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Research Contract Final Report to State

of California Air Resources Board

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Contract No: A8-053-30

Investigators: J.A. Nadel (Principal Investigator)

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EXECUTIVE SUMMARY

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This report describes a series of investigations on the relationship between inhalation of low levels of common air pollutants (ozone and sulfur dioxide) and the function of the airways of the lungs of volunteer human subjects. The airways of patients with asthma have long been known to be abnormally irritable, in the sense that they constrict intensely when even trace amounts of irritating materials are inhaled. This abnormal irritability is universal among patients with active asthma, suggesting that it may be fundamental to the pathogenesis of the disease, but its cause is unknown.

In previous work, we showed that a two-hour exposure to 0.6 parts per million (ppm) of ozone, a level that has been reported in the atmosphere over Los Angeles and that has been exceeded in the passenger cabins of commercial aircraft, increases the irritability of the airways of healthy, nonasthmatic human subjects, as assessed by the amount of constriction produced by inhalation of a test dose of a mildly irritating substance (histamine). The first part of the studies reported here was aimed at determining the lowest level of ozone that would have such an effect on the airways and, further, at determining whether repeated exposures, as might be experienced by a person living in the Los Angeles basin, caused a progressively greater increase in airway irritability. Our findings were that we could demonstrate no change in irritability at levels lower than 0.4 ppm of ozone, a level exceeded only in severe smog alerts, and that repeated exposures to ozone at 24-hour intervals caused no further increase in airway irritability. In fact, after the first exposure, additional exposures to ozone appeared to have progressively less effect, suggesting that tolerance may develop to this effect of ozone. This finding, of course, does not necessarily indicate that tolerance develops to ozone's other harmful effects.

In other studies described in this report, we examined whether volunteer subjects with very mild asthma -- who have the heightened airway irritability of that condition -- are more sensitive to inhalation of low concentrations of sulfur dioxide. Our findings showed that our subjects with asthma developed significant airway constriction, sometimes associated with the distressing symptoms of an asthmatic attack, on inhaling 1 or 3 ppm of sulfur dioxide, whereas healthy, nonasthmatic control subjects developed only mild airway narrowing on inhaling concentrations of 5 ppm or more. The presently approved Occupational Safety and Health Administration threshold limit value for occupational exposure to sulfur dioxide is 5 ppm as a time-weighted average over an eight-hour workshift. Since this standard is expressed as a time-weighted average, it allows brief exposure to considerably higher concentrations. Our findings indicate that workers with asthma may develop worsening symptoms even when the level of sulfur dioxide in the workplace is within the permitted range. Whether asthmatic people in the general population would develop symptoms on inhaling the very much lower levels of sulfur dioxide found in urban atmospheres is unknown.

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ABSTRACT

We have previously demonstrated that a 2-h exposure to 0.5-0.6 parts per million (ppm) of ozone increases bronchial reactivity to both histamine and methacholine in healthy nonatopic and atopic human subjects (1, 2). Studies completed within the past year have shown in 19 healthy adult subjects that a 2-h exposure to 0.4 ppm but not to 0.2 ppm of ozone significantly increased the rise in resistance provoked by inhalation of histamine. With three repeated 2-h exposures of 0.4 ppm of ozone on consecutive days, however, the bronchomotor response to histamine progressively decreased, ultimately returning to pre-exposure levels. Thus, we have shown that the threshold level of ozone causing an increase in bronchial reactivity in healthy human subjects is between 0.2 and 0.4 ppm and that tolerance to this effect of ozone develops with repeated exposures.

We also studied the relationship between bronchomotor responsiveness to histamine and the response to inhalation of sulfur dioxide (SO_2). In three subjects we were unable to show any change in the response to 5 ppm of SO_2 after a 2-h exposure to 0.6 ppm of ozone; but in a study of subjects with mild, asymptomatic asthma who had preexisting bronchial hyperreactivity to histamine, we showed a significant bronchomotor response to 10-min inhalation of 1, 3, and 5 ppm of SO_2 delivered via a mouthpiece, whereas normal and atopic subjects responded only to 5 ppm of SO_2. The response to SO_2 was blocked by pretreatment with atropine, suggesting the involvement of postganglionic cholinergic pathways, but it did not correlate with the response to histamine. Our results indicate that subjects with asthma develop bronchoconstriction on exposure to levels of SO_2 well below currently accepted standards for occupational exposure. The lack of correlation between responses to SO_2 and to histamine suggests that these agents exert their effects via different pathways.

This report was submitted in fulfillment of Contract Number A6-215-30 under the partial sponsorship of the California Air Resources Board. Work was completed on November 30, 1979.

TABLE OF CONTENTS

List of Figures

Conclusions

Recommendations

Disclaimer

Body of Report

Figures

References

Publications

Glossary

LIST OF FIGURES

Fig. 1-1: Bronchomotor response to histamine aerosol in three study groups before ozone exposure.

Fig. 1-2: Bronchomotor response to histamine aerosol before and after exposure to 0.2 ppm (Group I) and 0.4 ppm (Group II) of ozone.

Fig. 1-3: Effect of repeated 2-h exposures to 0.4 ppm of ozone on bronchomotor responsiveness to histamine in seven subjects.

Fig. 1-4: Change in bronchomotor responsiveness to histamine aerosol (bronchial reactivity) with consecutive days of exposure to 0.4 ppm of ozone.

Fig. 3-1: Mean + SD for SRaw following 5 min breathing filtered air and during the last 5 min of exposure to 1 ppm, 3 ppm, and 5 ppm of SO_2 in seven normal, seven atopic, and seven asthmatic subjects.

Fig. 3-2: Mean + SD for SRaw following 5 min breathing filtered air and during the last 5 min of exposure to SO_2 in 17 subjects following pretreatment with atropine and with saline placebo.

Fig. 3-3: Mean + SD for SRaw following 5 min breathing filtered air and during the last 5 min of exposure to 5 ppm of SO_2 following pretreatment with atropine and saline placebo in each of three subjects.

Fig. 3-4: Plot of the change in SRaw produced by breathing 5 ppm of SO_2 against the slope of each subject's histamine dose-response curve.

Fig. 3-5: Mean + SD for SRaw following 5 min breathing filtered air and during the last 5 min of exposure to 1 and 3 ppm of SO_2 in the control state and following pretreatment with atropine in one asthmatic subject.

CONCLUSIONS

The projects completed in this contract permit the following conclusions.

1. The threshold concentration of ozone causing an increase in bronchial reactivity to histamine after a 2-h exposure is between 0.2 and 0.4 ppm.

2. Tolerance to this effect of ozone develops with repeated 2-h exposures to 0.4 ppm on three consecutive days.

3. A 2-h exposure to a level of ozone known to cause an increase in bronchial reactivity to histamine caused no demonstrable change in bronchomotor responsiveness to 5 ppm of sulfur dioxide (SO_2) in three healthy human subjects.

4. Subjects with asthma, who have exaggerated bronchial reactivity to histamine, develop significant bronchoconstriction on inhaling 1, 3, and 5 ppm of SO_2, whereas normal and atopic subjects develop bronchoconstriction only on inhaling 5 ppm of SO_2.

5. The bronchomotor response to SO_2 is blocked by pretreatment with atropine sulfate aerosol, implying the involvement of postganglionic cholinergic pathways.

RECOMMENDATIONS

The finding that subjects with mild, currently asymptomatic asthma develop statistically significant bronchospasm, sometimes associated with marked dyspnea, on inhaling concentrations of sulfur dioxide (SO_2) well below accepted standards for occupational exposure implies that a subgroup of the population may be especially sensitive to common atmospheric pollutants. Further studies should be directed at determining the threshold concentration of SO_2 which can cause bronchoconstriction in subjects with asthma and at determining whether tolerance to this effect of SO_2 develops with repeated exposures.

Our finding that the response to SO_2 did not correlate with the response to histamine is surprising, for both are thought to cause bronchoconstriction through reflex parasympathetic pathways (3 , 4 , 5). Indeed, bronchial hyperreactivity itself is thought to depend on an exaggeration of activity in this reflex pathway (6 , 7 , 8). Additional studies should therefore be done to determine whether exposure to SO_2 affects other respiratory functions influenced by reflex mechanisms -- such as secretion from bronchial submucosal glands. Additional information on the mechanism of the response to SO_2 could also be learned by determining how the responses to agents acting at different sites in the parasympathetic reflex pathway are altered when tolerance to the bronchomotor effects of SO_2 has been induced by repeated exposures. Thus, a decrease in response to histamine aerosol but not to methacholine aerosol would imply that the responsiveness of afferent neural receptors in the airways had been affected, whereas a decrease in the response to both histamine and methacholine aerosols would imply either a decrease in airway mucosal permeability (reducing the amount of the agents that reached their sites of action in the airway) or a change in the sensitivity, number, or binding affinity of muscarinic receptors on airway smooth muscle. The finding that tolerance to SO_2 was associated with a decrease in responsiveness to citric acid but not to histamine or methacholine would imply that SO_2 exerted its effect through its conversion to acid (H_2SO_4, H_2SO_3) and that tolerance might be due to the development of local buffering mechanisms.

These recommendations for further study, which are described in greater detail in our application for renewal of this contract, are summarized in the following list:

1. Determination of the threshold of SO_2 causing bronchoconstriction in human subjects with mild, asymptomatic asthma.

2. Determination of the effects of repeated exposures to SO_2 in asthmatic subjects on bronchomotor responsiveness to histamine, methacholine, citric acid, and SO_2 itself.

3. Determination in animals of the effects of exposure to SO_2 and to ozone on secretion from bronchial submucosal glands.

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

The general purpose of the projects completed in the past year was to examine the relationships between exaggerated bronchial responsiveness to inhaled histamine -- often referred to as "nonspecific bronchial hyperreactivity" -- and the respiratory effects of common urban atmospheric pollutants. We first studied the induction of bronchial hyperreactivity by brief exposure to lower levels of ozone than we had previously studied and examined whether tolerance to this effect of ozone developed with repeated exposures. We then studied whether subjects with increased bronchial reactivity -- whether transiently induced by exposure to ozone or chronically associated with mild asthma -- develop bronchoconstriction on brief inhalation of low levels of sulfur dioxide (SO_2). The rationale behind each study, the methods used, and the results obtained will be discussed separately.

Project #1: Threshold of ozone causing an increase in bronchial reactivity in healthy subjects; the development of tolerance with repeated exposures.

