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TITLE OF CONTRACT: THE ROLES OF pH, TITRATABLE ACID AND SPECIFIC CHEMICAL COMPOSITION IN MEDIATING EFFECTS OF ACID AEROSOLS ON THE AIRWAYS

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Abstract

By exposing subjects with asthma to aerosols of buffered and unbuffered solutions of sulfuric and hydrochloric acid with a variety of concentrations of free hydrogen ion (pH) and total available hydrogen ion (titratable acidity - the sum of free hydrogen ion and hydrogen ion bound to buffers), we were able to study the relative importance of pH, titratable acidity and specific chemical composition in describing the bronchoconstrictor potency of acid aerosols. Aerosols of unbuffered acids caused little bronchoconstriction down to pH 2, whereas aerosols of buffered HCl and H_2SO_4 (buffered with glycine) caused titratable acidity-dependent bronchoconstriction in 7 of 8 subjects, suggesting that titratable acidity might be a more important determinant of bronchoconstrictor potency than is pH. We also studied the mechanism(s) by which sulfite aerosols cause bronchoconstriction. By comparing the bronchoconstrictor potency of sulfite aerosols inhaled at 3 different pHs (pH 4, pH 6.6, and pH 9) we examined the relative importance of the two different ionic forms of sulfite (HSO_3^- and $SO_3^=$) and of the sulfur dioxide gas with which these ions are in equilibrium. Our results suggested that HSO_3^- ion and/or SO_2 gas were likely to be important stimuli to bronchoconstriction, but that $SO_3^=$ ion was not. Finally, to determine if assessment of airway vascular permeability or morphologic evidence of airway injury would be more sensitive indicators of the effects of acid aerosols than is bronchconstriction, we examined the effects of exposure to a range of atmospheres containing aerosols of H_2SO_4 and of HCl on these parameters in male Hartley guinea pigs. Although we were able to cause bronchconstriction during exposure to acid aerosols at pH 1, no exposure condition caused increased tracheal vascular permeability or produced morphologic evidence of airway injury on light microscopy of Giemsa stained sections. We conclude that these parameters are not likely to be sensitive indicators of the short term effects of acid aerosol inhalation.

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Acknowledgement

This report was submitted in fulfillment of ARB contract A4-133-32, "The roles of pH, Titratable Acid and Specific Chemical Composition in Mediating Effects of Acid Aerosols in the Airways," under the sponsorship of the California Air Resources Board. Work was completed as of 7/1/86.

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Summary

Acid fog is a common phenomenon in California, but the health effects of exposure to acidic fog are unknown. Before studies evaluating such health effects can be intelligently designed and performed, basic information about the mechanisms by which acidic fog could cause adverse effects is required. Because subjects with asthma appear to be especially sensitive to the adverse health effects of acid pollutants, we examined the mechanisms by which simple acid aerosols cause bronchoconstriction in subjects with asthma. By having subjects inhale aerosols of buffered and unbuffered solutions of sulfuric and hydrochloric acid with a variety of concentrations of free hydrogen ion (pH) and total available hydrogen ion (titrable acidity -- the sum of free hydrogen ion and hydrogen ion bound to buffers), we were able to study the relative importance of pH, titrable acidity and specific chemical composition in describing the bronchoconstrictor potency of acid aerosols. Aerosols of unbuffered acids caused little bronchoconstriction down to pH 2, whereas aerosols of buffered HCl and H_2SO_4 (buffered with glycine) caused titrable acidity-dependent bronchoconstriction in 7 of 8 subjects, suggesting that titrable acidity might be a more important determinant of bronchoconstrictor potency than is pH. Buffered HCl was a slightly but significantly more potent bronchoconstrictor than was H_2SO_4, suggesting a role for specific chemical composition, as well.

Of note is the finding that inhalation of these large particle aerosols of unbuffered H_2SO_4 caused only mild bronchoconstriction when delivered at an H_2SO_4 concentration estimated to exceed 10 mg/m^3. This concentration of H_2SO_4 is 100x less than that shown to cause increases in pulmonary resistance when inhaled as a submicronic aerosol. This finding is consistent with the hypothesis that particle size may be an important determinant of the bronchoconstrictor potency of acid aerosols.

We also studied the mechanism(s) by which sulfite aerosols cause bronchoconstricton. By comparing the bronchoconstrictor potency of sulfite aerosols inhaled at 3 different pHs (pH 4, pH 6.6, and pH 9) we examined the relative importance of the two different ionic forms of sulfite (HSO_3^- and $SO_3^=$) and of the sulfur dioxide gas with which these ions are in equilibrium. Sulfite aerosols caused bronchoconstriction at all 3 pHs in 9 of 10 subjects. Sodium sulfite at pH 6.6 was more potent than at pH 9 in every subject. Since the concentration of $SO_3^=$ ions is approximately 10x higher at pH 9, these findings suggest that $SO_3^=$ ion is not an important stimulus to bronchoconstriction. Acetic acid aerosol with titratable acidity up to 200x that of the sulfite aerosol required to cause bronchoconstriction at pH 4 did not cause bronchoconstriction, suggesting that the effects of acidic sulfite solutions observed were not explained by acidity itself. Our results did not allow us to distinguish with certainty between the bronchoconstrictor potency of HSO_3^- ion and gaseous SO_2.

Finally, to determine if assessment of airway vascular permeability or morphologic evidence of airway injury would be more sensitive indicators of the effects of acid aerosols than is bronchoconstriction, we examined the effects of exposure to a range of atmospheres containing aerosols of H_2SO_4 and of HCl on bronchoconstriction during exposure to acid aerosols at pH 1. No exposure condition caused increased tracheal vascular permeability or produced morphologic evidence of airway injury on light microscopy of Giemsa stained sections. We conclude that these parameters are not likely to be sensitive indicators of the

short term effects of acid aerosol inhalation.

Conclusions

The projects completed in this contract permit the following conclusions:

1. Titratable acidity is an important determinant of the bronchoconstrictor effects of acid aerosols in subjects with asthma.

2. A large respirable aerosol (particle size approximately 6 microns, MMAD) may be a less potent stimulus to bronchoconstriction than a submicronic aerosol.

3. Sulfite aerosols are a potent stimulus to bronchoconstriction and this effect is potentiated by acid pH.

4. Sulfite aerosols (and sulfur dioxide) are not likely to cause bronchoconstriction by an effect of sulfite ($SO_3^=$) ion.

5. An alteration in airway pH is not likely to explain SO_2 or sulfite-induced bronchoconstriction.

6. Assessment of tracheal vascular permeability and light microscopic evidence of airway epithelial injury are not sensitive indicies of the effects of inhaled large particle acid aerosols on guinea pig airways.

Implications

1. Future characterizations of the composition of acid containing atmospheres, both in the environment and in the laboratory, should include measurement of titratable acidity as well as measurement of pH. If naturally occuring buffers are found to vary significantly among fog atmospheres, regulatory strategies will need to focus on the total acidity (titrable acidity) of acid fog rather than simply on the pH.

2. Our results suggest that large respirable aerosols of isotonic unbuffered acid solutions are not, by themselves, likely to cause significant bronchoconstriction in subjects with asthma, even in concentrations 100x higher than those found in naturally occuring atmospheres. However, several other factors that could contribute to adverse health effects of acid fog were not examined in our experiments. These include: a) The fact that natural fog is actually hypotonic (with only low concentrations of ions) and hypotonicity is itself a stimulus to bronchoconstriction -- fog acidity could potentiate the bronchoconstrictor effects of hypotonicity. b) The possibility that newly forming fogs may contain acid constituents that are concentrated in small particles. Small (e.g. submicronic) acid aerosols may be a more potent stimulus to bronchoconstriction than are the large respirable aerosols we studied. c) Acidic constituents other than those we studied may be more potent stimuli to bronchoconstriction. d) Fog often occurs at relatively low ambient temperatures. Acidity may potentiate the bronchoconstriction effects of airway cooling. These possibilities should be the focus of future studies of effects of acid fog on subjects with asthma.

3. Since sulfite containing aerosols are themselves a potent stimulus to bronchoconstriction, especially in the presence of low pH, sulfite needs to be evaluated as a possible co-factor in causing bronchoconstriction in the presence of acid fog.

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

Complex mixtures of acid pollutants occur commonly in California, especially along the coast where acidic pollutants mix with ambient fog (1). Present scientific evidence is insufficient to allow regulatory agencies to accurately predict the potential adverse health effects of these acidic mixtures. Before reasonable studies of the adverse effects of acidic pollutant mixtures can be designed and interpreted, basic information about the mechanisms by which acid aerosols can cause adverse effects needs to be obtained.

The experiments performed as part of this contract were designed to determine the relative importance of aerosol pH, titratable acid load, and specific chemical composition in mediating the adverse effects of acid aerosols on the airways. The best described clinically important adverse health effect of acid aerosols and acid precursors (e.g., sulfur dioxide) is bronchoconstriction in subjects with asthma (2,3,4,5). Therefore, the human studies were designed to determine the relative importance of each of these factors in the bronchoconstriction caused by acid aerosols in general and sulfite aerosols in particular in asthmatic subjects.

In addition, studies of the effects of acid aerosols on airway resistance, morphology and vascular permeability were performed in Male Hartley guinea pigs, to test the hypothesis that acid aerosols might cause airway inflammation and edema in the absence of bronchoconstriction.

PROJECT #1: Effects of aerosol pH and titratable acidity on induced bronchconstriction in subjects with asthma.

Rationale

Bronchoconstriction is one of the best described adverse effects of air pollution. Both sulfur dioxide and acid sulfate (especially sulfuric acid) have been shown to cause clinically significant bronchoconstriction in subjects with asthma (2-5). Since on deposition in the airways, both sulfur dioxide and acid sulfates are likely to at least transiently increase the concentration of hydrogen ions in the airway lumen, it has been suggested that the bronchoconstrictor effects of these pollutants may be mediated by changes in airway pH. This hypothesis is supported by the observation that ammonia produced in the oral cavity can reduce the bronchoconstrictor effects of inhaled sulfuric acid (6) and by the observation that sulfuric acid aerosol appears to be a more potent stimulus to bronchoconstriction in subjects with asthma than are aerosols of less acidic acid sulfates (5). This hypothesis has never been directly tested, however. In most previous studies of the bronchomotor effects of acid pollutants, the pH, and probably more importantly, the titratable acid load have neither been considered in experimental design nor discussed in the interpretation of results. It is thus not possible on the basis of existing data to accurately evaluate the role of alterations in airway pH in causing the bronchomotor effects of inhaled sulfur oxide and sulfate pollutants.

In short, before specific effects of different acidic air pollutants can be effectively studied, a basic question needs to be answered. That is, what are

the roles of the pH and of the titratable acid load of an inhaled aerosol in causing bronchoconstriction as distinct from specific effects of the chemical composition of the aerosol. The answer to this question may have important implications about the likely effects of a wide range of acid pollutants and of the complex and variable mixture of pollutants likely to be present in naturally occuring acid atmospheres.

Several pieces of evidence suggest that alterations in airway pH may be an important cause of bronchoconstriction even in the absence of specific air pollutants. Inhaled citric acid has been shown to cause bronchoconstriction both in people (7) and in Basenji-Greyhound dogs, the best characterized animal model of human asthma (8). Because of the buffering capacity present in airway fluid, unbuffered acids are likely to cause only very transient alterations in the pH at the airway surface, as has been shown when diluted HCl has been instilled directly into the airway lumen of dogs. Buffered acids, with greater titratable acidity, on the other hand may cause more significant alterations in airway surface pH. Thus, titratable acid load may be as important or more important than pH in determining the effects of inhaled acid aerosols on airway function.

METHODS

The subjects were 8 nonsmoking volunteers who were informed of the risks of the experimental protocol and signed consent forms approved by the Committee on Human Research of the University of California. 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. Since subjects in their everyday life often take bronchodilating substances such as asthma medications and caffeine in a variable fashion, we sought to standardize study conditions by having them withhold theophylline or sympathomimetic drugs within 12 hours and caffeine within 4 hours before any experiment. No subject took theophylline or sympathomimetic drugs within 12 hrs or consumed caffeine within 4 hrs before any experiment. All subjects denied having an upper respiratory tract infection within 6 weeks prior to the study.

The subjects came to the laboratory on 6 non-consecutive days during which they inhaled, on separate days, methacholine, unbuffered HCl, HCl and glycine at pH 2.0 and pH 3.5, unbuffered H_2SO_4, and H_2SO_4 and glycine at pH 2.0. To assess their response to inhalation of these aerosolized solutions, the subjects' 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, specific airway resistance (SRaw) (9). At the outset of each study day and after each challenge, five SRaw measurements were recorded, one every 30 seconds for 2 minutes, and the mean value calculated.

On the first study day, subjects filled out a questionnaire about their asthma and performed spirometry. To ensure that subjects possessed a key feature of asthma, namely airway hyperresponsiveness, they were screened with methacholine, a short acting cholinergic bronchoconstricting agent. Methacholine responsiveness was tested by measuring the subjects' SRaw before and after they inhaled 10 tidal breaths of phosphate buffered saline and doubling concentrations of methacholine (0.063, 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 mg/ml) delivered by a nebulizer (No. 646, DeVilbiss) with a dose metering device. The concentration of methacholine which provoked a 100% increase in SRaw from the post saline SRaw value (PC_{100}) was calculated by linear interpolation. In our laboratory, 95% of normal subjects required a concentration of aerosolized methacholine greater than 4 mg/ml to develop a 100% increase in SRaw. A value of less than 4 mg/ml is

bronchoconstriction highly associated with airway hyperresponsiveness.

On subsequent days, seated subjects inhaled during tidal breathing from a mouthpiece nebulized solutions for three minutes each, with SRaw measurements performed during a five minute interval between challenges. Subjects first inhaled neutral pH saline as a control and then successive acid solutions until their SRaw doubled or rose 6 units, whichever was greater, or until they reached the maximal acid concentration or lowest pH tested that day. The aerosols were generated by an ultrasonic nebulizer (EN 145 Mistogen, Time Meter Co., Lancaster, PA) and had a mass median aerodynamic diameter of 6.3 microns and a geometric standard deviation of 1.51 microns previously measured with a seven stage low flow cascade impactor. The liquid water content of the aerosol at the mouthpiece was determined by drawing a measured flow through a two stage silica gel filter, measuring the extracted water content gravimetrically, and correcting for the water content of fully humidified air. The nebulizer apparatus was arranged with the out flow port connected to a polyethylene t-piece, one limb of which was attached to a Hans Rudolph one way valve with a rubber mouth piece and the other limb to a 3 liter teflon tube to prevent dilution of the inspirate with room air.

The subjects were administered two different acids, HCl and H_2SO_4, in order to evaluate the role of specific chemical composition of acid aerosols in inducing airway effects. The pH at which these airway effects, namely cough and bronchoconstriction, might occur was investigated by administering aerosols of each acid unbuffered in normal saline at pH 7, 5, 4, 3, and 2. To determine if the amount of titratable acid (defined as the number of ml of 1 N NaOH required to titrate a 100 ml solution to pH 7.0) in the aerosolized acid solutions, in addition to the pH and specific chemical composition, was an important factor, the acids were also administered in solutions buffered with glycine at pH 2.0.

The buffer employed, glycine, is a zwitterion, existing in a dipolar state except in very acidic and basic solutions. Glycine was chosen because it is a physiologic substance without reported toxicity at the doses administered and performs as a stable buffer at the pH range tested. To determine whether glycine alone accounted for the airway effects of the buffered acid solution while minimizing the variation in reduction state of glycine, subjects were also exposed to HCl-glycine aerosols at pH 3.5. Compared to the most concentrated pH 2.0 HCl-glycine solution, the most concentrated pH 3.5 solution contained more glycine (20.3 vs 14.0 mg/ml) and considerably less titratable acidity (1.7 vs 12.1 ml of 1 N NaOH/100 ml). The titratable acidity of the unbuffered acids at pH 2.0 was 1.0.

The buffered solutions were prepared by making serial three-fold dilutions with normal saline of isotonic solutions of glycine and HCl or H_2SO_4 (all chemicals supplied by Fisher). Small amounts of 1 N HCl or 1 N H_2SO_4 were added to the dilutions to maintain their pH at 2.0 or 3.5. All solutions were isotonic (~300 mosm) as confirmed by measurement with a vapor pressure osmometer (No. 5700B, Wescor, Logan, UT) and were given in order of increasing buffer concentration and, hence, of increasing titratable acidity. The pH of all solutions was measured with a pH meter (No. 43, Beckman, Irvine, CA).

Expiratory airflow during the challenges was measured by having the subjects exhale through a heated pneumotachygraph (No. 3, Fleisch, Lausanne, Switzerland) and recording the generated signal on photosensitive paper (No. 1858 Visicorder, Honeywell, Denver, CO). Minute ventilation was recorded by integrating the signal from the pneumotachygraph with a respiratory integrator (No. FV 156, Validyne, Northridge, CA).

Coughs were counted throughout the experiment by an observer and recorded on a hand held tape recorder. In addition, coughs during inhalation of acid aerosols were correlated with increased expiratory flow measured with the pneumotachygraph. A significant cough reaction was defined as two coughs during any three minute period of aerosol inhalation.

To analyze the bronchoconstricting effects of HCl and H_2SO_4 in terms of titratable acidity, we plotted SRaw against the titratable acidity (log base 3 scale) of each concentration of buffered acid administered. The amount of titratable acidity which provoked a 50% rise in SRaw above the saline control value (PC_{50}) was calculated by log-linear interpolation between 2 points on the curve representing an increase of just less than and just greater than 50% over baseline. One subject (No. 8) experienced a 50% rise in SRaw after the first dose of acid aerosol and his PC_{50} was determined by linear extrapolation from his responses to subsequent doses. We used a Wilcoxon paired T test to evaluate the significance of differences in PC_{50} of the buffered acids at pH 2.0 and in the mean increases in SRaw provoked by the unbuffered acids. To assess the differences in mean ventilatory rates and baseline SRaw values among the acid aerosol challenges, we employed one-way analysis of variance. A p value $< .05$ was considered statistically significant.

RESULTS

Unbuffered acid aerosol inhalation produced mild rises in mean SRaw from the mean saline control values (Fig. 1). The mean ($\pm$ SE) percent increase in SRaw caused by HCl at pH 2 was 27.7 $\pm$ 14.3% and by H_2SO_4 at pH 2 was 14.8 $\pm$ 4.9%. Although one subject (No. 2) did react to HCl, but not H_2SO_4, at pH 2 with a large (120%) increase in SRaw, no significant difference between the bronchoconstricting effects of the two unbuffered acids was detected.

