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Air Pollution Effects on Nasal Function

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Abstract

We performed a series of studies to determine whether sulfur dioxide and ozone increase nasal symptoms, nasal resistance to airflow, or nasal responses to other stimuli. In the first study, we found that sulfur dioxide did not acutely increase nasal symptoms or resistance to airflow in 12 subjects with demonstrated nasal aresponsiveness to instillation of antigen or in 10 subjects with a history of nasal responsiveness to antigenic or nonantigenic stimuli. In a second study, we found that ozone tended to cause an increase in rhinorrhea, nasal congestion and sneezing, but this increase in symptoms was not statistically significant, was small when compared to the effects of intranasal antigen, and was not associated with a statistically significant increase in nasal resistance. Biochemical and cellular analysis of nasal lavage fluid from 8 of these subjects did not show a consistent or striking ozone-induced change in histamine, protein, or inflammatory cells in nasal secretions. Finally, results from our third study suggest that ozone augments nasal responsiveness to antigen in at least some subjects with allergic rhinitis.

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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.

Summary and Conclusions

In this series of investigations we examined the effect of sulfur dioxide and ozone on nasal symptoms and function in people with chronic allergic or nonallergic rhinitis. We considered this issue important for several reasons. Firstly, chronic rhinitis is the most common disease of the respiratory tract. As many as 40 million Americans are thought to have the condition, which is characterized by inflammation of the lining of the nose and by symptoms of nasal discharge, congestion, and sneezing for at least 30 minutes a day for two months or longer. The economic burdens and morbidity caused by chronic rhinitis are not trivial: according to one survey, allergic rhinitis caused 6 million bedridden days, 28 million restricted days, and 500 million dollars in physician and drug costs. In children, chronic rhinitis may contribute to developmental delay and infection of the sinuses and middle ear with ultimate hearing impairment.

A second reason to perform this study is that sulfur dioxide and ozone are almost ubiquitous in urban air. Such gaseous particles and water-soluble pollutants are efficiently removed from inspired air by the nose, which thereby protects the tracheobronchial tree and alveoli, but at the cost of itself being exposed to a large cumulative burden of pollutants.

From studies of the effects of pollutants on the lower respiratory tract, we hypothesized that sulfur dioxide and ozone can increase nasal resistance or increase nasal responses to other agents. It has been shown, for instance, that people with asthma may develop symptomatic attacks of bronchoconstriction after breathing sulfur dioxide at concentrations that have no detectable effect on people without asthma. This greater sensitivity to sulfur dioxide is thought to reflect a general airway "hyperirritability" in people with asthma. We have shown that a similar state of airway hyperirritability can be induced even in healthy people by brief exposure to levels of ozone that have been exceeded in California cities. Although this effect is associated with inflammation of the respiratory mucosa, the exact mechanisms of ozone-induced hyperirritability and of sulfur dioxide-induced bronchoconstriction remain to be elucidated.

If the nose is similar to the lower airways, then it too may develop states of hyperresponsiveness. This appears to be the case, for just as the bronchi are more reactive in people with asthma, the nose appears to be more reactive in people with allertic rhinitis; sneezing, nasal obstruction, and mucus secretion are all provoked by lower doses of histamine, methacholine, or ammonia vapor than in healthy subjects. If we were to find a population with increased nasal sensitivity to inhaled pollutants or with pollutant-induced nasal hyperresponsiveness to other agents, then the accessibility of the nose combined with recent advances in cytological and biochemical analysis may facilitate a better understanding of the mechanisms of airway responses in general. Even the finding that these pollutants do not change nasal resistance or reactivity would be of interest; it would then be important to investigate the differences in blood supply, innervation, cells, or chemical mediators which protect the nose from the effects of pollutants demonstrated in the lower airways.

Thus, these investigations are of environmental, epidemiologic, and physiologic importance. We proceeded with the investigations in three phases: 1) we determined the effects of sulfur dioxide on

nasal function in people with rhinitis 2) we determined the efects of ozone on nasal resistance and cellular content of nasal lavage fluid in people with rhinitis and 3) we determined the effects of ozone on nasal reactivity to allergen in subjects with allergic rhinitis.

1) Effect of sulfur dioxide on nasal function in subjects with symptoms exacerbated by allergens or by chemical or physical irritants: Design and results

To determine whether brief exposures to moderately high concentrations of sulfur dioxide causes acute increases in nasal symptoms and nasal resistance in subjects with chronic rhinitis, we studied 22 subjects who gave a clear history of chronic intermittent symptoms of nasal congestion, runny nose, or sneezing after breathing comon allergens or irritants. Nineteen of these subjects had some manifestation of allergy, such as a high serum IgE level, positive skin test responses to common allergens, or nasal symptoms distinctly exacerbated by common allergens. For each subject, we compared the nasal symptoms and resistance to airflow associated with breathing 4 ppm of sulfur dioxide for 10 minutes to those associated with breathing conditioned air.

