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16. Abstract (Limit: 200 words)

The investigators exposed asthmatic subjects to aerosols of sulfuric acid or saline with varying particle size and osmolarity. Aerosols of unbuffered sulfuric acid at pH2 did not cause bronchoconstriction in the subjects when inhaled during rest at a sulfate concentration of nearly 3mg/m^3. Neither osmolarity nor particle size appeared to influence this lack of bronchoconstrictor effect.

The investigators also studied whether there was a positive interaction between acidity and low temperature with regard to the potentiation of hypoosmolar aerosol-induced bronchoconstriction. They exposed asthmatic subjects to hypoosmolar aerosols of either sulfuric acid at pH2 or saline at pH 5.5 at either 7^o or 22^{o}C. No evidence of a positive interaction between acidity and low temperature was found.

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SUMMARY

Acid fog is a relatively frequent phenomenon in California but the health effects of exposure to such fog are unknown. Before studies evaluating potential adverse health effects of acid fog can be intelligently designed and performed, basic information about the mechanisms by which acid fog could cause such effects is required. Because subjects with asthma may be especially sensitive to the inhalation of air pollutants, we examined several mechanisms by which acid aerosols may cause or contribute to bronchoconstriction in such subjects. By having subjects with asthma inhale acid aerosols of sulfuric acid (H_2SO_4) or saline with varying particle size and osmolarity, we were able to study the relative importance of pH, particle size, and osmolarity with regard to the bronchoconstriction potency of aerosols. Aerosols of unbuffered H_2SO_4 at pH 2 did not cause bronchoconstriction in our subjects when inhaled during rest at a sulfate concentration of nearly 3 mg/m^3. Neither osmolarity nor particle size appeared to influence this lack of bronchoconstrictor effect.

We had reported previously that resting inhalation of a large respirable aerosol (mass media aerodynamic diameter (MMAD) 5-6 microns) of unbuffered H_2SO_4 caused only minimal bronchoconstriction in subjects with asthma at a concentration of >10 mg/m3. This concentration of H_2SO_4 is over 20 times that reported by investigators to cause bronchoconstriction when inhaled as a small particle aerosol (MMAD <1 micron). This apparent discrepancy in results was consistent with the hypothesis that particle size may be an important determinant of the bronchoconstrictor potency of acid aerosols. However, the absence of any measurable effect of H_2SO_4 aerosol in the study described in Project 1 of this report, whether inhaled as large (MMAD, 5 microns) or small (MMAD. 0.4 micron) particles, suggests that our previous failure to demonstrate significant bronchoconstrictor effects of H_2SO_4 was not simply due to administration of this acid in the form of large particles. It is important to point out some caveats to the essentially negative findings we report for Project 1. Since we studied resting subjects during a relatively brief

(16 min) exposure to H_2SO_4, we cannot exclude the possibility that a more significant bronchoconstrictor effect might result from exposure during exercise and/or more prolonged exposure. Furthermore, our results in no way rule out important adverse effects other than bronchoconstriction.

Because fog often occurs at relatively low ambient temperature and because the bronchoconstrictor effects of low temperature in subjects with asthma are well-described, we also studied whether there was a positive interaction between acidity and low temperature with regard to the potentiation of hypoosmolar aerosol-induced bronchoconstriction (Project 2 of this report). In a previous study, we reported that coexistent acidity can potentiate the bronchoconstrictor effect of a hypoosmolar aerosol. We hypothesized that cooling the inhaled hypoosmolar acid aerosol might cause an even greater potentiation of bronchoconstriction in subjects with asthma. By generating concentration-response curves during inhalation of hypoosmolar aerosols of either H_2SO_4 (pH 2) or saline (pH 5.5) at either 7° or 22° C, we were able to estimate whether acidity or low temperature had caused a significant shift to the left in the concentration-response curve for each subject.

While there were no statistically significant differences among the different exposure conditions in the mean concentration of aerosol required to induce significant bronchoconstriction (defined as a 100% increase in specific airway resistance), both acidity and low temperature did cause a shift to the left in the concentration-response curves of most subjects. However, we found no evidence of a positive interaction between acidity and low temperature with regard to this leftward shift of the concentration-response curves.

Our failure to find a significant positive interaction between acidity and low temperature suggests a relatively minor role for cold ambient temperature in acid fog-induced bronchoconstriction. Furthermore, the results of Project 2, utilizing a mouthpiece system, indicate that it should not be necessary to generate acid fogs at low temperature in order to study their potential adverse respiratory effects on freely breathing human subjects in our recently constructed exposure chamber.

CONCLUSIONS

The projects completed under this contract permit the following conclusions:

1. The particle size of sulfuric acid aerosols does not appear to be a major determinant of their bronchoconstrictor potency.

2. One previous finding that acidity potentiates the bronchoconstrictor effect of hypoosmolar aerosols with high liquid water content in subjects with asthma was not replicated when aerosols of much lower liquid water content were studied.

3. Clinically significant bronchoconstriction did not occur in subjects with mild-moderate asthma exposed to sulfuric acid aerosols while at rest. However, this finding does not rule out the possibility that significant bronchoconstriction may occur under other conditions, e.g., with exposure during exercise.

4. There does not appear to be a significant positive interaction between acidity and cold temperature with regard to the potentiation of hypoosmolar aerosol-induced bronchoconstriction in subjects with asthma.

5. Since acidity and cold temperature did not interact positively with regard to the potentiation of bronchoconstriction, it should not be necessary to generate acid fogs at low temperature in exposure chamber studies.

RECOMMENDATIONS

1. More information is needed to answer the question of whether varying the liquid water content of sulfuric acid aerosols has an effect on their bronchoconstrictor potency.

2. Studies of the bronchoconstrictor effect of sulfuric acid in subjects with asthma who are exposed during exercise should be conducted.

3. More monitoring of the chemistry of California acid fog should be performed, especially with regard to vapor phase nitric acid and acid sulfates other than sulfuric acid.

4. Since animal toxicological data suggest the enhancement of the toxic effects of oxidant pollutants by acid aerosols, experiments involving exposure of human subjects to acid fogs in sequence with ozone should be conducted.

DISCLAIMER

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

BODY OF REPORT

General Introduction

Complex mixtures of acid pollutants occur commonly in California, especially along the coast where the pH of ambient fog has been measured to be as low as 1.7 (1). Present scientific evidence is insufficient to allow regulatory agencies to accurately predict the potential adverse effects of acid aerosols, such as acid fog, on human respiratory health. Before definitive human exposure chamber studies of the health effects of acid fog can be designed and interpreted, basic information about the mechanisms by which acid aerosols can cause such effects needs to be obtained.

The experiments performed as part of this contract were designed to determine the importance of aerosol particle size and coexistent low temperature in mediating any adverse effects of acid fog on the respiratory tract. The best-described and most clinically important health effect of acid aerosols and acid precursors (e.g., sulfur dioxide) is bronchoconstriction in subjects with asthma (2-5). Thus, the studies performed under this contract were designed to determine the relative importance of the particle size and the temperature of inhaled acid aerosols in causing bronchoconstriction in subjects with asthma.

Project I:

Effect of Particle Size on Sulfuric Acid Aerosol-Induced Bronchoconstriction

Introduction

Acid aerosols currently are being considered for listing as a "criteria pollutant" by the U.S. Environmental Protection Agency and the California Air Resources Board. Such a listing would compel stricter regulation of ambient acid levels, that would entail considerable societal economic investment. Any judgement about criteria pollutant status for acid aerosols must involve review of controlled human exposure studies, an important component of the quantitative data base on the health effects of acid aerosols. In particular, controlled human studies contribute vital information regarding concentration-response relationships and effects on sensitive populations, such as individuals with asthma.

Sulfuric acid (H_2SO_4) is the most commonly found acid air pollutant across the nation and is the most widely studied. It is important to note that nitric acid (HNO_3) is a major contributor to the acidity of California acid fog. However, it is our hypothesis that the toxic effects of acid aerosols on the respiratory tract are due to hydrogen ion (H+) rather than to specific anions (6). Concentrations of H_2SO_4 many times greater than those encountered during even "worst-case" air pollution episodes have failed to cause normal subjects to develop significant decrements in pulmonary function in a number of studies. However, subjects with asthma are known to be considerably more sensitive to the bronchoconstrictor effects of certain pollutants (e.g., sulfur dioxide) than are normal subjects. Thus, the response of individuals with asthma to inhalation of H_2SO_4 aerosols has been the focus of considerable research interest.