Buffered acids at pH 2 (Fig. 2) produced much larger increases in SRaw than did the unbuffered acids. All subjects increased their SRaw by at least 50% from saline control values after HCl-glycine at pH 2 and 7 out of 8 subjects did so after H_2SO_4-glycine at pH 2. The mean pC_{50} of titratable acidity for HCl-glycine (2.2 ml of 1 N NaOH) was slightly, but significantly, lower than for H_2SO_4-glycine (3.5 ml of 1 N NaOH) ($p = 0.01$). The percent increase from pre-exposure SRaw induced by acid inhalation was significantly different ($p<0.05$) from that induced by saline only for HCl-glycine and H_2SO_4-glycine at pH 2.0. HCl solutions containing similar amounts of glycine buffer caused less bronchoconstriction at pH 3.5 than at pH 2 (Figure 3). In almost all challenges, cough was noted to precede the measured 50% increase in SRaw from saline control values and to occur during inhalation of unbuffered pH 2 aerosols regardless of bronchoconstricting response (Table 1).

The mean ($\pm$ SE) ventilation of the subjects during inhalation of the sets of H_2SO_4-glycine (10 $\pm$ 0.8 L/min), HCl-glycine at pH 2 (11.3 $\pm$ 1.7 L/min), and HCl-glycine at pH 3.5 (10.4 $\pm$ 0.9 L/min) were not significantly different. No significant difference was found among the subjects' baseline SRaw over the course of the study. All subjects demonstrated marked hyperresponsiveness to methacholine, requiring a concentration to achieve a 100% increase in SRaw ranging from 0.01 to 1.5 mg/ml with a mean ($\pm$S.D.) of .31($\pm$.53) mg/ml.

DISCUSSION

This study demonstrates that pH alone does not adequately characterize the bronchoconstrictor stimulus of inhaled acidic aerosols. Titratable acidity

appears to be a more important determinant of bronchoconstriction than is pH. Thus, naturally occurring buffers could have an important influence on any bronchoconstricting effect of acid fog. Furthermore, since HCl-glycine was a slightly but significantly stronger stimulus to bronchoconstriction than was H_2SO_4-glycine at pH 2, the effects of specific chemical composition must also be considered when evaluating the bronchoconstriction provoked by acid aerosols.

The lower bronchoconstricting potency of the unbuffered solutions, even at pH 2, compared to buffered solutions was not surprising given the latter's larger reservoir of hydrogen ions and, therefore, greater resistance to neutralization in the upper and lower airways. Though an independent effect of glycine cannot be excluded, it is unlikely to have alone accounted for the pH 2 buffers' bronchoconstricting effects given the relatively mild effects of equivalent amounts of glycine at pH 3.5.

A greater potency of HCl compared to H_2SO_4 per unit of titratable acidity was observed with the glycine buffered solutions but not with the unbuffered solutions. This phenomenon may, in part, be due to HCl's higher vapor pressure which might have become a significant factor at the increased acid concentration in the buffered solutions. HCl vapor could conceivably deposit more diffusely throughout the tracheobronchial tree than could acid in aerosol particles. Another possiblility is that specific interactions between the acids and glycine may have altered their properties as airway irritants.

Other features of acid aerosol exposure in addition to pH, titratable acidity, and specific chemical composition, may be important determinants of bronchoconstriction. Exercise is known to heighten the airway responsiveness of asthmatics to inhaled air pollutants (10). Koenig and colleagues observed changes in flow rates and resistance in asthmatics exposed to 100 ug/m^3 of sulfuric acid during 10 minutes of moderate exercise (3). The same subjects inhaling the same concentration of sulfuric acid for 30 minutes at rest preceding exercise experienced no significant decline in pulmonary function.

The size of the acid particle may also influence its bronchoconstricting potency. In particular, particles of submicronic diameter often have greater airway effects although the most potent particle size for H_2SO_4 is not known. Utell and coworkers employed a H_2SO_4 particle size of 1.0 um in another study in which mild asthmatics experience a decline in FEV1 and specific airway conductance after 16 minutes of exposure at rest to H_2SO_4 concentrations of 1000 and 450 ug/m^3 (5). H_2SO_4 concentration of 100 ug/m^3 had no significant effect on pulmonary function. In Koenig and associates' study showing bronchoconstriction with 100 ug/m^3 of H_2SO_4 after exercise, a particle size of 0.6 um was employed (4).

Experiments with guinea pigs exposed to sulfate aerosols showed an increase in airflow resistance with decreased particle size over a range from 2.5 microns MMAD to particles in the submicronic size ranges (11). In the present study, subjects inhaled acid particles with a mass median diameter of 6.3 microns. Although this study was not designed to simulate real world exposure conditions, we estimated (based on the gravimetrically measured liquid water content of the aerosol and the known concentration of H_2SO_4 in solution) that subjects inhaled in excess of 10 mg/m^3 H_2SO_4 during exposure to the unbuffered aerosol at pH2 with remarkably little bronchoconstriction. We speculate that the relatively large respirable acid particles we employed in the present sutdy are a weaker stimulus to bronchoconstriction than smaller (and therefore, more concentrated) acid particles. If this is the case, future monitoring of the acidity in fog should

include measurements of the distribution of acidity in particles of different size as well as overall fog acidity.

PROJECT #2: Chemical mechanism of sulfite-induced bronchoconstriction.

Purpose

The purpose of this study was to determine the relative roles of pH, titratable acid, sulfite anions and sulfur dioxide gas in mediating the bronchoconstriction caused by sulfite aerosols.

Rationale

We have shown that sulfur dioxide, a common air pollutant, causes bronchoconstriction in both healthy and asthmatic people when inhaled in concentrations that occasionally exist in workplaces and in ambient air (1,9). Sulfur dioxide is a highly water soluble polar molecule and is rapidly absorbed by the moist mucous membranes of the nose, mouth, and upper airways. When absorbed by water, sulfur dioxide enters the following equilibrium reactions:

$$SO_2 + H_2O \qquad H^+ + HSO_3^- \qquad 2H^+ + SO_3^=$$

The equilibrium constants for these reactions are:

$$K_1 = \frac{[H^+]\ [HSO_3^-]}{[SO2]} = 1.72 \times 10^{-2}$$

$$K_2 = \frac{[H+]\ [SO_3^=]}{[HSO_3^-]} = 6.24 \times 10^{-8}$$

Thus, at the pH of the airways (about 6.6) [12], the sulfur is predominantly in the form of bisulfite (HSO_3^-), sulfite ($SO_3^=$), and a third anion, metabisulfite ($S_2O_5^=$) in equilibrium with the other two.

Recently, others have shown that ingestion and inhalation of metabisulfite (which rapidly converts to sulfite in solution) can also cause bronchoconstriction in subjects with asthma. It has been suggested that a subset of persons with asthma are especially sensitive to ingested metabisulfite (13,14).

We hypothesize that sulfur dioxide induces bronchconstriction through the activity of one of three chemical forms: (1) through the sulfites formed by the dissolving of sulfur dioxide in water, (2) through the hydrogen ion liberated

when sulfur dioxide dissolves in water, or (3) through the activity of sulfur dioxide itself entering biochemical reactions. Additionally, it is possible that one of the sulfites might be more active than the others in causing bronchoconstriction.

The bronchoconstrictor effects of sulfur dioxide may be mediated by the sulfites formed. Sulfites are highly reactive chemically and react with a large number or organic molecules (14). A major reaction is the interaction with disulfite bonds to form S-sulfonates. Sulfites have been shown to bind to the walls of airways during lavage of rat lungs with sulfite-containing solutions (15) demonstrating that they are reactive within the airways. Sulfites have also been shown to increase the response of frog cutaneous pectoral muscle to acetylcholine by sulfonation of cholinergic recepter disulfide bonds (14). If sulfites sensitize cholinergic receptors in the airways in a similar manner, they might induce bronchoconstriction.

Alternatively, as suggested above, both sulfur dioxide and inhaled sulfites may cause bronchoconstriction by causing an alteration in airway pH. In the case of inhaled sulfites this may be a function of the aerosol pH, the titratable acid load or a combination of the two as discussed above.

Finally, sulfur dioxide itself may form complexes with many organic compounds and may directly initiate the chain that leads to bronchoconstriction.

In order to separate out these possibilities, we first compared the effects of inhaled sodium sulfite delivered at 3 different pH's. One, pH 9, was chosen to study the effects of sulfite ion ($SO_3^=$) in the absence of significant amounts of SO_2 gas or bisulfite (HSO_3^-). Because of the pKa of the equilibrium reactions, at pH 9 more than 90% of the sulfur in solution is present as sulfite. The second, pH 6.6, was chosen to study the effects of bisulfite, since at this pH nearly 90% of the sulfur in solution is bisulfite with only 10% as sulfite. Finally, since some SO_2 gas was generated during aerosolization of sulfite solutions at pH 6.6, we also studied the effects of sulfite solutions at pH 4, a pH that increased SO_2 generation more than 10 fold with little change in the relative concentration of bisulfite (99% vs 90%). To test the role of changes in airway pH in sulfite-induced bronchoconstriction we also studied the bronchoconstrictor effects of an acetic acid aerosol matched with sulfite at pH 4 for overall (titratable) acidity.

METHODS

The subjects were 10 non-smoking volunteers, all of whom signed consent forms approved by the committee on Human Experimentation of the University of California, San Francisco. All subjects had asthma as described above, and no subject took theophylline or sympathomimetic drugs within 12h or consumed caffeine within 4h before any experiment. All subjects denied symptoms of an upper respiratory infection within 6 weeks prior to being studied. No subject gave a history of sensitivity to ingestion of sulfite preservatives.

Each subject came to the laboratory on 7 separate days. On the first day we performed a methacholine dose-response curve as described above. On 3 other days we performed dose response curves to inhaled sodium sulfite aerosol at three different pH's (4.0, 6.6 and 9.0) as described below. To determine whether the effects of inhaled acidic sulfite aerosols we observed could be explained by effects of acidity itself, on another day we performed a dose-response curve to inhaled acetic acid aerosol at pH 4. Acetic acid buffered with sodium acetate

was chosen as a control acid because of its lack of known toxicity, aside from that contributed by its acidity, in the doses administered and its stable pH over the titrable acid concentrations tested. To ensure that any affects of inhaled sulfite we observed were not due to "specific sulfite sensitivity" we also performed a standard oral metabisulfite challenge on each subject. Finally, on another day we assessed each subject's sensitivity to inhaled sulfur dioxide gas.

To perform sodium sulfite dose-response curves, we measured specific airway resistance (SRaw) every 30 seconds for 5 min before and 5 min after each subject breathed an aerosol of first 0.9% sodium chloride (adjusted to the same pH as that day's sodium sulfite solutions by addition of either 1 N HCl or 1 N NaOH) for 1 min and then aerosols of sodium sulfite adjusted to the same pH with 1 N HCL in successively increasing concentrations (0.03, 0.1, 0.3, 1.0, 3.0, and 10.0 mg/ml). Solutions were inhaled at 10 min. intervals. The maximal average value of 4 consecutive measurements of SRaw was calculated during each 5 min measurement period and each study was continued until SRaw increased by 100% or 8 L x cmH_2O/L/S (whichever was greater) or the highest concentration of sodium sulfite (10 mg/ml) had been inhaled. The aerosol was generated from an ultrasonic nebulizer (as above) and inhaled during tidal breathing through a mouthpiece attached to a glass t-piece that was directly attached to the nebulizer outflow port without a respiratory valve. The outflow end of the t-piece was attached to a 2.5 liter teflon tube to prevent dilution of inspirate by room air. Each solution was adjusted to isotonicity (300 milliosmoles) by addition of the appropriate concentration of sodium chloride. We performed acetic acid dose-response curves in an identical fashion except that the subject inhaled successively increasing concentrations of acetic acid matched to the titratable acidity of the concentrations of sodium sulfite given at pH 4. Thus, for the highest concentration of acetic acid given the pH of 100 ml could be neutralized to pH 7 by addition of 4.83 ml of 1 N NaOH, exactly equivalent to the volume of NaOH required to neutralize 100 ml of the 10 mg/ml solution of sodium sulfite at pH 4.

To perform oral metabisulfite challenges we obtained the mean of 5 measurements of SRaw before and 30 min after each subject ingested first dextrose placebo and then 5, 15 and 50 mg of potassium metabisulfite enclosed in gelatin capsules. Published reports have shown that persons with specific sulfite sensitivity generally react to between 5 and 50 mg of ingested metabisulfite (13). In order to insure that the subjects we studied were sensitive to sulfur dioxide, we measured SRaw every 30 min for 5 min before and after each subject inhaled humidified filtered air (Dew point 18°C) or increasing concentrations of sulfur dioxide gas (0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 ppm) in humidified filtered air for 1 min. As for sodium sulfite challenges, sulfur dioxide was inhaled at 10 min intervals and values of SRaw were expressed as the highest average of 4 consecutive measurements. Each sulfur dioxide concentration was confirmed for each experiment by a pulsed flourescent SO_2 meter (Thermo-electron Corporation, Model #43).

To estimate the concentration of SO_2 generated during the aerosolization of the sodium sulfite solutions, we drew aerosols for 3 minutes from the mouthpiece throuh a Zefluor filter (No. P5PJ047, Gelman Science Inc., Ann Arbor, MI) to remove droplets and into the SO_2 analyzer. Teflon tubing and a polycarbonate filter holder (Nucleapore Corp., Pleasanton, CA) were employed to minimize SO_2 uptake by the apparatus. For each sodium sulfite solution at pH 4.0, 6.6, and 9.0 which released SO_2 in the concentration range detectable by our SO_2 analyzer (0.01-5.0 ppm), five SO_2 measurements were made and the mean value calculated.

For each sodium sulfite dose-response curve, the concentration of sodium sulfite (in mg/ml) required to increase SRaw by 100% above baseline was calculated by linear interpolation using log base 3 data (because solutions were inhaled in 3-fold increasing concentrations), and these values were called the provocative concentration of sodium sulfite (PC_{100}). Values of PC_{100} for methacholine and sulfur dioxide were calculated in a similar fashion using log base 2 data.

Results

Nine of ten subjects studies developed significant bronchoconstriciton (SRaw >100% above baseline) after inhalation of sulfite aerosols at all 3 pHs studied (Table 2). The one subject who did not develop bronchoconstriction from inhaling any concentration of sulfite at any pH was also the only subject who did not develop bronchoconstriction after inhaling up to 8 ppm SO_2 (Table 2). In each of the 9 subjects who developed sulfite-induced bronchoconstriction, this effect occurred at the lowest sulfite concentration for sulfite at pH 4, at an intermediate concentration at pH 6.6 and at the highest concentration at pH 9 (Table 2). The mean values for PC_{100} were 0.17 mg/ml at pH 4, 0.49 mg/ml at pH 6.6, and 2.1 mg/ml at pH 9 (Fig. 4). Only one subject developed bronchoconstriction after inhaling acetic acid aerosol at pH 4, and this effect occurred at a total (titratable) acidity 213 times higher than the acidity delivered in the concentration of sodium sulfite at pH 4 required to cause equivalent bronchoconstriction. No subject developed bronchoconstriction after oral ingestion of up to 50 mg of encapsulated potassium metabisulfite.

No sulfur dioxide gas was detectable during aerosolization of sodium sulfite at pH 9 except at the highest concentration (10 mg/ml) where we measured 0.02 ppm SO_2. Sulfur dioxide gas was detectable during aerosolization of sodium sulfite at pH 4 and pH 6.6 with 8-20 more SO_2 at pH 4. The relationship between SO_2 concentration and sulfite concentration was not linear, and the difference between the SO_2 concentrations at the 2 pH's was far less than that predicted by the equilibrium equations described above (Table 3). These findings imply that equilibrium conditions did not exist during aerosolization.

Discussion

The results of this study suggest that inhaled sulfite aerosols are a potent stimulus to bronchoconstriction in subjects with asthma. This effect of sulfite is not restricted to patients with a clinical history of sulfite sensitivity or to subjects who demonstrate sensitivity to oral ingestion of metabisulfite, since none of our subjects demonstrated such sensitivity.

The bronchoconstrictor effects of sodium sulfite aerosols were clearly pH dependent, with the greatest effects occurring at the most acid pH (pH 4) and the smallest effects at alkaline pH (pH 9). Nonetheless, acidity itself did not appear to be the stimulus to bronchoconstriction, since aerosols of acetic acid with a total acidity from 25-200 times that contained in the concentrations of sulfite at pH 4 required to cause bronchoconstriction were without effect, and the one subject who did develop bronchoconstriction after inhalation of sulfite only did so at an acetic acid concentration containing 213 times the acidity of the sulfite solution causing equivalent bronchoconstriction at pH 4. Rather, decreasing pH most likely increased sodium sulfite-induced bronchoconstriction by altering the relative concentrations of sulfite ($SO_3^=$) and bisulfite (HSO_3^-) and of SO_2 gas.

Of the two ionic forms of sulfite, our results suggest that bisulfite is either the more potent or the only bronchoconstrictor stimulus, since inhalation of sulfite solutions at pH 9, where most of the sulfur is in the form of sulfite, was the weakest stimulus to bronchoconstriction in every subject. The fact that bronchoconstriction did occur in 9 of 10 subjects after inhalation of concentrations of sodium sulfite at pH 9 not associated with any measurable generation of SO_2 gas implies that these ionic forms of SO_2 may themselves be capable of inducing bronchoconstriction. However, we cannot rule out the possibility that SO_2 was generated locally within the airways after deposition of the sulfite aerosol on the relatively acidic luminal surface (11).

The results of our experiment do not allow us to determine with certainty the relative importance of bisulfite ion and SO_2 gas itself as stimuli to bronchoconstriction. Sulfite aerosols at pH 4 and at pH 6.6 contained similar concentrations of bisulfite ion, but aerosolization of the solution at pH 4 resulted in generation of 8-20x as much SO_2 as did aerosolization of solutions at pH 6.6. Thus, if we had found no difference between the PC_{100} obtained for sulfite solutions at these two pH's we could have concluded that bisulfite rather than SO_2 gas was the relevant stimulus. Alternatively, if we had found a 10 fold or greater difference in PC_{100} we could have concluded that SO_2 itself was a more important stimulus to bronchoconstriction. However, the 3-fold difference we observed might be equally well explained by either hypothesis. If bisulfite is the more important stimulus, the 3-fold difference we observed could be explained by differences in local bisulfite concentrations when the ion is deposited primarily as large inhaled particles (at pH 6.6) or when the ion is formed locally from dissolved SO_2 gas (pH 4). Alternatively, if SO_2 gas is the more potent stimulus, our failure to observe a larger difference in the PC_{100} could be due to partial removal of the SO_2 generated during aerosolization in the extrathoracic airways.

Despite these limitations in interpretation, our results suggest that sulfite aerosols, such as those formed in the atmosphere by the interaction of liquid water and SO_2 might themselves be capable of causing bronchoconstriction, especially when they are inhaled at acid pH. The low pH of acid fog would thus potentiate the bronchoconstricting effects of its SO_2 and sulfite constituents.

PROJECT #3: Effects of acid aerosols on airway vascular permeability and morphologic evidence of airway epithelial injury in guinea pigs.