Introduction

Previous work in this laboratory has shown that a 2-h exposure to 0.6 ppm of ozone can increase bronchial reactivity to histamine and methacholine in healthy human subjects (1 , 2) and experimental animals (9). Because bronchial hyperreactivity may be important in the pathogenesis of asthmatic bronchospasm (10) and because it may contribute to the symptoms of cough and dyspnea in subjects without asthma (11), it is important to determine the threshold level of ozone which can be demonstrated to alter bronchial reactivity, and to determine the effects on bronchial reactivity of repeated exposure to levels which might be encountered in the urban atmosphere or in the air supplied to the passenger cabins of commercial airplanes (12,13).

Previous studies have demonstrated that the lowest concentration of ozone which significantly alters tests of maximal expiratory flow in exposed humans is between 0.3 and 0.4 ppm; but with repeated exposures to ozone, the changes in flow and in vital capacity become progressively smaller (14,15). Similarly, pretreatment of animals with sublethal doses of ozone protects them from subsequent exposure to much higher levels (16). This

acquired resistance to the effects of ozone has been described as "tolerance" and has been proposed as an explanation for the relative insensitivity of residents of Southern California to experimental exposure to ozone when compared to residents of western Canada (17). It is not known, however, whether tolerance also develops to the effects of ozone on bronchial reactivity.

The purposes of this study were to determine the minimum concentration of ozone that increases the bronchial response to histamine in healthy nonatopic subjects and to determine the effect of repeated exposure to this level on bronchial reactivity.

Materials and Methods

The subjects were 19 healthy, nonsmoking adult volunteers, 12 men and 7 women, 21-32 years of age, who were informed of the risks of the experimental protocol and who signed consent forms approved by the Academic Senate Committee on Human Experimentation of the University of California. Prior to entry into the study all subjects were classified as nonatopic on the basis of medical history and allergen skin tests: none of the subjects gave a personal or family history of hay fever, eczema, or asthma, and none had a positive response to prick skin tests with seven allergen mixes common to Northern California. Results from screening tests of pulmonary function -- spirometry, single-breath carbon monoxide diffusing capacity, single-breath oxygen test of distribution of inspired gas, and maximal expiratory flow-volume curves -- were normal in all subjects. None of the subjects had symptoms suggestive of a viral upper respiratory infection for at least one month prior to the study, and none was using antihistaminic or bronchodilator drugs at the time of the study.

Airway resistance (Raw) and thoracic gas volume (TGV) were measured using a constant-volume whole body plethysmograph (18). Bronchial reactivity was assessed by measuring the increase in specific airway resistance (SRaw) produced by inhalation of 10 breaths of histamine aerosol (16 mg/ml). Baseline values were obtained before inhaling histamine by measuring Raw and TGV five times at 30-s intervals; values of SRaw (Raw x TGV) were calculated and averaged. After inhaling histamine (10 breaths, 16 mg/ml solution), SRaw was measured at 30-s intervals for 5 min, and the three highest consecutive measurements were averaged.

A solution of histamine (16 mg/ml) was prepared daily from a stock supply of histamine diphosphate, dissolved in normal saline and buffered with sodium bicarbonate to a pH of 7.0. The histamine was delivered as an aerosol from a DeVilbiss No. 40 glass nebulizer equipped with a dosemetering device (19). This device consists of a breath-activated solenoid valve and a timing circuit in series with a compressed oxygen source at 20 psi as described previously (1). The response to histamine inhalation was a rise in SRaw of less than 7.5 L x cm H_2O/L/s in all subjects.

Ozone exposures were conducted in a chamber (12 x 10 x 8 ft) previously described (1). Subjects alternated 15-min periods of rest with exercise on a bicycle ergometer at a workload that doubled resting minute volume of ventilation. During exercise, subjects wore a nose clip. Ozone was generated by passing 100% oxygen into a commercial ozonator (Welsbach Ozonator, Model T-408). The concentration of ozone was monitored continuously using an ultraviolet ozone analyzer (Dasibi, Model No. 1003 AH), whose calibration was confirmed by a potassium iodide method (20) before and after the study was conducted. Room temperature and relative humidity were monitored during all exposures (21.8 ± 0.2°C and 55.3 ± 1.5% mean ± SE, respectively). All concentrations of ozone are expressed by volume ± greatest deviation from mean.

Upon entry into the study, the subjects were divided into three groups. Group I (three men and four women) underwent a 2-h exposure to 0.2 ± 0.02 ppm of ozone (mean ± greatest deviation from mean). Group II (five men and two women) underwent a 2-h exposure to 0.4 ± 0.05 ppm of ozone on each of three consecutive days. Group III (four men and one woman) served as a control group and had bronchial reactivity determined in the same manner as Group II but were not exposed to ozone.

Group I was studied over four days. On the first two days, the bronchomotor response to inhalation of histamine aerosol (16 mg/ml) was determined at 9:30 AM and again at noon. On the third day, baseline SRaw and bronchial reactivity to inhaled histamine were again determined at 9:30 AM, and the subject then underwent a 2-h exposure to 0.2 ± 0.02 ppm of ozone. After exposure, SRaw was measured at 10-min intervals until it reached its pre-exposure baseline; bronchial reactivity to inhaled histamine was then reassessed. The response to inhaled histamine was measured for the final time at 9:30 AM on the following day.

The protocol for studying subjects in Group II was similar to that used for the subjects in Group I but differed in that a level of 0.4 ± 0.05 ppm was used for ozone exposure and in that the subjects were exposed on each of three consecutive days (study days 3, 4, and 5), rather than on the third study day alone. Measurement of bronchial reactivity to inhaled histamine was repeated 24 h (study day 6) and one week (study day 7) after the final exposure to ozone.

In Group III, bronchial reactivity to inhaled histamine was determined at 9:30 AM and again at noon on each of four consecutive days. This group was not exposed to ozone.

Statistical Analysis

Differences in bronchial reactivity to histamine among groups and changes in bronchial reactivity caused by ozone exposure within a group were analyzed by comparing the increases in SRaw produced by inhalation of histamine aerosol. The pre-exposure bronchial reactivity of groups I and II (i.e., the bronchial reactivity measured twice on the first two study days

and at 9:30 AM on the third study day) and the bronchial reactivity of the control group measured on the same study days were compared by a two-way analysis of variance with replicates. The change in bronchial reactivity caused by the single exposure to ozone in Group I and by the first exposure in Group II was analyzed by Student's t-test for paired data, comparing the mean response obtained immediately prior to ozone exposure to that obtained afterward. In order to determine the number of individuals in whom a significant increase in bronchial reactivity occurred following exposure to ozone, the rise in SRaw produced by histamine inhalation after ozone exposure was compared to the five bronchial responses to inhaled histamine prior to ozone exposure by the procedures outlined by Grubs for outlying observations (21). The change in bronchial reactivity following repeated exposures to ozone was analyzed using a linear regression, with day of exposure as the independent variable and the rise in SRaw provoked by histamine as the dependent variable. The control group's data was analyzed in a similar fashion. The slopes were then compared to zero using an analysis of variance, and to each other using Student's t-test. The daily pre-ozone response to inhaled histamine was also analyzed using a linear regression with the study day as the independent variable.

Results

The baseline bronchomotor response to inhalation of histamine aerosol (10 breaths, 16 mg/ml) did not differ in the three groups of subjects (Fig. 1-1). In Group I, the mean of the five bronchial responses to inhalation of histamine aerosol obtained before ozone exposure (days 1, 2, and 9:30 AM on day 3) was a rise in SRaw of 2.3 ± 0.22 L x cm H_2O/L/s (from 5.1 ± 0.17 to 7.4 ± 0.27 L x cm H_2O/L/s). In Group II, the mean response to inhalation of histamine on comparable study days was 2.1 ± 0.22 L x cm H_2O/L/s (from 4.0 ± 0.22 to 6.1 ± 0.36 L x cm H_2O/L/s). The mean of the first five responses to inhaled histamine aerosol obtained in Group III was a rise in SRaw of 2.8 ± 0.29 L x cm H_2O/L/s (from 5.2 ± 0.3 to 8.0 ± 0.29 L x cm H_2O/L/s). All values are means ± SE. The differences observed were not significant ($p > 0.30$).

The 2-h exposure to 0.2 ppm of ozone in Group I did not alter the bronchial response to inhaled histamine aerosol (Fig. 1-2). The mean bronchial response to inhaled histamine aerosol of the seven subjects immediately prior to ozone exposure (day 3, 9:30 AM) was a change in SRaw of 2.0 ± 0.17 L x cm H_2O/L/s (from 5.3 ± 0.14 to 7.3 ± 0.37 L x cm H_2O/L/s). Following ozone exposure, the mean bronchial response to inhaled histamine aerosol was a change in SRaw of 1.8 ± 0.22 L x cm H_2O/L/s (from 4.6 ± 0.15 to 6.4 ± 0.31 L x cm H_2O/L/s). In no subject was the response to histamine immediately following ozone exposure significantly greater than the five baseline responses obtained prior to exposure ($p > 0.5$). Thus, neither the group as a whole nor any individual showed a significant change in bronchial reactivity following exposure to 0.2 ppm of ozone for 2 h.

The 2-h exposure to 0.4 ppm of ozone significantly increased bronchial reactivity to inhaled histamine aerosol (Fig. 1-2). The mean response of

the seven subjects immediately prior to the first exposure to ozone (day 3, 9:30 AM) was a change in SRaw of 2.0 ± 0.24 L x cm H_2O/L/s. Following the 2-h exposure to 0.4 ppm of ozone, histamine inhalation provoked a significantly greater mean rise in SRaw of 4.4 ± 0.31 L x cm H_2O/L/s (from 4.1 ± 0.25 to 8.5 ± 0.49; $p < 0.025$). In five subjects, the bronchomotor response to histamine was greater immediately after ozone exposure than it had been before exposure; and in four of these subjects, the difference was significant ($p < 0.025$). Thus, both the group as a whole and four out of the seven individuals had a significant increase in bronchial reactivity following exposure to 0.4 ppm of ozone.

Repeated exposures to 0.4 ppm had a progressively smaller effect on bronchial reactivity (Fig. 1-3). The daily change in bronchial reactivity was calculated for each subject by subtracting the change in SRaw provoked by histamine aerosol immediately before that day's exposure from the one provoked after exposure (change in reactivity = ΔSRaw after exposure - ΔSRaw before exposure). The change in bronchial reactivity for each subject was then correlated to the day of exposure by linear regression. The regression coefficient (slope) of the line generated was negative and significantly different from zero ($p < 0.02$; Fig. 1-4). In no subject exposed to 0.4 ppm of ozone was the response to histamine after the second or third exposure to ozone greater than the response after the first exposure. In order to assure that the progressive fall in the change in reactivity implied by the negative coefficient was not an artefact due to systematic increase in pre-exposure bronchial reactivity, the daily pre-ozone rise in SRaw provoked by inhaled histamine aerosol was correlated to the day of exposure. The regression coefficient did not differ from zero. The data from the control group was analyzed in an identical manner. When the change in bronchial reactivity (change in reactivity = ΔSRaw at noon - ΔSRaw at 9:30 AM) was correlated with the day of the study, the regression coefficient for the control group did not differ from zero ($p > 0.5$) but did differ from the regression coefficient for the group repeatedly exposed to 0.4 ppm ($p < 0.025$). Thus, repeated 2-h exposures to 0.4 ppm of ozone at 24-h intervals had progressively smaller effects on bronchial reactivity.