Utell and coworkers observed a significant decrease in specific airway conductance (SGaw) in adults with asthma exposed at rest to 450 $\mu g/m^3$ H_2SO_4 (5). Similarly, Koenig and coworkers observed a significant decrease in forced expiratory volume (FEV1) in 10 adolescents with asthma exposed during both rest and exercise to 100 $\mu g/m^3$ H_2SO_4 (4). On the basis of these findings, it has been suggested that sulfuric acid aerosols, like sulfur dioxide, may be an important stimulus to bronchoconstriction in patients with asthma. However, the effects of sulfuric acid on lung function in the studies cited above were small. Furthermore, in the study by Utell and coworkers, a more than two-fold increase in the concentration of sulfuric acid (from 450 to 1000 $\mu g/m^3$) did not increase the magnitude of the change in lung function observed (5). In two previous studies designed to evaluate the bronchoconstrictor effects of acid fog, we have found only minimal increases in specific airway resistance (SRaw) in subjects with asthma.exposed for brief periods at rest to up to 40 mg/m^3 H_2SO_4 (6,7). The slope of the dose-response relationship for virtually all known stimuli to clinically significant bronchoconstriction in subjects with asthma is relatively steep. Thus, our failure to observe a marked bronchoconstrictor response despite a nearly hundred-fold increase in the magnitude of the concentration suggested to us that H_2SO_4 was not likely to be a clinically important stimulus to bronchoconstriction. However, the size of the particles we used in our previous studies (5-6 micron mass median aerodynamic diameter (MMAD)), our use of sodium chloride to maintain isoosmolarity, and our use of a short exposure duration (3 min) were all important differences from the exposure conditions studied by other investigators, and from the exposure conditions likely to occur in the

was generated. Only subjects who developed ≥ 100% increase in specific airway resistance (SRaw) continued in the study. On 5 subsequent, randomly separated days, subjects inhaled one of 5 aerosols: large particle (~5 micron MMAD), isoosmolar (300 mOsm) H_2SO_4 at pH 2; (0.005M); large particle, hypoosmolar (30 mOsm) H_2SO_4 at pH 2; large particle, hypoosmolar saline at pH 5.5 (the pH of H_2O in equilibrium with atmospheric CO_2); small particle (~0.4 micron MMAD) H_2SO_4 at pH 2 (0.005M); small particle saline at pH 5.5. The aerosol challenges were randomly ordered, occurred at the same time on separate days, and were performed in a single-blind fashion. The aerosols were inhaled for 16 min through a mouthpiece during tidal breathing at rest. Subjects were not exposed to aerosol on days when their baseline SRaw's were < 50% or > 150% of their usual baselines. In order to reduce neutralization of inhaled aerosol by oral ammonia, the subjects brushed their teeth and gargled with antiseptic mouthwash prior to each challenge. To assess airway responses of the subjects to the inhaled aerosols, airway resistance (Raw) and thoracic gas volume (Vtg) were measured in a constant volume body plethysmograph (No. 09103, Warren E. Collins, Braintree, MA) and expressed as the product of Raw and Vtg, SRaw. Five measurements of SRaw, one every 30 sec, were made before and after each aerosol challenge. Coughs were counted throughout the experiment by an observer and recorded on a small portable tape recorder. Throat and respiratory symptoms were assessed by a post-exposure questionnaire with an 11-point rating scale for each of 9 symptoms.

Aerosol was generated by an ultrasonic nebulizer (Mistogen EN 145, Time Meter Co., Lancaster, PA). The fan of the nebulizer was disconnected, and compressed air at a flow rate of 15 L/min was

entrained through the nebulizer. The compressed air was humidified via a cascade humidifier if large particles were to be generated and left dry if small particles were to be generated. The output of the nebulizer was diluted with 30 L/min of humidified air to maintain large particles or dry air to create small particles. The diluted nebulizer output was then directed into an "aging chamber," an approximately 16 ft x 1.5 in section of coiled polyethylene tubing. The subjects inhaled aerosol through a mouthpiece connected to a plastic T-piece (with a 2 L outflow reservoir) that was directly attached to the downstream end of the aging chamber without a respiratory valve. The subjects wore noseclips while they inhaled aerosol. The temperature of the inhaled aerosol was measured continuously at the mouthpiece and recorded after each minute of exposure.

The oscillation amplitude of the ultrasonic nebulizer was adjusted to yield a concentration of H_2SO_4 at the mouthpiece of 2.9 mg/m^3. The nebulizer output setting was the same for both H_2SO_4 and saline aerosols. For both large and small particle aerosols, we calculated the delivered H_2SO_4 concentration by measuring the concentration of sulfate ion. The airstream was drawn across either glass fiber filters (Gelman Sciences, Ann Arbor, MI) with an effective retention of 0.3 microns for the large particles or cellulose membrane filters (Nuclepore, Pleasanton, CA) with a 0.22 micron pore size for the small particles. The airstream was drawn across the filters at a flow rate of 6 L per min for 5 min. Each filter was washed with 10 cc of distilled H_2O that was drawn across the filter while it was still in the filter cassette by continuous vacuum. The sulfate concentration of 2-3 ml aliquots of the filter wash was measured by ion chromatography (4000i, Dionex, Sunnyvale, CA), using a Dionex AS4A HPIC

column, a Dionex AMMS-1 suppressor column, and an eluant containing 0.0056 M sodium bicarbonate and 0.0048 M sodium carbonate. The sulfate concentration of the sample divided by the known air sample volume yielded the sulfate concentration of the aerosol.

The particle sizes of the aerosols delivered at the mouthpiece were measured by a phase/Doppler particle analyzer (Aerometrics, Mountain View, CA). Because the lower limit of the phase/Doppler particle analyzer was 0.3 micron, the particle size of the small particle aerosols were measured by using a laser particle counter (Model μLPC-1001, Particle Measuring Systems, Boulder, CO.).

Each solution was prepared and its pH was measured (pH Meter No. 43, Beckman, Irvine, CA) immediately before nebulization. The osmolarity of each solution was measured with a vapor-pressure osmometer (No 5700B, Wescor, Logan, UT).

To determine whether there were significant differences among the subjects' airway responses to inhalation of the 5 aerosols, we compared the mean change in SRaw from pre-exposure baseline values after inhalation of each aerosol using a 2-way analysis of variance. The mean values of baseline SRaw before administration of each aerosol were compared using a 2-way analysis of variance. To analyze the symptoms experienced after each aerosol by each subject, we grouped the 9 symptom scores into 3 categories: a) lower respiratory symptoms (chest pain, chest tightness, wheezing, shortness of breath, cough, and sputum production); b) throat irritation; and c) non-respiratory symptoms (back pain and headache). To determine whether there were significant differences among the subjects' reported symptoms following inhalation of the 5 aerosols, we compared the symptom category scores also by

means of a 2-way analysis of variance. To determine whether there were significant differences among the subjects' cough responses to inhalation of the 5 aerosols, we compared cough frequencies again using a 2-way analysis of variance. Finally, the mean temperatures of the inhaled aerosols during each 16 min exposure period for each of the 5 solutions were compared by a 2-way analysis of variance. A p value of ≤ 0.05 was considered statistically significant.

Results

None of the 11 subjects developed an increase in SRaw of $\geq 15\%$ after inhalation of any of the 5 aerosols. In fact, the SRaw of most subjects decreased slightly from their pre-exposure baseline values following inhalation of all 5 aerosols. The mean changes in SRaw (in L X cm H_2O ÷ L/s) from pre-exposure baseline values were as follows: -1.15 ± 0.52 for large particle, hypoosmolar H_2SO_4; -1.22 ± 0.42 for large particle, hypoosmolar saline; -1.93 ± 0.50 for small particle H_2SO_4; and -1.42 ± 0.41 for small particle saline (Fig. 1). There were no significant differences in mean change in SRaw among the 5 aerosols. There were no significant differences in pre-exposure baseline SRaw among the 5 aerosols.

Only 1 subject experienced as much as "moderate" (symptom score 6 on a 0-10 scale) throat irritation after inhaling the large, hypoosmolar H_2SO_4 aerosol. One other subject experienced "moderate" (symptom scores 4-5) chest tightness, wheezing and shortness of breath after inhaling the large, hypoosmolar aerosol. The mean scores for throat

irritation and respiratory symptoms (chest pain, chest tightness, wheezing, cough, sputum production, shortness of breath) were not significantly different among the 5 aerosols (Table 2). Subjects rarely coughed during any of the 5 exposures. There were no significant differences in cough frequency among the 5 aerosols.