Purpose

The major goal of these experiments was to determine whether examination of endpoints other than bronchoconstriction (i.e. vascular permeability and epithelial injury) would be more sensitive indicies of adverse airway effects of acid pollutants.

Methods and Results

Studies were performed in Male Hartley guinea pigs weighing 300-500 grams. Guinea pigs were fed and watered ad libitim, housed in hanging steel mesh cages, and fed standard guinea pig chow. Animals were exposed to acid aerosols by

several different methods. Initially, animals were exposed to aerosols of either HCl or H_2SO_4 generated by either a Devilbiss #646 (MMAD 1.2 microns) or an ultrasonic nebulizer (MMAD 6.3 Microns) for 3 min intervals at progressively decreasing pH from pH 7 to pH 1. As above, acid aerosols were generated from freshly prepared solutions of HCl or H_2SO_4 in isotonic sodium chloride (osmolarity 300 milliosmoles). The gravimetrically determined water content of aerosol-containing air was approximately 20 gm/m^3 for the experiments with the Devilbiss nebulizer and 60 gm/m^3 for experiments with the ultrasonic nebulizer. Specific airway resistance (SRaw) was measured before and at frequent intervals for 5 min after exposure to each aerosol using the method of Agrawal (17). Studies were continued until SRaw increased by more than 100% or a pH of 1 had been inhaled. At the end of each experiment the animal was anesthetized with pentabarbitol (25 mg/kg) and injected with 50 mg/kg Evans blue dye in a jugular vein. Thirty min later the animal was given a lethal dose of pentabarbitol and perfused systemically through the aorta with phosphate buffered saline for 2.5 min at a pressure of 100 mmHg. Both lungs and a 2 mm section of the trachea just proximal to the carina were removed for fixation in 2% gluteraldehyde and subsequent morphologic evaluation. A 1 cm section of trachea just proximal to this was removed, weighed, and placed in formamide at 60°C for 24h for extraction of Evans blue dye. Evans blue content was then quantified on the basis of spectrophotometric adsorbance at 620nm and normalized for tracheal wet weight. Values were initially compared to those obtained in unexposed animals. Evans blue dye entirely binds to plasma proteins. After intravascular perfusion, the residual Evans blue content in the trachea reflects the amount of plasma protein extravasation that has occurred. Evans blue content is thus an index of vascular permeability to proteins which is often an early manifestation of tissue injury and inflammation. In our initial pilot studies, no animals developed increases in SRaw after inhalation of aerosols at pH 2 or higher, thus each animal was exposed to all pH's including pH 1. Nonetheless, no animal demonstrated increased tracheal Evans blue content. Light microscopic evaluation of 2.5 micron giemsa stained sections revealed no evidence of epithelial injury or inflammation after these short term exposures.

To determine wheter evaluation of airway morphology and Evans blue extravasation would be sensitive indicators of the effects of longer exposures to acid aerosols, we then performed additional experiments in guinea pigs exposed to aerosols of HCl and H_2SO_4 in a whole body exposure chamber for 30 min periods. For these experiments aerosols of acid solutions at pH 2.0, pH 1.5 and pH 1.0 were generated with either an ultrasonic nebulizer (particle MMD ~ 6.3 microns) or a Babbington solisphere (MMD ~ 3.0 microns). As above aerosols were generated from freshly prepared solutions of HCl and H_2SO_4 in isotonic sodium chloride. The liquid water content of exposure atmospheres from each nebulizer was gravimetrically determined to be in excess of 50 gm/m^3. In order to detect any transient increases in vascular permeability that might have occurred during acid exposure in some animals we inserted a jugular venous catheter prior to exposure and injected Evans blue dye during acid exposure. As in the shorter term exposures, bronchoconstriction only occurred after exposure to aerosols at pH 1, and no animal demonstrated evidence of increased Evan's blue extravasation or morphologic evidence of epithelial injury or airway inflammation.

Discussion

On the basis of these experiments we conclude that measurements of airway vascular permeability and morphologic evidence of airway epithelial injury in guinea pigs are not likely to be sensitive indicies of adverse airway effects from inhalation of large particle acid aerosols.

TABLE 1

TITRATABLE ACIDITY AND pH REQUIRED TO PROVOKE BRONCHOCONSTRICTION AND COUGH

SUBJECTS	HCL		H_2SO_4		HCL-GLYCINE pH 2.0		H_2SO_4-GLYCINE pH 2.0		HCL-GLYCINE pH 3.5	
	Cough	BC	Cough	BC	Cough	BC	Cough	BC	Cough	BC
1	pH 2.0	NA	NA	NA	4.3	12.1	6.6	NA	NA	NA
2	pH 3.0	pH2.0	pH 2.0	NA	0.8	4.3	1.1	2.7	1.7	0.5
3	pH 2.0	NA	pH 2.0	NA	0.8	4.3	1.1	2.1	1.7	NA
4	pH 2.0	NA	pH 2.0	NA	0.8	1.9	1.1	6.6	0.5	NA
5	pH 2.0	NA	pH 2.0	NA	0.8	12.1	1.1	NA	1.7	NA
6	pH 2.0	NA	pH 2.0	NA	0.8	1.9	1.1	19.8	0.5	NA
7	pH 2.0	NA	pH 2.0	NA	1.9	4.3	1.1	19.8	0.2	NA
8	pH 2.0	NA	pH 2.0	NA	0.8	0.8	1.1	1.5	0.5	1.7

The pH or titratable acidity (ml of 1N NaØH to neutralize 100 ml solution) of the solution which provokes at least 2 coughs.

The pH or titratable acidity which provokes bronchoconstriction (at least a 50% increase in SRaw).

Not achieved

In units of titratable acidity (ml of 1N NaOH to neutralize 100 ml solution)

TABLE 2

PROVOCATIVE CONCENTRATIONS OF SODIUM SULFITE Na_2SO_3, METHACHOLINE (Mch), SULFUR DIOXIDE (SO_2), AND ACETIC ACID REQUIRED TO INCREASE SPECIFIC AIRWAY RESISTANCE BY 100% ABOVE BASELINE IN PROJECT 2

Subject #	Mch (mg/ml)	pH4	pH6.6	pH9	SO_2 (ppm)	Acetic Acid *
1	0.1	0.52	1.65	5.26	1.2	N/A
2	2.2	N/A §	N/A	N/A	N/A	N/A
3	0.09	0.032	0.18	1.2	0.55	6.8*
4	0.50	0.42	0.81	2.13	2.16	N/A
5	0.19	0.14	0.37	1.08	2.13	N/A
6	0.14	0.11	0.32	1.57	2.27	N/A
7	0.45	0.22	0.49	2.01	1.7	N/A
8	0.10	0.14	0.74	3.71	2.6	N/A
9	0.16	0.11	0.32	1.2	2.11	N/A
10	0.84	0.26	0.51	3.6	1.77	N/A
MEAN		0.17	0.49	2.1		

*Acetic acid at pH4 was inhaled in concentrations matched for titratable acidity to the concentrations of Na_2SO_3 inhaled at pH4. The reported value of 6.8 is expressed in Na_2SO_3 equivalents. Thus, 6.8 is equivalent to 6.8 mg/ml Na_2SO_3 at pH4.

§ Not achieved.

+ Calculated from $\log_3$ values.

TABLE 3

SO_2 concentrations measured in aerosols of sodium sulfite

Na Sulfite (mg/ml)	SO_2 (ppm)*		
	pH 4.0	pH 6.6	pH 9.0
0.03	0.12 ± .05	< 0.01†	< 0.01
0.1	0.50 ± .08	0.02 ± .01	< 0.01
0.3	1.37 ± .27	0.08 ± .04	< 0.01
1.0	3.42 ± .20	0.19 ± .03	< 0.01
3.0	> 5.0‡	1.38 ± .30	< 0.01
10.0	> 5.0	> 5.0	0.02 ± .01

* Values are mean ± SD (n = 5)

† 0.01 ppm is the lower detection limit of our SO_2 analyzer

‡ 5.0 ppm is the upper detection limit of our SO_2 analyzer

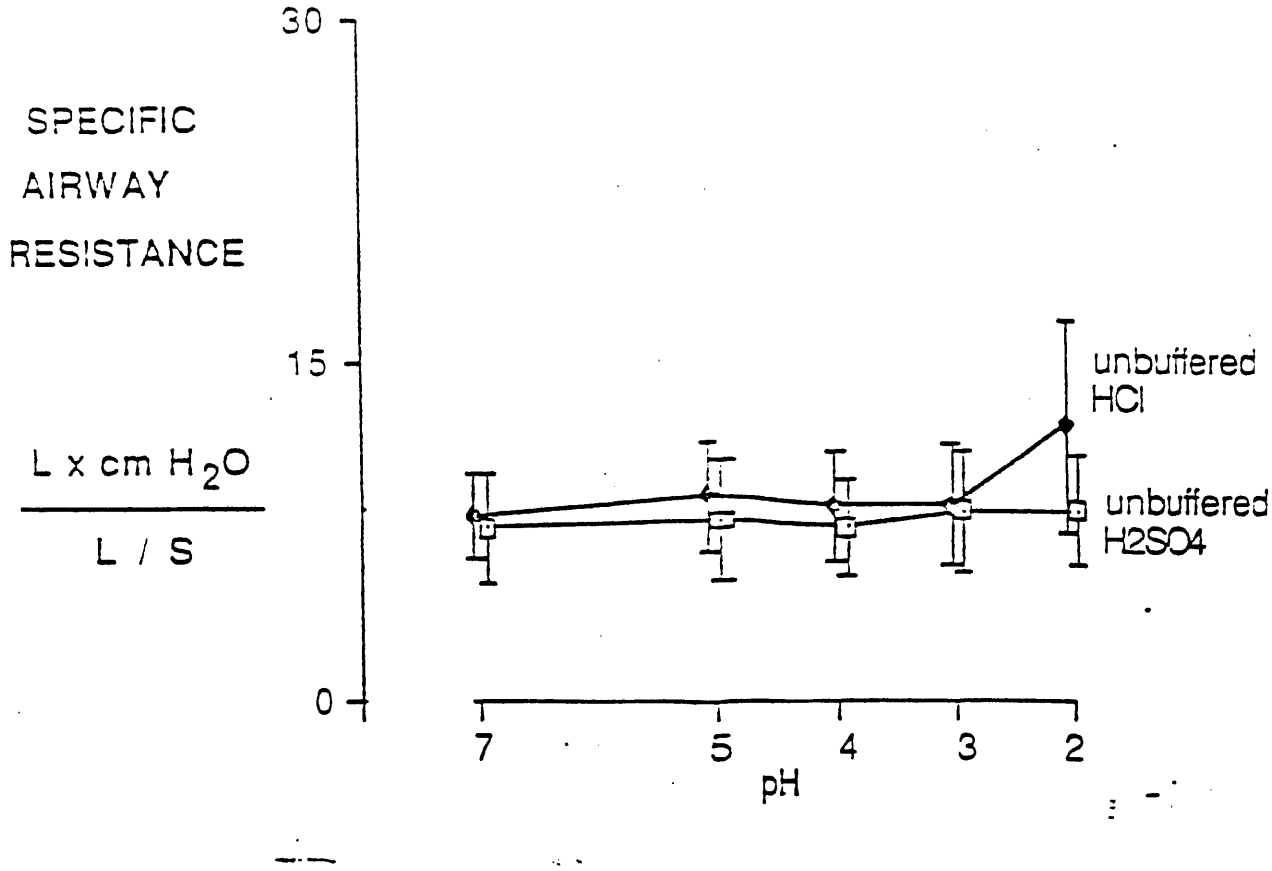

FIGURE 1

Mean ± SEM values of specific airway resistance after inhalation of ultrasonic aerosols of isotonic neutral saline (pH 7) and progressively more acidic solutions of unbuffered HCl and H_2SO_4 in 8 subjects with asthma.

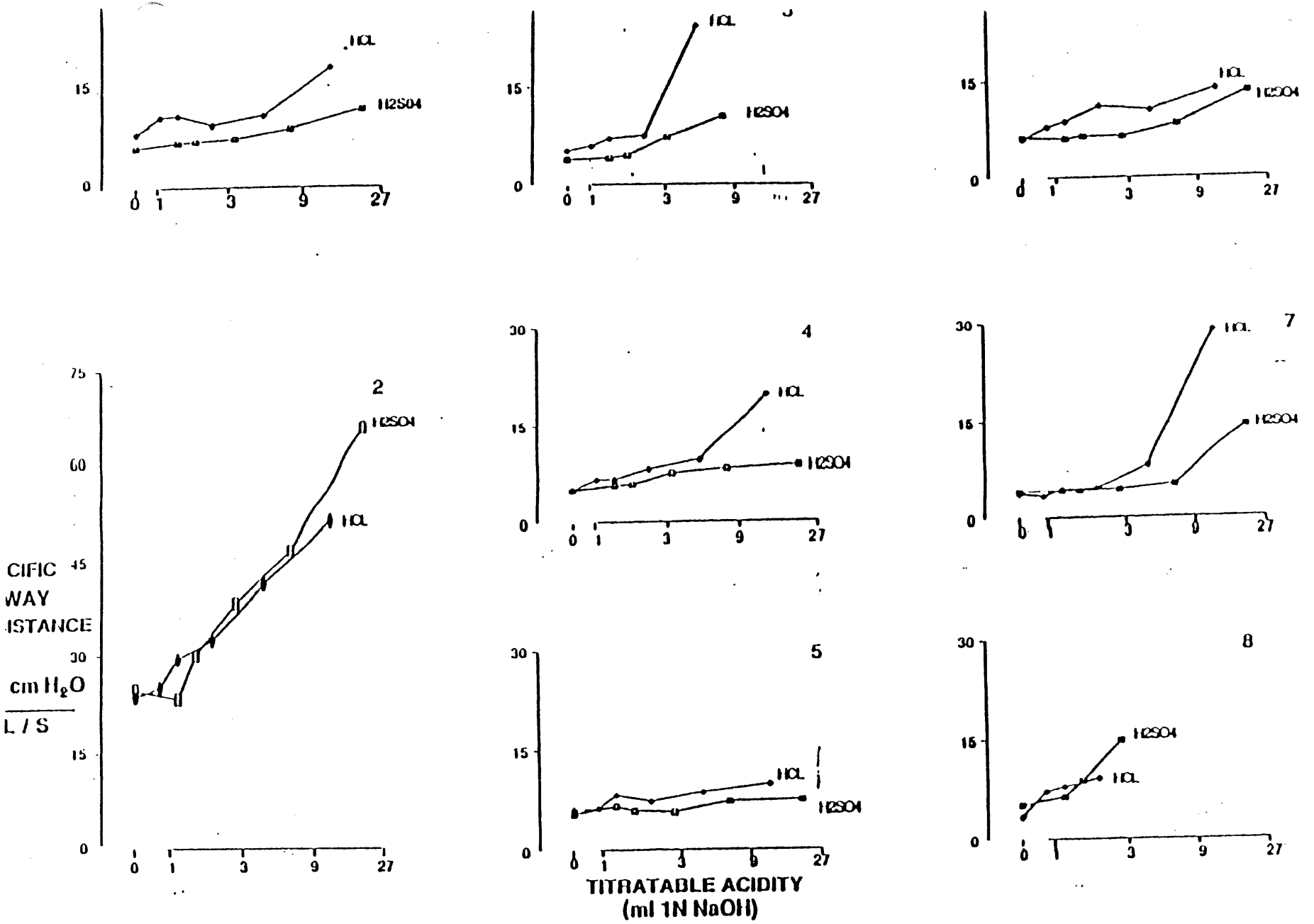

FIGURE 2

Specific airway resistance before and after inhalation of ultrasonic aerosols of HCl or H_2SO_4 buffered with increasing concentrations of glycine at pH 2 to yield increasing amounts of titratable acidity. Individual dose-response curves are shown for each of 8 subjects. The units of titratable acidity are ml of 1 N NaOH required to buffer 100 ml of the test solution to pH 7.

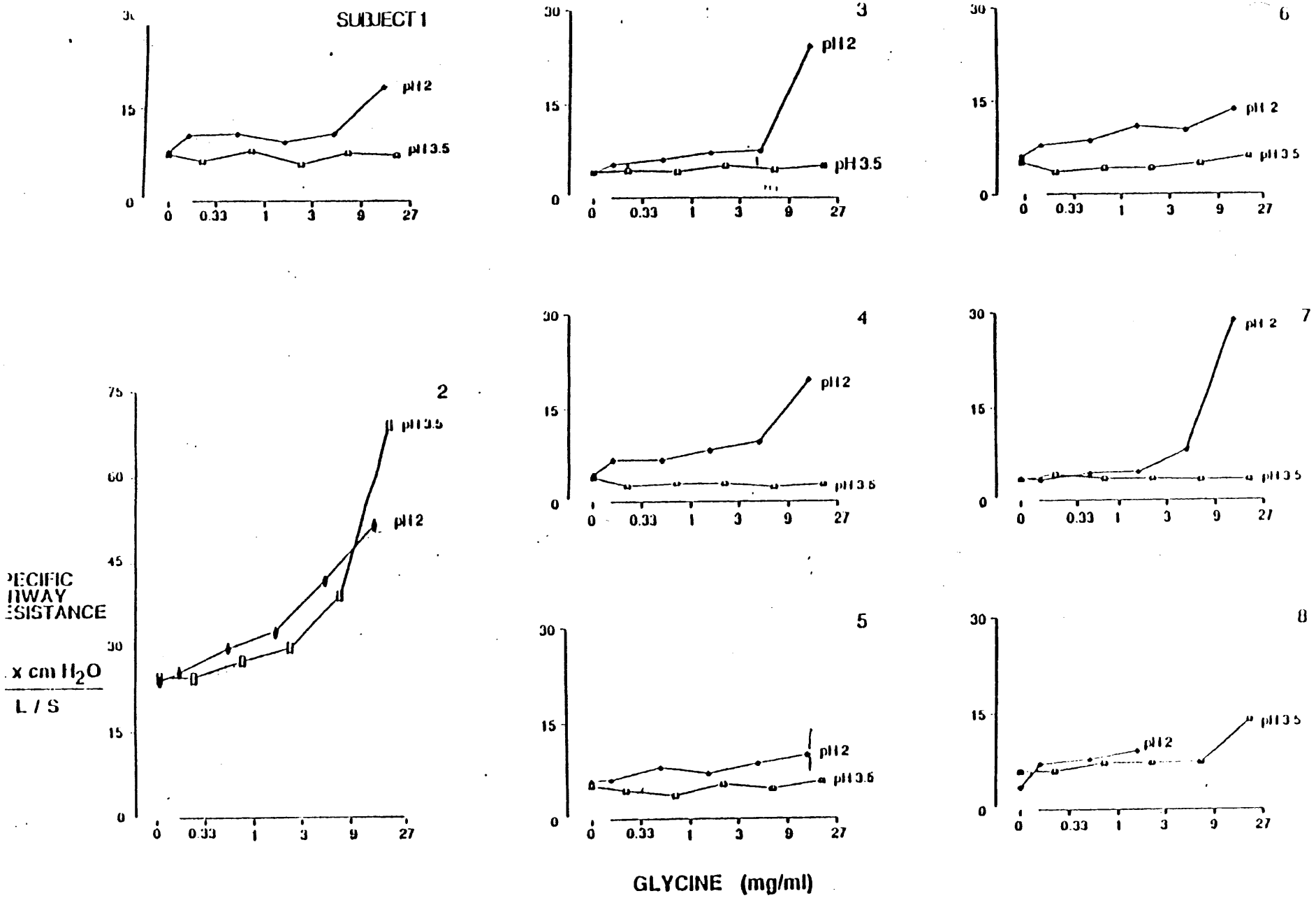

FIGURE 3

Specific airway resistance before and after inhalation of ultrasonic aerosols of HCl buffered with increasing concentrations of glycine at pH 2 and at pH 3.5. Individual dose-response curves are shown for each of 8 subjects.