Discussion

This study shows that in healthy, nonatopic, nonsmoking adults, a 2-h exposure to 0.4 but not to 0.2 ppm of ozone increases the bronchomotor response to inhalation of histamine. Repeated exposures to 0.4 ppm of ozone at 24-h intervals, however, did not produce a further increase in bronchial reactivity; on the contrary, the response to histamine progressively decreased, and in no subject was the rise in SRaw provoked by histamine greater after the second or third exposure to ozone than it had been after the first exposure. The progressive decrease in reactivity does not appear to have been caused simply by repeated inhalation of histamine itself, for there was no systematic change in reactivity in the control group, who were not exposed to ozone and in whom the response to histamine was assessed twice each day. Thus, under the conditions of our study, the threshold level of ozone causing an increase in bronchial reactivity is between 0.2 and 0.4 ppm, and tolerance to this effect of ozone develops with repeated exposures.

The threshold concentration of ozone causing changes in tests of lung volume, distribution of ventilation, and maximal expiratory flow is similar to the threshold concentration causing an increase in bronchial reactivity. Exposures for 2 h to concentrations of 0.37 ppm or higher have repeatedly been shown to cause decreases in vital capacity and alterations in tests of airway caliber that suggest bronchoconstriction (22,23,24,25,26). Studies of the effects of lower concentrations have produced conflicting results. Goldsmith and Nadel found a significant rise in Raw in two of four subjects exposed to 0.1 ppm for 1 h (23) and von Nieding and associates reported changes in Raw and in the alveolar-arterial oxygen difference [$(A-a)dO_2$] in healthy volunteers performing intermittent light exercise during a 2-h exposure to the same low concentration (27). Other work, however, has shown no change in the pulmonary function of asthmatic subjects (28) or in the pulmonary function and arterial oxygen tension of normal subjects (29) performing exercise during exposure to 0.2 ppm.

The time course of the development of tolerance to ozone's effect on bronchial reactivity is also similar to that observed in other studies in which tolerance has been demonstrated. Both the symptoms of airway irritation and the changes in pulmonary function that are produced by a single exposure decrease with repeated exposures on three to five consecutive days (14,15,30). In all of these studies, the peak change occurred after the first or second exposure and returned to near control or pre-exposure levels after the third, fourth, or fifth exposure.

That the threshold concentration and time course of the development of tolerance should be so similar in studies analyzing changes in symptoms, lung volumes, airway caliber, and bronchomotor responsiveness suggests that all of the changes may depend on the same underlying mechanism. The mechanism underlying ozone's various effects on the respiratory system is unknown, but because the response to histamine appears to involve stimulation of afferent receptors in the airways (3,4,6,7), we have suggested that the damage ozone causes in the airway epithelium somehow alters the accessibility or sensitivity of afferent receptors (1,9).

An increase in the spontaneous activity of airway receptors could account for the symptoms of substernal pain and cough produced by ozone exposure (13,26) and, by reflexly increasing tonic vagal efferent activity, could also account for the changes in pulmonary function tests that suggest an increase in bronchial smooth muscle tone (23,24). An increase in the sensitivity of these receptors would account for the heightening of the reflex component of the bronchomotor response to histamine (1,9).

Some support for the concept that the activity of afferent airway receptors is altered by exposure to ozone is supplied by human and animal studies of the effects of ozone exposure on the ventilatory response to exercise. Follinsbee and co-workers showed in humans that exposure to ozone caused no change in minute ventilation at any level of exercise but that ventilation was achieved with a more rapid, shallow pattern of breathing (31). They speculated that these effects might be due to stimulation of irritant receptors in the airways. In animals, Lee and his co-workers similarly showed that exposure to ozone caused dogs to breathe more rapidly and shallowly on exercise and on rebreathing carbon dioxide (32). They further showed that ozone exposure increased the ventilatory response

to inhalation of histamine and prostaglandin $F_{2\alpha}$ (33). All of these effects were abolished by cooling blockade of the vagus nerves.

There is evidence, however, that not all of ozone's effects are due to a change in the activity of afferent receptors. We have shown that exposure to ozone increases bronchial reactivity to methacholine as well as to histamine (2), and there is persuasive evidence that the bronchomotor response to methacholine does not depend on reflex pathways. Studies in animals have shown no significant effect of vagal blockade on the bronchoconstriction induced by acetylcholine (34,35) and little direct effect of cholinergic agonists on the rate of discharge from vagal sensory endings in the airways (36). In our study of bronchial hyperreactivity in five atopic subjects, we found that aerosolized hexamethonium blocked the response to inhaled histamine but had no significant effect on the response to methacholine (14). Our findings suggested that bronchial hyperreactivity may be due to a change in the characteristics of the efferent parasympathetic pathway at a site distal to the ganglia, possibly at the smooth muscle itself.

The two hypotheses are not mutually exclusive: an increase in the activity or sensitivity of afferent receptors (suggested by the changes in the control of breathing) and an increase in postganglionic cholinergic sensitivity (suggested by the increased bronchial reactivity to methacholine) may both be caused by ozone exposure. A possible unifying hypothesis is that ozone increases airway epithelial permeability, so that a greater concentration of an inhaled agonist reaches its site of activity, whether that site is afferent receptors in the airway or airway smooth muscle iself. Direct evidence for this hypothesis was provided by Matsamura's study of guinea pigs, where he showed that prior exposure to ozone increased the rate of appearance of radioactivity in the blood after tracheal instillation of radiolabeled albumin (37). A change in epithelial permeability alone, however, could not account for the increased responsiveness to agonists injected parenterally (38,39).

The fact that tolerance develops to the effects of ozone on airway resistance, lung volumes, and respiratory symptoms does not necessarily imply that tolerance also develops to ozone's other harmful effects. In studies of animals, for example, it has been shown that an initial ozone exposure induced a tolerance against pulmonary edema but that no protection was afforded against the cytotoxic effects of ozone (40). The effects of ozone on cellular function or lung defense mechanisms were not studied in our investigation.

Project #2: The effect of exposure to 0.6 ppm of ozone on the subsequent response to inhalation of SO_2.

Introduction

Previous studies have demonstrated that a 2-h exposure to 0.6 ppm of ozone produces an increased responsiveness to inhaled histamine aerosol in most normal and atopic individuals and that this effect appears to involve postganglionic cholinergic pathways (1 , 2). Other work has shown that

SO_2 in concentrations of 5 ppm or greater produces an increase in airways resistance which also appears to be mediated by cholinergic reflex pathways (5,41). This project was designed to assess whether a 2-h exposure to 0.6 ppm of ozone increases bronchomotor responsiveness to inhaled SO_2 as well as to inhaled histamine.

Materials and Methods

A pilot study was performed in three healthy adults (two men and one woman), 30-33 years of age. All SO_2 exposures were performed with the subject seated in a body plethysmograph. Sulfur dioxide was delivered from a calibrated tank (500 ppm) and mixed in a glass mixing chamber with air delivered from a compressed air source at 1 L/s. The air was filtered through a HEPA filter (Mine Safety Appliances, No. 81857) before entering the chamber. Sulfur dioxide levels were measured continuously with a pulsed fluorescent analyzer (Thermo-Electron Corporation, Model 43). All tubing in contact with the gas mixture was made of Teflon. The mixture was delivered to the subject using a "blow-by" system connected via a glass T-piece to a mouthpiece mounted in the body plethysmograph.

Measurements of Raw and of TGV were obtained by standard plethysmograph techniques (18). To correct for the effects of lung volume on Raw, SRaw was calculated for each measurement (SRaw = Raw x TGV).

Each subject was exposed to 1, 5, and 10 ppm of SO_2 for 10-min periods in succession separated by 10 min of recovery. Measurements of Raw and TGV were obtained and SRaw calculated prior to each exposure and at 2½-min intervals during each exposure. Each subject then underwent a 2-h exposure to 0.6 ppm of ozone in an exposure chamber using the protocol previously described in this report. Immediately following ozone exposure, SRaw was measured at 10-min intervals until it returned to baseline (≈30 min). The exposure to 1, 5, and 10 ppm of SO_2 was then repeated. In one subject, the response to 10 breaths of histamine aerosol (16 mg/ml) was assessed prior to the initial SO_2 exposure and immediately following the ozone exposure using the protocol described earlier in this report.

For each subject, the response to each concentration of SO_2 inhaled after ozone exposure was compared to the pre-exposure response by using a one-tailed Student's t-test.

Results

Two subjects demonstrated significant increases in SRaw during exposure to 5 and 10 ppm of SO_2 before and after ozone exposure. The third subject significantly increased SRaw only at 10 ppm of SO_2 both before and after ozone exposure. The magnitude of the increases in SRaw in response to a particular concentration of SO_2 was quite similar before and after ozone exposure in all three subjects (Table 2-1). The one subject in whom histamine reactivity was assessed before and after ozone exposure demonstrated

a marked increase in histamine reactivity following ozone exposure. Before exposure, SRaw increased from 3.35 to 3.51 L x cm H_2O/L/s. After exposure, SRaw increased from 3.10 to 6.16 L x cm H_2O/L/s.

Table 2-1: Effect of exposure to ozone on the subsequent response to inhaled SO_2

Before ozone exposure

Subject	Baseline	1 ppm*	5 ppm	10 ppm
DS	2.93**	3.37	4.05	3.55
CU	4.32	4.28	4.89	6.82
NM	3.82	4.19	4.80	4.56

After ozone exposure

Subject	Baseline	1 ppm*	5 ppm	10 ppm
DS	2.84**	2.58	4.22	4.37
CU	4.47	4.31	4.69	5.58
NM	3.71	3.36	3.49	3.88

*ppm refers to concentration of SO_2 inhaled.
**All values are in L x cm H_2O/L/s (units of SRaw).

Discussion

This small pilot study did not demonstrate any increase in responsiveness to SO_2 exposure following a 2-h exposure to 0.6 ppm of ozone, despite a definite increase in histamine reactivity in at least one subject. These results suggest that it will be difficult to document any effect of pre-exposure to 0.6 ppm of ozone on the bronchomotor response to SO_2 in a small study using human volunteers.

Project #3: Bronchomotor response to inhaled SO_2 in asthmatic, atopic, and normal subjects.

