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COMPARISON OF NORMAL AND ASTHMATIC SUBJECTS' RESPONSES TO SULFATE POLLUTANT AEROSOLS

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ABSTRACT

Epidemiological studies support an association between elevated levels of sulfates and acute respiratory disease. To determine if these pollutants produce airway hyperreactivity, 16 normal and 17 asthmatic subjects inhaled a control NaCl aerosol and the following sulfates: ammonium sulfate, sodium bisulfate, ammonium bisulfate, and sulfuric acid. A Lovelace generator produced particles with an average MMAD of ~ 1.0 μm ($\sigma_g \approx 2.0$) and concentrations of 0.1 and 1.0 mg/m^3. By double-blind randomization, all subjects breathed these aerosols for a 16-minute period. To determine if sulfate inhalation caused increased reactivity to a known bronchoconstrictor, all subjects inhaled carbachol following each 16-minute exposure. Before, during, and after exposure, we performed pulmonary function studies. When compared to NaCl, sulfate (1 mg/m^3) produced significant reductions in airway conductance and flow rates in asthmatics. The two most sensitive asthmatics demonstrated changes even at 0.1 mg/m^3 sulfate. To a far more significant degree, the bronchoconstrictor action of carbachol was potentiated by sulfates more or less in relation to their acidity in normals and asthmatics.

INTRODUCTION

Recent epidemiologic studies have emphasized the relationship between elevated levels of air pollutants and symptoms in asthmatic subjects (FINKLEA et al., 1974). The identification of the harmful agent or agents responsible for provoking such symptoms remains incomplete. Cohen and co-workers (1972) reported that acute asthmatic episodes correlated with elevations in the levels of a number of atmospheric pollutants including sulfur dioxide, suspended sulfates, and nitrates, while in a second study the risk of asthmatic attack correlated with the combined sulfate-nitrate levels (FRENCH, 1975). Experimentally, short-duration exposures to relatively high concentrations of sulfuric acid aerosols have been effective in increasing airway resistance in humans (AMDUR, SILVERMAN AND DRINKER, 1952) and slowing mucociliary clearance in donkeys (SCHLESINGER, LIPPMANN, ALBERT, 1978) but other investigators have failed to detect airway changes at comparable exposure levels (AVOL et al., 1975; SACKNER et al., 1978) These inconsistent findings and the lack of definitive studies in large groups of susceptible individuals indicate a great deal more data are needed before the significance of the pollutant sulfates can be established.

Prior epidemiologic reports have also noted a correlation between acute respiratory illness and elevated levels of pollutants (LEVY, GENT AND NEWHOUSE, 1977). Utell and co-workers (1979) recently demonstrated that particulate nitrate produced no effect either in normals or asthmatics after short-term inhalation. However, when subjects with uncomplicated influenza infection were exposed to an identical nitrate concentration, they uniformly demonstrated transient reduction in airway conductance and diminution in forced expiratory flow rates lasting up to 3 weeks following infection (UTELL, et al., 1980). They concluded that a healthy population normally unresponsive to a common air pollutant, when beset with even a nonpneumonic respiratory infection, developed transient airway dysfunction after inhalation of a pollutant.

Following these initial studies with nitrates, we began a series of acute inhalation studies in normal and asthmatic subjects with acidic sulfate aerosols. In addition, we have examined airway reactivity following inhalation of the parasympathomimetic agent carbachol to identify any potentiation of airway reactivity after sulfate exposure. These studies form the basis of this report.

EXPERIMENTAL APPROACH

For the acute studies, we evaluated airway responses to inhaled sulfate aerosols in a population of 16 healthy laboratory workers of mean age 27 years and 17 asymptomatic asthmatics of mean age 26 years. All subjects were nonsmokers. Although the asthmatics utilized either intermittent or daily bronchodilators, this therapy was discontinued 24 hours prior to testing and none required chronic corticosteroid therapy. As a prerequisite to the diagnosis of asthma and inclusion in this study, the asthmatics demonstrated an abnormal increase in airway resistance after inhaling carbachol. All participants completed a self-administered, modified respiratory ques-

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tionnaire and gave informed consent.