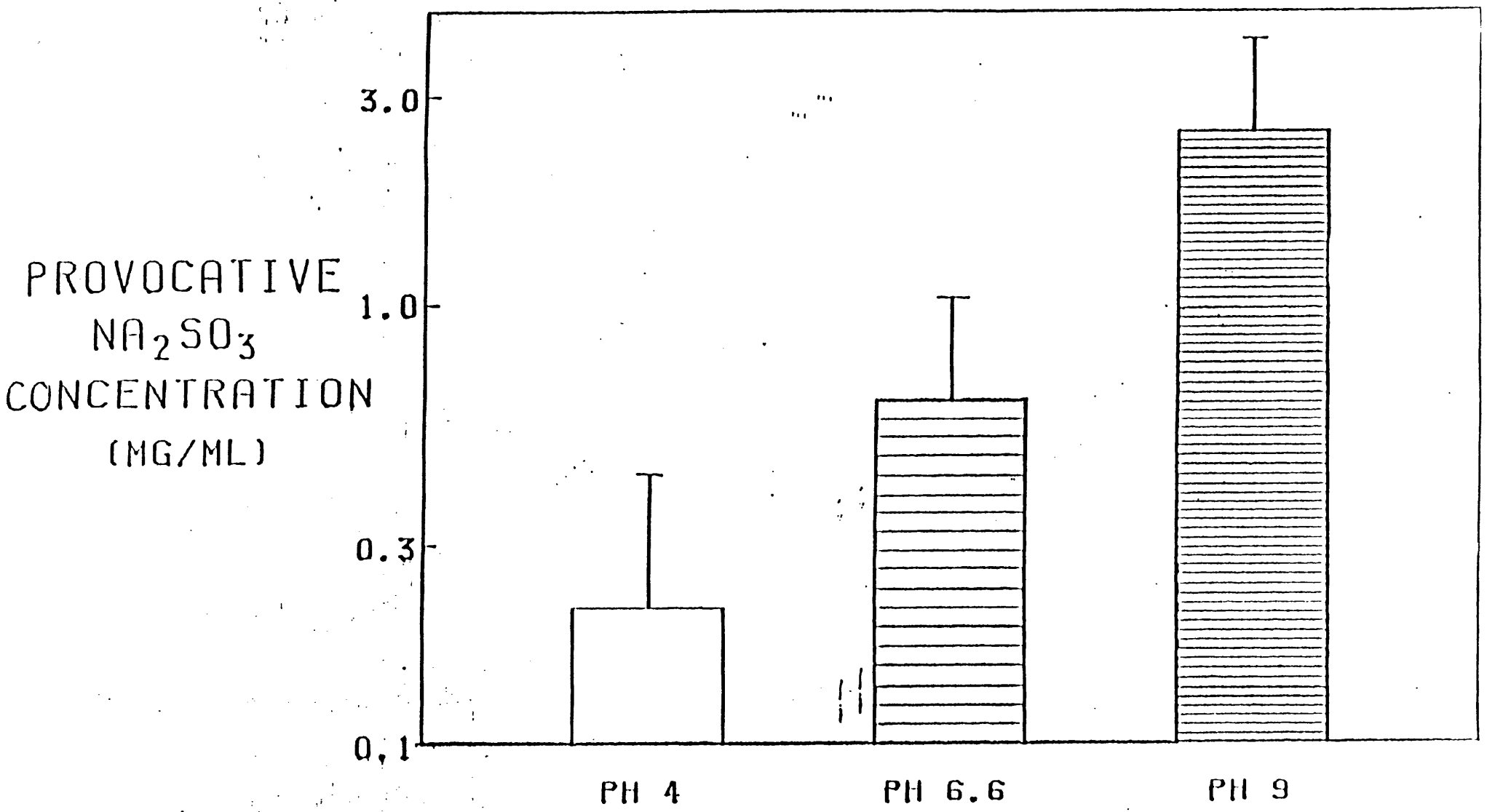

FIGURE 4

Mean ± SEM values for the provocative concentration of Na_2SO_3 required to increase specific airway resistance by 100% above baseline in 9 subjects with asthma. Na_2SO_3 was inhaled by each subject as an ultrasonic aerosol at each of 3 pH's (pH 4, pH 6.6, and pH 9) on 3 separate days.

References

1. Jacobs DJ, Waldman JW, Munger JW, Hoffman MR. Chemical composition of fogwater collected along the California coast. Environ Sci Technol 1985; 19:730-736.

2. Sheppard D, Wong WS, Uehara CF, Nadel JA, Boushey HA. Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulfur dioxide. Am Rev Respir Dis 1980; 122:873-8.

3. Sheppard D, Saisho A, Nadel JA, Boushey HA. Exercise increases sulfur dioxide-induced bronchoconstriction in asthmatic subjects. Am Rev Respir Dis 1981; 123:486-91.

4. Koenig JQ, Pierson WF, Horike M. The effects of inhaled sulfuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis 1983; 128:221-5.

5. Utell MJ, Morrow PE, Speers DM, Darling J, Hyde RW. Airway responses to sulfate and sulfuric acid aerosols in asthmatics. Am Rev Respir Dis 1983; 128:444-50.

6. Larson TV, Frank B, Covert DS, Holub D, Morgan MS. Measurement of respiratory ammonia and the chemical neutralization of inhaled sulfuric acid aerosol in anesthetized dogs. Am Rev Respir Dis 1982; 125:502-6.

7. Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyperreactivity in normal subjects following upper respiratory tract infection. Am Rev Respir Dis 1976; 113:131-9.

8. Hirshman CA, Peters J, Downes H, Leon D, Lynn RK, Butler J, Hanifin JM. Citric acid airway constriction in dogs with hyperreactive airways. J Appl Physiol 1983; 54:1101-7.

9. Dubois AB, Botello SY, Comrol JH Jr. A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease. J Clin Invest 1956; 35:327-35.

10. Sheppard D, Saisho A, Nadel JA, Boushey HA. Exercise increases sulfur dioxide-induced bronchoconstriction in asthmatic subjects. Am Rev Resp Dis 1981; 123:486-91.

11. Amdur MO, Bayles J, Ugro V, Underhill DW. Comparative irritant potency of sulfate salts. Environ Res 1978; 16:1-8.

12. Bodem CR, Lampton LM, Miller DP, Tarka EF, Everett ED. Endobronchial pH. Am Rev Respir Dis 1983; 127:39-41.

13. Stevenson D, Simon R. Sensitivity to ingested metabisulfites in asthmatic subjects. J Allergy Clin Immunol 1981; 68:26-32.

14. Petering DH, Shih NT. Biochemistry of bisulfite-sulfur dioxide. Environ Res 1975; 9:55-65.

15. Dahl HR, Funt GM, Hahn FF. Binding of sulfite-introduced ^{35}S to the pleura and walls of conducting airways in rat lungs. Toxicol Lett 1981; 8:295-8.

16. Steinacker A. Sulphonation of cholinergic receptor disulphide bond increases response to ACh. Nature 1979; 278:358-60.

17. Agrawal KP. Specific airway conductance in guinea pigs: normal values and histamine-induced fall. Respir Physiol 1981; 43:23-30.

PUBLICATIONS

The Role of Titratable Acidity in Acid Aerosol-induced Bronchoconstriction. Fine, J.M., Gordon, T., Thompson, J.E. and Sheppard, D. (1987) Am. Rev. Respir. Dis. 135: 826-830.

The Roles of pH and Ionic Species in Sulfur Dioxide- and Sulfite-Induced Bronchoconstriction. (1987) Am Rev. Respir Dis. 136: 1122-1126.

Symptomatic Bronchoconstriction after Short-Term Inhalation of Sulfur Dioxide. (1987) Am Rev. Respir Dis. 136: 1117-1121.

APPENDIX

The role of titratable acidity in acied aerosol-induced bronchoconstriction. J.M. Fine, T. Gordon, J.E. Thompson, D. Sheppard. (1987) Am Rev. Respir. Dis. 135: 826-830. (Attached).

The roles of pH, ionic species, and sulfur dioxide in sulfite-induced bronchoconstriction. J.M. Fine, T. Gordon, D. Sheppard. (1987) Am. Rev. Respir. Dis. 136: 1122-1126. (Attached).

Symptomatic Bronchoconstriction after short-term inhalation of sulfur dioxide. J.R. Balmes, J.M. Fine, D. Sheppard. (1987) Am Rev. Respir. Dis. 136: 1117-1121. (Attached).

The Role of Titratable Acidity in Acid Aerosol-induced Bronchoconstriction[1-3]

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SUMMARY We evaluated the importance of pH, titratable acidity, and specific chemical composition in acid aerosol-induced bronchoconstriction in 8 asthmatic subjects. We administered aerosols of HCl and H_2SO_4 at pH 2.0 in an unbuffered state and buffered with glycine. The buffered acids were given in order of increasing titratable acidity (defined as the number of ml of 1 N NaOH required to neutralize 100 ml of acid solution to pH 7.0). Each set of buffered or unbuffered acid aerosols was given on a separate day and each aerosol was inhaled through a mouthpiece during 3 min of tidal breathing. Bronchoconstriction was assessed by measurement of specific airway resistance (SRaw) before and after inhalation of each aerosol. SRaw increased by more than 50% above baseline in 1 of 8 subjects after inhalation of unbuffered HCl and in no subjects after inhalation of unbuffered H_2SO_4, even at pH 2.0. In contrast, SRaw increased by greater than 50% in all 8 subjects after inhalation of HCl and glycine at pH 2.0 and 7 of 8 subjects after inhalation of H_2SO_4 and glycine at pH 2.0. The mean titratable acidity required to increase SRaw by 50% above baseline was calculated for each challenge by linear interpolation; these values for H_2SO_4 and glycine (5.1 ml of 1 N NaOH) and HCl and glycine (2.2 ml of 1 N NaOH) were slightly, but significantly, different ($p = 0.01$) and were considerably higher than the titratable acidity of the unbuffered acids at pH 2 (1.0 ml of 1 N NaOH). These results demonstrate that titratable acidity and specific chemical composition as well as pH must be considered when evaluating the airway responses of asthmatics to acid aerosols.

AM REV RESPIR DIS 1987; 135:826-830

Introduction

Acid aerosols are commonly encountered in the workplace and in polluted outdoor air. Sulfuric acid is a common constituent of ambient acid aerosols and has been shown to cause bronchoconstriction when inhaled by animals (1) and by persons with asthma (2, 3). Two pieces of indirect evidence suggest that the bronchoconstrictor response to sulfuric acid may be due to its acidic properties. First, sulfuric acid-induced bronchoconstriction is diminished as the concentration of the weak base ammonia is increased in the oral cavity (4). Second, aerosols of acidic sulfate solutions with lower acidity than sulfuric acid are less potent stimuli to bronchoconstriction (3). Moreover, the bronchoconstrictor potency of these sulfate aerosols is roughly proportional to their acidity. Despite these observations, the significance of acidity itself as a bronchoconstrictor stimulus has not been directly examined. The recent finding that California fog can have a pH as low as 1.7 (5) has focused attention on the possible adverse health effects of breathing such acid fog, especially on potentially sensitive segments of the population, such as patients with asthma. Because naturally occurring acid fog contains complex mixtures of pollutants, we thought it would be important to characterize the general mechanisms by which acids can cause bronchoconstriction before embarking on studies of simulated fogs.

The acidity of fog has generally been quantified by direct measurements of pH, reflecting the concentration of free hydrogen ion present in fog liquid. However, in the presence of naturally occurring buffers, pH does not accurately describe the total pool of available hydrogen ion. This available hydrogen ion pool is better described on the basis of titratable acidity (expressed as the amount of strong base required to neutralize a given volume of acid solution to pH 7.0).

Because of the considerable buffering capacity of airway surface liquid, inhalation of free hydrogen ion, in the form of unbuffered acid aerosols, will cause only a transient decrease in airway pH. Inhalation of buffered acids at the same pH would be expected to cause a more persistent decrease in airway surface pH because of their larger pool of available hydrogen ion (described by titratable acidity). We therefore reasoned that if inhaled acid aerosols cause bronchoconstriction by altering airway surface pH, then the titratable acidity of these aerosols might be a more important determinant of bronchoconstrictor potency than would pH per se. To test this hypothesis we studied the bronchoconstrictor effects of aerosols of buffered and unbuffered acid solutions in subjects with asthma.

Methods

The subjects were 8 nonsmoking volunteers who were informed of the risks of the experimental protocol and signed consent forms approved by the Committee on Human Research of the University of California. 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 sympathomimetic drugs within 12 h or consumed caffeine within 4 h before any experiment. All subjects denied having an upper respiratory tract infection within 6 wk prior to the study.

The subjects came to the laboratory on 6 nonconsecutive days. Each day they were tested in a single blind fashion with a different set of aerosols containing one of the following: methacholine, HCl, HCl buffered with glycine at pH 2.0 and pH 3.5, H_2SO_4, and H_2SO_4 buffered with glycine at pH 2.0. To assess their response to inhalation of these aerosolized solutions, the subjects' airway resistance (Raw) and thoracic gas volume (Vtg) were measured in a constant volume body plethysmograph (No. 09103; Warren E. Collins,

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Braintree, MA) and expressed as the product of Raw and Vtg, specific airway resistance (SRaw) (6). At the outset of each study day and 1 min after each challenge, 5 SRaw measurements were recorded, 1 every 30 s for 2 min, and the mean value calculated.

On the first study day, subjects performed spirometry and underwent a methacholine challenge. Methacholine responsiveness was tested by measuring each subject's SRaw before and after inhalation of 10 tidal breaths of phosphate buffered saline and doubling concentrations of methacholine (0.063, 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 mg/ml) delivered by a Devilbiss No. 646 nebulizer (Devilbiss Co, Somerset, PA) with a dose-metering device calibrated to deliver 0.01 ml per breath (7). The concentration of methacholine which provoked a 100% increase in SRaw from the post saline SRaw value (PC_{100}) was calculated by linear interpolation.

On subsequent days, subjects inhaled nebulized solutions from a mouthpiece for 3 min each, with SRaw measurements performed during a 5-min interval between challenges. Subjects first inhaled neutral pH saline as a control and then successive acid solutions until their SRaw doubled or increased 6 L × cm H_2O/L/s, whichever was greater, or until they reached the maximal acid concentration or lowest pH tested that day. The subjects were administered 2 different acids, HCl and H_2SO_4, in order to evaluate the role of specific chemical composition of acid aerosols in inducing airway effects. The pH at which these airway effects, namely cough and bronchoconstriction, occurred was investigated by administering aerosols of each acid unbuffered in normal saline at pH 5, 4, 3, and 2. To determine if the amount of titratable acid (defined as the number of ml of 1 N NaOH required to titrate a 100 ml solution to pH 7.0) in the aerosolized acid solutions, in addition to the pH and specific chemical composition, was an important factor, the acids were then administered in solutions buffered with glycine at pH 2.0. We chose to give buffered acids at pH 2.0 after first demonstrating with the unbuffered acids that this level of acidity could be safely inhaled by each subject.

The buffer employed, glycine, is a zwitterion, existing in a dipolar state except in very acidic and basic solutions. To determine whether glycine alone accounted for the airway effects of the buffered acid solution while minimizing the variation in reduction state of glycine, subjects also inhaled aerosols of HCl and glycine at pH 3.5. Compared to the most concentrated pH 2.0 HCl and glycine solution administered, the most concentrated pH 3.5 solution given contained more glycine (20.3 vs 14.0 mg/ml) and considerably less titratable acidity (1.7 vs 12.1 ml of 1 N NaOH).

The buffered solutions were prepared by making serial 3-fold dilutions with normal saline of stock isotonic solutions of glycine with either HCl or H_2SO_4 (all chemicals supplied by Fisher Scientific, Fairlawn, New Jersey). In this fashion, solutions with 33%, 11%, 3.7%, and 1.2% of the HCl or H_2SO_4 and glycine contained in the stock isotonic solutions were prepared. Small amounts of 1 N HCl or 1 N H_2SO_4 were then added to the dilutions to maintain their pH at 2.0 or 3.5. All solutions were isotonic (300 mosm) as confirmed by measurement with a vapor pressure osmometer (No. 5700B; Wescor, Logan, UT) and were given in order of increasing buffer concentration and, hence, of increasing titratable acidity. The pH of each solution was measured with a pH meter (No. 43; Beckman, Irvine, CA).

The aerosols were generated by an ultrasonic nebulizer (EN 145 Mistogen; Time Meter Co., Lancaster, PA). Using a low flow, seven-stage cascade impactor (In-Tox Products, Albuquerque, NM), the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were determined for aerosols of isotonic saline at pH 7.0 and unbuffered and buffered acids at pH 2.0. There was no constant relationship among pH, buffer capacity, and particle size. The MMAD ranged from 5.3 microns with a GSD of 1.8 microns (H_2SO_4 and glycine) to 6.2 microns with a GSD of 1.6 microns (HCl). With the various solutions, the nebulizer had a mean (± SD) liquid water output measured gravimetrically of 5.74 (0.16) g per min.

Expiratory airflow during the challenges was measured by having the subjects exhale through a heated pneumotachograph (No. 3; Fleisch, Lausanne, Switzerland) and registering the generated signal on photosensitive paper (No. 1858 Visicorder; Honeywell, Denver, CO). Minute ventilation was recorded by integrating the signal from the pneumotachograph with a respiratory integrator (No. FV 156; Validyne, Northridge, CA) and was corrected to body temperature and pressure saturated.

Coughs were counted throughout the experiment by an observer and recorded on a hand-held tape recorder. In addition, coughs during inhalation of acid aerosols were correlated with increased expiratory flow measured with the pneumotachograph. A significant cough reaction was defined as 2 coughs during any 3-min period of aerosol inhalation.