The particle size (numerical mass diameter (geometric standard deviation = GSD)) of the large particle, hypoosmolar H_2SO_4 aerosols was 2.7 (1.7) microns and of the small particle H_2SO_4 aerosols, 0.25 (1.5) microns. The calculated MMAD's were 5.0 and 0.4 respectively. The sulfate concentrations (mean ± SE) at the mouthpiece of the aerosols were as follows: 2.8 ± 0.2 mg/m^3 for the large particle aerosols and 2.9 ± 0.2 mg/m^3 for the small particle aerosols. There were no significant differences in mean temperatures of the inhaled aerosols among the 5 exposures.

Discussion

In the present study, inhalation of an aerosol of H_2SO_4 at a concentration more than 30 times higher than that commonly encountered in polluted urban air failed to cause a significant increase in SRaw, cough, or respiratory symptoms in subjects with asthma. The concentration studied was 6 times higher than the concentration previously shown to cause a small but significant decrease in specific airway conductance (the reciprocal of SRaw) during resting exposure using a similar protocol (5). The absence of any measurable effect of H_2SO_4 was consistent across all aerosol exposures, whether they were large (5 micron MMAD) or small (0.4

micron MMAD) particles, and whether the particles were hypoosmolar (30 mOsm) or isoosmolar (300 mOsm). The results of this study suggest that our previous failure to demonstrate significant bronchoconstrictor effects of H_2SO_4 (6,7) was not simply due to administration of this acid in large, isoosmolar particles. In addition, they suggest that our previous finding that acidity potentiates the bronchoconstrictor effect of hypoosmolar aerosols (7) may not be relevant to environmental exposures. Together these findings suggest that clinically significant bronchoconstriction is extremely unlikely to occur as a result of resting exposure to respirable sulfuric acid aerosols. However, exposure to such aerosols during moderate exercise in which the effective dose is increased due to increased minute ventilation may prove to be capable of inducing significant bronchoconstriction. Combined or sequential exposures to acid aerosols and oxidant pollutants may interact positively to cause clinically significant toxicity. Such toxicity may be manifested by effects other than bronchoconstriction.

Previous controlled exposure studies of the effects of H_2SO_4 involving subjects with asthma have generated conflicting data. Utell and coworkers exposed 17 subjects with asthma to 3 concentrations of H_2SO_4 (100, 450, and 1000 $\mu g/m^3$) through a mouthpiece during 16 min of tidal breathing at rest (5). While these investigators found significant decreases in the mean changes in SGaw after 450 and 1000 $\mu g/m^3$ H_2SO_4 as compared to those after control NaCl aerosols at the same concentrations, these decreases were small (19% and 21% respectively), and there was no obvious concentration-response relationship over this concentration range.

The only controlled human exposure study of H_2SO_4 to observe a significant effect at a concentration as low as 100μg/m^3 was that of Koenig and coworkers (4). These investigators exposed 10 adolescent subjects who were characterized by sensitivity to one or more aeroallergens (confirmed by specific inhalation challenge), elevated serum IgE levels, and documented exercise-induced bronchospasm (EIB) to 100 μg/m^3 of either H_2SO_4 or NaCl aerosol through a mouthpiece. Duration of exposure was 30 min at rest followed by 10 min of moderate exercise (minute ventilation ~40 L). There were no significant differences from baseline values in pulmonary function after exposure at rest. Immediately after exposure, the mean FEV1 was significantly reduced from the baseline value. However, this reduction in FEV1 was slight (8%) and transient (it was no longer present at 4-5 min after exercise). The particle size (MMAD 0.4 micron, GSD 1.5) of the H_2SO_4 aerosol studied by Koenig et al. was similar to that studied by us. However, the protocol followed by these investigators was clearly different from that of the current study in that the exposure duration was longer, and that exposure with exercise as well as at rest occurred. The subjects studied by Koenig and coworkers also differed from those studied by us, primarily in terms of age and their selection for the presence of EIB.

In an experiment designed to examine the effects of H_2SO_4 aerosol on mucociliary clearance, Spektor and coworkers also reported effects on pulmonary function (9). Ten adults with asthma were exposed to 110, 319, and 971 μg/m^3 of H_2SO_4 (MMAD 0.5 micron) for 1 h via nasal mask. The 971 μg/m^3 exposure caused a slight but significant change in SGaw (10% decrease) in 6 out of the 10 subjects. There were no effects on pulmonary function at the 2 lower H_2SO_4 concentrations.

Not all controlled human exposure studies of the effects of H_2SO_4 aerosols have demonstrated significant changes in pulmonary function. Sackner and coworkers reported no alterations in FEV1, FVC, or total respiratory resistance in 5 adults with asthma exposed at rest to H_2SO_4 aerosols (MMAD 0.1 micron) at 10, 100, and 1000 $\mu g/m^3$ for 10 min through a mouthpiece system (10). More recently, Linn and coworkers exposed 27 adults with asthma to H_2SO_4 aerosols (MMAD 0.6 micron) at 122, 242, and 410 $\mu g/m^3$ in an exposure chamber for 1 hr during which the subjects exercised (mean minute ventilation 42 L) and rested during alternate 10 min periods (11). Physiologic and symptomatic changes attributable to H_2SO_4 exposure were small and not statistically significant. The effect of exercise on pulmonary function was much greater than that of H_2SO_4.

On the basis of the small but significant decrements in lung function demonstrated by Utell and coworkers, and by Koenig and coworkers, it has been suggested that patients with asthma are a segment of the population that is especially sensitive to sulfuric acid aerosols, and that bronchoconstriction in these patients due to exposure to sulfuric acid in polluted air is a significant public health problem. However, the failure to demonstrate a clear-cut and consistent dose-response relationship over the range of concentrations previously studied (100-1000 $\mu g/m^3$) together with our failure to demonstrate any meaningful bronchoconstrictor response to concentrations 30 (in the present study) to 400 (in a previous study) times those encountered in polluted air suggests that more dose-response data, including time as well as concentration, are needed to assess the likelihood of adverse respiratory effects

occurring in patients with asthma exposed to ambient levels of acid pollutants.

The finding that patients with asthma are not particularly sensitive to any bronchoconstrictor effect of acid aerosols is surprising and flies in the face of conventional wisdom. Indeed, at the outset of these studies we and others fully expected that acid aerosols would be quite potent as stimuli to bronchoconstriction. However, a probable explanation for the lack of bronchoconstrictor effect is the considerable buffering capacity of the airway lining fluids. Thus, in a study conducted by Jones and coworkers, even instillation of a large volume of hydrochloric acid into the airways of dogs caused only a transient decrease in pH (12). We speculate that this buffering capacity of the airways has evolved as a protective mechanism against the well-recognized phenomenon of recurrent gastric aspiration that occurs in most normal humans.

It is important to point out some caveats to the essentially negative findings we report. Since we studied resting subjects during a relatively brief exposure to H_2SO_4, we cannot exclude the possibility that a more significant bronchoconstrictor effect might result from exposure during exercise and/or more prolonged exposure. Furthermore, our results in no way rule out important adverse effects other than bronchoconstriction.

While the bronchoconstrictor potency of H_2SO_4 appears weak from the available controlled human exposure data, there are several epidemiological studies which provide evidence linking acute exposure to acid aerosols to pulmonary function decrements and respiratory symptoms (13-15). Unfortunately, it has not been possible in these studies to distinguish the effects of acid aerosols from those of co-pollutants such as ozone or sulfur dioxide. Other factors such as high temperature and

humidity may also play a role in the acute respiratory morbidity associated with summer acid haze episodes. It has been hypothesized that there may be a positive interaction between acid aerosols and gaseous pollutants such as ozone with regard to the induction of respiratory tract toxicity (16). This hypothesis deserves further investigation before any conclusion about the potential for adverse health effects due to ambient H_2SO_4 is reached.

Another unresolved issue is whether the HNO_3 present in appreciable concentrations in California acid fog should be of special concern due to its relatively high vapor pressure. Vapor phase HNO_3 may allow more distal deposition than would occur with aerosol alone. Unfortunately, the fact that HNO_3 is more volatile than H_2SO_4 also makes it harder to study. Nonetheless, it will be necessary in the future to expose subjects to aerosols containing HNO_3.

Project II:

Effect of Temperature on Hypoosmolar Sulfuric Acid Aerosol-Induced Bronchoconstriction

Introduction

Fog often occurs at relatively low ambient temperatures. The bronchoconstrictor effects of low temperature in subjects with asthma are well-described (17-18). Thus, fog conditions provide the opportunity for acidity and low ambient temperatures to interact positively with regard to the induction of bronchoconstriction. Furthermore, in a previous study, we found that acidity can potentiate the effect of another bronchoconstrictor stimulus present during fog conditions, hypoosmolarity (7). Seven of 12 subjects in that study demonstrated a shift to the left in the output-response curve generated during inhalation of doubling outputs of hypoosmolar acid aerosols at pH 2 as compared to the curve generated during inhalation of doubling outputs of hypoosmolar saline at pH 5.5. We hypothesized that cooling the inhaled hypoosmolar acid aerosol in such an experimental system might cause a further shift to the left in the output-response curve in subjects with asthma.

