JCI The Journal of Clinical Investigation

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A G Togias, … , P S Norman, L M Lichtenstein

J Clin Invest. 1985;76(4):1375-1381. https://doi.org/10.1172/JCI112113.

Research Article

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Nasal Challenge with Cold, Dry Air Results in Release of Inflammatory Mediators

Possible Mast Cell Involvement

Alkis G. Togias, Robert M. Naclerio, David Proud, James E. Fish, N. Franklin Adkinson, Jr., Anne Kagey-Sobotka, Philip S. Norman, and Lawrence M. Lichtenstein

Division of Clinical Immunology, Departments of Medicine and Otolaryngology, The Johns Hopkins University School of Medicine at The Good Samaritan Hospital, Baltimore, Maryland 21239

Abstract

The purpose of our study was to assess the effect of cold, dry air (CDA) on the nasal mucosa of selected individuals in relation to the release of inflammatory mediators associated with mast cells. 12 subjects with a history of nasal symptoms of rhinorrhea and congestion upon cold or dry environmental exposure were challenged by nasal breathing of CDA and warm, moist air (WMA). Each subject was tested on two occasions with the order of the challenges reversed. Symptom scores were recorded, and the levels of histamine, prostaglandin (PG) D_2, kinins, and [^{3}H]-*N*-α-tosyl-L-arginine methyl ester (TAME)-esterase activity in nasal lavage fluids were measured.

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Introduction

The allergic response clearly involves the action of mediators released from mast cells and/or basophils upon exposure to the appropriate antigen (1–4). Frequently, however, the symptoms of rhinitis, urticaria, or asthma cannot be related directly to allergen exposure. In these latter cases, it is not clear if the same mediators contribute to the symptomatology of the reaction or, if they do, how they are released from mast cells and/or basophils. The fact that several agents including complement proteins (C3a and C5a) and formyl-methionine–containing peptides can induce release of mediators from basophils and/or mast cells in vitro (5–8) suggests that other than antigenic stimuli may activate these cells in vivo, possibly accounting for the pathophysiology of some of the above clinical entities.

Perhaps the most intensively studied of these nonallergen-related conditions is exercise-induced bronchospasm (and its laboratory correlate, hyperventilation of cold, dry air [CDA]).[1] This condition is experienced by most asthmatic patients, and controversy exists among investigators regarding its pathogenesis. Attempts to establish the involvement of mast cell mediators have led to ambiguous results (9–17). Recently, low levels of histamine and another mediator, neutrophil chemotactic factor, have been found in the circulation of asthmatic patients when they exercise while breathing cold air (9, 10, 16, 17), but not upon breathing warm and moist air (WMA) (18). The well-documented difficulties in measuring low levels of plasma histamine (19), the failure of other investigators to detect increased histamine levels under similar conditions (11, 12, 15), and the inability to obtain similar results in eucapnic hyperventilation with CDA (18), however, indicate the need for further confirmation of these findings.

In the present study, we have used a different model, nasal airway challenge, to ascertain whether CDA can induce in vivo mediator release in the upper respiratory tract. Using a similar model in previous studies on pollen-sensitive patients, we found that, after antigen challenge, nasal secretions contained elevated levels of histamine, prostaglandin D_2 (PGD_2), bradykinin, and lysylbradykinin, as well as an enzyme(s) displaying [^{3}H]-*N*-α-tosyl-L-arginine methyl ester (TAME)-esterase activity (20–22). We now report that the same mediators are found when selected patients undergo nasal challenge with cold, dry air.

Methods

Subjects. Two groups of volunteers between the ages of 18 and 45 yr were studied. Group I consisted of 12 individuals selected by virtue of having symptoms of rhinorrhea, nasal congestion, and/or sneezing when exposed to cold and/or dry environments. Some of these patients also reported a history of seasonal or perennial rhinitis. Group II consisted of five individuals, all of whom claimed to be asymptomatic on exposure to cold and/or dry environments. All subjects were tested intradermally with a panel of 10 aeroallergens. During the trial, symptoms usually lasted for only a few minutes after challenge, although, occasionally, minor local symptoms persisted for hours. Subjects were able to perform routine activities upon leaving the laboratory, and no episodes of wheezing, acute otitis, or sinusitis occurred. These studies were approved by the Johns Hopkins University Joint Committee on Clinical Investigations, Baltimore, MD, and informed consent was obtained from all subjects.