To analyze the bronchoconstricting effects of HCl and H_2SO_4 in terms of titratable acidity, we plotted SRaw against the titratable acidity of each concentration of buffered acid administered. Because the experiment was conducted with roughly 3-fold increases in titratable acidity, the data were plotted on a log base 3 scale. The amount of titratable acidity that provoked a 50% rise in SRaw above the saline control value (PC_{50}) was calculated by log-linear interpolation between 2 points on the curve representing an increase of just less than and just greater than 50% over baseline. One subject (Subject 8) experienced a 50% increase in SRaw after the first dose of acid aerosol and his PC_{50} was determined by linear extrapolation from his responses to subsequent doses. Since the data were not normally distributed, we used a Wilcoxon paired-sample test to evaluate the significance of differences in PC_{50} of the

TABLE 1

SUBJECT CHARACTERISTICS

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV, (L)	FEV, (% pred)	FVC (L)	FVC (% pred)	Methacholine Responsiveness* (mg/ml)	Medications
1	F	23	166	57	2.83	89%	3.63	95%	1.5	
2	M	29	188	68	1.89	41%	4.09	71%	0.07	β-agonist inhaler
3	F	26	153	73	2.28	80%	3.52	104%	0.01	theophylline
4	F	24	175	61	3.35	94%	4.66	107%	0.14	β-agonist inhaler
5	F	26	163	55	2.8	90%	3.54	94%	0.1	
6	F	24	155	75	2.92	99%	3.61	102%	0.36	β-agonist inhaler
7	F	22	168	66	3.61	108%	4.9	121%	0.09	theophylline β-agonist inhaler
8	M	28	173	86	4.25	102%	5.41	105%	0.5	theophylline β-agonist inhaler

* Concentration of methacholine required to produce a 100% increase in SRaw from saline control value calculated by linear interpolation.

buffered acids at pH 2.0 and in the mean increases in SRaw provoked by the unbuffered acids. To assess the differences in mean ventilatory rates and baseline SRaw values among the acid aerosol challenges, we employed a two-way analysis of variance. A p value < 0.05 was considered statistically significant.

Results

All of the subjects had clinically stable asthma during the course of the study. Their baseline characteristics are listed in table 1. All of the subjects displayed increased methacholine responsiveness compared with 19 normal subjects tested in our laboratory who had a PC_{100} greater than 4.0 mg/ml.

No significant difference was found among the subjects' baseline SRaw values during the course of the study. The mean (± SE) ventilation of the subjects during inhalation of the sets of H_2SO_4 and glycine (10.09 ± 0.9 L/min), HCl and glycine at pH 2.0 (12.6 ± 1.8 L/min), and HCl and glycine at pH 3.5 (11.3 ± 1.0 L/min) were not significantly different. The mean ventilation during unbuffered acid inhalation, HCl (11.1 ± 3.0 L/min) and H_2SO_4 (11.3 ± 2.4 L/min), were also not significantly different.

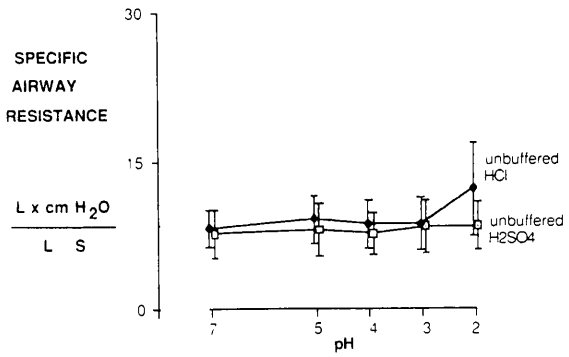

Fig. 1. Mean ± SEM values of specific airway resistance after inhalation of ultrasonic aerosols of isotonic neutral saline (pH 7.0) and progressively more acidic solutions of unbuffered HCl and H_2SO_4 in 8 subjects with asthma.

Unbuffered acid aerosol inhalation caused little increase in SRaw over baseline values (figure 1). The mean (± SE) percent increase in SRaw caused by HCl at pH 2.0 was 27.7 ± 14.3% and by H_2SO_4 at pH 2.0 was 14.8 ± 4.9%. Although one subject (Subject 2) did react to HCl, but not H_2SO_4, at pH 2.0 with a large (120%) increase in SRaw, no significant group difference between the bronchoconstricting effects of the two unbuffered acids was detected.

Buffered acids at pH 2.0 (figure 2) produced much larger increases in SRaw

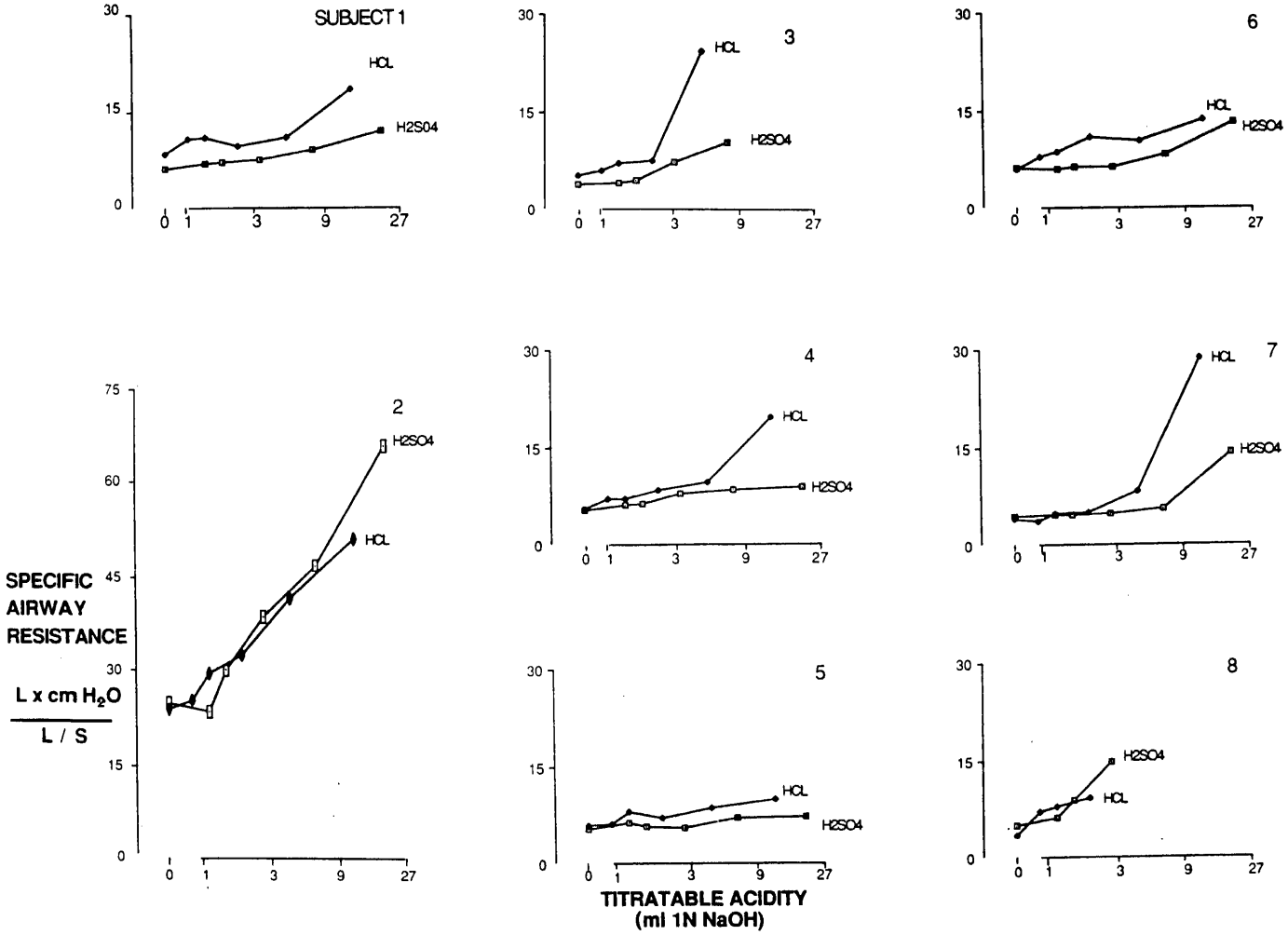

Fig. 2. Specific airway resistance before and after inhalation of ultrasonic aerosols of HCl or H_2SO_4 buffered with increasing concentrations of glycine at pH 2.0 to yield increasing amounts of titratable acidity. Individual dose-response curves are shown for each of 8 subjects. The units of titratable acidity are ml of 1 N NaOH required to buffer 100 ml of the test solution to pH 7.0.

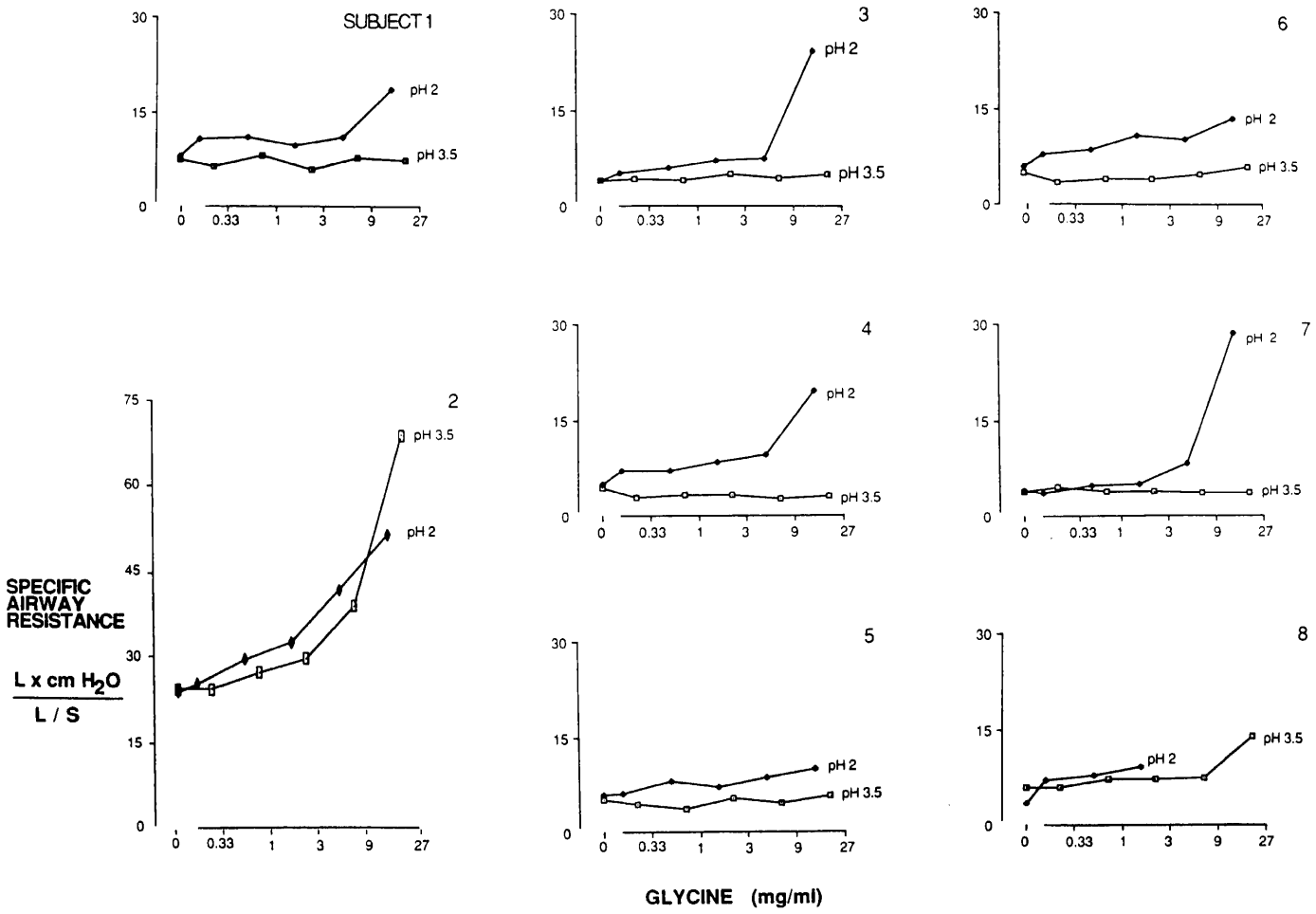

Fig. 3. Specific airway resistance before and after inhalation of ultrasonic aerosols of HCl buffered with increasing concentrations of glycine at pH 2.0 and at pH 3.5. Individual dose-response curves are shown for each of 8 subjects.

than did the unbuffered acids. All subjects increased their SRaw by at least 50% from saline control values after HCl and glycine at pH 2.0 and 7 out of 8 subjects did so after H_2SO_4 and glycine at pH 2.0. The mean PC_{50} of titratable acidity for HCl and glycine (2.2 ml of 1 N NaOH) was slightly, but significantly, lower than for H_2SO_4 and glycine (5.1 ml of 1 N NaOH) ($p = 0.01$). HCl solutions containing similar amounts of glycine buffer caused less bronchoconstriction at pH 3.5 than at pH 2.0 (figure 3). In almost all challenges, cough was noted to precede the measured 50% increase in SRaw from saline control values and to occur during inhalation of unbuffered pH 2.0 aerosols regardless of the bronchomotor response.

Discussion

The results of this study suggest that the acidity of inhaled large particle aerosols can itself be a stimulus to bronchoconstriction. The bronchoconstrictor potency of acidic aerosols is 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 2.0), increasing amounts of titratable acidity caused increasing severity of bronchoconstriction for the two chemically distinct acid aerosols studied. The free hydrogen ion concentration (pH) of the large respirable aerosols we studied was a weak stimulus to bronchoconstriction, with little effect down to pH 2.0.

The relationship we observed between titratable acidity and bronchoconstrictor potency fits with the notion that acid aerosols cause bronchoconstriction at least in part by altering airway surface pH. The alterations in airway pH caused by inhalation of unbuffered acids are presumably quite transient due to the considerable buffering capacity of airway surface liquid (8). Inhalation of buffered acids would be expected to cause a more persistent alteration of airway surface pH due to gradual release of available hydrogen ion from the buffer.

The greater potency of HCl compared to H_2SO_4 per unit of titratable acidity was observed with the glycine buffered solutions but not with the unbuffered solutions. This finding implies that the effects of specific chemical composition must also be considered when evaluating the bronchoconstriction provoked by acid aerosols. The increased potency of HCl may, in part, be due to its higher vapor pressure which might have become a significant factor at the increased acid concentration in the buffered solutions. HCl vapor could conceivably deposit more distally than HCl inhaled in large aerosol particles. Another possibility is that specific interactions between the acids and glycine may have altered their properties as airway irritants.

To ensure that the relationship between increasing titratable acidity and increasing bronchoconstrictor potency that we observed for buffered acids at pH 2.0 was not merely due to a bronchoconstrictor effect of increasing concentrations of glycine buffer, we also examined the effects

of inhalation of aerosols of increasing concentrations of glycine with considerably less titratable acidity at pH 3.5. At each glycine concentration examined, the aerosol with less titratable acidity caused less bronchoconstriction (figure 3). It is thus unlikely that glycine itself was responsible for the bronchomotor effect of buffered acids we observed.

We have presumed that the increases in specific airway resistance measured were primarily due to narrowing of intrathoracic airways as a response to locally deposited acidic droplets. We recognize, however, that this measurement of lung function is also affected by changes in extrathoracic airway caliber, including the caliber of the larynx. Since we did not directly measure laryngeal caliber in this study, we cannot be certain that some or all of the effects on SRaw we observed were not due to laryngeal narrowing. Although the panting maneuver we employed during measurements of SRaw tends to overcome laryngeal narrowing, some decreases in laryngeal caliber have been shown to persist even during panting (9).

This study was not designed to simulate natural exposures to acid aerosols, but rather to examine the role of acidity itself as a bronchoconstrictor stimulus. Thus, the conditions of exposure we studied (isotonic particles of relatively uniform diameter delivered at high concentration through a mouthpiece) are quite different from those encountered in the workplace or the general environment. It is therefore not possible to extrapolate directly from our results to predict the effects of environmental exposure to acid aerosols. Nonetheless, we were impressed by how weak a bronchoconstrictor stimulus unbuffered acid aerosols were under the conditions we studied. On the basis of measurements of water content at the mouthpiece during simulated tidal breathing, we estimated that during inhalation of sulfuric acid at pH 2.0, our subjects were exposed to in excess of 10 mg/m^3 sulfuric acid. Despite this high concentration, the 14.8% increase in SRaw we observed was less than the increase in SRaw reported by Utell and coworkers (3) in resting subjects exposed to a sulfuric acid concentration more than 10-fold lower (1 mg/m^3). One major difference between that study and the present one is the particle size employed. Whereas in this study we examined the effects of large respirable particles (MMAD 6.0 microns for H_2SO_4), such as those that predominate in acid fog, Utell and coworkers studied small sulfuric acid particles (MMAD 1.0 micron), such as those that predominate in the atmosphere in the absence of fog. The discrepancy between our results is consistent with the hypothesis that particle size is an important determinant of the bronchoconstrictor potency of acid aerosols. This hypothesis is further supported by evidence in guinea pigs that decreasing particle size of sulfuric acid aerosols over a range from 2.5 to 0.1 microns MMAD is associated with increasing bronchoconstrictor potency (10). However, our study and that of Utell and coworkers were performed in different subjects and with different experimental protocols; for example, our subjects breathed the acid aerosol for 3 min, whereas the subjects in the study of Utell and coworkers breathed their aerosol for 16 min. The role of particle size in the bronchoconstrictor response of human subjects with asthma to acid aerosols thus needs to be directly studied before one can conclude that large respirable fog particles are a weaker stimulus than are the smaller acid particles present in the absence of fog.

The results of this study have implications for design of future studies and for atmospheric monitoring of acid pollutants. 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 measurements of coexistent buffers and of titratable acidity in addition to measurements of pH.

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References

1. Amdur MO. The respiratory response of guinea pigs to sulfuric acid mist. Arch Indust Health 1958; 18:407–14.

2. Koenig JQ, Pierson WE, Horike M. The effects of inhaled sulfuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis 1983; 128:221–5.

3. Utell MJ, Morrow PE, Speers DM, Darling J, Hyde RW. Airway responses to sulfate and sulfuric acid aerosols in asthmatics. Am Rev Respir Dis 1983; 128:444–50.

4. Mariglio JA, Utell MJ, Morrow PE, Speers DM, Gibb FR, Platner J. Endogenous oral ammonia inhibits sulfuric acid aerosol induced bronchoconstriction in exercising asthmatics (abstract). Am Rev Respir Dis 1983; 127(Suppl:A161).

5. Jacobs DJ, Waldman JW, Munger JW, Hoffman MR. Chemical composition of fogwater collected along the california coast. Environ Sci Technol 1985; 19:730–6.

6. Dubois AB, Botelho SY, Comroe JH Jr. A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease. J Clin Invest 1956; 35:327–35.

7. Chai H, Farr RS, Froehlich L, *et al.* Standardization of bronchial inhalation challenge procedures. J Allergy Clin Immunol 1975; 56:323–7.