Methods

The subjects were 22 non-smoking volunteers who were informed of the risks of the experimental protocol and who signed consent forms approved by the Committee on Human Research of the University of California, San Francisco. All subjects had asthma as defined by a history of recurrent episodes of wheezing, chest tightness, and reversible airway obstruction previously documented by a physician. No subject took theophylline or B-adrenergic agonists within 24 hours or consumed

caffeine within 4 hours before any experiment. No subject took oral corticosteroids during the study period. All subjects denied having an upper respiratory tract infection within 6 weeks prior to the study. Subject characteristics are listed in Table 3. Predicted values are those of Knudson and coworkers (8).

On the initial study day, baseline spirometry (No. 822, Ohio Medical Products, Madison, WI) was performed and a screening dose-response curve to inhaled hypoosmolar (30 mOsm) saline aerosol (pH 5.5, the pH of H_2O in equilibrium with atmospheric CO_2) was generated. Only subjects who developed bronchoconstriction after inhaling this aerosol were continued in the study. One of 22 subjects initially screened was excluded on this basis. On 4 subsequent, randomly separated days, a dose-response curve was generated for inhalation of one of the following hypoosmolar (30 mOsm) aerosols: H_2SO_4 (pH 2) at 7° C; H_2SO_4 (pH 2) at 22° C; saline (pH 5.5) at 7° C; and saline (pH 5.5) at 22° C. The aerosol challenges were randomly ordered, occurred at the same time on each day, and were performed in a single-blind fashion. Subjects were not exposed to aerosol on days when their baseline SRaw was < 50% or > 150% of their usual baseline value. To assess each subject's airway response to these challenges, his or her airway resistance (Raw) and thoracic gas volume (Vtg) were measured in a constant volume body plethysmograph (No. 09103, Warren E. Collins, Braintree, MA) and expressed as the product of Raw and Vtg, SRaw. Coughs were counted throughout the experiment by an observer and recorded on a small portable tape recorder. Throat, lower respiratory, and nonrespiratory symptoms were assessed by a post-exposure questionnaire with an 11-point rating scale for each of 9 symptoms.

Aerosol was generated by an ultrasonic nebulizer (Mistogen EN 145, Time Meter Co., Lancaster, PA). The oscillation amplitude was varied to yield 5 approximately doubling gravimetrically calibrated output settings (Table 4). Aerosol was generated with air that was brought to the desired temperature by passage through a heat exchanger (Thermomix 1480/Frigomix 1496, B. Braun, West Germany) consisting of an 80 cm stainless steel tube cooled on its external surface by circulating chilled ethylene glycol and then humidified by passage through a cascade humidifier. The subjects inhaled aerosol through a mouthpiece connected to a plastic t-piece (with a 2L outflow reservioir) that was directly attached to the outflow port of the nebulizer via a 30 cm X 2 cm insulated polyvinyl chloride tube without a respiratory valve. The subjects wore noseclips while they inhaled aerosol. The temperature of the inhaled aerosol was measured continuously at the mouthpiece and recorded after each minute of exposure.

To confirm that doubling the nebulizer output resulted in a doubling of the available aerosol at the mouthpiece, we measured the ambient aerosol concentration at the mouthpiece gravimetrically by drawing the airstream across glass fiber filters (Gelman Sciences, Ann Arbor, MI) with an effective retention of 0.3 microns. The filter holder was capped and the entire apparatus was weighed before and immediately after each sample was obtained. Because the ambient aerosols were fully saturated with water and the sampling system and airstream were at room temperature, these measurements of filter weight change, divided by the known air sample volume, yielded the effective aerosol concentration. Because the cold aerosols were not at room temperature, they were not measured by this gravimetric method. However, we also measured the

sulfate concentration of the filter samples for both ambient and cold H_2SO_4 aerosols and determined the concentration of H_2SO_4 in the airstream, using a modified barium perchlorate-Thorin colorimetric assay. From the sulfate concentration of the aerosol, effective aerosol concentration of the cold H_2SO_4 aerosol was calculated.

Responsiveness to the screening hypoosmolar saline aerosol was tested by measuring the SRaw of each subject every 30 seconds for 2 minutes before and then 2 minutes beginning 1 minute after he or she inhaled doubling outputs of aerosol. Each aerosol concentration was inhaled during tidal breathing for 3 minutes. The mean value of 5 consecutive measurements of SRaw was calculated during each 2-minute measurement period. Each challenge was continued until SRaw increased by 100% or 10 L X cm H_2O/L/s, whichever was greater. We chose this level of increase in SRaw as the endpoint based on our experience with many inhalation challenge tests. Such an increase usually is associated with respiratory symptoms. Subjects who did not develop such an increase in SRaw by the fifth and final dose (aerosol concentration ~93 g/m^3) were excluded from further study.

In an identical fashion, concentration-response curves were generated for the 4 randomly ordered aerosols. Each solution was prepared and its pH was measured (pH Meter No. 43, Beckman, Irvine, CA) immediately before nebulization. The osmolarity of each solution was measured with a vapor-pressure osmometer (No. 5700B, Wescor, Logan, UT). The particle size of the aerosols delivered at the mouthpiece were measured with a low-flow. 7-stage cascade impactor (In-Tox Products, Albuquerque, NM).

environment. Thus, in the present study we employed the same exposure duration as that reported by Utell and co-workers (16 min), but we extended the dose-response curve by examining a concentration of H_2SO_4 approximately 3 times higher (2.9 mg/m^3) than the highest concentration they studied.To determine whether differences in particle size or the presence of isoosmolar saline had prevented us from observing a clinically important bronchoconstrictor response, we compared the effects of large (~5 micron MMAD) and small (~0.4 micron MMAD) as well as isoosmolar (~300 mOsm) and hypoosmolar (~30 mOsm) particles.

Methods

The subjects were 11 non-smoking volunteers who were informed of the risks of the experimental protocol and who signed consent forms approved by the Committee on Human Research of the University of California, San Francisco. All subjects had asthma as defined by a history of recurrent episodes of wheezing, chest tightness, and reversible airway obstruction previously documented by a physician. No subject took theophylline or B-adrenergic agonists within 24 hours or consumed caffeine within 4 hours before any experiment. No subject took oral corticosteroids during the study period. All subjects denied having an upper respiratory tract infection within 6 weeks prior to the study. Subject characteristics are listed in Table 1. Predicted values for the spirometer parameters described are those of Knudson and coworkers (8).

On the initial study day, baseline spirometry (No. 822, Ohio Medical Products, Madison, WI) was performed and a screening dose-response curve to inhaled methacholine (0.063, 0.125, 0.25, 0.5, 1.0, and 2.0 mg/ml)

To analyze the bronchoconstrictor effects of each aerosol for each subject, we plotted SRaw against the provocative aerosol concentration (in g/m^3). Because the experiment was conducted with roughly doubling increases in aerosol concentration, the data were plotted using a log base 2 abscissa. For each aerosol concentration-response curve, the aerosol concentration required to increase SRaw by 100% above baseline was calculated by log-linear interpolation, and this value was called the provocative aerosol concentration (PC_{100}). To determine whether there were significant differences among the subjects' airway responses to inhalation of the 4 hypoosmolar aerosols, we compared PC_{100}s using a 2-way analysis of variance. In 3 subjects, SRaw did not increase by 100% during the randomized exposure to ambient hypoosmolar saline. In another subject, SRaw did not increase by 100% during the exposure to either cold hypoosmolar saline or ambient hypoosmolar H_2SO_4. Four subjects did not complete the study protocol for personal reasons. The results from these 8 subjects were excluded from the data analysis. The mean values of baseline SRaw before administration of each aerosol were compared using a 2-way analysis of variance. To analyze the symptoms experienced after each aerosol by each subject, we grouped the 9 symptom scores into three categories: a) lower respiratory symptoms; b) throat irritation; and c) nonrespiratory symptoms (back pain and headache). To determine whether there were significant differences among the subjects' reported symptoms following inhalation of the 4 aerosols, we compared the symptom category scores, by means of a 2-way analysis of variance. To determine whether there were significant differences among the subjects' cough responses to inhalation of the 4 aerosols, we compared cough frequencies, again using a 2-way analysis of variance. Finally, the mean

temperatures of the inhaled aerosols during each 3-minute exposure period for each of the 4 aerosols were compared by a 1-way analysis of variance. A p value of ≤ 0.05 was considered statistically significant.