Challenges. Compressed air (MG Industries, Long Beach, CA) was delivered through a pediatric air mask placed over the nose. Subjects

This paper is publication number 616 from the O'Neill Research Laboratories at the Good Samaritan Hospital. Address reprint requests to Dr. Togias, Division of Clinical Immunology.

Received for publication 15 June 1984 and in revised form 17 January 1985.

J. Clin. Invest.

0021-9738/85/10/1375/07 $1.00
Volume 76, October 1985, 1375–1381

1. *Abbreviations used in this paper:* CDA, cold, dry air; PG, prostaglandin; PGD_2, PGE_2, $PGF_{1\alpha}$, and $PGF_{2\alpha}$, prostaglandins D_2, E_2, $F_{1\alpha}$, and $F_{2\alpha}$; TAME, [^{3}H]-*N*-α-tosyl-L-arginine methyl ester; TNAC, total nasal airway conductance.

were instructed to breathe by inhaling through the nose and exhaling through the mouth. Each air challenge lasted for 15 min. The flow, measured by a Matheson 605 flowmeter, was set at 12.5 liters/min. To obtain warm and moist air, compressed air was passed through a coiled plastic tube immersed in warm water (60°C), and subsequently through a bottle containing distilled water at 60°C. The WMA had a temperature of 28°–32°C, measured 10 cm distal to the mask with a temperature probe connected to a Bailey Bat-12 digital thermocouple thermometer. Relative humidity, measured by the dry and wet bulb thermometer technique, was ~98.5%. CDA was obtained by passing compressed air through a coiled plastic tube immersed in a dry ice/alcohol bath (−60°C). The final temperature varied between −3° and −10°C. The relative humidity was <10%.

Collection of nasal secretions. Nasal lavages were performed as previously described (20–22). 2½ ml of saline at 37°C were instilled in each nostril and expelled after ~10 s. The mixture of mucus and saline (gel and sol phase) was vigorously shaken for 10 s, resulting in partial solubilization of the gel phase. 1-ml aliquots from each sample, to be assayed for kinins, were immediately made 40 mM in EDTA. Upon conclusion of the experiment, all aliquots were centrifuged at 27,750 *g* at 4°C for 15 min for further separation of the sol and gel phase. The sol phase was used in the assays, since we have previously shown that the distribution of histamine, kinins, and PGD_2 in nasal washes is not different between the two phases (20–22). Aliquots obtained for kinin determination were stored at −80°C until assayed. Aliquots for TAME esterase(s) and PGD_2 determinations were stored at −20°C. The samples for histamine were mixed with 8% $HClO_4$ at a ratio of 4:1 and centrifuged for 10 min at 1,000 *g*. Supernatants were stored at 4°C before assay.

Symptom scores. Total nasal airway conductance (TNAC) was measured several times during each challenge procedure (Fig. 1) using anterior rhinometry. However, TNAC dropped significantly ($P < 0.05$) after the seven nasal washes performed before challenge, confirming earlier observations that even slight manipulations in the nose can cause significant changes in these measurements (23). We concluded, therefore, that TNAC would not be a useful parameter, and so subjective symptoms scores were used to provide the clinical parameter for our studies.

Symptom scores were obtained from the subjects several times during each challenge. They reported three symptoms/symptom groups; rhinorrhea, nasal congestion, and a composite of other symptoms (nasal or pharyngeal tickling, watery eyes, etc.) on a scale from 0 to 3. To calculate differences, the scores from all three symptom categories were added at each evaluation time. Thus, scores ranged from zero to a maximum of nine. Sneezes were also counted.