Introduction

Sulfur dioxide, a common air and industrial pollutant, has been shown to produce bronchoconstriction in most normal individuals during brief exposures to concentrations of 5 ppm or greater (41,42). This response can be blocked by pretreatment with atropine in humans and by atropine or cooling of the vagus nerves in cats (5), suggesting that it is mediated via parasympathetic reflex pathways.

Increased activity in parasympathetic reflex pathways also appears to be involved in the increased bronchomotor responsiveness to a variety of inhaled agents noted in subjects with asthma or seasonal rhinitis (6, 7), so one would expect that these subjects would also be abnormally sensitive to the bronchoconstrictor effect of SO_2. We undertook this study to ascertain whether individuals with asthma and/or seasonal rhinitis manifest increased bronchomotor responsiveness to SO_2, whether the response in these individuals, as well as in normal subjects, can be blocked by pretreatment with atropine, and whether responsiveness to SO_2 can be predicted from the response to another agent which causes bronchoconstriction through reflex parasympathetic pathways: histamine aerosol (3, 4).

Materials and Methods

The subjects were 21 nonsmoking volunteers, 15 men and 6 women, 24-37 years of age, who were informed of the risks of the experimental protocol and who signed consent forms approved by the Committee on Human Experimentation of the University of California. We classified seven subjects, five men and two women, 24-34 years of age, as normal; they had no history of asthma or seasonal rhinitis and had no reaction to skin prick tests with eight mixes of antigens common to Northern California. Results from screening tests of pulmonary function -- spirometry, single-breath carbon monoxide diffusing capacity, single-breath oxygen test of distribution, and maximal expiratory flow-volume curve -- were normal in all subjects. We also classified seven subjects, five men and two women, 24-37 years of age, as atopic; each had a history of seasonal rhinitis but not asthma, had positive reactions to skin prick tests to two or more of the eight antigen mixes, and had normal results of pulmonary function testing as described above. The asthmatic group consisted of seven subjects, five men and two women, 23-35 years of age; each subject had a history of recurrent episodes of wheezing, chest tightness, and reversible airways obstruction previously documented by a physician. All had two or more positive skin prick tests. Five had normal results of pulmonary function testing as described above. Two had mild airways obstruction at the time of testing, manifested by a ratio of forced expired volume in one second to forced vital capacity (FEV_1/FVC) of 65% and 68%, and by slight maldistribution of ventilation on the single-breath oxygen test.

Measurements of Raw and TGV were performed using a constant-volume, whole-body plethysmograph (18). Airflow was measured with a heated pneumotachograph (Fleisch #2) and a differential pressure transducer (Validyne DP45). Mouth pressure was measured with a pressure transducer (Statham P23 DB) and box pressure with a differential pressure transducer (Statham 11227). The electrical output from these transducers was amplified and displayed on an oscilloscope (Electronics for Medicine DR-12). Permanent recordings were obtained with a rapid writing device. The slopes of the tracings were measured directly from these recordings.

On the first study day, bronchomotor reactivity to inhaled histamine aerosol was assessed in each subject by measuring the rise in SRaw provoked by

serially increasing doses of inhaled histamine aerosol. Histamine solutions were prepared daily from a stock supply of histamine diphosphate and were buffered with sodium bicarbonate to a pH of 7.0. Histamine solutions were delivered as aerosols from a glass nebulizer (DeVilbiss, No. 40) equipped with a Rosenthal-French nebulization dosimeter (Lab for Applied Immunology, No. D-2-014). This device consisted of a breath-activated solenoid valve and a timing circuit in series with a compressed oxygen source at 20 psi. The solenoid was set to remain open for 0.6 s from the onset of inspiration during which time the oxygen was allowed to flow through the nebulizer and dispersed an average of 0.008 ml of the solution with each breath. The volume median droplet diameter of the aerosol under similar conditions was reported as 3.2 μm (43). Baseline Raw and TGV were measured five times; values of SRaw were calculated and averaged. Dose-response curves were obtained by having each subject inhale 10 breaths each of successively increasing doses of histamine aerosol administered at 5-min intervals. The initial concentration used was 0.25 mg/ml, and subsequent concentrations were increased in twofold increments. Measurements of Raw and TGV were taken every 30 s for 5 min after inhalation of each concentration. Values of SRaw were calculated, and the three highest consecutive values at each concentration were averaged. Subjects inhaled increasing concentrations of histamine until SRaw increased 120% from baseline or a concentration of 16 mg/ml was reached. We then linearized the data by plotting log SRaw against the histamine concentration inhaled and obtained the slope of each dose-response curve by linear regression.

Each subject returned to the laboratory for exposure to SO_2 on three subsequent days separated by at least 48 h. Subjects were seated in a body plethysmograph, and SO_2 was delivered from a calibrated tank (500 ppm) and mixed in a glass mixing chamber with air delivered from a compressed air source at 1 L/s. The air was filtered through a HEPA filter (Mine Safety Appliances, No. 81857) before entering the mixing chamber. Levels of SO_2 were measured continuously with a pulsed fluorescent analyzer (Thermo-Electron Corporation, Model 43). All tubing in contact with the gas mixture was made of Teflon. The gas mixture was delivered to the subject using a "blow-by" system connected via a glass T-piece to a mouthpiece mounted in the body plethysmograph.

Prior to each exposure, the subject was told s/he would be breathing either SO_2 or air. Five baseline measurements of Raw and TGV were obtained, and values of SRaw were calculated and averaged. The subject then breathed SO_2-free air from the "blow-by" apparatus for 5 min, and five more measurements of Raw and TGV were obtained. To control for possible effects on bronchomotor tone of breathing air from the apparatus, the averaged value of SRaw after 5 min of breathing SO_2-free air was considered the pre-exposure baseline; this value did not differ significantly from the baseline before exposure to SO_2-free air in any study group. Then SO_2 was delivered to the mixing chamber to obtain a concentration of 1, 3, or 5 ppm, and the subject breathed this gas mixture for 10 min. After 1, 2½, 5, 7½, and 10 min of exposure, the subject was instructed to switch to the plethysmograph mouthpiece, and four measurements of Raw and TGV were obtained at the end of successive tidal volume breaths. The 12

values obtained during the last 5 min of exposure were averaged to obtain the exposure value of SRaw. During 21 exposures in nine subjects, we continuously measured airflow in the gasline leaving the plethysmograph using a pneumotachygraph (Fleisch, No. 2) and a differential pressure transducer (Validyne DP45). This signal was then integrated electrically and electrically balanced to offset the continuous baseline flow of 1 L/s to obtain continuous measurements of the subject's tidal volume. Both flow and volume tracings were amplified and displayed on an oscilloscope (Electronics for Medicine DR-12) and recorded with a rapid writing device. A continuous record of tidal volume, respiratory rate, and cough frequency was thus obtained. All subjects were exposed to each concentration of SO_2 except one asthmatic subject (ER) who became severely symptomatic during exposure to 3 ppm and was therefore not exposed to 5 ppm. Each subject's response to each SO_2 exposure was analyzed for statistical significance using Student's t-test.

Each subject who had a significant increase in SRaw ($p < 0.05$) during any exposure (17 subjects) was then studied on two additional days at least 48 h apart. On the first day, five baseline values of SRaw were obtained, and the subject then inhaled 0.1 mg/kg of atropine sulfate aerosol (10 mg/ml) from a DeVilbiss #40 nebulizer and the dose-metering device described earlier. The subject repeated the full exposure protocol outlined above 20 min later, breathing a concentration of SO_2 which had previously resulted in a significant increase in SRaw (5 ppm in every subject except asthmatic subject ER). On the final study day, the same protocol was repeated, but the subject inhaled an aerosol of an identical volume of quinine-flavored saline placebo instead of atropine.

Because the responses to SO_2 inhalation were not normally distributed, the group responses were analyzed using nonparametric tests. In each group, SRaw during exposure to each concentration of SO_2 was compared to baseline SRaw using the Wilcoxon T-test. Differences among the three study groups for change in SRaw (ΔSRaw) at each exposure concentration were analyzed using the Kruskal-Wallis rank order analysis of variance. This test is 95% as powerful as the standard parametric analysis of variance and is preferable for data that are not normally distributed (44). Differences between specific groups were then analyzed using a nonparametric equivalent of the Neuman-Keuls multiple range test (44). To correct for the difference in group size at 5 ppm caused by not exposing the most responsive asthmatic subject (ER) to this concentration, it was assumed that his response would have at least equaled the mean response of the other asthmatic subjects to 5 ppm. The baseline values for SRaw among groups prior to each exposure level were also compared using the Kruskal-Wallis test. To assess the importance of baseline SRaw in determining the response to SO_2 exposure, we plotted the rise in SRaw produced by SO_2 against baseline SRaw for each subject at each level of SO_2 exposure.

To assess the relationship between bronchomotor responsiveness to inhaled histamine aerosol and responsiveness to SO_2, we plotted histamine reactivity as described by the slope of each subject's histamine dose-response curve (log SRaw vs dose of histamine) against the subject's change in SRaw

during exposure to the highest concentration of SO_2 (5 ppm in every subject except ER). We compared baseline values for SRaw and the change in SRaw during SO_2 exposure after pretreatment with atropine sulfate to the baseline values and the change in SRaw during SO_2 exposure after pretreatment with placebo using Wilcoxon's T-test.

Results

Response of SO_2. Asthmatic subjects had significant increases in SRaw during exposure to every SO_2 concentration tested (Table 3-1). During exposure to 1 ppm, four of seven asthmatic subjects, zero of seven normal subjects, and one of seven atopic subjects developed significant increases in SRaw. Two asthmatic subjects with very significant increases in SRaw ($p < 0.0005$) complained of chest tightness and developed audible wheezing. As a group, the asthmatic subjects had a significant increase in SRaw ($p = 0.05$), whereas the normal and atopic subjects did not (Fig. 3-1). The overall difference among groups at 1 ppm did not reach statistical significance ($0.05 < p < 0.1$).

During exposure to 3 ppm, seven of seven asthmatic subjects, one of seven normal subjects, and two of seven atopic subjects developed significant increases in SRaw. One asthmatic subject (ER) increased his SRaw nearly fourfold and became severely dyspneic during exposure. As a group, the asthmatic subjects significantly increased SRaw ($p < 0.01$), whereas the normal subjects and the atopic subjects did not. The difference among groups was highly significant ($p < 0.001$). The multiple range test revealed highly significant differences between asthmatic subjects and normal subjects ($p < 0.001$) and between asthmatic subjects and atopic subjects ($p < 0.001$) but no difference between normal subjects and atopic subjects.