For all exposures, the apparatus illustrated in Figure 1 was used. The aerosols were dispersed by a Lovelace nebulizer using approximately 1% (w/v) solute concentration. The aerosols included sodium bisulfate ($NaHSO_4$), ammonium sulfate ($(NH_4)_2SO_4$), ammonium bisulfate (NH_4HSO_4), sulfuric acid (H_2SO_4) and sodium chloride (NaCl). Slight solute adjustments were made to keep SO_4 levels constant but these produced no significant change in operating conditions or aerosol characteristics. The aerosols produced had a range of MMADs of between 0.5 - 1 μm (σ_g 1.5 - 2.2). The aerosols were diluted with anhydrous air so that the concentration was 1 mg/m^3 for the high sulfate and 0.1 mg/m^3 for the low sulfate exposures. The relative humidity of the aerosol was kept between 20 and 25% to assure all aerosols consisted of dry particles (CHARLSON, et al., 1978; AHLBERG AND WINCHESTER, 1978). The diluted aerosol was brought to Boltzmann equilibrium before entering the "bag-in-box" exposure chamber by passing it through a deionizer.

For each exposure the subject inhaled the aerosol through a one-way respiratory valve by which the expired aerosol was diverted to an absolute filter (Fluorpore) sampling at 50 l/min through a loose coupling. This sampling arrangement provided no respiratory resistance to the individual. It is designed not to unseat the respiratory valve, yet provide sufficient capacity to sample each expiration. For the 16 minute exposure, the chamber was refilled after about 8 minutes; this refilling required approximately 1 - 2 minutes.

Before and after each exposure, the subject's pulmonary function was assessed in an air-conditioned, intergrated flow, pressure-corrected body plethysmograph. Flow at the mouth was measured with a Fleisch (No. 7322) pneumotachograph and lung volume changes were derived from the box pressure signal. Flow-volume curves were displayed on a Tektronix storage oscilloscope (Model 5103N) and photographed. Airway resistance and thoracic gas volume were measured during panting (DuBOIS, BOTELHO AND COMROE, 1956). Airway resistance was corrected for lung volume and expressed as its reciprocal, specific airway conductance (SGaw).

Two types of expiratory flow-volume curves were obtained. The subject first inspired to approximately 60% of vital capacity and then expired forcefully to residual volume (RV), while expiratory flow versus lung volume was recorded to obtain a partial expiratory flow-volume (PEFV) curve. Then the subject inspired maximally to total lung capacity (TLC) and again forcefully expired to RV in order to record the maximum expiratory flow-volume (MEFV) curve. From MEFV and PEFV curves, the maximum flow rate at 40% and 60% of TLC were measured. Forced vital capacity (FVC) was determined directly from the MEFV curve and an electronic timer permitted calculation of FVC in one second (FEV_1).

At the beginning of each study, a dose-response curve to inhaled carbachol was constructed for all subjects. A D-31 nebulizer was used to generate 0.025-1.0% carbachol solutions in 80% aqueous propylene glycol. The carbachol aerosol had an average MMAD of 0.8 μm (σ_g = 2.2). Five maximal inhalations, each with 5 second breath holds, constituted a test exposure. Carbachol concentrations of 0.00, 0.025, 0.05, 0.10, 0.25, 0.50, and 1.0% were used sequentially until SGaw decreased by more than 40% of the control value. Exposures were repeated at approximately 10 minute intervals. With a positive response, MEFV and PEFV curves were recorded and the testing stopped.

On day 2, the asthmatics were reassessed at the concentrations previously determined to produce a 20-30% decrease in SGaw i.e., when the decrease in specific airway conductance was equivocal. Normal subjects were uniformly unresponsive to carbachol in concentration less than 1%, consequently they were routinely examined at the 1% concentration and all parameters recorded before and after carbachol exposure.

During the next two days, in a double-blind, randomized fashion, the subjects were exposed to the NaCl and several sulfate aerosols, with at least a 3-hour separation between exposures. The technician conducting the exposures was provided coded containers of the test materials and directions as to the sequence of administration. Following each aerosol evaluation, the subjects were again exposed to carbachol at the day 2 level to determine if the aerosol had a potentiating effect on the bronchoconstrictor. Additional details of the physiological testing methods and protocol have been published elsewhere (UTELL, et al., 1979).

Deposition was determined from analyses of aerosol concentrations in the chamber (inspired) air and the expired aerosol collected on filter samples. The filters were immersed in distilled water and duplicate aliquots analyzed for ^+Na using a flame photometer (IL Model 143). For sulfate analysis, duplicate 5 ml aliquots were analyzed by a flash-volatilization technique (HUNT-ZICKER AND HOFFMAN, 1978) using an S_2* analyser (Meloy Model SA285E) and Hewlett-Packard Automation System (Model 385A). Particle size analyses of the aerosols were performed using an 8-stage impactor (MERCER, TILLERY AND NEWTON, 1970.)