TABLE 1

SUBJECT CHARACTERISTICS (Project 1)

Subject	Sex	Age (yrs)	Ht (cm)	FEV_1 (L)	FEV_1 (% pred)	FVC (L)	FVC (% pred)	Baseline SRaw * $\left(L \times \frac{cmH_2O}{L/S}\right)$	Methacholine Responsiveness † (mg/ml)	Medications
1	M	27	178	3.84	105	5.52	122	9.2	0.15	B-agonist inhaler beclomethasone
2	F	22	155	3.38	116	4.21	121	5.2	0.15	B-agonist inhaler theophylline
3	M	33	183	4.25	96	5.55	101	5.9	0.21	B-agonist inhaler theophylline
4	F	32	158	2.51	90	3.58	107	4.2	0.09	B-agonist inhaler
5	M	32	188	2.21	47	5.09	87	13.1	0.17	B-agonist inhaler beclomethasone
6	M	34	180	2.05	57	4.39	98	10.1	0.70	B-agonist inhaler
7	M	34	178	4.04	97	5.33	104	3.7	0.26	B-agonist inhaler
8	M	28	183	2.61	57	5.55	99	19.4	0.58	B-agonist inhaler beclomethasone
9	M	24	183	2.49	53	4.40	80	12.3	0.16	B-agonist inhaler theophylline beclomethasone
10	F	23	163	1.86	70	2.58	81	3.3	0.07	B-agonist inhaler
11	F	28	158	2.26	78	3.29	95	5.4	0.16	B-agonist inhaler beclomethasone

* mean of pre-exposure baseline values from 5 separate days

† concentration of methacholine required to produce a 100% increase in SRaw above baseline calculated by linear log interpolation.

TABLE 2

MEAN SYMPTOM SCORES, PROJECT I

		Isoosmolar H_2SO_4 Large	Hypoosmolar H_2SO_4 Large	Hypoosmolar NaCl Large	H_2SO_4 Small	NaCl Small
Symptom Category	Throat	0.9 (0.4)	0.7 (0.3)	0.9 (0.5)	0.7 (0.5)	0.7 (0.3)
	Respiratory	1.8 (0.7)	3.0 (1.5)	1.6 (0.7)	1.9 (0.7)	1.7 (0.9)
	Non-Respiratory	0.7 (0.6)	0.2 (0.1)	0.4 (0.3)	0.5 (0.3)	0.7 (0.4)

Mean scores (SEM) for 3 symptom categories, throat (maximum score 10), respiratory (maximum score 60), and non-respiratory (maximum score 20), after inhalation of 5 aerosols.

TABLE 3

SUBJECT CHARACTERISTICS (Project 2)

Subject	Sex	Age (yrs)	Ht (cm)	FEV_1 (L)	FEV_1 (% pred)	FVC (L)	FVC (% pred)	Baseline SRaw * $\left(L \times \frac{cmH_2O}{L/S}\right)$	Methacholine Responsiveness † (mg/ml)	Medications
1	M	27	178	3.84	105	5.52	122	7.2	0.15	B-agonist inhaler beclomethasone
2	F	22	155	3.38	116	4.21	121	5.3	0.15	B-agonist inhaler theophylline
3	M	33	183	4.25	96	5.55	101	3.9	0.21	B-agonist inhaler
4	F	32	158	2.51	90	3.58	107	6.3	0.09	B-agonist inhaler
5	M	35	183	4.65	107	6.72	124	4.9	0.76	B-agonist inhaler beclomethasone
6	F	30	173	2.51	77	4.12	104	10.9	0.24	B-agonist inhaler
7	M	34	178	4.04	97	5.33	104	3.9	0.26	B-agonist inhaler
8	M	32	185	4.65	102	5.75	101	4.0	0.55	B-agonist inhaler
9	M	24	183	2.49	53	4.40	80	8.5	0.16	B-agonist inhaler beclomethasone
10	F	23	163	1.86	70	2.58	81	4.0	0.07	B-agonist inhaler
11	F	28	165	1.84	60	2.87	77	12.4	<0.32	B-agonist inhaler theophylline
12	M	22	175	3.58	99	4.87	115	5.3	nd	B-agonist inhaler
13	M	31	173	2.80	71	3.99	81	4.9	0.10	B-agonist inhaler

* mean of pre-exposure baseline values from 4 separate days

† concentration of methacholine required to produce a 100% increase in SRaw above baseline calculated by linear log interpolation.

TABLE 4

EXPOSURE CHARACTERISTICS (Project 2)

Nebulizer Setting	Gravimetric Aerosol Concentration (g/m^3)		H_2SO_4 Concentration (mg/m^3)		Temperatures (°C)	
	Ambient	Cold	Ambient	Cold	Ambient	Cold
1	6.07	4.08	2.97	2.0	21.5	6.2
2	12.45	13.62	6.10	6.67	21.6	6.9
3	24.35	22.63	11.93	11.09	21.7	7.0
4	45.96	46.88	22.52	22.97	21.9	7.9
5 *	93.40	91.93	45.77	45.05	22.7	9.5

*Only 2 subjects required inhalation of aerosol at this setting in order to increase their specific airway resistance (SRaw) by 100% from baseline values during exposure to one of the 4 hypoosmolar aerosols.