Experimental protocols. Fig. 1 presents the two experimental protocols used; they differ only by the reversal in the order of WMA and CDA challenges. All subjects were tested using both protocols. Before challenge, seven initial washes were performed over a period of 12 min. Samples obtained from the first and seventh nasal washes were saved and subsequently assayed for mediators. The first challenge (either with WMA or CDA) began 10 min after the seventh wash. Nasal lavages were repeated 10 and 20 min after the challenge. After a further 10-min interval, the second challenge was performed, and nasal washes were obtained again 10 and 20 min after challenge. The time intervals between challenges

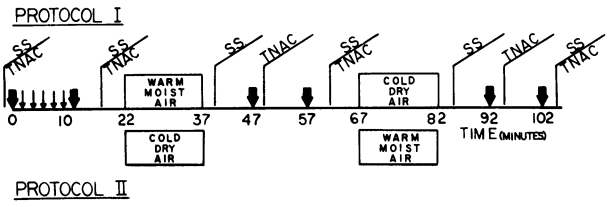

Figure 1. Experimental protocols used to assess whether CDA induces release of inflammatory mediators. WMA serves as a control. Thick arrows indicate nasal lavages used for mediator measurements. Thin arrows indicate discarded nasal lavages. SS, symptom scores.

and nasal lavages were arbitrarily selected in the design of the study. The levels of mediators in the seventh wash were considered baseline for the rest of the study. For reasons of simplification, this approach was chosen as an alternative to the use of the second lavage after the first challenge as the baseline for the challenge to follow. When the results were analyzed by both methods, the differences in the statistical significance were trivial (data not shown).

Mediator assays. Histamine, PGD_2, kinins, and TAME esterase activity were measured as previously described (20–22). In brief, acidified samples were assayed for histamine by an automated spectrofluorometric assay sensitive to ≥1 ng/ml of histamine with a precision of <5% (24).

To assess the specificity of the assay, we incubated three nasal lavage samples obtained after CDA challenge with diamine oxidase, at a final concentration of 0.1 U/ml for 1 h at 37°C. This treatment resulted in 70–80% reduction in the spectrofluorometric readings as compared with the untreated samples. Histamine standards treated similarly led to the same percent reduction. PGD_2 was measured by a competitive radioimmunoassay sensitive to 50 pg/ml (25, 26). Cross-reaction of PGE, PGE_2, thromboxane B_2, 6-keto-$PGF_{1\alpha}$, and $PGF_{2\alpha}$ was <1% in each case. PGD_2 was measured in the complete set of samples from two group I and one group II subjects and only in three samples from each one of the rest of the experiments, i.e., the seventh preliminary wash (baseline), the first wash after CDA, and the first wash after WMA provocation. Data from the three complete series of samples and from numerous nasal provocations with antigen (20) indicated that PGD_2 levels closely followed the pattern of release of the other mediators. The concentration of kinins in nasal washes was determined by a specific, competitive radioimmunoassay capable of detecting 20 pg/ml of kinin (22). Enzyme(s) displaying arginine esterase activity were assayed essentially according to the radiochemical method of Imanari and colleagues (27), which is based on the liberation of ^3H-labeled methanol from the synthetic substrate ^3H-TAME. Serial dilutions of nasal lavage samples obtained after CDA challenge from four different subjects resulted in linear decrease in the concentration of all mediators.

Statistical analysis. Nonparametric statistics were used. For evaluation of the differences in mediator levels and symptom scores between baseline and CDA challenge, WMA and CDA challenge, as well as between baseline and WMA challenge, the Wilcoxon Matched-Pairs Signed-Ranks test was applied. The same test was used when comparing the mediator and symptom score levels after CDA challenge between protocols I and II. For the correlation analysis between net increase of different mediators and symptom scores after CDA challenge in comparison to base-line levels, we used the Spearman rank correlation coefficient. In addition, multiple correlation analysis was performed using the Kendall coefficient of concordance test. Kruskall Wallis one-way analysis of variance was used to compare base-line mediator levels among subjects of both groups on both test days.