Cardinal features of experimental design included the use of: (a) a double-blind testing procedure to minimize the role of psychological factors and eliminate investigator bias; (b) sodium chloride control aerosol to determine the specific or non-specific nature of the responses; (c) standardized carbachol evaluation for acquiring baseline information on inherent airway irritability and for revealing possible potentiation effects; and (d) dose-response studies to determine if time-concentration relationship exist.

RESULTS

A. Carbachol Inhalation:

By design, all asthmatics studied demonstrated increased airway reactivity to carbachol as compared to normals. Breathing 0.25% carbachol, the asthmatic group demonstrated a 54 ± 2.8 (SE) percent fall in SGaw whereas normal subjects with 1% carbachol decreased 8 ± 3.3% ($p < .001$). All asthmatics demonstrated a fall in SGaw of 40% or more with 0.25% or less carbachol inhalation, approximately 1/16 the dose necessary to produce the same change in normals.

B. Normal Subjects Exposed to Acidic Sulfates: (NaCl; $NaHSO_4$, NH_4HSO_4, $(NH_4)_2SO_4$ and H_2SO_4).

At 1 mg/m^3 (as H_2SO_4), none of the inhaled sulfates produced significant decreases in SGaw when compared to NaCl. However, all sulfates produce small but significant changes in flow at 60% TLC on MEFV and at 60% and 40% TLC on PEFV curves ($p < 0.05$). The effect of sulfate aerosol exposure on airway response to 1% carbachol is shown in Figure 2. The bronchoconstrictor action of carbachol was potentiated by the sulfate aerosols more or less in relation to their activity.

C. Asthmatic Subjects Exposed to Acidic Sulfates: (NaCl, $NaHSO_4$, NH_4HSO_4, and H_2SO_4).

High (1 mg/m^3) and low (0.1 mg/m^3) dose response studies were performed in 17 asthmatics. No asthmatic developed symptoms after any aerosol exposure. At 1 mg/m^3, H_2SO_4 exposure produced a significant decrease in mean SGaw ($p < .001$) compared to pre-exposure or NaCl control values; NH_4HSO_4 inhalation caused a significant decrease in mean SGaw ($p < .001$) compared to the pre-exposure value. The FEV_1 significantly decreased after H_2SO_4 and NH_4HSO_4. All sulfates produced a decrease in flow rates on maximum and partial expiratory flow-volume curves. In the asthmatic group, prior inhalation of H_2SO_4 significantly enhanced the effect of carbachol on conductance and all flow rates compared to NaCl and carbachol or carbachol alone (Figure 3).

In contrast, low level sulfate exposure (0.1 mg/m^3) produced no changes in SGaw or flow rates at 60% and 40% TLC on maximum or partial flow volume curves compared to NaCl control values. However, the two asthmatics most responsive to the high dose H_2SO_4 inhalation exhibited a potentiation effect of H_2SO_4 on carbachol (SGaw and flow rates) but significant changes did not occur for the asthmatic group as a whole.

D. Deposition Studies:

The normal subjects showed a mean sulfate deposition of 55 percent with total deposition of 115-338 µg of sulfate. For the asthmatic group, deposition characteristics were similar for both high and low sulfate aerosols (Figure 4) and resembled that of the normals. At 1 mg/m^3, asthmatic subjects deposited between 75-325 µg of sulfate; at the lower dose, deposition ranged between 6-63 µg of sulfate.

DISCUSSION

The present study demonstrated significant responses in normal and asthmatic subjects to short-term inhalation of acidic sulfates at 1 mg/m^3 for 16 minutes. Although normals demonstrated no reduction in airway conductance, the bronchoconstrictor action of carbachol was significantly potentiated by the prior inhalation of sulfuric acid or ammonium bisulfate. The decrease in flow rates following sulfate exposure was quite small. Indeed, since sodium chloride inhalation often resulted in improvement in flow rates, the statistical technique of comparing sulfate to chloride responses resulted in significance despite the relatively small reduction in flow.

On the other hand, the high level sulfate exposure in asthmatics produced definite but asymptomatic airway obstruction. With prior sulfuric acid inhalation, the carbachol response was significantly potentiated. The absence of similar findings in other short-term inhalation studies in asthmatics (AVOL, et al., 1979; SACKNER, et al., 1978) requires explanation. Several possibilities exist. First, our asthmatics were selected by the demonstration of increased airway reactivity to the non-specific agent carbachol. Perhaps they represent a group more likely to respond to specific inhaled pollutants. Second, mouth breathing compared to nose breathing (AVOL, et al., 1979) may result in greater sulfate exposure and deposition in the bronchial airways. Third, Sackner and associates (1978) studied a group of asthmatics utilizing daily oral corticosteroids to control bronchospasm. These steroids may have blunted the response to these inhaled pollutants. In contrast, none of our asthmatics required this medication. Finally, our selection of pulmonary parameters to assess response included airway conductance and partial expiratory flow rates; these appeared very sensitive to small changes in airway function induced by inhaled agents.

