentration %), respectively.

There was a good correlation between UND W-PD₂₀ and cough threshold in the AA inhalation challenge ($r_s = 0.527$; $p < 0.001$; Fig. 1, A). However, there was no relationship either between UND W-PD₂₀ and histamine-PC₂₀, or between cough threshold in the AA inhalation challenge

and histamine-PC₂₀ ($r_s = 0.384$; $p > 0.1$; and $r_s = 0.308$; $p > 0.05$, respectively) (Fig. 1, B and C).

Effect of furosemide on AA, UND W, and histamine inhalation challenges

In study 2, neither bronchoconstriction nor bronchodilatation after inhalation of furosemide was observed. The mean value and standard error of the mean of the cough threshold in AA inhalation challenge after placebo inhalation was 0.38 ± 0.10 log(concentration %) ($n = 12$). After furosemide inhalation the cough threshold in the AA inhalation challenge increased to 0.70 ± 0.11 log(concentration %) ($p < 0.01$; Fig. 2). PD₂₀ in UND W inhalation challenge after placebo inhalation was 3.96 ± 0.89 ml ($n = 12$), and after

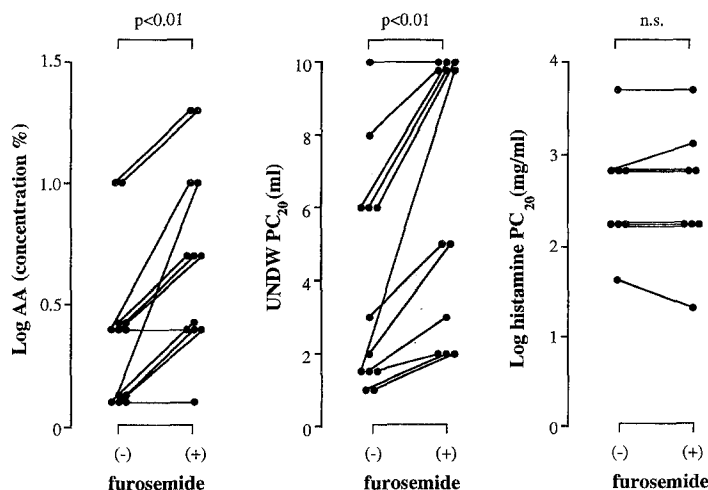


FIG. 2. Changes in the threshold dose causing cough in AA inhalation challenge, UNDW-PC₂₀, and histamine-PC₂₀ after placebo or furosemide inhalation. (Furosemide (-), Without pretreatment of furosemide; furosemide (+), with pretreatment of furosemide; n.s., not significant.)

furosemide inhalation PD₂₀ was significantly higher than that after placebo inhalation (6.58 ± 1.07 ml; $p < 0.01$; Fig. 2). The mean value and standard error of the mean of PC₂₀ in the histamine inhalation challenge after placebo inhalation was 2.97 ± 2.77 log(mg/ml) ($n = 8$), and PC₂₀ in histamine inhalation challenge after furosemide inhalation was not significantly higher than that after placebo inhalation (3.0 ± 2.77 log(mg/ml); $p > 0.1$; Fig. 2).

DISCUSSION

In persons with asthma wheezing, which is mainly dependent on bronchoconstriction, and cough are the most common complaints.²¹ However, the mechanism of hyperresponsiveness to bronchoconstriction and cough in these patients is still unclear. Accumulating data suggest that the two airway reflexes of cough and bronchoconstriction have separate afferent neural pathways,²² and they may have a differing sensitivity to inhibitory drugs.^{23, 24} In the recent report of Eschenbacher et al.,⁶ hyperosmolarity appeared to be the stimulus causing both cough and bronchoconstriction with low ion concentrations inducing cough but not bronchoconstriction. These findings suggest that bronchoconstriction and cough are produced by stimulation of different populations of afferent nerve endings, that is, irritant receptors and cough receptors. Other reports, however, suggest that common mechanisms are involved in cough and bronchoconstriction in persons with asthma.^{25, 26}

Cough, a reflex response of the respiratory tract, is produced by inflammatory, mechanical, chemical, and thermal stimulation of the cough receptors,^{27, 28} which are believed to be the fine sensory nerve endings of the trigeminal, glossopharyngeal, superior laryngeal, and vagal nerves. Cough is one of the most common complaints associated with respiratory diseases and is sometimes the only important symptom in childhood asthma. Previously, the evaluation of hyperresponsiveness of cough receptors was examined by the inhalation of capsaicin, which stimulates sensory nerve endings directly,^{4, 5} or various acids.^{1, 2, 15} Although it is not yet certain why inhalation of low-concentrated acid can induce cough in children with asthma, or osmolar changes in airway luminal fluid may stimulate cough receptors in mucosa and submucosa directly or indirectly.^{6, 7}

On the other hand, inhaled UNDW does not appear to act directly on smooth muscles in airways, and the changes in osmolarity and ion composition of the periciliary fluid of airway epithelium are the most important factors in the bronchoconstriction induced by UNDW inhalation.^{29, 30} Inhaled UNDW may induce rapid ionic and osmolar changes in airway fluid that, in turn, may affect the activation of mast cells and other inflammatory cells or the stimulation of sensory nerve endings,^{31, 32} resulting in bronchoconstriction.