Results

The clinical response to nasal challenge with CDA was primarily characterized by rhinorrhea and nasal congestion, usually apparent within the first 10 min of the challenge and lasting for not more than 10 min after the end of the challenge. Sneezing was not a frequent finding and was, therefore, not used as a measurable clinical parameter.

Data obtained from a representative challenge of a cold-symptomatic (group I) subject are shown in Fig. 2. As we have reported previously (20), there may be significant levels of one or more mediator in the initial wash before challenge, but these can be reduced by repeated lavages, such that a low, stable baseline exists in the wash just before the first air challenge. In protocol I, WMA did not result in any response in terms of the mediators measured in the two subsequent nasal washes, nor were clinical symptoms experienced. In contrast, CDA evoked

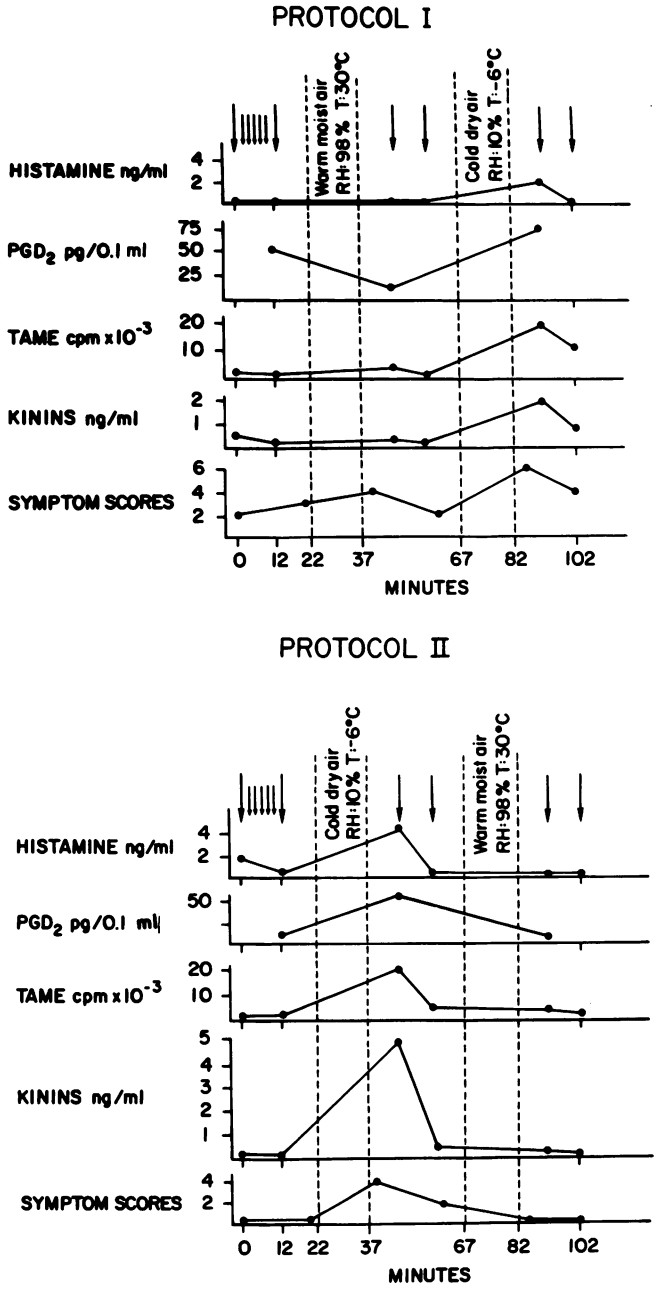

Figure 2. Nasal challenge of a CDA responder challenged according to both protocols. RH, relative humidity; T, temperature.

a significant increase in histamine, TAME-esterase activity, PGD_2, and kinins in nasal fluids. Moreover, CDA elicited clinical symptoms as shown in Fig. 2. After reversing the order of challenge (protocol II), similar results were obtained; symptoms and mediator release occurred after CDA challenge but not with WMA.