These studies suggest the existence of dose-response relationship for inhaled pollutants. At the lower sulfate level, the asthmatic group showed no enhancement of airway reactivity to carbachol or airway responsiveness to sulfate per se. However, the two asthmatics most responsive to high dose sulfate, were sensitive to the lower dose and exhibited striking potentiation of the carbachol response with prior sulfuric acid inhalation. Therefore, even within a non-reactive group certain individuals may demonstrate bronchoconstrictor responses. We believe that future studies, using other time-concentration protocols with the approach described, will prove useful in further qualifying the differences in normal and susceptible populations toward air pollutants.

ACKNOWLEDGEMENT

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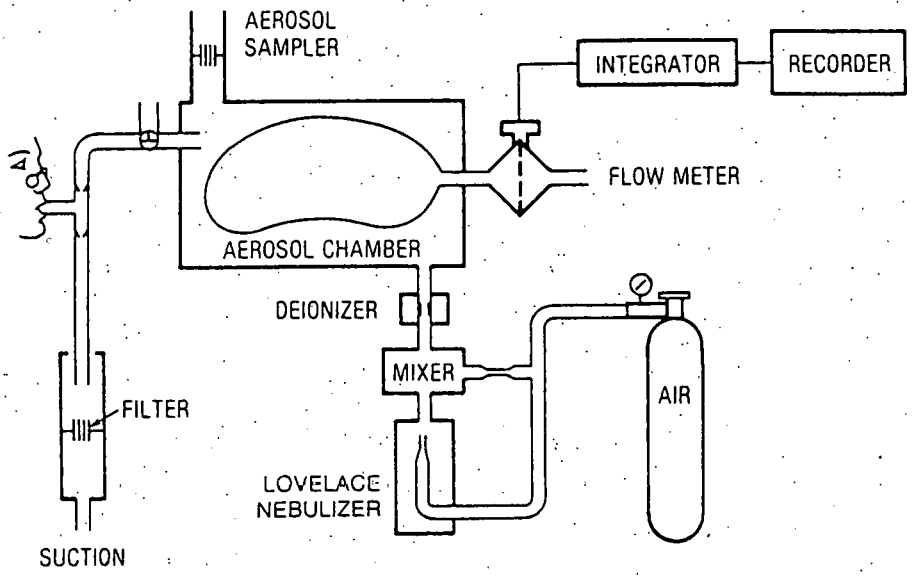
ILLUSTRATIONS

FIG. 1. Diagram of apparatus used to generate aerosols.

FIG. 2. Changes in SGaw in normal subjects after sulfate aerosols. Using the NaCl post-exposure values as a control, sulfate aerosols produced a decreased SGaw before and after carbachol challenge. The most significant changes were seen with H_2SO_4 ($p < 0.01$) and NH_4HSO_4 ($p < 0.01$) aerosols following carbachol.

FIG. 3. Mean changes in SGaw after carbachol, without prior aerosol exposure, after NaCl, and after 1 mg/m^3 H_2SO_4 exposure. Prior inhalation of H_2SO_4 significantly potentiated ($p < 0.001$) the effect of carbachol on SGaw compared to NaCl and carbachol or carbachol alone. Bars represent standard error of mean.

FIG. 4. Deposition data in high and low dose sulfate studies. Bars represent standard error of mean.



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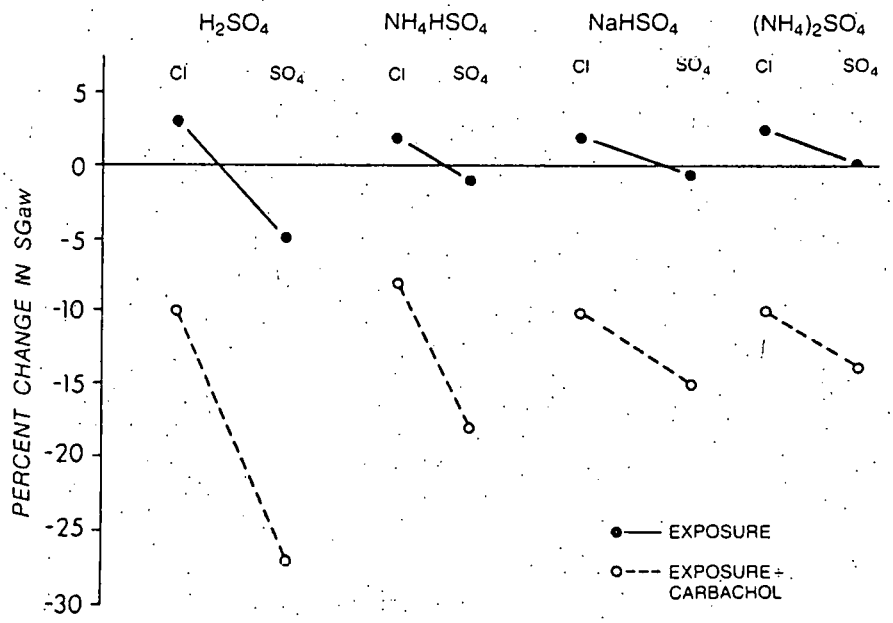