During exposure to 5 ppm, six of six asthmatic subjects, five of seven normal subjects, and five of seven atopic subjects had significant increases in SRaw. Four of the six asthmatic subjects, but no other subjects, complained of chest tightness and wheezing. In all groups, the rise in SRaw was significant, but the difference in ΔSRaw among groups was highly significant ($p < 0.001$). The multiple range test again revealed significant differences between the asthmatic and normal groups ($p < .01$) and between the asthmatic and atopic groups ($p < .005$) but not between the atopic and normal groups.

Baseline SRaw was not significantly different among groups prior to any exposure, and a plot of ΔSRaw against baseline SRaw at each exposure concentration revealed no apparent correlation. In the 21 exposures during which recordings of tidal volume, respiratory rate, and cough frequency were obtained, no clear-cut relationship of any of these parameters to responsiveness to SO_2 was apparent. No subject demonstrated a single tidal volume greater than 40% of his/her vital capacity during any of these exposures.

Table 3-1: SRaw during exposure to air and to 1, 3, and 5 ppm of SO_2

Normal subjects

Subject	Air	1 ppm SO_2	Air	3 ppm SO_2	Air	5 ppm SO_2
SB	5.03*	4.90	5.63	4.81	4.62	5.13
NT	2.48	3.09	2.97	3.43[2]	3.10	4.23[2]
LJ	5.18	4.81	5.94	5.93	6.27	6.54
JA	6.26	6.43	5.41	5.74	5.93	7.85[6]
CI	7.28	6.62	4.75	4.09	4.82	5.71[1]
CW	6.54	6.56	5.60	5.10	5.33	6.56[4]
NH	9.79	8.46	6.17	6.26	7.69	8.34[1]
mean±SD	6.08±2.25	5.84±1.72	5.21±1.08	5.05±1.03	5.39±1.44	6.34±1.46[3]

Atopic subjects

Subject	Air	1 ppm SO_2	Air	3 ppm SO_2	Air	5 ppm SO_2
SG	4.84	4.98	5.24	4.58	5.01	5.95[3]
EH	5.36	5.57	5.71	6.35[1]	5.61	8.45[6]
AH	6.61	7.64	5.29	5.98	4.82	5.44[2]
BF	2.86	3.40[2]	5.09	6.54[6]	5.51	11.40[6]
ND	7.36	6.80	7.14	6.63	7.44	7.45
PR	2.94	2.51	5.36	4.36	2.98	3.35
MK	4.06	3.94	3.31	3.28	3.90	4.95[4]
mean±SD	4.86±1.73	4.98±1.84	5.31±1.12	5.39±1.31	5.04±1.41	6.71±2.65[3]

Asthmatic subjects

Subject	Air	1 ppm SO_2	Air	3 ppm SO_2	Air	5 ppm SO_2
ER	4.59	8.49[6]	9.52	34.22[6]	----------	
JB	8.83	9.85	6.84	8.62[4]	11.54	16.40[6]
SW	5.88	10.26[6]	4.30	7.15[6]	5.44	29.37[6]
LG	4.09	5.16[5]	4.52	6.45[6]	4.07	14.29[6]
SG	4.57	5.18[1]	5.79	7.12[4]	5.76	11.07[6]
SZ	6.31	5.84	5.90	6.86[2]	4.46	7.26[6]
KS	7.27	7.19	9.10	10.28[1]	6.87	7.86[1]
mean±SD	5.93±1.70	7.42±2.15[1]	6.56±2.07	11.53±10.09[3]	6.36±2.73	14.38±8.16[2]

*units of SRaw are L x cm H_2O/L/s

[1] $p<.05$ [2] $p<.025$ [3] $p<.01$ [4] $p<.005$ [5] $p<.001$ [6] $p<.0005$

Effects of atropine pretreatment. Only one of 17 subjects had a significant increase in SRaw during SO_2 exposure following pretreatment with inhaled atropine sulfate, whereas 15 of 17 had a significant increase following pretreatment with placebo. The difference between these two exposure responses was highly significant ($p < 0.0005$; Fig. 3-2). Baseline SRaw following inhalation of atropine was significantly lower than baseline following inhalation of placebo ($p < .025$); but in three subjects, baseline SRaw was almost the same after inhalation of atropine as it was after inhaling placebo (± 0.25 L x cm H_2O/L/s). In all three subjects, SRaw increased significantly during SO_2 exposure after treatment with placebo but not after treatment with atropine (Fig. 3-3).

Relationship of SO_2 response to histamine reactivity. Plotting the rise in SRaw provoked by the highest concentration of SO_2 (5 ppm in all but one subject) against the slope of each subject's histamine dose-response curve revealed no apparent correlation for any of the three study groups plotted separately or for the pooled data plotted on a single graph (Fig. 3-4).

Discussion

The presently approved Occupational Safety and Health Administration (OSHA) threshold limit value (TLV) for occupational exposure to SO_2 is 5 ppm as a time-weighted average over an 8-h work shift (45). Since this standard is expressed as a time-weighted average, it allows brief exposures to considerably higher concentrations. Previous studies by Frank and others have shown that normal subjects develop bronchoconstriction during exposure to 5 ppm of SO_2 but have not consistently shown bronchoconstriction at lower concentrations (41,42). This study confirms these observations in normal subjects, suggesting that our methods of exposure were comparable, and demonstrates that bronchoconstriction develops in asthmatic subjects at considerably lower exposure concentrations (1 and 3 ppm) and is of significantly greater magnitude. The magnitude of the increase in SRaw and the development of significant dyspnea and wheezing that occurred in a few asthmatic subjects at 1 and 3 ppm and in most at 5 ppm suggest that SO_2-induced bronchoconstriction might be clinically significant at concentrations considerably lower than those permitted in the workplace. The rapid onset of bronchoconstriction and its prompt reversal after treatment with isoproterenol in a few subjects suggest that this response to SO_2 is caused by a change in the tone of airway smooth muscle.

When measurements of Raw are used to infer changes in airway smooth muscle tone, however, it is conceivable that apparent differences in muscle responsiveness might actually reflect differences in baseline airway caliber. Since resistance is inversely proportional to the fourth power of the radius when airflow is laminar, a similar reduction in the radius of a narrow airway will cause a greater change in airway resistance than the same reduction in the radius of a dilated airway. Differences in baseline airway caliber among subjects in our study may have contributed to the

increased responsiveness seen in a few of our asthmatic subjects, but this factor cannot be the sole explanation for our findings. Although the mean values for baseline SRaw differed slightly among groups for all exposure concentrations, none of these differences approached statistical significance. Furthermore, when baseline SRaw was plotted against the rise in SRaw during SO_2 exposure for each exposure concentration, no correlation was apparent. Finally, from the table of individual responses (Table 3-1) it is clear that several asthmatic subjects had very dramatic increases in SRaw during SO_2 exposure in spite of baseline SRaw's considerably lower than those measured in normal or atopic subjects who had no response during exposure to the same concentrations.

Studies by Nadel and others (5) have shown that the bronchoconstriction induced by SO_2 in normal subjects can be blocked by pretreatment with atropine, suggesting that this effect is mediated via parasympathetic pathways. These same workers have shown that the bronchomotor response to SO_2 in cats can be blocked by cooling of the vagus nerves and that constriction of the lower airways of tracheostomized cats will occur following insufflation of SO_2 into an anatomically separated laryngeal pouch (5). This suggests the involvement of a reflex mechanism mediated through the vagus nerves. Our finding that pretreatment with atropine blocks the exaggerated bronchomotor response to inhalation of SO_2 in asthmatic subjects suggests that this abnormally great response is mediated via parasympathetic pathways as well. Since atropine caused a statistically significant fall in SRaw, one could argue that its effect on the subsequent response to SO_2 merely reflects a change in baseline airway caliber. But several observations make this an unlikely explanation for our findings. In one asthmatic subject, for example, atropine caused a small change in baseline SRaw but completely abolished the large responses to inhalation of 1 and 3 ppm of SO_2 (Fig. 3-5). It would be difficult to explain the difference between a fourfold increase and absolutely no change on the basis of the small differences in baseline SRaw. In three subjects, there was by chance very little difference between the values obtained for SRaw after treatment with atropine or saline placebo. Despite similar values for baseline SRaw, each subject developed a significant increase in SRaw during inhalation of SO_2 following saline pretreatment but not following pretreatment with atropine (Fig. 3-3).

Asthmatic subjects manifest abnormally increased bronchomotor reactivity to a variety of stimuli, including inhaled histamine. Simonsson and coworkers have suggested that this may be due to an increase in the activity of vagally mediated reflexes (6). Evidence supporting this hypothesis includes the finding that hexamethonium (a specific ganglionic blocking agent), atropine, and cooling blockade of the vagus nerves all reduce the bronchomotor response to histamine (3 , 4). The observation that histamine stimulates rapidly adapting receptors with afferent fibers in the vagus nerves provides further support for this hypothesis (36). If the exaggerated bronchomotor responses to SO_2 and to inhaled histamine are both mediated via parasympathetic reflex pathways, one might expect similar responsiveness to both stimuli in any individual. Although no subject with normal histamine responsiveness was hyperreactive to SO_2 in our

study, the bronchomotor response to these two agents was not well correlated (Fig. 3-4). There are a number of possible explanations for this observation. The major differences in responsiveness to SO_2 among subjects with roughly equivalent histamine reactivity might be explained by differences in detoxification or scrubbing out of SO_2 in the upper airways so that different quantities are delivered to the larynx or intrathoracic airways. Another possible explanation is that there are differences in the site of deposition of inhaled histamine aerosol and SO_2, suggesting that the abnormality responsible for hyperreactivity might not be uniformly distributed throughout the airways. Finally, these differences could reflect stimulation of different populations of afferent receptors by SO_2 and histamine, a possibility suggested by early work by Widdicombe on the activity of airway afferent fibers in cats (46,47). This study does not provide insights into which of the above mechanisms, if any, explains our observation.