Based on our previous experience with the antigen challenge model, we consider a threefold increase over baseline in the levels of a mediator in one of the two postchallenge nasal washes as an indication of a positive response to the preceding challenge. In 17 out of the 24 studies performed in group I subjects, a positive response to CDA with at least three of the four mediators was obtained. In five other studies, the response to CDA was positive for two of four mediators. One trial resulted in elevation in only one mediator, and in one no positive response in either the levels of any mediator or of symptom scores occurred. A positive response to WMA was observed in only one subject, which was considerably less than that obtained after CDA challenge in the same subject.

Histamine data for each individual in group I during warm and cold air challenge are illustrated in Fig. 3. During protocol I, most base-line values were below the limit of detection of the fluorometric assay (1 ng/ml). The amount of histamine recovered from nasal washes after WMA was unchanged from baseline. However, when the patients were exposed to CDA, 9 out of 12 showed at least a threefold increase over baseline in both protocols. This effect was different from both the baseline ($P < 0.01$) and the effect of WMA challenge ($P < 0.01$). When the order of challenge was reversed, these results were reproduced. A similar pattern was found with the individual data obtained from the measurement of the other mediators, as well as of symptom scores.

A summary of mediator levels and symptom scores obtained in group I subjects is presented in Fig. 4 *A*. Regardless of the order of challenge, CDA exposure produced significant increases (P values ranging from <0.01 to <0.001) over baseline in the level of each mediator, whereas WMA did not cause significant changes in the levels of histamine, PGD_2, or TAME-esterase activity. The kinin levels, however, were increased over baseline ($P < 0.02$ for protocol II and $P < 0.01$ for protocol I) after the WMA challenge, but this effect was trivial compared with the values obtained after CDA challenge (mean group values of kinin levels after WMA: 0.20 ng/ml vs. 3.4 ng/ml after CDA in protocol I and 0.32 ng/ml vs. 2.1 ng/ml in protocol II). The subjective symptom scores followed a pattern identical to that of the mediator levels ($P < 0.01$). Mediator levels and symptom scores in the first nasal lavage (data not shown) were usually higher than those in the seventh wash (baseline). Only with histamine did this difference reach significance for both groups and protocols (P values ranging from <0.01 to =0.05). Nevertheless, CDA challenge in the cold-symptomatic group of subjects resulted in every instance in levels significantly higher than those obtained in the first wash (for histamine: $P < 0.02$ in both protocols).

Although there were marked differences in individual responses in terms of the increment in mediators, paired analysis of the levels of mediators after CDA challenge between the first

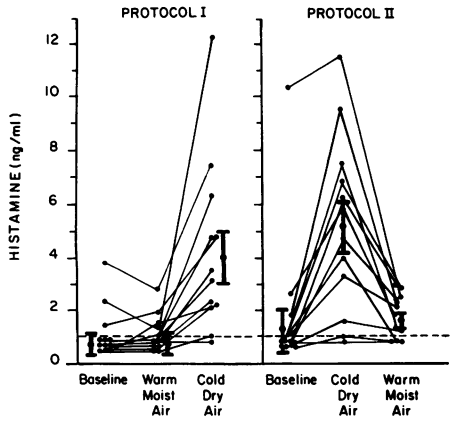

Figure 3. Individual histamine data from group I subjects, all tested twice, using both protocols. Dashed line indicates level of sensitivity of the histamine assay. Mean values±SEM are depicted.