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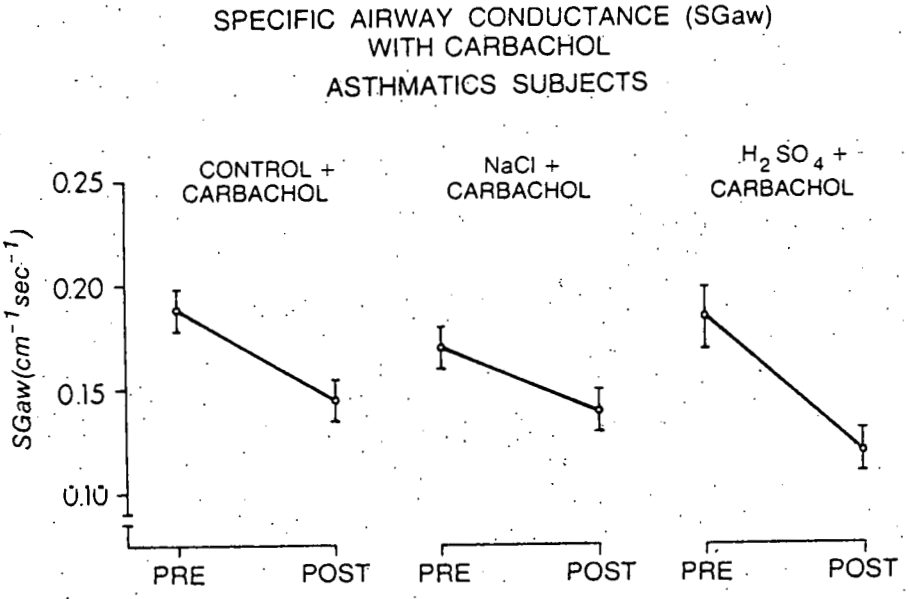

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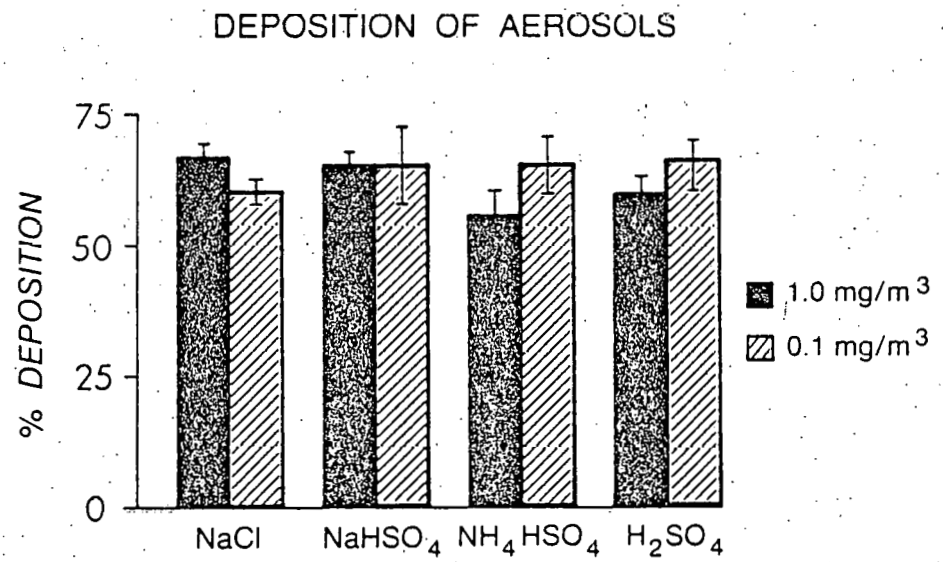

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