8. Jones JG, Berry M, Hulands GH, Gawley JCW. The time course and degree of change in alveolar capillary membrane permeability induced by aspiration of hydrochloric acid and hypotonic saline. Am Rev Respir Dis 1978; 118:1007–13.

9. Sekizawa K, Sasaki H, Tamotsu T. Laryngeal resistance immediately after panting in control and constricted airways. J Appl Physiol 1985; 58:1164–9.

10. Amdur MO, Bayles J, Ugro V, Underhill DW. Comparative irritant potency of sulfate salts. Environ Res 1978; 16:1–8.

The Roles of pH and Ionic Species in Sulfur Dioxide- and Sulfite-Induced Bronchoconstriction[1-3]

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SUMMARY Sulfur dioxide (SO_2) and sulfites are well-described causes of bronchoconstriction in persons with asthma that are chemically related and, therefore, may share a common mechanism of action. When either sulfur species dissolves in aqueous solutions, a pH-dependent equilibrium is established predominantly among bisulfite ion (HSO_3^-), sulfite ion ($SO_3^=$), and SO_2. In addition, hydrogen ions may be released. To assess the relative bronchoconstricting potencies of these chemical forms and the role of acidity caused by the release of hydrogen ions in SO_2- and sulfite-induced bronchoconstriction, we administered to 10 asthmatic subjects 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 solutions. Subjects inhaled increasing concentrations of aerosolized Na_2SO_3 at each pH during 1 min of tidal breathing. Subjects also breathed 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. Bronchoconstrictor response was assessed by measuring specific airway resistance (SRaw) before and after each challenge. Nine of the 10 subjects developed bronchoconstriction after inhaling the Na_2SO_3 aerosols at all 3 levels of pH 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 levels of pH: 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. These results suggest that bronchoconstriction provoked by inhalation of sodium sulfite aerosols may be caused by the SO_2 gas released or by bisulfite ions, but not by sulfite ions. Furthermore, SO_2 and bisulfite are not likely to cause bronchoconstriction merely as a result of an alteration in airway pH.

AM REV RESPIR DIS 1987; 136:1122-1126

Introduction

Sulfur dioxide (SO_2) and sulfites have been widely described as inducers of bronchoconstriction when inhaled or ingested by persons with asthma (1-7). These agents are fairly ubiquitous: SO_2 is a common air pollutant, and sulfites including metabisulfite ($S_2O_5^=$), bisulfite (HSO_3^-), and sulfite ($SO_3^=$) have been popular food and drug preservatives. In addition, sulfites are also formed in the atmosphere as a reaction product of SO_2 and water droplets.

Although the mechanism by which inhaled SO_2 and sulfites induce bronchoconstriction is unknown, clues are provided by their common chemistry. When dissolved in water, such as in the moist mucous membrane lining of the airways where SO_2 is avidly absorbed (8, 9), these sulfur substances enter into equilibrium with one another. SO_2 and metabisulfite convert to bisulfite (pKa 1.86 and 0.09, respectively) (10, 11), and bisulfite in turn enters into equilibrium with sulfite ion (pKa 7.2) (12). These reactions are accompanied by the release of hydrogen ions.

We hypothesize that inhaled SO_2 and sulfites induce bronchoconstriction either by acting as acids by releasing hydrogen ions and altering the pH of the airway lining or by themselves directly entering into biochemical reactions. In addition, either SO_2 or one of the sulfites formed may be more active than the others in causing bronchoconstriction. The purpose of the present study was to determine the relative contributions of bisulfite, sulfite ion, SO_2, and change in airway pH to bronchoconstriction caused by inhalation of these sulfur species.

Methods

The subjects were 10 nonsmoking volunteers who were informed of the risks of the experimental protocol and 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 sympathomimetic drugs within 12 h or consumed caffeine within 4 h before any experiment. All subjects denied having an upper respiratory tract infection within 6 wk prior to the study. No subject gave a history suggestive of sensitivity to ingested sulfite preservatives. Subject characteristics are listed in table 1.

In order to distinguish the effects of SO_2, bisulfite ion, and sulfite ion, we first compared the effects of inhaled sodium sulfite delivered at 3 different levels of pH. One, pH 9, was chosen to study the effects of sulfite ion ($SO_3^=$) in the absence of significant amounts of SO_2 gas or bisulfite (HSO_3^-). Because of the pKa of the equilibrium reactions, more than 90% of the sulfur in solution at pH 9 is present as sulfite. The second, pH 6.6, was chosen to study the effects of bisulfite, since at this pH nearly 80% of the sulfur in solution is bisulfite, with only approximately 20% as sulfite. In addition, pH 6.6 is the recorded pH of human airways (13). Finally, since some SO_2 gas was generated during aerosolization of sulfite solutions at pH 6.6, we also studied the effects of sulfite solutions at pH 4, a pH calculated to increase SO_2 generation by more than 2 orders of magnitude with little change in the relative concentration of bisulfite (99 versus 80%). To test the role of change in airway pH as would be induced by the release of hydrogen ions by dissolved SO_2 and sulfite, we also studied the bronchoconstricting effects of buffered acetic acid aerosol matched with sulfite at pH 4 for overall (titratable) acidity.

Each subject came to the laboratory on 7 separate days. Concentration-response curves were performed to methacholine on the first day and to inhaled sodium sulfite aerosol at

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TABLE 1

SUBJECT CHARACTERISTICS

Subject No.	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (L)	FVC (L)	Medications	Methacholine Responsiveness* (mg/ml)	SO_2 Responsiveness† (ppm)
1	F	26	163	55	2.8	3.54	–	0.1	1.2
2	F	23	166	57	2.83	3.63	–	2.2	> 8‡
3	F	22	168	66	3.61	4.9	β-agonist inhaler	0.09	0.55
4	M	34	178	61	3.83	5.34	–	0.5	2.16
5	M	29	185	68	2.09	4.25	β-agonist inhaler	0.19	2.1
6	F	24	175	61	3.35	4.66	β-agonist inhaler	0.14	2.27
7	M	23	183	84	3.8	5.32	β-agonist inhaler	0.45	1.7
8	M	30	173	91	2.84	3.8	β-agonist inhaler	0.16	2.6
9	M	30	185	91	4.36	5.49	β-agonist inhaler	0.16	2.11
10	F	26	160	52	3.14	3.4	β-agonist inhaler	0.84	1.77

* Concentration of methacholine calculated by log-linear interpolation to produce a 100% increase in specific airway resistance (SRaw) after inhalation of isotonic saline. The provocative concentration for normal subjects > 4.0 mg/ml.

† Concentration of sulfur dioxide (SO_2) calculated by log-linear interpolation to produce a 100% increase in SRaw from baseline control value.

‡ SRaw increased by less than 100% after inhalation of 8 ppm SO_2, the highest concentration studied.

3 different levels of pH (4.0, 6.6, and 9.0) on 3 subsequent days as described below. To determine whether the observed effects of inhaled acidic sulfite aerosols could be explained by the effects of their acidity alone, on another day we performed a concentration-response curve to inhaled buffered acetic acid aerosol at pH 4. The challenges with the different sets of sulfite solutions and buffered acetic acid were randomly ordered and performed in a single-blind fashion. To ensure that any effects of inhaled sulfite we observed were not due to "specific sulfite sensitivity," we also performed an oral metabisulfite challenge on each subject. Finally, on another day we assessed each subject's sensitivity to inhaled SO_2 gas. 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, specific airway resistance (SRaw) (14).

Methacholine responsiveness was tested by measuring the SRaw of each subject every 30 s for 2 min before and for 2 min beginning 1 min after they inhaled 10 deep breaths of phosphate-buffered saline and doubling concentrations of methacholine (0.063 to 4.0 mg/ml) delivered by a nebulizer (No. 646; DeVilbiss, Somerset, PA) with a dose-metering device (15). The concentration of methacholine that provoked a 100% increase in SRaw from the post-saline baseline values (PC_{100}) was calculated by log-linear interpolation. Ninety-five percent of normal subjects tested in our laboratory have PC_{100} values for methacholine greater than 4.0 mg/ml.

To perform sodium sulfite concentration-response curves, we measured specific airway resistance every 30 s for 5 min before and 5 min after each subject breathed first an aerosol of 0.9% sodium chloride (adjusted to the same pH as the subsequent sulfite solutions with 1 N HCl or 1 N NaOH) and then aerosols of sodium sulfite (0.03, 0.1, 0.3, 1.0, 3.0, and 10.0 mg/ml adjusted to the same pH with 1 N HCl) for 1 min each. Solutions were inhaled during tidal breathing at 10-min intervals. The maximal average value of 4 consecutive measurements of SRaw was calculated during each 5-min measurement period. Each study was continued until SRaw increased by 100% or 8 L × cm H_2O/L/s (whichever was greater) or the highest concentration of sodium sulfite (10 mg/ml) had been inhaled.

Aerosol was generated by an ultrasonic nebulizer (Mistogen EN 145; Time Meter Co., Lancaster, PA), which had a fluid output measured gravimetrically of 4.65 g/min. Using a low flow, seven-stage cascade impactor (Intox Products, Albuquerque, NM), the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined for aerosols of pH 7.0 isotonic saline, pH 4.0 acetic acid and sodium acetate, and pH 4.0 and pH 9.0 sodium sulfite. The MMAD of these aerosols were similar, ranging from 5.6 μ with a GSD of 1.7 μ (saline) to 6.1 μ with a GSD of 1.6 μ (pH 9.0 sodium sulfite). The subjects inhaled aerosol through a mouthpiece connected to a glass T-piece that was directly attached to the nebulizer outflow port without a respiratory valve. The outflow end of the T-piece was attached to a 2.5-L teflon tube to prevent dilution of inspirate by room air.

In an identical fashion, we performed concentration-response curves with acetic acid buffered with sodium acetate. The subjects inhaled successively increasing concentrations of pH 4 buffered acetic acid matched to the titratable acidity of the concentrations of sodium sulfite given at pH 4. Titratable acidity was defined as the concentration of available hydrogen ions and recorded as the amount of base required to titrate a given acid solution to neutral pH. Thus, for the highest concentration of buffered acetic acid given, the pH of 100 ml could be neutralized to pH 7 by addition of 4.8 ml of 1 N NaOH, equivalent to the volume of NaOH required to neutralize 100 ml of the 10-mg/ml solution of sodium sulfite at pH 4.

Each solution was prepared and its pH was measured (pH Meter No. 43; Beckman Instruments, Irvine, CA) immediately before nebulization. Each solution also was adjusted to isotonicity (300 milliosmoles), as verified with a vapor-pressure osmometer (No. 5700B; Wescor, Logan, UT), by the addition of an appropriate amount of sodium chloride (all chemicals supplied by Fisher Scientific, Fair Lawn, NJ).

To perform oral metabisulfite challenges, we obtained the mean of 5 measurements of SRaw before and 30 min after each subject ingested first dextrose placebo and then 5, 15, and 50 mg of potassium metabisulfite enclosed in gelatin capsules. In order to insure that the subjects we studied were sensitive to SO_2, we measured SRaw every 30 s for 5 min before and after each subject inhaled humidified filtered air or increasing concentrations of SO_2 gas (0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 ppm) in humidified filtered air for 1 minute during eucapneic hyperpnea at 40 L/min. As in the sodium sulfite challenges, sulfur dioxide was inhaled at 10-min intervals, and values of SRaw were expressed as the highest average of 4 consecutive measurements.

To achieve the desired concentration of SO_2, we mixed metered flows of SO_2 from a calibrated tank (529 ppm) and air from a compressed air source in a 3-L glass mixing chamber. Before entering the mixing chamber, the air was humidified in a bubble humidifier and passed through a high-efficiency particulate absorption filter (Model 9FP-A-42-4505; ALF Co., Carpinteria, CA) to remove any water particles added during humidification. All tubing in contact with the gas mixture was made of teflon or glass. To document that temperature and relative humidity of the inspired air were relatively constant at 22° C and 75%, respectively, we measured continuously temperature and dew point with a digital humidity analyzer equipped with a mirrored dew point hygrometer and a platinum temperature probe (No. 911; E G and G, Waltham, MA). We measured continuously SO_2 concentrations from a needle just proximal to the mouthpiece with a pulsed fluorescent SO_2 analyzer (No. 43: Thermo-Electron Corp., Hopkinton, MA).

Subjects inhaled SO_2 from a mouthpiece attached to a Koegel one-way valve (E. Koegel, San Antonio, TX). To measure ventilation, a heated pneumotachygraph (Fleisch No. 3, Lausanne, Switzerland) was attached to the outflow tubing and its signal was integrated

with a respiratory integrator (No. FV156; Validyne, Northridge, CA) and recorded on photosensitive paper (Visicorder No. 1858; Honeywell, Denver, CO).

During each period of hyperpnea, the subjects controlled their expired tidal volume by following a linear voltmeter signal driven by the respiratory integrator and regulated their respiratory rate by keeping time with a metronome. End-tidal carbon dioxide tension was maintained at resting levels by measuring expired carbon dioxide percentage with a carbon dioxide analyzer (No. LB-1; Beckman, Fullerton, CA) and delivering a metered flow of 100% carbon dioxide into the inflow tubing.

To estimate the concentration of SO_2 gas generated during the nebulization of the sodium sulfite solutions, we entrained aerosols for 3 min from the mouthpiece through a Zefluor filter (No. P5PJ047; Gelman Science Inc., Ann Arbor, MI) to remove droplets and into the SO_2 analyzer. Teflon tubing and a polycarbonate filter holder (Nucleopore Corp., Pleasanton, CA) were employed to minimize SO_2 uptake by the apparatus. For each sodium sulfite solution at pH 4.0, 6.6, and 9.0 that released SO_2 in the concentration range detectable by our SO_2 analyzer (0.01 to 5.0 ppm), 5 SO_2 measurements were made, and the mean value was calculated.

For each sodium sulfite concentration-response curve, the concentration of sodium sulfite (in mg/ml) required to increase SRaw by 100% above baseline was calculated by linear interpolation using log base 3 data (because solutions were inhaled in 3-fold increasing concentrations), and these values were called the provocative concentration of sodium sulfite (PC_{100}). Values of PC_{100} for sulfur dioxide were calculated in a similar fashion using log base 2 data.

To determine whether there were significant differences among the subjects' airway responses to inhalation of sulfite solutions at pH 4.0, 6.6, and 9.0, we compared PC_{100} values using a 2-way analysis of variance followed by a Newman-Keuls multiple range test. The mean baseline SRaw values of the subjects before administration of aerosols of sodium sulfite at the different levels of pH and of buffered acetic acid were compared using a two-way analysis of variance.

Results

Nine of 10 subjects studied developed significant bronchoconstriction (SRaw > 100% above baseline) after inhalation of sulfite aerosols at all 3 levels of pH studied (figure 1). The one subject (Subject 2) who did not develop bronchoconstriction from inhaling any concentration of sulfite at any pH was also the only subject who did not develop bronchoconstriction after inhaling as much as 8 ppm SO_2 (table 1). In each of the 9 subjects who developed sulfite-induced bronchoconstriction, a 100% increase in SRaw occurred at the lowest sulfite concentration at pH 4.0 and at the highest concentration at pH 9 (figure 2). The mean values for PC_{100} were 0.17 mg/ml at pH 4, 0.49 mg/ml at pH 6.6, and 2.10 mg/ml at pH 9 and were significantly different ($p < 0.01$). Only one subject developed bronchoconstriction after inhaling acetic acid aerosol at pH 4; this effect occurred at a total (titrable) acidity more than 70 times higher than the acidity delivered in the concentration of sodium sulfite at pH 4 required to cause equivalent bronchoconstriction. No subject developed bronchoconstriction after oral ingestion of as much as 50 mg of encapsulated potassium metabisulfite. The mean baseline specific airway resistances of the subjects before inhaling the various pH sulfite aerosols and acetic acid aerosols were not significantly different.

No sulfur dioxide gas was detectable during aerosolization of sodium sulfite at pH 9 except at the highest concentration (10 mg/ml) where we measured 0.02 ppm SO_2 (table 2). Sulfur dioxide gas was detectable during aerosolization of sodium sulfite at pH 4 and pH 6.6. Aerosols of pH 4.0 solutions yielded approximately 20 times more SO_2 than did those of pH 6.6 solutions at concentrations of 0.1, 0.3, and 1.0 mg/ml. The relationship between SO_2 concentration and sulfite

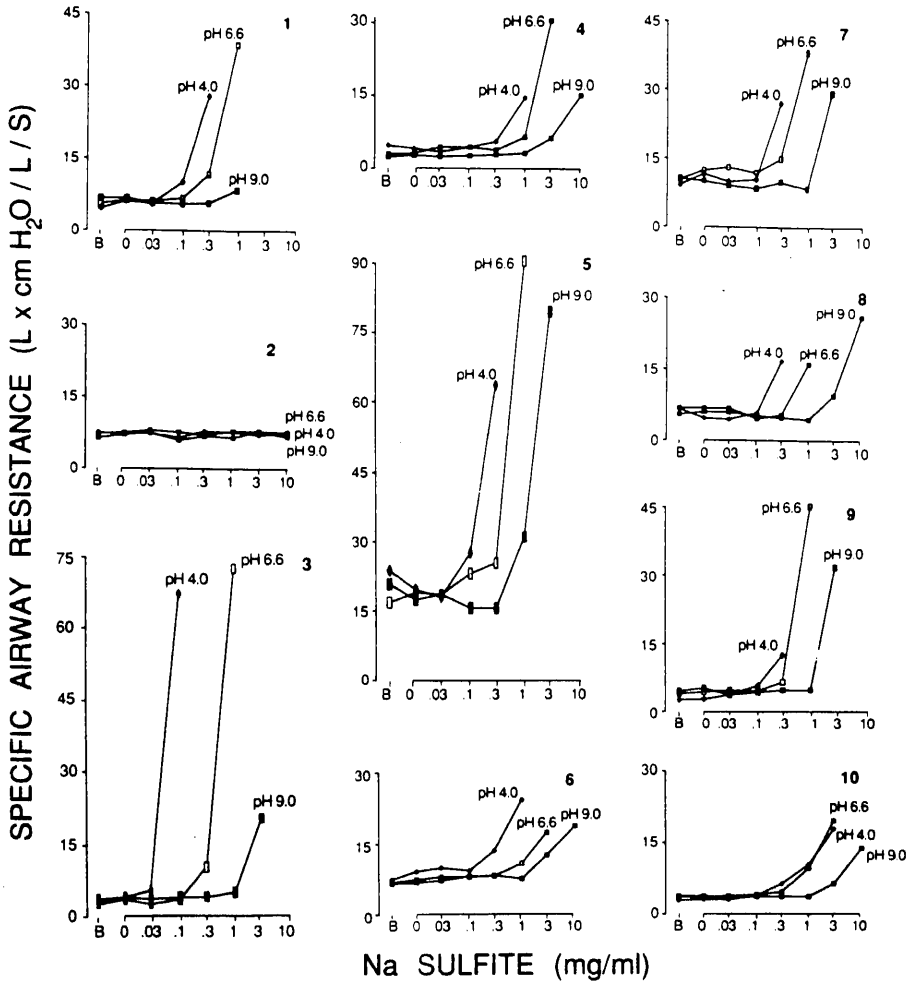

Fig. 1. Specific airway resistance before and after inhalation of increasing concentrations of nebulized sodium sulfite at pH 4.0, pH 6.6, and pH 9.0. Individual concentration-response curves are shown for each of 10 subjects.