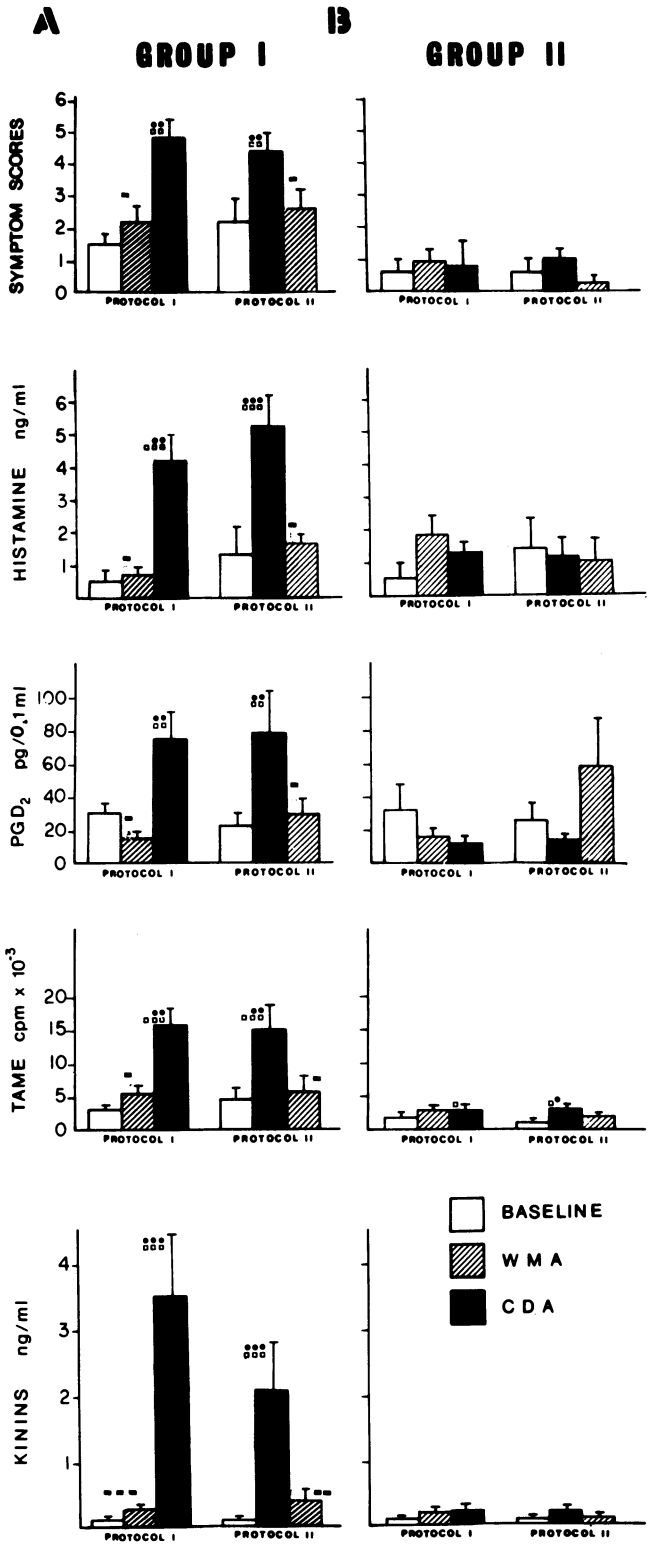

Figure 4. Summary of mediator levels and symptom scores obtained during the study. (*A*) Group I: individuals with history of sensitivity to dry and/or cold environmental exposure, $n = 12$. CDA vs. baseline: □□, $P < 0.01$; □□□, $P < 0.001$. CDA vs. WMA: ●●, $P < 0.01$; ●●●, $P < 0.001$. WMA vs. baseline: ■ NS; ■■, $P < 0.02$; ■■■, $P < 0.01$. (*B*) Group II: individuals without history of sensitivity to cold and/or dry environments, $n = 5$. CDA vs. baseline: □, $P = 0.05$. CDA vs. WMA: ●, $P < 0.05$. No other comparisons were statistically different.

and the second protocol showed no statistically significant differences.

The negative results of challenges in group II subjects indicate that responses to CDA do not occur in all individuals (Fig. 4 *B*). Only in the case of TAME-esterase activity was a difference noted in the levels obtained after CDA as compared with WMA ($P = 0.05$). Even in this instance, the level of this enzymatic activity after CDA in group II subjects was much lower than that measured after CDA in group I subjects. Analysis of variance indicated that mediator levels between both groups and both test days in the first and the seventh (baseline) wash were not significantly different, thus eliminating the possibility of differences in base-line concentration of mediators between the two groups.