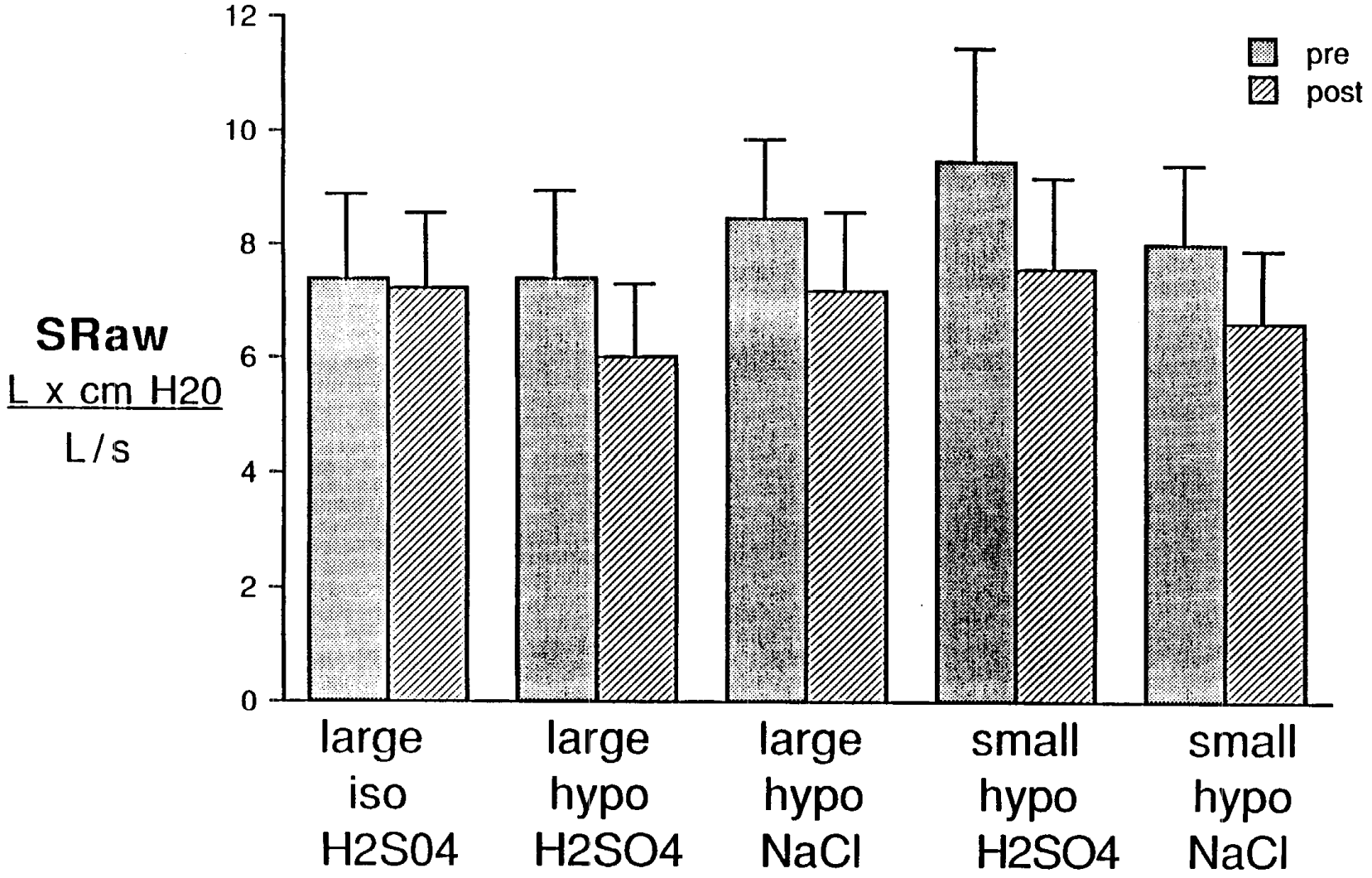
TABLE 5

MEAN SYMPTOM SCORES AND COUGH FREQUENCIES, PROJECT II

		Cold NaCl	Cold H2SO4	Ambient NaC1	Ambient H2SO4
Symptom Category	Throat	1.8 (0.6)	2.7 (0.8)	1.9 (0.7)	2.9 (0.8)
	Respiratory	19.7 (2.8)	21.5 (2.5)	19.2 (0.7)	16.7 (2.9)
	Non-Respiratory	1.9 (1.4)	6.5 (2.0)	6.0 (2.1)	4.4 (2.1)
Cough Frequency		5.0 (1.4)	6.5 (2.0)	6.0 (2.1)	4.4 (2.1)

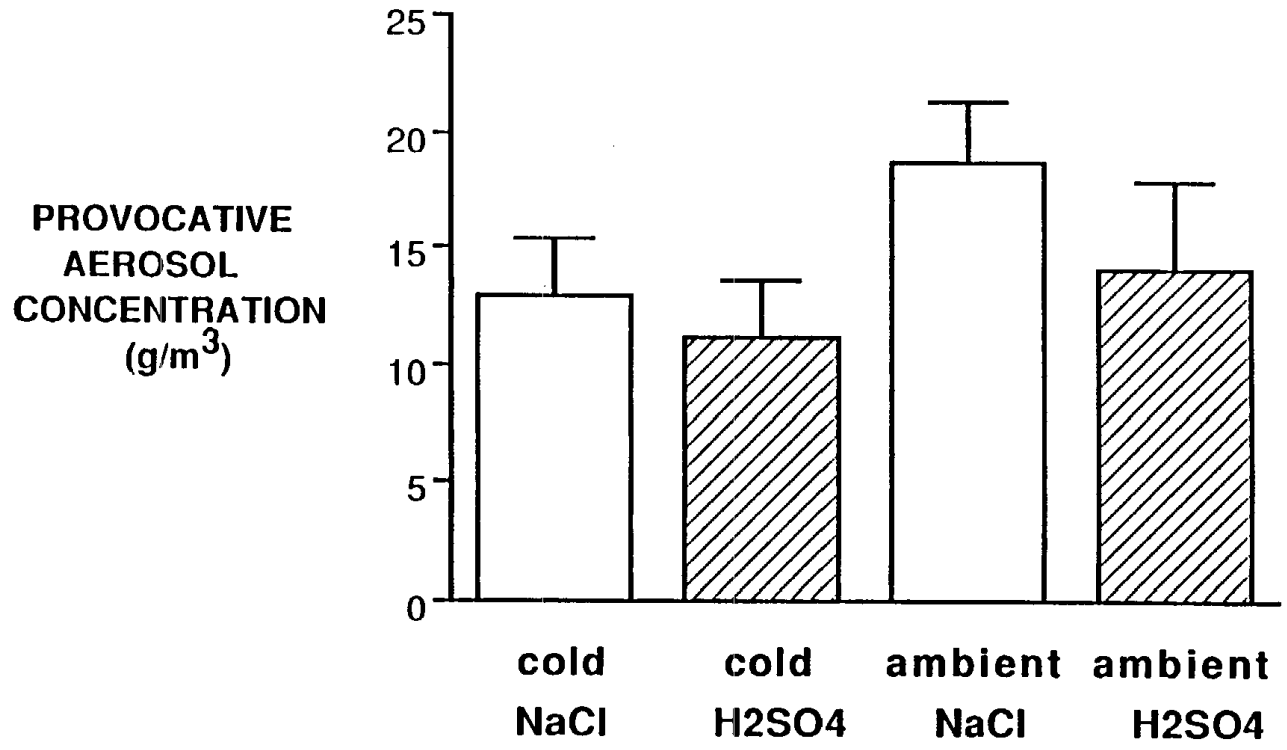
Mean scores (SEM) for 3 symptom categories, throat (maximum score 10), respiratory (maximum score 60), and non-respiratory (maximum score 20), and mean cough frequencies (SEM) after inhalation of 4 hypoosmolar aerosols.

FIGURE 1



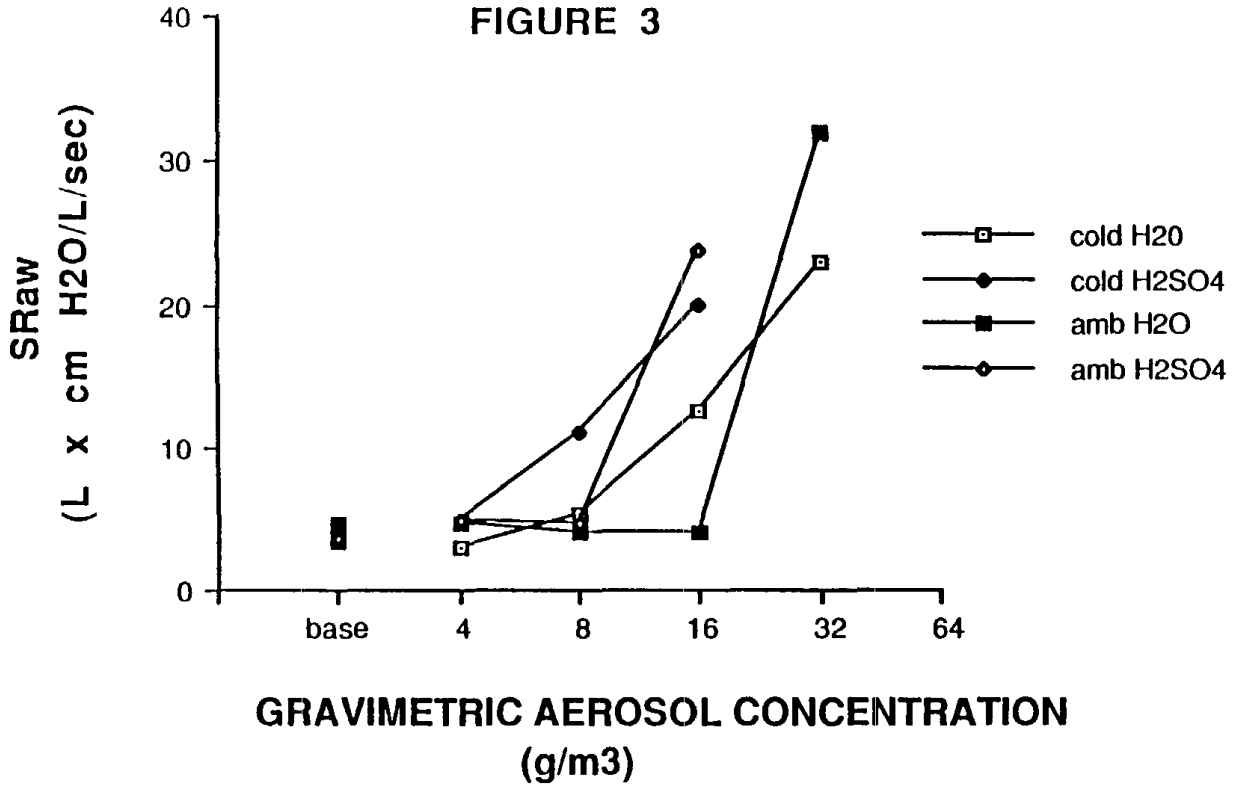
Mean specific airway resistance (SRaw) data for 11 subjects pre- and post-exposure to 5 different aerosols.

FIGURE 2



Mean aerosol concentration required to produce a 100% increase in specific airway resistance (SRaw) from baseline values (provocative aerosol concentration, PC100) during inhalation of 4 hypoosmolar aerosols by 13 subjects.

FIGURE 3



Concentration-response curve for each of 4 hypoosmolar aerosols inhaled by one subject (#10), Project 2.