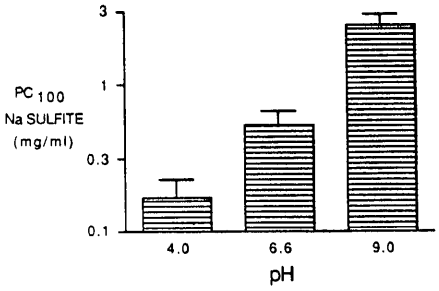

Fig. 2. Mean ± SEM values for the provocative concentration of sodium sulfite required to increase specific airway resistance by 100% above baseline in 9 subjects with asthma. Each subject inhaled on separate days sodium sulfite aerosols at pH 4.0, pH 6.6, and pH 9.0.

TABLE 2

SO_2 CONCENTRATIONS MEASURED IN AEROSOLS OF SODIUM SULFITE*

Sodium Sulfite (mg/ml)	SO_2 (ppm)		
	pH 4.0	pH 6.6	pH 9.0
0.03	0.12 ± 0.05	< 0.01†	< 0.01
0.1	0.50 ± 0.08	0.02 ± 0.01	< 0.01
0.3	1.37 ± 0.27	0.08 ± 0.04	< 0.01
1.0	3.42 ± 0.20	0.19 ± 0.03	< 0.01
3.0	> 5.0‡	1.38 ± 0.30	< 0.01
10.0	> 5.0	> 5.0	0.02 ± 0.01

* Values are mean ± SD (n = 5).
† 0.01 ppm is the lower detection limit of our SO_2 analyzer.
‡ 5.0 ppm is the upper detection limit of our SO_2 analyzer.

concentration was not linear, and the difference between the SO_2 concentrations at the 2 levels of pH was far less than that predicted by the equilibrium equations described above. These findings imply that equilibrium conditions did not exist during aerosolization.

Discussion

The results of this study confirm the reports of other investigators that inhaled sulfite aerosols are a stimulus to bronchoconstriction in subjects with asthma (4, 5, 7). This effect of sulfite is not restricted to patients with a clinical history of sulfite sensitivity or to subjects who demonstrate sensitivity to oral ingestion of metabisulfite because none of our subjects demonstrated such sensitivity.

The bronchoconstrictor effects of sodium sulfite aerosols were clearly pH-dependent, with the greatest effects occurring at the most acid pH (pH 4) and the smallest effects at alkaline pH (pH 9). Although inhalation of acid aerosols has been shown to induce bronchoconstriction in asthmatic subjects (16–18), acidity itself did not appear to be the stimulus to bronchoconstriction in this study; 8 of 9 subjects were unaffected by inhalation of aerosols of acetic acid with a total acidity from 20 to 70 times that contained in the concentrations of sulfite at pH 4 required to cause bronchoconstriction. The one subject who did develop bronchoconstriction after inhalation of acetic acid only did so at a concentration containing 70 times the acidity of the pH 4 sulfite solution causing equivalent bronchoconstriction. Rather than exerting a direct effect, decreasing pH most likely increased sodium sulfite-induced bronchoconstriction by altering the relative concentrations of sulfite ($SO_3^=$), bisulfite (HSO_3^-), and SO_2 gas.

Between the 2 ionic forms of sulfite, our results suggest that bisulfite is either the more potent or the only bronchoconstrictor stimulus, since inhalation of sodium sulfite solutions at pH 9, where most of the sulfur is in the form of sulfite, was the weakest stimulus to bronchoconstriction in every subject. The fact that bronchoconstriction did occur in 9 of 10 subjects after inhalation of concentrations of sodium sulfite at pH 9 not associated with any measurable generation of SO_2 gas implies that these ionic forms of sulfur oxide may themselves be capable of inducing bronchoconstriction. However, we cannot rule out the possibility that SO_2 was generated locally within the airways after deposition of the sodium sulfite aerosol on the relatively acidic luminal surface.

The results of our experiment do not allow us to determine with certainty the relative importance of bisulfite ion and SO_2 gas as stimuli to bronchoconstriction. Sodium sulfite aerosols at pH 4 and at pH 6.6 contained similar concentrations of bisulfite ion, but aerosolization of the solution at pH 4 resulted in generation of approximately 20 times as much SO_2 as did aerosolization of solutions at pH 6.6. Thus, if we had found no difference between the PC_{100} obtained for sodium sulfite solutions at these 2 pH levels, we could have concluded that bisulfite rather than SO_2 gas was the relevant stimulus. Alternatively, if we had found an approximately 20-fold difference in PC_{100}, we could have concluded that SO_2 itself was a more important stimulus to bronchoconstriction. However, the 3-fold difference we observed might be equally well explained by either hypothesis. If bisulfite is the more important stimulus, the 3-fold difference we observed could be explained by differences in local bisulfite concentrations when the ion is deposited primarily as large inhaled particles (at pH 6.6) or when the ion is formed locally from dissolved SO_2 gas (pH 4). Alternatively, if SO_2 gas is the more potent stimulus, our failure to observe a larger difference in the PC_{100} could be due to partial removal in the extrathoracic airways of the SO_2 generated during aerosolization.

Although we cannot determine whether SO_2 or bisulfite is the more potent bronchoconstricting stimulus, biochemical and *in vitro* evidence points toward bisulfite. Sulfites are highly reactive with proteins (19) and have been shown to avidly bind to rat airways lavaged with sulfite solution (20). A common target for sulfite binding are disulfide bonds, which can play a critical role in a variety of cell membrane receptors (21). By sulfonating specific disulfide bonds in the neuromuscular junction, sulfites have been shown to increase acetylcholine release and responsiveness in frog pectoral muscle (22, 23). One potential mechanism, therefore, by which sulfites would cause bronchoconstriction is by altering receptors for acetylcholine as well as for other mediators that modulate contraction of airway smooth muscle (24).

Despite the limitations in interpretation, our results are of possible clinical relevance in at least 2 respects. First, our finding that most patients with asthma develop bronchoconstriction after inhalation of sodium sulfite aerosols suggests that the practice of identifying patients thought to have "specific sulfite sensitivity" on the basis of a bronchoconstrictor response to inhaled sulfites is not valid, even if the specific conditions of aerosolization do not generate significant concentrations of SO_2. Second, our results suggest that sulfite aerosols, such as those formed in the atmosphere by the interaction of liquid water and SO_2, are capable of causing bronchoconstriction, especially when inhaled at acid pH.

Acknowledgment

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References

1. Sheppard D, Wong WS, Uehara CF, Nadel JA, Boushey HA. Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulfur dioxide. Am Rev Respir Dis 1980; 122:873–8.

2. Sheppard D, Saisho A, Nadel JA, Boushey HA. Exercise increases sulfur dioxide-induced bronchoconstriction in asthmatic subjects. Am Rev Respir Dis 1981; 123:486–91.

3. Stevenson DD, Simon RA. Sensitivity to ingested metabisulfite in asthmatic subjects. J Allergy Clin Immunol 1981; 68:26–32.

4. Werth GR. Inhaled metabisulfite sensitivity. J Allergy Clin Immunol 1983; 70:143.

5. Koepke JW, Selner JC, Christopher K, Glover G. Inhaled metabisulfite sensitivity. J Allergy Clin Immunol 1984; 73:135.

6. Goldfarb G, Simon R. Provocation of sulfite sensitive asthma. J Allergy Clin Immunol 1984; 73:135.

7. Schwartz HJ, Chester EH. Bronchospastic responses to aerosolized metabisulfite in asthmatic subjects: potential mechanisms and clinical implications. J Allergy Clin Immunol 1984; 74:511–3.

8. Frank NR, Yoder RE, Brain JD, Yokoyama E. SO_2 (^{35}S labelled) absorption by the nose and mouth under conditions of varying concentration and flow. Arch Environ Health 1969; 18:315–22.

9. Speizer FF, Frank NR. The uptake and release of SO_2 by the human nose. Arch Environ Health 1966; 12:725–8.

10. Huss A, Eckert C. Equilibria and ion activities in aqueous sulfur dioxide solutions. J Phys Chem 1977; 81:2268–70.

11. Connick RE, Tam MT, von Deuster E. Equi-

librium constant for the dimerization of bisulfite ion to form $S_2O_5^{2-}$. J Inorg Chem 1982; 21:103–7.

12. Hayon E, Treinin A, Wilf J. Electronic spectra, photo-chemistry and autoxidation mechanism of the sulfite-bisulfite-pyrosulfite systems. The SO_2^-, SO_3^-, SO_4^-, and SO_5^- radicals. J Am Chem Soc 1972; 94:47–57.

13. Bodem CR, Lampton LM, Miller DP, Tarka EF, Everett ED. Endobronchial pH. Am Rev Respir Dis 1983; 127:39–44.

14. Dubois AB, Botelho SY, Comroe JH. A new method for measuring airway resistance in man using a body plethysmograph: values in normal subjects and in patients with respiratory disease. J Clin Invest 1956; 35:327–35.

15. Chai H, Farr RS, Froehlich L, *et al.* Standardization of bronchial inhalation challenge procedures. J Allergy Clin Immunol 1975; 56:323–7.

16. Fine JM, Gordon T, Thompson JE, Sheppard DS. The role of titratable acidity in acid aerosol-induced bronchoconstriction. Am Rev Respir Dis 1987; 135:826–30.

17. Koenig JQ, Pierson WE, Horike M. The effects of inhaled sulfuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis 1983; 128:221–5.

18. Utell MJ, Morrow PE, Speers DM, Darling J, Hyde RW. Airway responses to sulfate and sulfuric acid aerosols in asthmatics. Am Rev Respir Dis 1983; 128:444–50.

19. Petering DH, Shih NT. Biochemistry of bisulfite-sulfur dioxide. Environ Res 1975; 9:55–65.

20. Dahl AR, Furst GM, Hahn FF. Binding of sulfite-introduced ^{35}S to the pleura and walls of conducting airways in rat lungs. Toxicol Lett 1981; 8:295–8.

21. Strauss WL. Sulfhydroxyl groups and disulfide bonds: modifications of amino acid residues in studies of receptor structure and function. In: Venter JC, Harrison LC, eds. Membranes, detergents, and receptor solubilization. New York: Allan R Liss, 1984; 85–97.

22. Steinacker A. Presynaptic effects of sodium bisulfite at the frog neuromuscular junction. J Neurosci Res 1982; 7:313–9.

23. Steinacker A. Sulphonation of cholinergic receptor disulphide bond increases response to ACh. Nature 1979; 278:358–60.

24. Steinacker A. A suggested mechanism of action for sulfite sensitivity. J Allergy Clin Immunol 1985; 77:116–7.

Symptomatic Bronchoconstriction after Short-Term Inhalation of Sulfur Dioxide[1,2]

JOHN R. BALMES, JONATHAN M. FINE, and DEAN SHEPPARD

SUMMARY We studied the relationship between duration and concentration of exposure in SO_2-induced bronchoconstriction in 8 asthmatic subjects. On separate days, we administered SO_2 in humidified air through a mouthpiece at 2 concentrations (0.5 and 1.0 ppm) for 3 time periods (1, 3, and 5 min) during eucapnic hyperpnea (60 L/min). Humidified air was administered for 5 min as a control. Bronchoconstriction was assessed by measurement of specific airway resistance (SRaw). The magnitude of the bronchoconstrictor response to both concentrations of SO_2 increased progressively over the 3 time periods studied. The mean (± SE) increase in SRaw (in L × cm H_2O/L/s) and percent increase above baseline (in parentheses) after each exposure to SO_2 were as follows: 2.5 ± 0.3 (34%) after 0.5 ppm for 1 min; 7.5 ± 4.7 (93%) after 1.0 ppm for 1 min; 13 ± 3.2 (173%) after 0.5 ppm for 3 min; 31.4 ± 7.4 (395%) after 1.0 ppm for 3 min; 19.6 ± 4.0 (234%) after 0.5 ppm for 5 min; 44.1 ± 9.8 (580%) after 1.0 ppm for 5 min; 3.5 ± 1.5 (46%) after humidified air for 5 min. For the group, the increases in SRaw caused by inhalation of both concentrations of SO_2 for 1 min were small. However, 2 of 8 subjects did develop large increases in SRaw and chest tightness after inhalation of 1.0 ppm for 1 min. Seven of 8 subjects developed wheezing, chest tightness, or dyspnea and used an inhaled bronchodilator after inhalation of 0.5 ppm for 3 and 5 min and 1.0 ppm for 3 minutes. Two subjects were unable to complete the 5-min exposure to 1.0 ppm because of symptomatic bronchoconstriction. These results suggest that short-term (i.e., ≤ 3 min) inhalation of near ambient concentrations of SO_2 can cause clinically significant bronchoconstriction. If these effects are confirmed in freely breathing subjects, pollution control strategies may need to be devised to prevent such short-term peak concentrations of SO_2 from occurring in ambient air.

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Introduction

Symptomatic bronchoconstriction in persons with asthma is the most widely recognized adverse health effect caused by near ambient concentrations of the common air pollutant sulfur dioxide (SO_2). This effect has been observed in numerous studies when subjects with asthma have inhaled SO_2 in concentrations of 0.4 ppm or greater during exercise or voluntary hyperpnea (1-13). Sulfur-dioxide-induced bronchoconstriction has thus become a major issue in state and federal efforts to minimize the likelihood of adverse health effects from SO_2 in ambient air. The current primary National Ambient Air Quality Standard for SO_2 is based on a 24-h average concentration (14). Yet, SO_2-induced bronchoconstriction occurs after exposures of 5 min or less (2, 3, 6, 7, 9, 10, 12). Peak short-term ambient concentrations of SO_2 can be many times higher than the 24-h averages, especially in the vicinity of large point sources (15). Thus, recent regulatory attention has focused on the need for a short-term standard for SO_2.

Previously published studies have reported that maximal bronchoconstriction for SO_2 occurs within 10 min of the onset of exposure (13) and that the duration of exposure beyond 10 min does not significantly influence the magnitude of the response (16). However, the effect of exposure duration on the bronchoconstrictor response to inhalation of SO_2 for time periods less than 10 min has not been systematically examined. This issue has major social and economic implications. For example, if exposure to SO_2 for periods as short as 1 min caused bronchoconstriction as severe as that caused by exposure for 10 min or more, regulatory strategies would need to be devised to prevent even very brief exposures to peak SO_2 concentrations such as those that occur from drifting SO_2 plumes in the vicinity of point sources (15). Such strategies could be considerably more cumbersome and expensive than those required to prevent exposures for 10 min or longer.

The purpose of this study was to examine the bronchoconstrictor effects of inhalation of SO_2 for very short time periods (1 to 5 min) and to determine the duration-response relationship for 2 concentrations of SO_2 (0.5 and 1.0 ppm) within the range encountered in SO_2 plumes (14, 15).

Methods

The subjects were 8 nonsmoking volunteers who were informed 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 used theophylline or β-adrenergic agonists within 12 h or consumed caffeine within 4 h before any experiment. No subject received oral or inhaled corticosteroid preparations during the study period. All subjects denied having an upper respiratory tract infection within 6 wk prior to the study.

Each subject came to the laboratory on 8 nonconsecutive days. On the initial day, baseline lung function and methacholine responsiveness were measured. On each subsequent day, the subject was exposed in a single-blind fashion to one of the following: humidified air for 5 min or SO_2 in 1 of 6 different exposure conditions. Each inhalational exposure occurred at the same time on a separate day. To assess the response to these exposures, the subject's 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, specific airway resistance (SRaw). Prior to and 1 min after each inhalational exposure, 5 SRaw measurements were recorded, 1 every 30 s for 2 min, and the mean value was calculated.

On the first study day, spirometry (No. 822; Ohio Medical Products, Madison, WI) was performed, and methacholine responsiveness was tested by measuring the subject's SRaw before and after inhalation of 10 breaths of phosphate-buffered saline and doubling concentrations of methacholine (0.063 to 4.0

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TABLE 1
SUBJECT CHARACTERISTICS

Subject No.	Sex	Age (yr)	Height (cm)	Weight (kg)	FEV_1 (L)	FEV_1 (% pred)	FVC (L)	FVC (% pred)	Methacholine Responsiveness* (mg/ml)	Medications
1	F	28	165	52	2.52	79	3.38	88	< 0.06†	Theophylline, β-agonist inhaler
2	F	24	175	61	3.37	94	4.53	104	0.14	β-agonist inhaler
3	M	31	185	84	4.36	94	5.49	96	0.16	Theophylline, β-agonist inhaler
4	M	32	185	87	3.89	85	6.83	121	1.0	β-agonist inhaler
5	M	23	180	82	3.84	83	5.63	104	1.7	β-agonist inhaler
6	M	35	178	61	3.83	93	5.34	105	0.50	β-agonist inhaler
7	M	30	173	91	2.80	71	3.99	81	0.10	β-agonist inhaler
8	M	39	183	84	4.47	106	5.84	106	1.8	β-agonist inhaler, beclomethasone inhaler

* Concentration of methacholine required to produce a 100% increase in specific airway resistance above baseline calculated by linear log interpolation.
† Subject responded to the first dose of methacholine (0.063 mg/ml) with > 100% increase in SRaw.

mg/ml) delivered by a Devilbiss no. 646 nebulizer (Devilbiss Co., Somerset, PA) with a dose-metering device calibrated to deliver 0.01 ml per breath (17). The concentration of methacholine that provoked an increase of 100% in SRaw from the postsaline value (PC_{100}) was calculated by linear interpolation. Subject characteristics are described in table 1. Predicted values for the spirometric parameters described are those of Knudson and coworkers (18).

On subsequent days, the subject inhaled in random order either humidified air for 5 min or SO_2 and 1 of 2 concentrations (0.5 and 1.0 ppm) for 1 of 3 exposure durations (1, 3, and 5 min). To achieve the desired concentration of SO_2, metered flows of SO_2 from a calibrated tank (529 ppm) and air from a compressed air source were mixed in a 3-L glass mixing chamber. Before entering the mixing chamber, the air was partially humidified in a bubble humidifier and passed through a high-efficiency particulate absorption filter (Model 9FP-A-42-4505; ALF Co., Carpinteria, CA) to remove any water particles added during humidification. All tubing in contact with the gas mixture was made of teflon or glass. The temperature and relative humidity of the inspired air were measured continuously with a digital humidity analyzer equipped with a mirrored dew point hygrometer and a platinum temperature probe (No. 911; E G and G, Waltham, MA). SO_2 concentrations were measured continuously from a needle just proximal to the mouthpiece with a pulsed fluorescent SO_2 analyzer (No. 43; Thermo-Electron Corp., Hopkinton, MA).