The correlations in Table I indicate significant relationships between symptom scores and the net increments in the concentrations of the three mediators measured in absolute weight units. Moreover, there was a significant correlation in the magnitude of elevation between the different mediators. Multiple correlation analysis between all four mediators and symptom scores was also highly significant ($P < 0.001$).

Although many individuals in group I had positive skin tests, we cannot make any conclusions concerning the relationship between skin-test sensitivity and CDA responsiveness.

Discussion

The CDA nasal challenge model has allowed us to demonstrate, for the first time, the local release of inflammatory mediators in the respiratory mucosa in vivo after a physical stimulus. The clinical response to CDA as well as the concomitant release of mediators was reproducible and specific for the above stimulus. Symptom scores were a useful clinical parameter for assessing the magnitude of the response, although, as is often the case when subjective symptoms are related to an objective measurement, the correlations between mediator levels and symptoms were low.

Of the mediators measured, histamine can be confidently attributed to basophils and mast cells. PGD_2 is the major cyclooxygenase product of mast cells, and this is likely its source in the nasal mucosa. Studies with human lung tissue have shown that, after antigenic stimulation, essentially all of the PGD_2 released is derived from the mast cells (24). Furthermore, until now, only human platelets and rat adrenocortical cells have been shown to produce PGD_2, and the amount produced by human platelets is extremely small when compared with activated mast cells (28–30). The fact that TAME-esterase activity and kinins relate statistically to each other and to histamine and PGD_2 (Table I) is consistent with their possible association with mast cell activation. The TAME-esterase activity detected in the nasal antigen challenge model (20–22) probably reflects the activity of several enzymes. A portion of this, however, resembles the major neutral protease described from human mast cells (31). It is not known whether the profile of TAME-esterase obtained after CDA challenge is similar. Kininogenase activity has been detected in human mast cells (32) and basophils (33). It is attractive to suggest, therefore, that these enzymes may contribute to kinin generation in the nose, although alternative mechanisms can also be postulated (22). No completely satisfactory explanation can be given for the slight increase in the kinin levels

Table I. Correlations between Mediators and Symptom Scores in Group I Subjects Using Pooled Data from Both Protocols

	Δ symptom scores	Δ Histamine	Δ PGD_2	Δ Tame
Δ Histamine	rs: 0.43 $P < 0.05$			
Δ PGD_2	rs: 0.49 $P < 0.02$	rs: 0.47 $P < 0.02$		
Δ Tame	rs: 0.36 $P < 0.08$	rs: 0.48 $P < 0.01$	rs: 0.40 $P < 0.05$	
Δ Kinins	rs: 0.40 $P < 0.05$	rs: 0.63 $P < 0.001$	rs: 0.42 $P < 0.05$	rs: 0.86 $P < 0.001$

Δ, Net increase over base-line values. rs, Spearman Rank correlation coefficient.

after WMA challenge, apart from the possibility that warm air may induce a vasodilatory reflex through which kininogen and, possibly, plasma kallikrein may diffuse from plasma into the upper airway surface.

A disadvantage of our system is the variable dilution of mediators created by the nasal lavage, so that the absolute amount of mediators released topically in the nasal mucosal surface cannot be determined. This may contribute to the weak correlations between the absolute quantities of mediators found in nasal secretions (Table I). Interestingly, however, these correlations are not much poorer than those obtained in vitro between histamine and PGD_2 release from purified human lung mast cells stimulated with anti-IgE (34).

Group I subjects were selected based on a history of sensitivity to cold and/or dry environmental exposure and do not reflect the frequency of this type of response in the general population. Group II subjects, with a negative history of sensitivity to similar conditions, did not show any clinical response to CDA. The slight increase in TAME-esterase activity after CDA in this group suggests that a spectrum of responses to this stimulus might be expected in the general population. The definite difference between the two groups, however, indicates that the phenomenon we are describing is not ubiquitous and that it might well represent a form of hyperreactivity of the nasal mucosa. It has been reported that conditions such as vasomotor rhinitis, nasal mastocytosis, and nonallergic rhinitis with eosinophilia are associated with nasal sensitivity to climatic changes (35–37). It has also been reported that when volunteers breathed or hyperventilated cold air through their nose, nasal irritation and increased nasal airway resistance occurred (38–40). The CDA challenge model may give useful insights into these conditions and may help to characterize the large number of poorly defined patients with rhinitis.