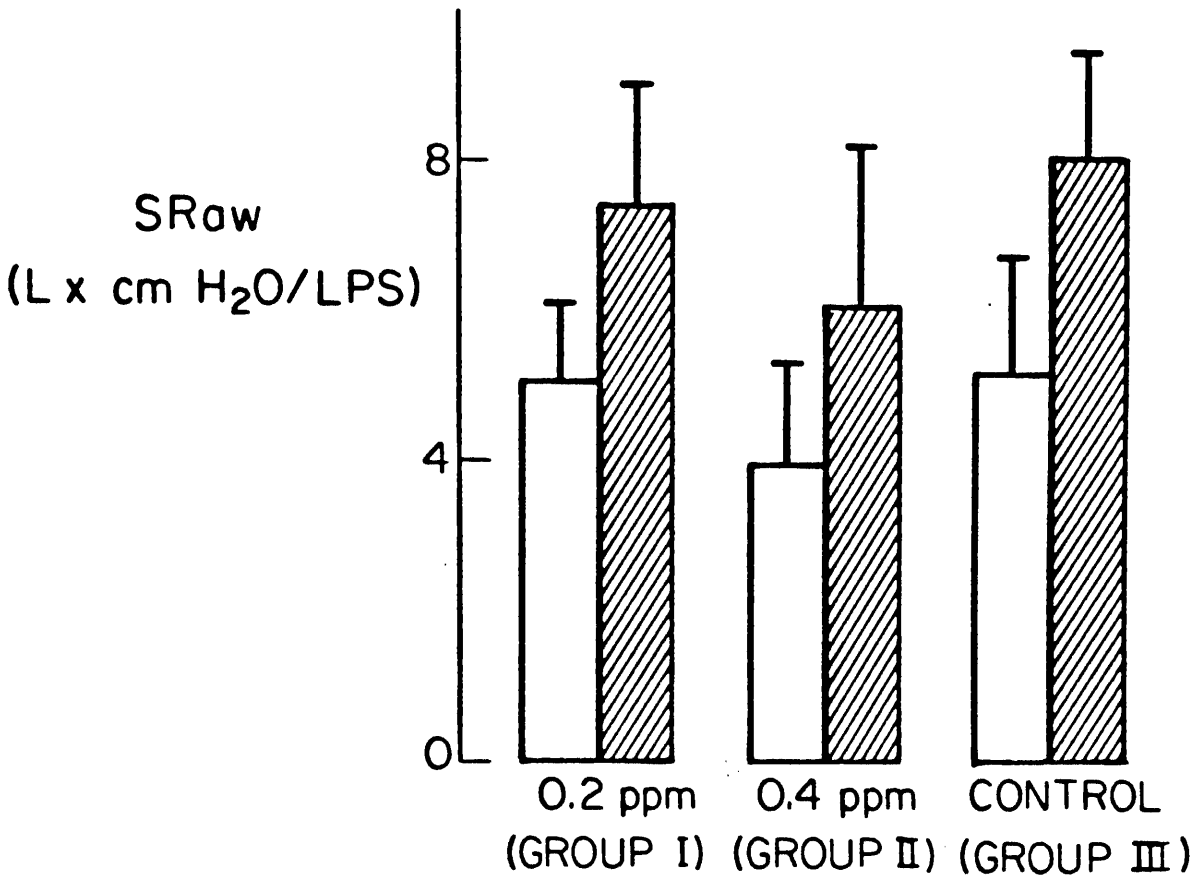

Fig. 1-1: Bronchomotor response to histamine aerosol in three study groups before ozone exposure. Open bars = baseline values for for SRaw (mean + SE); crosshatched bars = mean values obtained after inhalation of histamine aerosol. Neither the mean values for baseline SRaw nor the rises provoked by histamine differed significantly between groups prior to ozone exposure ($p > 0.30$). For further discussion, see text.

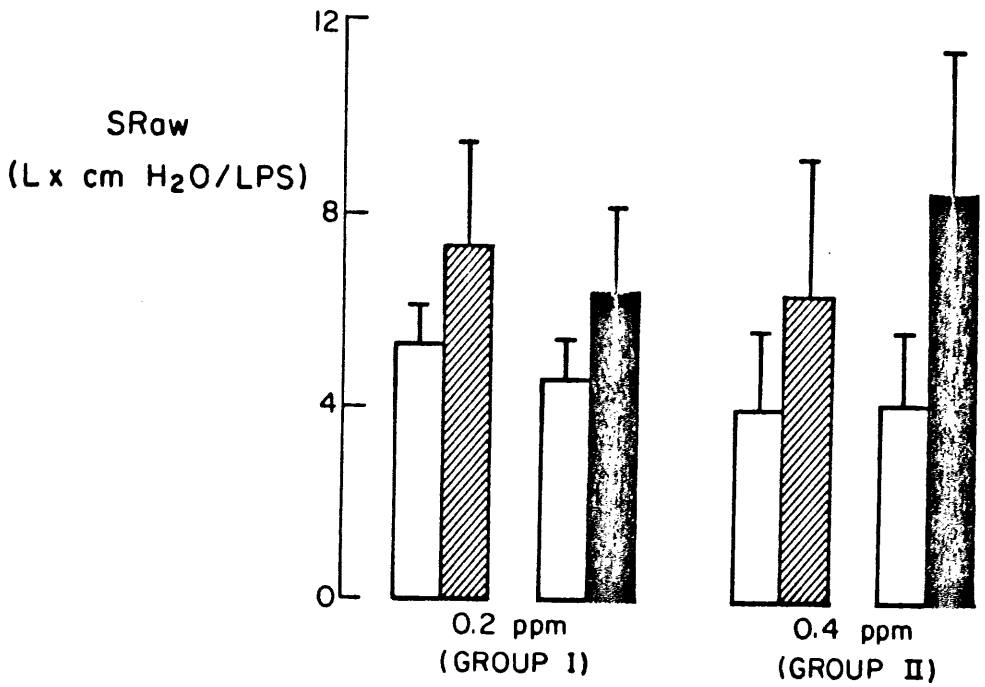

Fig. 1-2: Bronchomotor response to histamine aerosol before and after exposure to 0.2 ppm (Group I) and 0.4 ppm (Group II) of ozone. Open bars = baseline values for SRaw (mean + SE); crosshatched bars = mean values obtained after inhalation of histamine aerosol before exposure to ozone; solid bars = mean values obtained after inhalation of histamine aerosol following exposure to ozone. The rise in SRaw provoked by histamine after ozone exposure is not significantly greater than control in the group exposed to 0.2 ppm ($p > 0.50$) but is significantly increased in the group exposed to 0.4 ppm ($p < 0.025$).

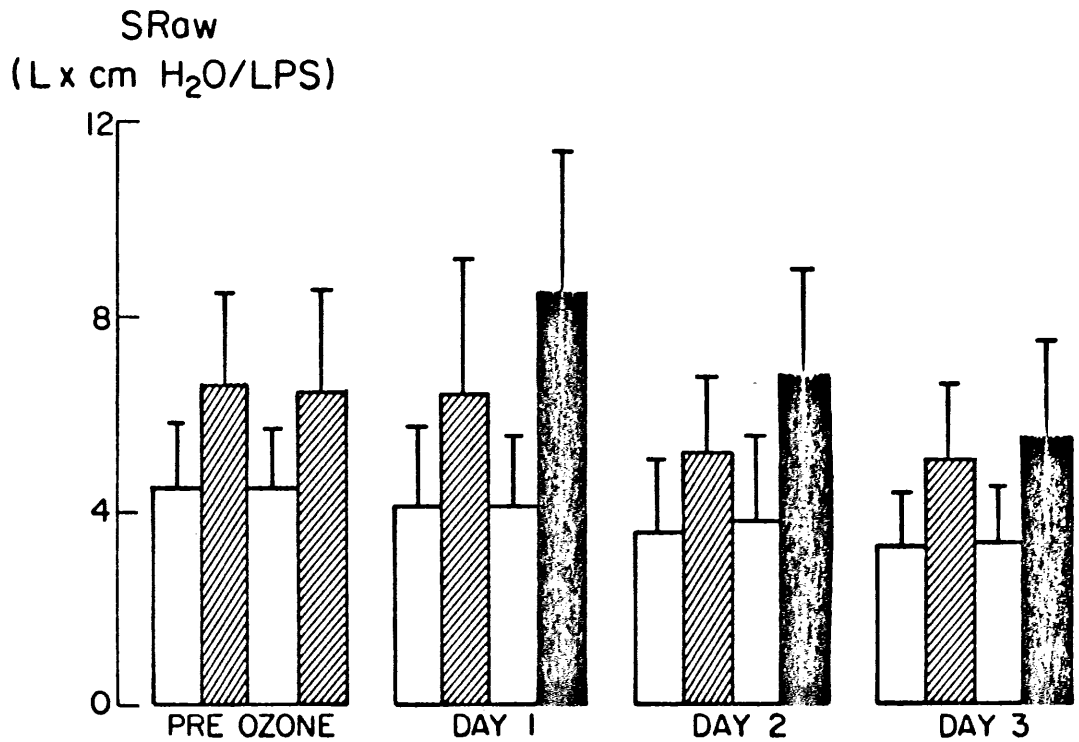

Fig. 1-3: Effect of repeated 2-h exposures to 0.4 ppm of ozone on bronchomotor responsiveness to histamine in seven subjects. Open bars = baseline values for SRaw (mean + SE); cross-hatched bars = mean values obtained after inhalation of histamine aerosol before exposure to ozone; solid bars = mean values obtained after inhalation of histamine aerosol following exposure to ozone. The rise in SRaw provoked by histamine after ozone exposure is significantly greater than control on the first day but not on subsequent days of exposure.