In UNDW-induced bronchoconstriction, however, the mechanism of airway mast cell activation

is not necessarily involved. Verapamil, a known calcium antagonist that was expected to antagonize mast cell degranulation, did not inhibit UNDW-induced bronchoconstriction.³³ Recently we reported that inhaled furosemide protected against UNDW-induced bronchoconstriction not only in atopic asthma but also in nonatopic asthma, indicating that UNDW-induced bronchoconstriction may not be caused by its stimulating action on airway mast cells.¹⁴ Rather, a rapid ion transport across epithelial cells induced by ionic or osmolar changes in airway fluid may play a role in stimulating sensory nerve endings in luminal mucosa and submucosa or epithelial sensory nerve endings in luminal mucosa and submucosa or epithelial cell swelling,³⁴ resulting in an FEV₁ decrease during UNDW inhalation challenge in persons with asthma.

Considering these results, we speculated that one of the common mechanisms of hyperresponsiveness against UNDW-induced bronchoconstriction and AA-induced cough in children with asthma may be ionic or osmotic changes in luminal fluid in the trachea. The results of our study show that there is a relationship between UNDW-PD₂₀ and cough threshold in AA inhalation challenge, whereas there is no relationship either between UNDW-PD₂₀ and histamine-PC₂₀ or between cough threshold and histamine-PC₂₀. In addition, both UNDW-PD₂₀ and cough threshold in the AA inhalation challenge were attenuated by furosemide inhalation, whereas histamine-PC₂₀ was not.

Furosemide, a loop diuretic agent, has been shown to inhibit Na⁺-K⁺-Cl⁻ cotransporter in the thick ascending loop of Henle,³⁵ and also to inhibit the secretion of Cl⁻ into the bronchial lumen by blocking the cotransport of Na⁺ and Cl⁻ on the basolateral membrane of epithelial cells.³⁶ Recently furosemide inhalation was shown to prevent bronchoconstriction in adults with asthma. Bianco et al.⁹⁻¹¹ reported that inhaled furosemide inhibited the bronchoconstriction induced by allergen, exercise, and nebulized distilled water in subjects with asthma. It has been suggested that furosemide may not act directly at the airway smooth muscle level because of its lack of effect against both histamine- and methacholine-induced bronchoconstriction.^{12, 13} It is noteworthy that inhaled furosemide inhibited the cough induced by low chloride-content solutions but not by capsaicin, indicating that it may act by inhibiting the cough reflex indirectly, perhaps by changing local Cl⁻

concentrations in the vicinity of epithelial cough receptors.⁸

From these results we speculated that the hyperresponsiveness to UNDW-induced bronchoconstriction and AA-induced cough in children with asthma may be due to an activation of ion transport system across the airway epithelium, and that the successive effects of inhaled furosemide on these airway responses may be explained by its most potent effect, that is, ion transport inhibition. Epithelial cells probably show a rapid Cl⁻ secretion on the luminal side of mucosa, both after UNDW inhalation, which may cause an osmolar decrease in airway fluid, and also after AA inhalation, which may cause an increase of protons in airway fluid. It is possible to consider that the second messengers, Ca⁺⁺ and cAMP, may increase in airway epithelial cells in persons with asthma because most chemical mediators induced by allergic reactions stimulate the production of these second messengers, which activate ion channels in airway epithelial cells.^{37, 38} Under these conditions, ion transport systems in airway epithelial cells of persons with asthma may become hyperresponsive to nonspecific stimuli (i.e., UNDW and AA), which are likely to cause rapid ionic or osmolar changes.

Consequently, we hypothesized that a common mechanism is at work in the hyperresponsiveness to UNDW-induced bronchoconstriction and that of AA-induced cough in children with asthma, a common mechanism that may be based on an activated ion transport system across airway epithelium. However, we cannot measure pH or osmolar changes directly in airway fluid during provocation tests in children with asthma. Further investigations will be needed to clarify the common mechanisms involved in UNDW-induced bronchoconstriction and AA-induced cough in childhood asthma.

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