There are similarities and differences between the results obtained with the CDA challenge system and the findings after nasal antigen challenge. Mediators detected during nasal antigen challenge (20) were also present after CDA provocation. We have not measured peptide leukotrienes in this study, although we have recently reported the generation of these mediators after antigen challenge in the nose (41). The response to CDA is marked by a very low incidence of sneezing, a shorter duration of nasal symptoms, and lower levels of mediators than in the antigen challenge. Only two of twelve subjects in group I consistently sneezed after breathing CDA. In contrast, sneezing has been a major clinical parameter measured in the antigen challenge model, where it correlates significantly with the release of all mediators (20–22). The cooling of the nasal mucosa and thus of the surface nerve endings during CDA challenge could be a reason for this difference.

Although the pathophysiologic mechanisms by which CDA causes mediator release are unknown, it is unlikely that CDA has a toxic effect on mediator containing cells of the nasal mucosa, because certain subjects do not have a clinical response to CDA or release mediators. In addition, the release of PGD_2, which requires active synthesis, is unlikely to be associated with a lytic event. It has been shown that human basophils and mast cells release mediators when exposed, in vitro, to a hyperosmolar environment (42, 43). The large volume (~150 liters) of CDA that passes through the nasal cavities during challenge may lead to water evaporation and an increase in the osmolarity of the extracellular fluid surrounding the mucosal mast cells that is sufficient to induce mediator release. Such a mechanism has been previously suggested by Anderson et al. (44) in in vivo studies using the exercise-induced asthma model. Alternatively, as postulated for exercise-induced asthma (45, 46), it is possible that respiratory heat loss is responsible for mediator release, although it should be noted that lowering the temperature of the medium to 0°–20°C does not cause histamine release from lung mast cells in vitro. Our studies were not designed to provide information on the pathogenesis of CDA effect, hence we have no data to support either one of the above hypotheses.

While there are clearly differences between our nasal challenge system and the model of exercise-induced asthma, it is interesting to speculate that there may be some similarities in the pathogenesis of the response in each case. Some investigators have reported increased levels of histamine and neutrophil chemotactic factors in the serum of patients postexercise, and suggest that exercise-induced asthma is due to mediator release from mast cells (9, 10, 16, 17). Other investigators have failed to detect such mediators (11, 12, 15), while yet others report similar increases after exercise of nonasthmatic individuals (13, 14). We feel that our finding of mediator release in the upper respiratory tract supports the hypothesis that mast cell mediator release is involved in exercise-induced asthma. The recent demonstration that bronchial challenge of asthmatic patients with hyperosmolar solutions results in bronchoconstriction (47, 48) makes it tempting to speculate that increased extracellular osmolarity may play a role in mediator release.

In conclusion, we have presented the first clear demonstration, in vivo, that physical stimulation of the human respiratory tract causes the local release of inflammatory mediators that are most likely mast cell derived. The appearance of these mediators correlated with nasal symptomatology, and it is possible that they contribute to its induction. We believe that we have de-

veloped a reproducible and flexible model that can provide further insight into the pathogenesis of several upper and lower respiratory tract disorders.

Acknowledgments

The authors would like to thank Ms. Indira Nimmagadda for her technical assistance and Mrs. Denise Jones and Mrs. Karen Nelson for typing the manuscript.

Dr. Naclerio is the recipient of Teacher Development Investigator Award 1K07 NS00811 01 from the National Institute of Neurological and Communicative Disorders and Stroke. This work is supported by grants AI07290, HL32272, AI04866, and AI20135 from the National Institutes of Health.

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