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Balmes JR, Fine JM, Christian D, Sheppard D. Effects of particle size on sulfuric acid aerosol-induced bronchoconstriction. Am Rev Respir Dis 1988; 136: A167. (Manuscript in preparation).

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APPENDIX

Bronchoconstrictor stimuli in acid fog. Environmental Health Perspective. J.R. Balmes, J.M. Fine, D. Sheppard. In Press. (Attached).

EXECUTIVE SUMMARY

Acid fog is a relatively frequent phenomenon in California but the health effects of exposure to such fog are unknown. Because subjects with asthma may be especially sensitive to the inhalation of air pollutants, we examined several mechanisms by which acid aerosols may cause or contribute to airway narrowing in such subjects. By having subjects with asthma inhale various aerosols, we were able to study the relative importance of acidity, particle size, and ionic strength with regard to the ability of these aerosols to cause airway narrowing. Sulfuric acid aerosols did not cause airway narrowing in our subjects when inhaled during rest at a concentration of nearly 3 mg/m^3. Neither ionic strength nor particle size appeared to influence this lack of airway narrowing effect. Since we studied resting subjects during a relatively brief (16 min) exposure to sulfuric acid, we cannot exclude the possibility that a more significant airway narrowing effect might result from exposure during exercise and/or more prolonged time periods. Furthermore, our results in no way rule out important adverse effects other than airway narrowing.

Because fog often occurs at relatively cool temperature and because the airway narrowing effects of cool temperature are well-described, we also studied whether cool temperature and acidity worked together to enhance the airway narrowing that occurs with the inhalation of aerosols of low ionic strength. While we did find that both cool temperature and acidity tend to cause enhanced airway narrowing when inhaled separately, we found no evidence that these stimuli can cause an even greater effect when inhaled together. Our failure to find such an effect suggests a relatively minor role for cool temperature in any airway narrowing induced by acid fog. Furthermore, it should not be necessary to generate acid fogs at cool temperature in order to study their potential adverse respiratory effects on subjects in our recently constructed exposure chamber.

Potential Bronchoconstrictor Stimuli in Acid Fog

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Running head: Bronchoconstrictor stimuli in acid fog

ABSTRACT

Acid fog is complex and contains multiple stimuli that may be capable of inducing bronchoconstriction. These stimuli include: sulfuric and nitric acids (the principal inorganic acids present); sulfites (formed in the atmosphere as a reaction product of sulfur dioxide and water droplets); fog water itself (a hypoosmolar aerosol); the organic acid hydroxymethanesulfonate (the bisulfite adduct of formaldehyde); and gaseous pollutants (e.g., sulfur dioxide, oxides of nitrogen, ozone). Given this complexity, evaluation of the respiratory health effects of naturally occurring acid fog requires assessment of the bronchoconstrictor potency of each component stimulus and possible interactions among these stimuli. We summarize the results of three studies that involve characterization of the bronchoconstrictor potency of acid fog stimuli and/or their interaction in subjects with asthma. The results of the first study indicate that titratable acidity appears to be a more important stimulus to bronchoconstriction than is pH. The results of the second study demonstrate that sulfite species are capable of inducing bronchoconstriction, especially when inhaled at acid pH. The results of the third study suggest that acidity can potentiate hypoosmolar fog-induced bronchoconstriction.

key words: acid fog, asthma, sulfuric acid, sulfite, nitric acid, hypoosmolar aerosol

Complex mixtures of atmospheric pollutants occur commonly throughout the United States, particularly in areas where acidic pollutants mix with ambient fog. Naturally occurring fog has recently been shown to be quite acidic, with pH values as low as 1.7 (1). The major ions present in acid fog are hydrogen, sulfate, nitrate, ammonium and chloride (1, 2), suggesting that the low pH is in large part due to the presence of sulfuric (H_2SO_4) and nitric (HNO_3) acids. The buffering capacity of naturally occurring acid fogs have not been adequately measured. However, the presence of weak organic acids may allow an increase in the total titratable acidity of such fogs at any given pH. The potential adverse health effects of breathing acid fog have not been adequately characterized.

Acid fog contains multiple stimuli that may be capable of inducing bronchoconstriction. These stimuli include: sulfuric and nitric acids (the principal inorganic acids present); sulfites (formed in the atmosphere as a reaction product of sulfur dioxide (SO_2) and water droplets); fog water itself (a hypoosmolar aerosol); the organic acid hydroxymethanesulfonate (the bisulfite adduct of formaldehyde); the airway cooling capacity of fog droplets that are cooler than body temperature; and gaseous pollutants (e.g., SO_2, oxides of nitrogen, ozone). Given this complexity, evaluation of the potential bronchoconstrictor effects of naturally occurring acid fog first requires assessment of the mechanisms of action of each component stimulus and then requires examination of possible interactions among these stimuli. Mechanisms of action of component stimuli and initial characterization of interactions between stimuli are easily studied using artificial conditions (i.e., mouthpiece exposures) that allow one to examine several doses of the stimulus of interest and to more tightly control stimuli that are not of interest. We report here, in summary fashion, the results of three studies that involve characterization of the bronchoconstrictor potency of acid fog stimuli and/or their interaction.

The Role of Titratable Acidity in Acid Fog-induced Bronchoconstriction

The first study directly examined the significance of acidity itself as a bronchoconstrictor stimulus (3). We hypothesized that buffered acid fogs(with a greater available pool of hydrogen ions) would cause more bronchoconstriction than unbuffered acid fogs at the same pH. Since the airway lining fluid has a considerable capacity to buffer acid, we reasoned that inhalation of buffered acid would cause a more

persistent decrease in airway pH. If a change in pH is the primary mechanism by which bronchoconstriction occurs following inhalation of acid fog, then buffered acids should have more potent bronchoconstrictor effects than unbuffered acids.

Fogs of HCl and H_2SO_4 in an unbuffered state and buffered with glycine at pH 2 were administered to 8 non-smoking subjects with mild asthma. The buffered acids were given in order of increasing titratable acidity (defined as the number of ml of 1N NaOH required to neutralize 100 ml of acid solution to pH 7). Each set of buffered or unbuffered acid fogs was given on a separate day and each fog was inhaled through a mouthpiece during 3 min of tidal breathing. A dense fog (liquid water content approximately 90 g/m^3) was generated by an ultrasonic nebulizer that produced particles in the large respirable size range (MMAD 5.3 - 6.2 microns). Bronchoconstriction was assessed by measurement of specific airway resistance (SRaw) before and after inhalation of each fog.

The subjects' asthma remained stable throughout the study and there were no significant deviations in baseline SRaw. SRaw increased by more than 50% above baseline in only 1 of 8 subjects after inhalation of unbuffered HCl and in no subjects after inhalation of unbuffered H_2SO_4, even at pH 2. In contrast, SRaw increased by greater than 50% in all 8 subjects after inhalation of HCl and glycine at pH 2 and 7 of 8 subjects after inhalation of H_2SO_4 and glycine at pH 2. The mean titratable acidity required to increase SRaw by 50% above baseline was calculated for each challenge by log-linear interpolation; the values for buffered H_2SO_4 (5.1 ml of 1N NaOH) and buffered HCl (2.2 ml of 1N NaOH) were slightly, but significantly different ($p < 0.01$) and were considerable higher than the titratable acidity of the unbuffered acids at pH 2 (1.0 ml of 1N NaOH). The results of this study suggest that the acidity of inhaled large particle fogs can itself be a stimulus to bronchoconstriction. The bronchoconstrictor potency of acid fogs appears to be related to their total available hydrogen ion concentration (titratable acidity) and not merely to their free hydrogen ion concentration (pH) since, at a constant pH (pH 2), increasing amounts of titratable acidity caused increasing severity of bronchoconstriction for the two chemically distinct acid fogs studied. The greater potency of HCl compared to H_2SO_4 per unit of titratable acidity that was observed with the glycine-buffered solutions may have been due to the higher vapor pressure of HCl which could have allowed greater distal deposition.

Because the conditions of exposure of this study (isoosmolar particles of relatively uniform diameter (MMAD 5-6 microns) delivered at a high concentration through a mouthpiece) were quite different from those encountered in the environment, it is not possible to extrapolate directly from our results to predict the effects of environmental exposure to acid fogs. Nonetheless, we were impressed by how weak a bronchoconstrictor stimulus unbuffered acid fogs were under the conditions we studied. Since titratable acidity appears to be a more important stimulus to bronchoconstriction than is pH, atmospheric monitoring during episodes of natural (or experimental) acid fog should include measurement of coexistent buffers and/or titratable acidity in addition to measurement of pH.