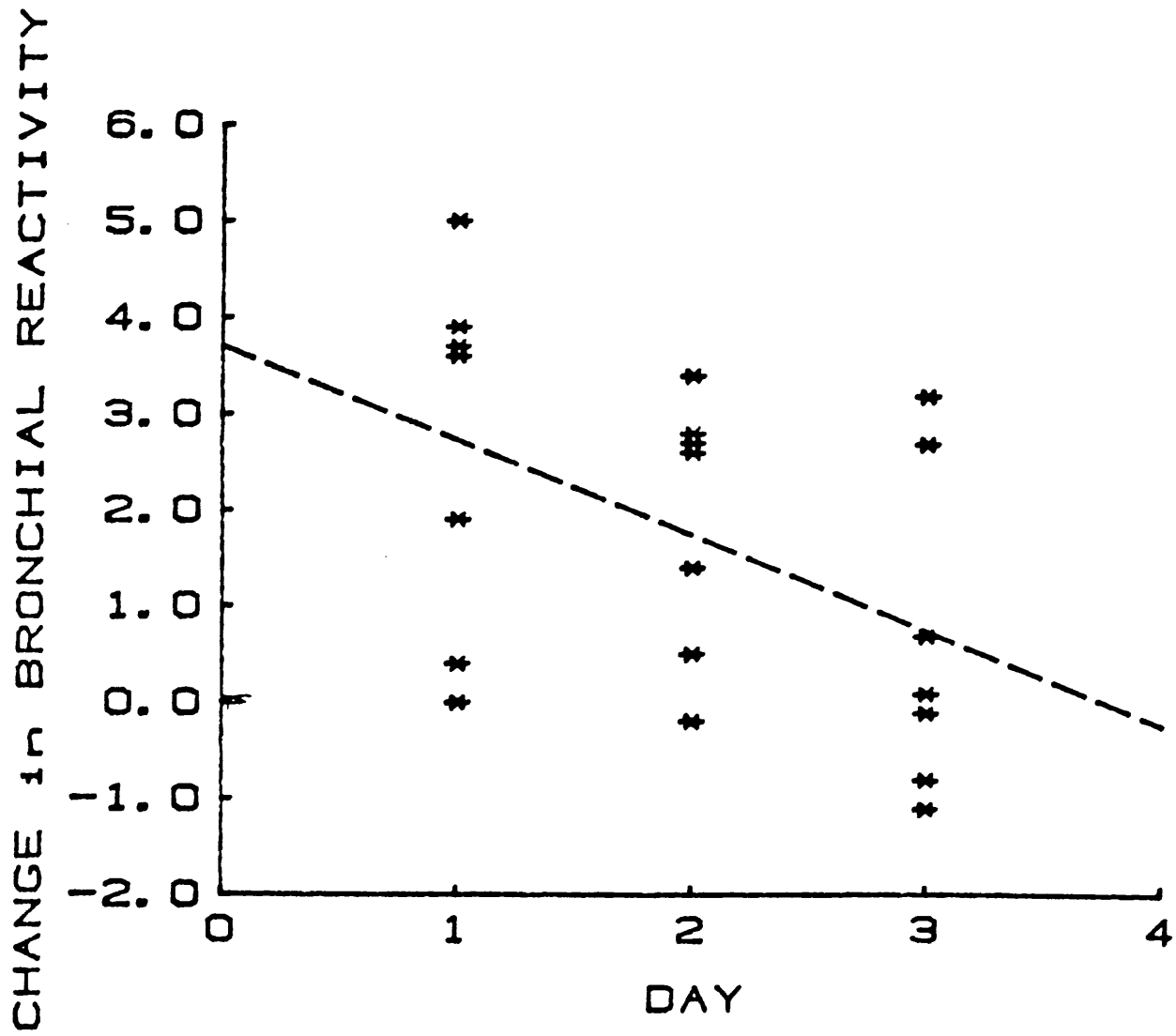

Fig. 1-4: Change in bronchomotor responsiveness to histamine aerosol (bronchial reactivity) with consecutive days of exposure to 0.4 ppm of ozone. The change in bronchial reactivity caused by ozone exposure in each subject was calculated as the difference between the rise in SRaw provoked by histamine after ozone exposure and that provoked before ozone exposure. Days 1 to 3 refer to three consecutive days of 2-h exposures to 0.4 ppm of ozone. The slope of the line generated by linear regression is negative and differs significantly from zero ($p < 0.02$), indicating a progressive decrease in the change in bronchial reactivity caused by ozone exposure.

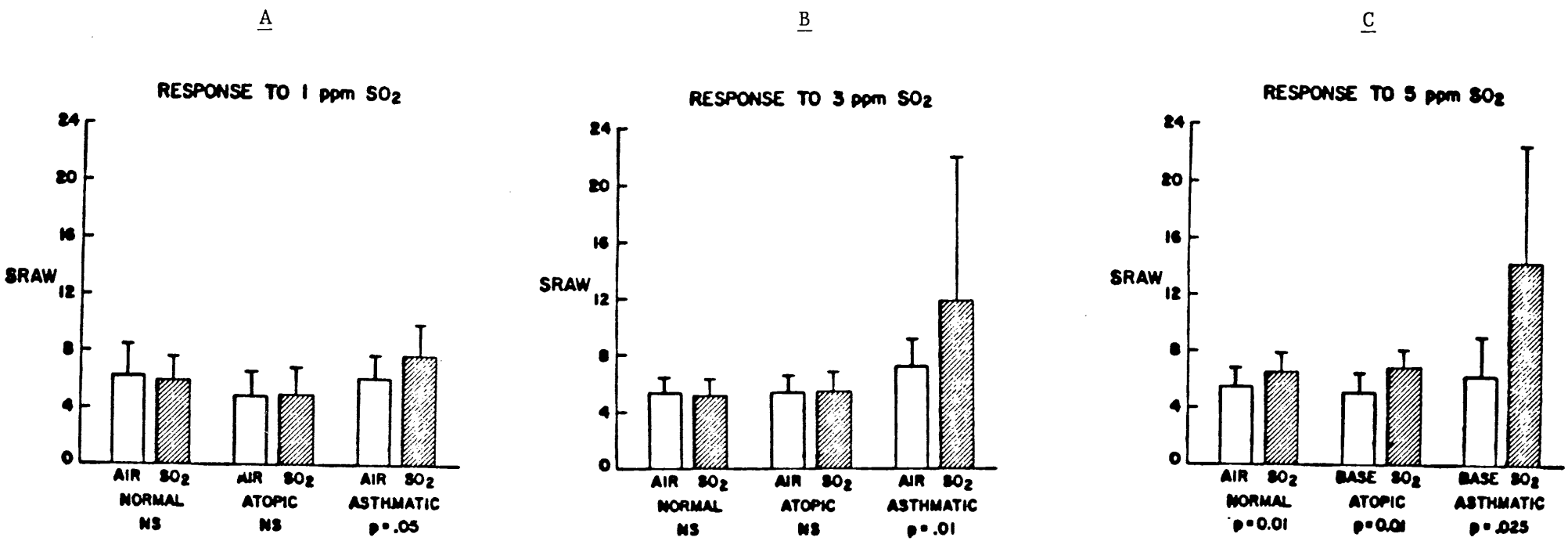

Fig. 3-1: Mean + SD for SRaw following 5 min breathing filtered air (open bars) and during the last 5 min of exposure (crosshatched bars) to 1 ppm (A), 3 ppm (B), and 5 ppm (C) of SO_2 in seven normal, seven atopic, and seven asthmatic subjects.* The p values represent the significance of the difference between values of SRaw obtained following exposure to air and during SO_2 exposure for each study group at each exposure concentration.

*One asthmatic subject developed severe bronchoconstriction during exposure to 3 ppm and was therefore not exposed to 5 ppm. Thus, the graphs for asthmatic subjects at 5 ppm include data only from six subjects.

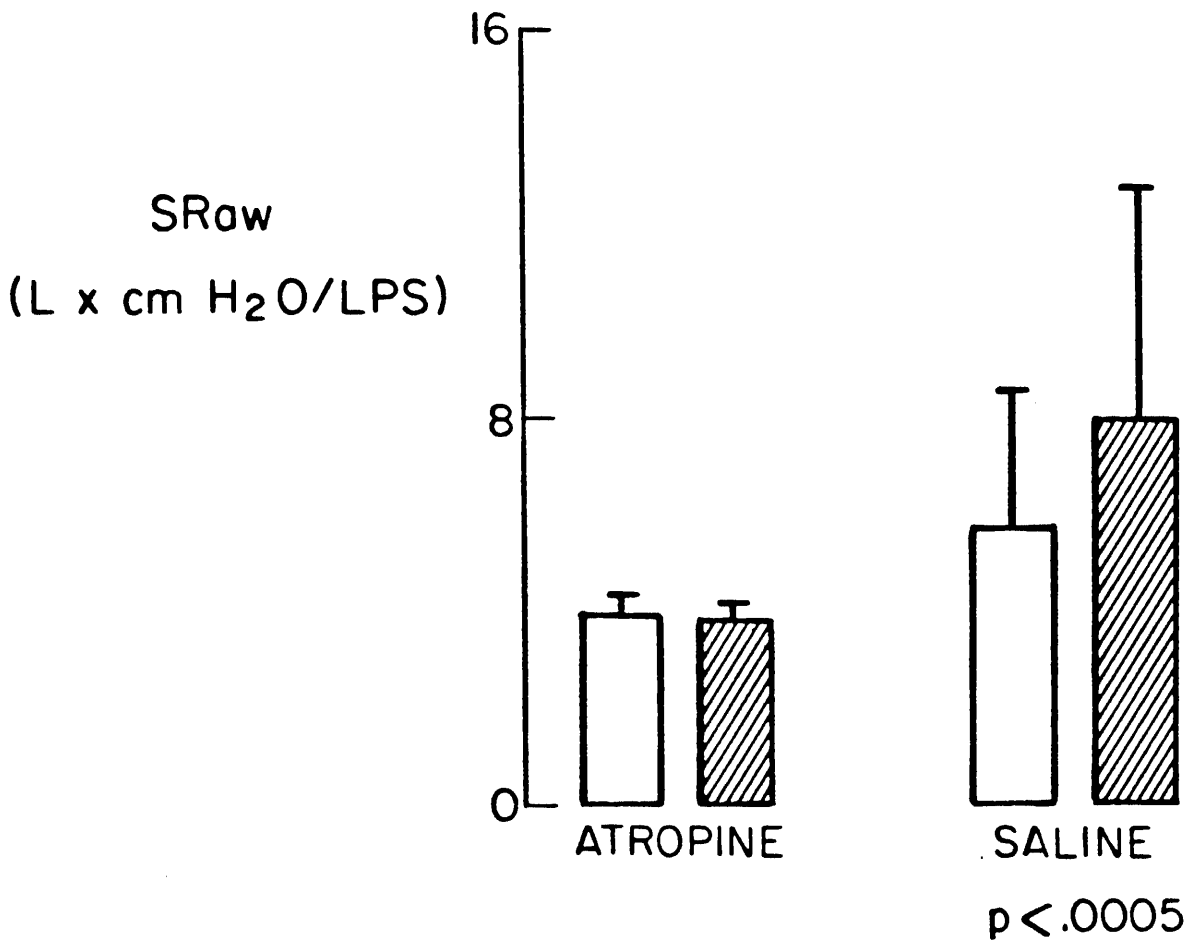

Fig. 3-2: Mean + SD for SRaw following 5 min breathing filtered air (open bars) and during the last 5 min of exposure to SO_2 (crosshatched bars) in 17 subjects following pretreatment with atropine and with saline placebo. The concentration of SO_2 was 5 ppm in 16 subjects and 1 ppm in one subject who developed severe bronchoconstriction during exposure to 3 ppm. The p value represents the significance of the difference between values of SRaw obtained following exposure to air and during SO_2 exposure under each experimental condition.