The subject inhaled humidified air and SO_2 from a mouthpiece attached to a Koegel one-way valve (E. Koegel, San Antonio, TX) under conditions of eucapnic hyperpnea at 60 L/min. To measure minute ventilation, a heated pneumotachygraph (No. 3; Fleisch, Lausanne, Switzerland) was attached to the outflow tubing, and its generated signal was integrated with a respiratory integrator (No. FV156; Validyne Corp., Northridge, CA) and recorded on photosensitive paper (No. 1858 Visicorder; Honeywell, Denver, CO).

During each period of hyperpnea, the subject controlled expired tidal volume by following a calibrated linear voltmeter signal driven by the respiratory integrator and regulated respiratory rate by keeping time with a metronome. End-tidal CO_2 tension was measured with a CO_2 analyzer (No. LB-1; Beckman Instruments, Fullerton, CA) and maintained in the range of 4.5 to 5% by addition of a metered flow of 100% CO_2 into the inflow tubing.

Because the data were not normally distributed, Wilcoxon's paired sample test was employed to determine whether the mean increases in SRaw after the various inhalational exposures were significantly different from

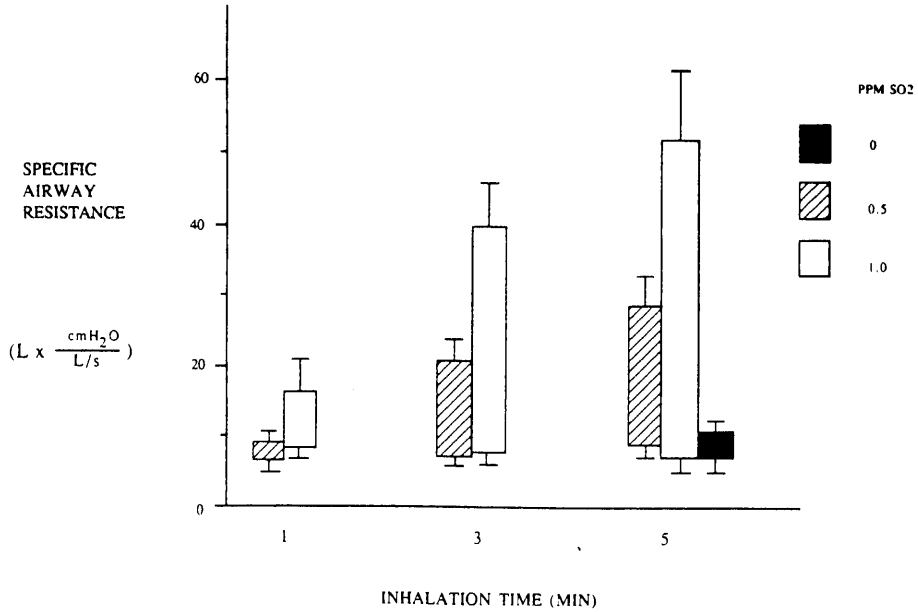

Fig. 1. Mean values for specific airway resistance before (bottom of bars − SEM) and after (top of bars + SEM) inhalation of 0.5 and 1.0 ppm SO_2 for 1, 3, and 5 min, and before (bottom of bars − SEM) and after (top of bars + SEM) inhalation of filtered, humidified air for 5 min, in 8 subjects with asthma.

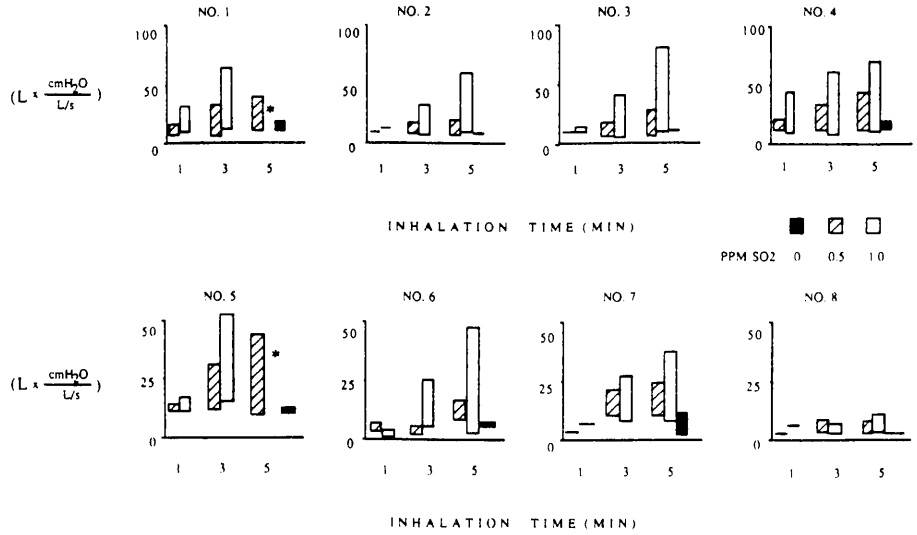

Fig. 2. Individual values for specific airway resistance, before (bottom of bars) and after (top of bars) inhalation of 0.5 and 1.0 ppm SO_2 for 1, 3, and 5 min, and before (bottom of bars) and after (top of bars) inhalation of filtered, humidified air for 5 min, for each of 8 subjects with asthma. Note that the scale of the ordinate axes on the top row is different from that of the bottom row. Asterisk indicates missing data because of inability of subject to tolerate inhalation of 1.0 ppm SO_2 for 5 min.

the mean baseline values. To assess the differences in mean increases in SRaw provoked by each of the 6 different exposure situations, Friedman's randomized block analysis of variance by ranks followed by a nonparametric equivalent of the Newman-Keuls multiple range test was employed. Because this test requires that there be equal numbers of data in each group, the data for the 5 min-exposure to 1.0 ppm SO_2 were excluded from this analysis as only 6 of the 8 subjects in the study were able to tolerate this exposure. In order to evaluate the data for the 5-min exposure to 1.0 ppm SO_2, Wilcoxon's paired sample test was employed on data generated for the 6 subjects who completed all exposures. To assess the differences in mean ventilatory rates, temperature and dew point of the inspired air, and baseline SRaw values among the various exposures, two-way analysis of variance was employed. A p value of < 0.05 was considered statistically significant.

Results

Inhalation of both concentrations of SO_2 for all 3 time periods studied caused significant increases in SRaw above baseline (figure 1). However, the effects of 1-min exposures were primarily confined to 2 subjects (Subjects 1 and 5), both of whom developed chest tightness and a marked increase in SRaw after a 1-min exposure to 1.0 ppm SO_2 (figure 2). After each 3- and 5-min exposure, 7 of 8 subjects developed large increases in SRaw associated with wheezing, chest tightness, or dyspnea and requested inhaled bronchodilator therapy. Two subjects (Subjects 1 and 5) were unable to tolerate more than 3 min of exposure to 1.0 ppm SO_2 because of the development of symptomatic bronchoconstriction and thus did not complete the 5-min exposure to that concentration.

Over the time periods and concentrations employed in this study, the increases in SRaw caused by SO_2 were both time and concentration dependent. Thus, for both concentrations, SRaw increased significantly more after 3 min (173% for 0.5 ppm and 395% for 1.0 ppm) than after 1 min (34% for 0.5 ppm and 93% for 1.0 ppm) and also increased significantly more after 5 min (234% for 0.5 ppm and 580% for 1.0 ppm) than after either shorter time period (figure 1). For the 3- and 5-min time periods, the effect of 1.0 ppm was significantly greater than that of 0.5 ppm.

Most subjects developed small increases in SRaw after 5 min of hyperpnea with humidified, filtered air (mean increase, 46% above baseline) (figure 2) that were significantly less than the increases caused by inhalation of both concentrations of SO_2 for 5 min ($p < 0.025$).

No significant differences were found among the mean baseline SRaw values prior to the various inhalational exposures. There were also no significant differences in the mean minute ventilation of the subjects and in both the mean temperature and mean dew point of the inspired air during the various exposures (table 2).

Discussion

The results of this study show that hyperpnea through a mouthpiece with concentrations of SO_2 as low as 0.5 ppm for periods as brief as 3 min can cause symptomatic bronchoconstriction requiring bronchodilator therapy in subjects with asthma. Hyperpnea with 1 ppm SO_2 for 5 min uniformly caused symptomatic bronchoconstriction requiring therapy. Two subjects were unable to tolerate more than 3 min of inhalation of 1.0 ppm SO_2.

Most previous studies of SO_2-induced bronchoconstriction have examined the effects of exposures of 5 min or greater (1–13). On the basis of these studies, it has become clear that the magnitude of the bronchoconstrictor response to SO_2 is primarily dependent on the concentration of SO_2 in inspired air and the minute ventilation at which it is inhaled (3, 7, 19, 20). The magnitude of bronchoconstriction produced by exposure durations as long as 5 h has not been consistently larger than that produced by 5- to 10-min exposures (21), suggesting that most of the bronchoconstrictor effect of SO_2 occurs within the first few minutes of exposure. One previous study systematically examined the significance of exposure duration by comparing the bronchoconstrictor effects of 1 ppm SO_2 inhaled during exercise at identical work rates (producing a minute ventilation of 41 L/min) in the same subjects for exposure durations of either 10 or 30 min (16). The bronchoconstrictor response to 10- and 30-min exposures was the same, confirming that the exposure time required for a maximal bronchoconstrictor response to SO_2 is 10 min or less. However, on the basis of these data, it

TABLE 2

EXPOSURE CHARACTERISTICS

Concentration of SO_2 (ppm)	Exposure Duration (min)	Mean Ventilation (L/min)	Mean Temperature of Inspired Air (°C)	Mean Dew Point of Inspired Air (°C)
0.5	1	65.6	23.5	17.3
0.5	3	62.2	22.3	17.3
0.5	5	63.1	22.9	17.5
1.0	1	64.1	21.8	17.3
1.0	3	61.3	21.0	17.3
1.0	5	61.1	22.1	17.5
0	5	62.9	22.6	17.4

was not possible to determine the relationship between exposure duration and response for shorter periods of exposure. In the present study, the magnitude of SO_2-induced bronchoconstriction was clearly shown to increase directly with the duration of exposure for periods of 5 min or less. This relationship was true for both concentrations of SO_2 studied. Because of the method of exposure studied (eucapnic hyperpnea) and the large magnitude of the responses observed (preventing 2 subjects from even completing 5-min exposures), we were unable to directly examine the role of exposure duration for longer exposure periods. Nonetheless, on the basis of these and previously reported data (16), it seems likely that the maximal response to SO_2 occurs after 5 to 10 min of exposure.

The possible clinical significance of the effects of 3- and 5-min exposures to SO_2 we observed deserves comment. The changes in SRaw caused by both concentrations of SO_2 were quite large. Seven of 8 subjects developed wheezing, chest tightness, or dyspnea after the 3-min exposures to both concentrations of SO_2 and the 5-min exposure to 0.5 ppm, which caused them to request bronchodilator therapy. In 2 subjects, the severity of the response prevented completion of the 5-min exposure to 1 ppm. One other subject was unable to maintain a minute ventilation of 60 L/min throughout that exposure. All 6 subjects completing the 5-min exposure to 1 ppm SO_2 again developed chest tightness, wheezing, and obvious respiratory distress and requested treatment. These responses are qualitatively similar to the maximal acute bronchoconstrictor responses we have observed from other nonimmunologic stimuli (e.g., hyperpnea with cold, dry air, inhalation of aerosols of distilled water or hypertonic saline and inhalation of pharmacologic bronchoconstrictors such as histamine or methacholine).

In the present study, subjects breathed through a mouthpiece while wearing a noseclip, thereby insuring oral breathing. This method of exposure was chosen because we wanted to examine the effects of very short exposure durations and thought it was important to closely regulate ventilation during these exposures. If we had increased ventilation with exercise rather than with voluntary hyperpnea, ventilation would have been changing at a variable rate throughout the 1-min exposure. The short-term exposures we studied roughly mimic the exposures of strenuously exercising persons to drifting plumes of SO_2 such as those that occur in the vicinity of point sources. However, it has previously been shown that mouth-only exposure exaggerates the bronchoconstrictor effects of SO_2 since the mouth is a less efficient scrubber of SO_2 than is the nose (2–4, 6, 22). Therefore, it is possible that the magnitude of bronchoconstriction we observed was greater than would have occurred after similar exposures during oronasal breathing. In at least one previous study at exercise work rates sufficient to achieve the ventilatory rate used in this study (60 L/min), the difference between the magnitude of bronchoconstriction caused by oral and oronasal breathing was small but statistically significant (3).

The findings in the present study that 3-min exposures to SO_2 concentrations of 0.5 and 1.0 ppm caused significant bronchoconstriction are consistent with the results of a previous study designed to determine whether tolerance to the bronchoconstriction induced by a low concentration of SO_2 in subjects with asthma develops with repeated exposure. In that study (8), we showed that an initial inhalation of 0.5 ppm for 3 min at ventilatory rates of 30 to 50 L/min caused a significant mean increase in SRaw (104%) in the 8 subjects tested. The larger effect of a 3-min exposure to 0.5 ppm SO_2 seen in the present study (a 173% increase in SRaw) is most likely due to the higher minute ventilation employed (60 L/min compared to 30 to 50 L/min).

The early time course of SO_2-induced bronchoconstriction has important regulatory implications. The Clean Air Act requires that the U. S. Environmental Protection Agency set National Ambient Air Quality Standards in order to protect the health of the most sensitive segments of the population (14). Symptomatic bronchoconstriction, such as that provoked by exposures to 0.5 ppm SO_2 as brief as 3 min in the present study, can reasonably be considered an adverse health effect (23). Although prolonged SO_2 levels in excess of 0.5 ppm are rare with current U.S. air quality, 5- to 10-min peaks exceeding this level occur in the vicinity of point sources such as smelters and power plants (14). If the results of the present study are confirmed for subjects exposed to SO_2 during unencumbered breathing, then the Clean Air Act would appear to require an SO_2 standard designed to protect persons with asthma from 3-min exposures to 0.5 ppm.

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References

1. Sheppard D, Wong WS, Uehara C, Nadel JA, Boushey HA. Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulfur dioxide. Am Rev Respir Dis 1980; 122:873–8.

2. Kirkpatrick MB, Sheppard D, Nadel JA, Boushey HA. Effect of the oronasal breathing route on sulfur dioxide-induced bronchoconstriction in exercising asthmatic subjects. Am Rev Respir Dis 1982; 125:627–31.

3. Bethel RA, Erle DJ, Epstein J, Sheppard D, Nadel JA, Boushey HA. Effect of exercise rate and route of inhalation on sulfur dioxide-induced bronchoconstriction in asthmatic subjects. Am Rev Respir Dis 1983; 128:592–6.

4. Bethel RA, Epstein J, Sheppard D, Nadel JA, Boushey HA. Sulfur dioxide-induced bronchoconstriction in freely breathing, exercising, asthmatic subjects. Am Rev Respir Dis 1983; 128:987–90.

5. Koenig JQ, Pierson WE, Horike M, Frank R. A comparison of the pulmonary effects of 0.5 ppm versus 1.0 ppm sulfur dioxide plus sodium chloride droplets in asthmatic adolescents. J Toxicol Environ Health 1983; 11:129–39.

6. Linn WS, Venet TG, Shamoo DA, Respiratory effects of sulfur dioxide in exercising asthmatics: influence of upper respiratory defenses. Am Rev Respir Dis 1983; 127:278–83.

7. Linn WS, Shamoo DA, Spier CE. Respiratory effects of 0.75 ppm sulfur dioxide in exercising asthmatics: influence of upper respiratory defenses. Environ Res 1980; 30:340–8.

8. Sheppard D, Epstein J, Bethel RA, Nadel JA, Boushey HA. Tolerance to sulfur dioxide-induced bronchoconstriction in subjects with asthma. Environ Res 1983; 30:412–9.

9. Linn WS, Shamoo DA, Venet TG, Bailey RM, Wightman LH, Hackney JD. Comparative effects of sulfur dioxide exposures at 5° C and 22° C in exercising asthmatics. Am Rev Respir Dis 1984; 129:234–9.

10. Linn WS, Shamoo DA, Venet TG, Spier CE, Valencia LM, Anzar UT, Hackney JD. Combined effect of sulfur dioxide and cold in exercising asthmatics. Arch Environ Health 1984; 39:339–46.

11. Schacter EN, Witek TJ, Beck GJ, *et al.* Airway effects of low concentrations of sulfur dioxide: dose-response characteristics. Arch Environ Health 1984; 39:34–42.

12. Linn WS, Shamoo DA, Anderson KR, Whynot JD, Arol EL, Hackney JD. Effects of heat and humidity on the responses of exercising asthmatics to sulfur dioxide exposure. Am Rev Respir Dis 1985; 131:221–5.

13. Roger LJ, Kehrl HR, Hazuch M, Horstman DH. Bronchoconstriction in asthmatics exposed to sulfur dioxide during repeated exercise. J Appl Physiol 1985; 59:784–91.

14. U.S. Environmental Protection Agency. Air quality criteria for particulate matter and sulfur oxides. Research Triangle Park, NC: Environmental Criteria and Assessment Office, 1982.

15. Lott RA. SO_2 concentrations near tall stacks. Atmos Environ 1985; 19:1589–99.

16. Kehrl HR, Roger LJ, Hazucha MJ, Horstman DH. Differing response of asthmatics to SO_2 exposure with continuous and intermittent exercise. Am Rev Respir Dis 1987; 135:350–5.

17. Chai H, Farr RS, Froehlich L, *et al.*. Standardization of bronchial inhalation challenge procedures. J Allergy Clin Immunol 1975; 56:323–7.

18. Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the maximum expiratory flow-volume curve with growth and aging. Am Rev Respir Dis 1983; 127:725–34.

19. Linn WS, Bailey RM, Shamoo DA, Venet TG, Wrightman LH, Hackney JD. Respiratory responses of young adult asthmatics to sulfur dioxide exposure under simulated ambient conditions. Environ Res 1982; 29:220–32.

20. Linn WS, Venet TG, Shamoo DA, *et al.* Respiratory effects of sulfur dioxide in heavily exercising asthmatics: a dose-response study. Am Rev Respir Dis 1983; 127:278–83.

21. Linn WS, Arol EL, Shamoo DA, *et al.* Asthmatics' response to 6-hr sulfur dioxide exposures on two successive days. Arch Environ Health 1984; 39:313–9.

22. Speizer FE, Frank NR. A comparison of changes in pulmonary flow resistance in healthy volunteers acutely exposed to SO_2 by mouth and by nose. Br J Ind Med 1966; 23:75–9.

23. American Thoracic Society. Guidelines as to what constitutes an adverse health effect, with special reference to epidemiologic studies of air pollution. Am Rev Respir Dis 1985; 131:666–8.