The Roles of pH and Ionic Species in SO_2 and Sulfite-induced Bronchoconstriction

SO_2 and sulfites are well-described inducers of bronchoconstriction in individuals with asthma which are chemically related and, therefore, may share a common mechanism of action. When dissolved in aqueous solution, such as in the airway lining fluid, these sulfur oxide species enter into equilibrium with one another. SO_2 and metabisulfite convert to bisulfite (pKa 1.86 and 0.09, respectively) and bisulfite, in turn, enters into equilibrium with sulfite ion (pKa 7.2). These reactions are accompanied by the release of hydrogen ions. We hypothesized that inhaled SO_2 induces bronchoconstriction through one of 3 possible mechanisms: 1) the formation of sulfites by the dissolving of SO_2 in water; 2) the entry of SO_2 itself into biochemical reactions; or 3) the liberation of hydrogen ion by the dissolving of SO_2 in water. Additionally, it is possible that one of the sulfites might be more active than the others in causing bronchoconstriction.

To test these possibilities, we challenged 10 non-smoking subjects with mild asthma with aerosols of sodium sulfite or acetic acid at various pHs and with SO_2 gas. We administered nebulized sodium sulfite (Na_2SO_3) solutions at pH 9 (containing 95% sulfite), at pH 6.6 (containing 80% bisulfite) and at pH 4 (containing 99% bisulfite but greater than an order of magnitude more SO_2 than the pH 6.6 solution). Subjects inhaled increasing concentrations of aerosolized Na_2SO_3 at each pH during 1 min of tidal breathing. Subjects also inhaled buffered acetic acid aerosols with the same acidity as the pH 4 Na_2SO_3 solutions to control for the airway effects of acid aerosols. To assess sensitivity to SO_2 gas, subjects inhaled increasing concentrations

of SO_2 during eucapneic hyperpnea. Bronchoconstriction was assessed by measurement of SRaw before and after each challenge.

Again, the subjects' asthma remained stable throughout the study and there were no significant deviations in baseline SRaw. Nine of the 10 subjects developed bronchoconstriction after inhaling the Na_2SO_3 aerosols at all 3 pHs and the SO_2 gas. The mean concentration of Na_2SO_3 solution calculated to increase SRaw by 100% above baseline was significantly different ($p < 0.01$) at the various pHs: pH 4 (0.17 mg/ml) < pH 6.6 (0.49 mg/ml) < pH 9 (2.10 mg/ml). Only 1 subject responded to the acetic acid aerosol.

The results of this study confirm the reports of other investigators that inhaled sulfite aerosols are a stimulus to bronchoconstriction in subjects with asthma (5-7). This effect is not restricted to individuals with a clinical history of sulfite sensitivity because none of our subjects had such a history. The bronchoconstrictor potency of sodium sulfite aerosols was clearly pH dependent, with the greatest effect occurring at the most acid pH and the least effect at alkaline pH. However, acidity itself did not appear to be the stimulus to bronchoconstriction because most subjects were unaffected by inhalation of acetic acid with a titratable acidity many times greater than that contained in the concentration of sulfite at pH 4 required to produce bronchoconstriction. Rather than exerting a direct effect, decreasing pH most likely increased sodium sulfite-induced bronchoconstriction by altering the relative concentrations of sulfite, bisulfite and SO_2 gas.

Since bronchoconstriction occurred in 9 of 10 subjects after inhalation of concentrations of sodium sulfite at pH 9 not associated with measurable generation of SO_2 gas, it appears that sulfite species are themselves capable of inducing bronchoconstriction. While at pH 9 there may have been some oxidation of sulfite to sulfate, the absolute magnitude of this conversion would have been small since the rate coefficient for this reaction (3×10^{-3} sec $^{-1}$) corresponds to a sulfite lifetime approximately 1000 times the residence time of sulfite aerosol in our system (8). Because the airways are lined with water and SO_2 is rapidly converted to sulfites in an aqueous environment, it is possible that bisulfite ion is the primary species responsible for SO_2-induced bronchoconstriction. In addition, stable inorganic sulfite species have been found in plumes and effluents from power plants and smelters (9-11). While the artificial conditions and high sulfite concentrations

employed do not allow extrapolation to such environmental exposures, the results of this study suggest that sulfite-containing aerosols could be stimuli to bronchoconstriction, especially when inhaled at acid pH.

Acidity Potentiates Bronchoconstriction Induced By Hypoosmolar Fogs

Naturally occurring fogs are usually hypoosmolar with respect to body fluids (including airway lining fluid). Inhalation of hypoosmolar aerosols is well established as a potent stimulus to bronchoconstriction (12-15). Thus, we thought it would be important to characterize the nature of the interaction, if any, between hypoosmolarity and acidity in causing bronchoconstriction in subjects with asthma. Because of the limited bronchoconstrictor effects of unbuffered acid fogs demonstrated in the initial study described above (3), we hypothesized that acidity would be more likely to potentiate the bronchoconstriction induced by hypoosmolarity than to have a significant independent effect.

To test this hypothesis, we studied in 12 non-smoking subjects with mild asthma the bronchoconstrictor effects of fogs that varied with regard to both their osmolarity and acidity. We administered the following fogs: hypoosmolar saline (30 mOsm) at pH 5.5; 3 hypoosmolar acids (H_2SO_4, HNO_3 and a 1:1 mixture of H_2SO_4 and HNO_3, all 30 mOsm) at pH 2; and isoosmolar H_2SO_4 (300 mOsm) at pH 2. Because the airstream was fully saturated with water and the generated fogs were dense, off-gassing of nitric acid vapor was negligible (16, 17). Each fog was administered on a separate day and was inhaled through a mouthpiece during tidal breathing. SRaw was measured before and after the subjects inhaled fog from an ultrasonic nebulizer for 3 min in up to 5 doubling nebulizer outputs.

Again, the subjects' asthma remained stable throughout the study and there were no significant deviations in baseline SRaw. For each fog challenge, an output-response curve was generated and the nebulizer output required to increase SRaw by 100% above baseline (PO_{100}) was calculated. Mean values of PO_{100} were significantly lower for each of the hypoosmolar acids than for hypoosmolar saline (1.65 + 0.43 g/min (mean + SEM) for saline compared to 0.95 + 0.11, 1.05 + 0.20 and 0.90 + 0.14, for H_2SO_4, HNO_3 and a 1:1 mixture of the two acids, all p values < 0.025). Mean values of PO_{100} did not differ among the 3 acids studied. For 7 of 12 subjects, all 3 acids caused a clearcut leftward shift in the output-response curve from the curve

generated for hypoosmolar saline fog. Isoosmolar H_2SO_4 did not increase SRaw by 100% in any subjects, even at the maximal nebulizer output which delivered a concentration of H_2SO_4 in excess of 40 mg/m^3.

The results of this study suggest that acidity can significantly potentiate the bronchoconstriction caused by inhalation of a hypoosmolar fog in subjects with asthma. Since each of the 3 hypoosmolar, acidic solutions studied (H_2SO_4, HNO_3 or a 1:1 mixture of the two acids) had an equivalent bronchoconstriction-potentiating effect, the specific chemical composition of the solution did not appear to be an important factor. As we reported in the initial study described above, large particle aerosols (MMAD 5-6 microns) of unbuffered isotosmolar H_2SO_4 caused little bronchoconstriction.

Again, the conditions of exposure we studied were quite different from those encountered in the environment. The liquid water content of the fogs (ranging from 6 to 87 g/m^3) was many times higher than that which has been measured during even "worst case" natural fog conditions (2 g/m^3) (18). In addition, the H_2SO_4 concentrations we studied were many times higher than those encountered in natural fog. Despite the high concentrations of water and H_2SO_4 used in this study, our results might be relevant to possible adverse health effects of acid fog. The exposures used were brief (3 min) and occurred during resting tidal breathing. It is possible that longer exposures, especially during exercise, might lead to significant bronchoconstriction during exposure to fogs with lower water content and/or acid concentrations. In any case, the results of this study suggest that the interaction of acidity and osmolarity needs to be considered in the design and interpretation of studies of the respiratory health effects of acid fog.

The studies summarized above contribute to the characterization of the bronchoconstrictor potency of several stimuli present in acid fogs in individuals with asthma. Titratable acidity appears to be a more important stimulus to bronchoconstriction than is pH and unbuffered acid fogs have only weak bronchoconstrictor effects. However, unbuffered acidity can potentiate the bronchoconstriction caused by inhalation of a hypoosmolar fog. Finally, sulfite species are themselves capable of inducing bronchoconstriction, especially when inhaled at acid pH and bisulfite ion may be the primary species responsible for SO_2-induced bronchoconstriction.

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