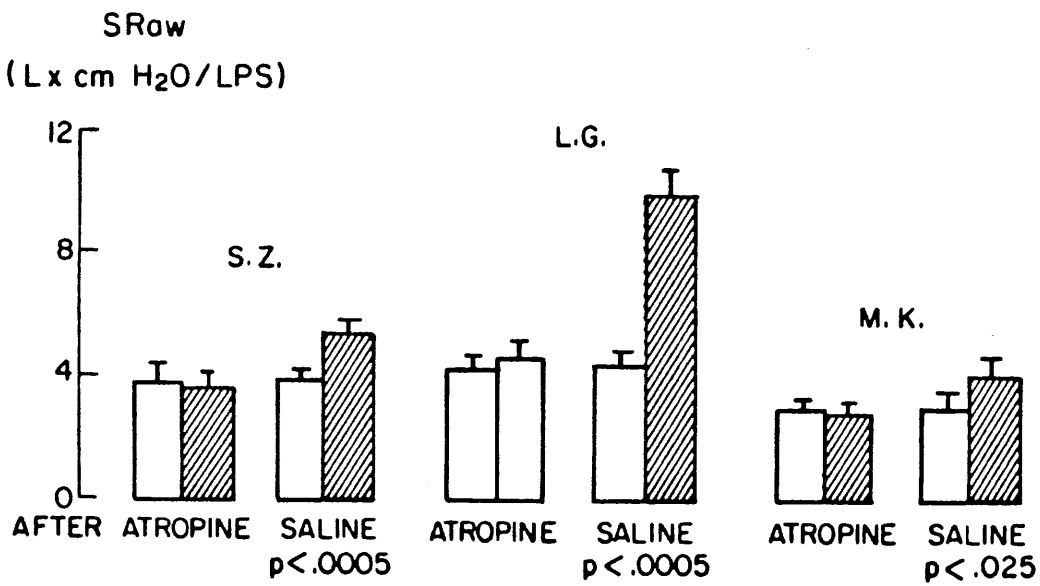

Fig. 3-3: Mean + SD for SRaw following 5 min breathing filtered air (open bars) and during the last 5 min of exposure to 5 ppm SO_2 (crosshatched bars) following pretreatment with atropine and saline placebo in each of three subjects. After pretreatment with atropine, exposure to SO_2 did not cause a significant increase in SRaw in any of these subjects. The p values refer to the significance of the increase in SRaw during SO_2 exposure after pretreatment with saline.

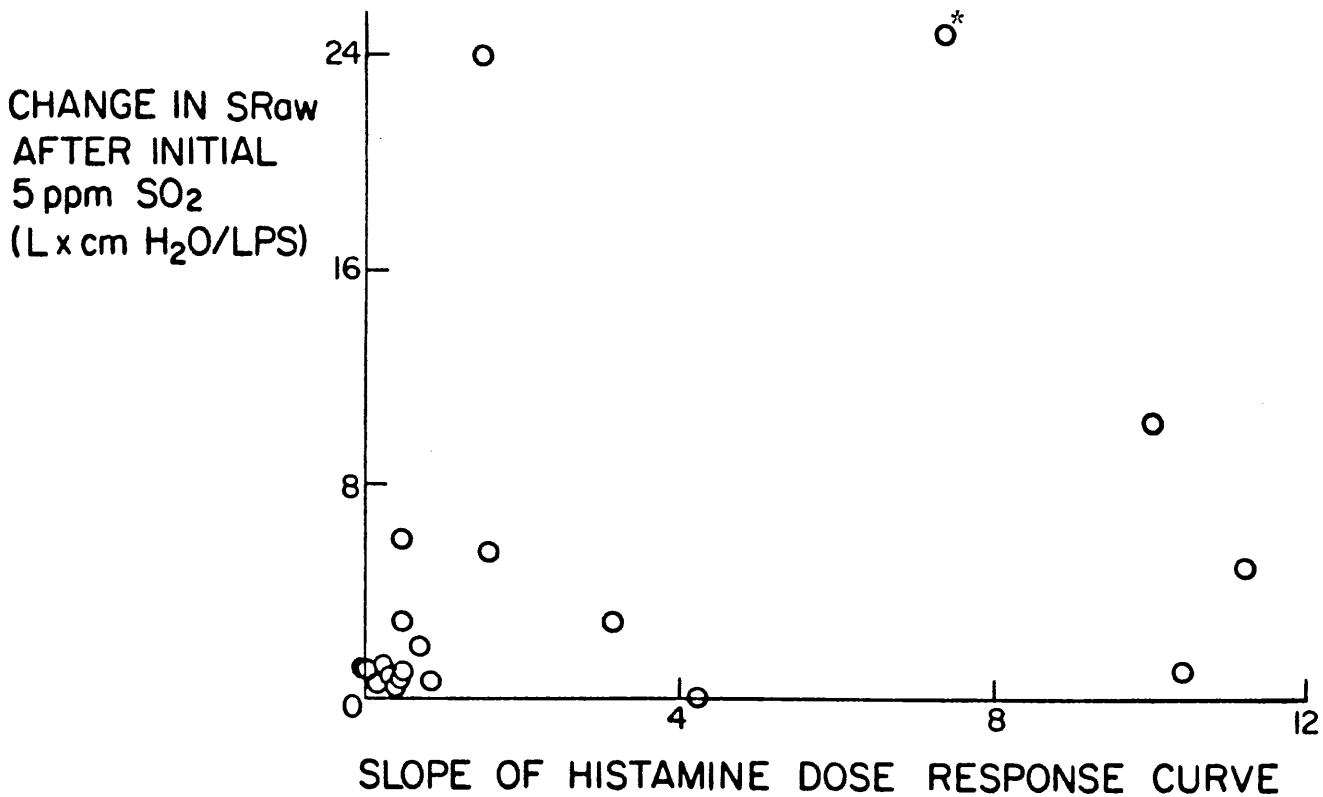

Fig. 3-4: Plot of the change in SRaw produced by breathing 5 ppm of SO_2 against the slope of each subject's histamine dose-response curve (see Methods). The highest value for the slope of the histamine dose-response curve for any normal subject was 0.85. The lowest value for any asthmatic subject was 1.48. There is no significant correlation between these two measurements ($p > 0.05$).

*This subject developed severe bronchoconstriction during exposure to 3 ppm of SO_2 and was thus not exposed to 5 ppm. The y coordinate of this point therefore represents this subject's change in SRaw during exposure to 3 ppm.

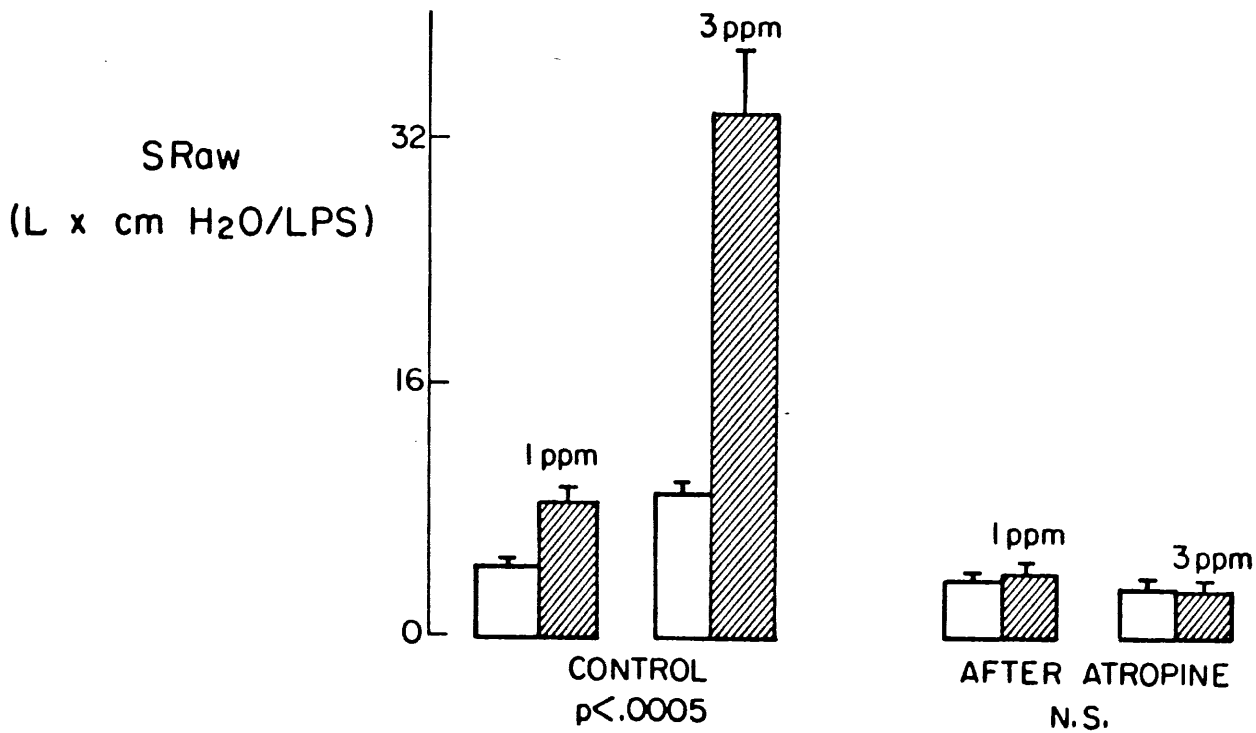

Fig. 3-5: Mean + SD for SRaw following 5 min breathing filtered air (open bars) and during the last 5 min of exposure to 1 and 3 ppm of SO_2 (cross-hatched bars) in the control state and following pretreatment with atropine in one asthmatic subject (ER). The p value represents the significance of the difference between values of SRaw following exposure to air and during SO_2 exposure at each concentration of SO_2 under each experimental condition.

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GLOSSARY

1. Alveolar-arterial oxygen difference [(A-a)dO_2]= the difference between the mean value for alveolar oxygen tension (calculated by the alveolar gas equation) and measured arterial oxygen tension.

2. Bronchial reactivity = the responsiveness of airway smooth muscle, as reflected by changes in tests of airway caliber (airway resistance, maximal expiratory flow, or forced expiratory volume in one second) provoked by inhalation of an irritating material (usually an aerosol of a solution of histamine or methacholine).

3. Bronchial hyperreactivity = exaggerated responsiveness of airway smooth muscle, as measured by the tests described above.

4. Dosimeter = a dose metering device, consisting of a nebulizer connected to a 20-psi pressure source through a solenoid valve. At the onset of inspiration, the valve opens for a preset interval (usually 0.6 s), so that the volume of solution aerosolized with each breath is constant.

5. Raw = airway resistance (cm H_2O/L/s).

6. TGV = thoracic gas volume (L).

7. SRaw = the product of Raw and TGV (L x cm H_2O/L/s). Because bronchoconstriction may cause an increase in either Raw or TGV alone and because a simple decrease in lung volume causes Raw to increase, the use of SRaw is both more sensitive and more specific for changes in airway caliber.

8. Threshold = the lowest dose of an agent causing a measurable change in the function monitored.

9. Tolerance = the induction of resistance to the toxic effects of high doses of an agent by prior exposure to lower doses of the same agent.