We found that the increase in nasal resistance and nasal symptoms after subjects quietly breathed 4 ppm SO2 was not greater than that after subjects breathed only filtered conditioned room air. We conclude that brief exposure to SO2 at a concentration of 4 ppm or less is unlikely by itself to cause significant nasal dysfunction in most subjects with chronic rhinitis and that the presence of the mechanisms of immediate hypersensitivity in the nasal mucosa does not necessarily confer nasal sensitivity to sulfur dioxide. We also infer from our findings that healthy subjects without rhinitis are unlikely to suffer acute nasal effects after breathing similar or smaller concentrations of sulfur dioxide.

2) Effect of ozone on nasal resistance, nasal reactivity, and cellular content of nasal lavage fluid in people with allergic rhinitis: Design and results

To determine whether concentrations of ozone which cause cough, chest pain, and increased bronchial constriction and responsiveness in healthy people also cause nasal discomfort, increased nasal resistance, and increased nasal responsiveness in subjects with allergic rhinitis, we performed18 experiments on 16 subjects who gave a clear history of allergic rhinitis. Nasal symptom scores and nasal resistances were measured immediately before and after a 2-hour exposure to ozone and a 2-hour sham exposure. Also, to determine if breathing ozone causes a significant change in the cellular and histamine content of nasal secretions, we studied nasal lavage fluid obtained before and after the ozone and sham exposures in 8 of the experiments.

We found that breathing 0.55 ppm of ozone for 2 hours does not cause a significantly greater increase in nasal symptoms, nasal resistance, or nasal lavage fluid cells, protein or histamine than does breathing conditioned air alone. We infer from these findings that subjects without rhinitis are unlikely to suffer acute nasal effects after breathing similar or smaller concentrations of ozone.

3) Effect of ozone on nasal sensitivity to allergen in subjects with allergic rhinitis: Design and results

To determine whether exposure to levels of ozone that have been shown to cause bronchial inflammation and constriction also alters nasal sensitivity to inhaled allergens in people with allergic rhinitis, we compared the effects on the nose of increasing concentrations of antigen after ozone exposure to those after sham exposure in 10 subjects with allergic rhinitis. More specifically, we compared the concentration of antigen which caused symptoms and a doubling of the nasal resistance (the "provocative concentration") on the ozone day to the concentration required to cause similar changes on the control day. We found that 3 of the 10 subjects developed nasal symptoms and resistance at a concentration of antigen that was only one tenth the concentration required to cause the same changes on the control day. No subject developed symptoms or restriction at a smaller provocative concentration on the control day than on the ozone day. This suggests that ozone-induced byperreactivity to allergen is a real finding, although the results in this small sample will have to be corroborated in a larger study.

In summary, our final findings are:

1) In both allergic and non-allergic subjects who develop nasal symptoms after exposure to allergic or physical and chemical stimuli, sulfur dioxide did not cause an acute increase in nasal symptoms or nasal resistance to airflow.

2) In subjects with allergic rhinitis, concentrations of ozone which are rarely exceeded in urban air did not cause a significantly greater increase in rhinorrhea, nasal congestion, and sneezing than did conditioned air alone. The increase in symptoms, moreover, was small when compared to the symptoms caused by allergens, and was not associated with a significant increase in nasal resistance to airflow.

3) In some subjects with allergic rhinitis, ozone increased the nasal sensitivity to allergen.

4) Neither sulfur dioxide nor ozone appeared to be associated with a consistent, acute increase in total cells, inflammatory cells, protein, or histamine in the nasal secretions. This finding must be taken in the context of the marked variation of these indices even in the control state.

Recommendations

On the basis of this and other work performed under contract with the California Air Resources Board, we suggest that the following work be considered:

1) Determine whether subjects with allergic rhinitis respond to 3-fold or 5-fold smaller concentrations of antigen after breathing ozone than they do after breathing air. The current study was designed to detect a big difference in responsiveness to antigen (on the order of subjects sensitized to 10-, 100-, or 1000- fold smaller doses of antigen). We found that only a few subjects became this much more sensitive to antigen. By modifying the antigen dose response curves to include dilutions of, say, 1:625, 1:125, 1:25, 1:5, and 1:1, one may find more subjects sensitized by ozone to allergen, albeit to a smaller degree. If so, one may more reasonably conclude that ozone-induced hyperresponsiveness is a general--not idiosyncratic--phenomenon in the large population with allergic rhinitis. We would caution that developing the methods to perform such necessarily refined and reproducible antigen dose-response curves in the nose may be very time-consuming, for methods to extract, measure, preserve, and deliver antigens are not at all standardized. We would also caution that the enhanced responsiveness seen after exposure to 0.5 ppm of ozone may not occur after exposure to lower concentration of ozone.

2) Determine whether air pollutants such as ozone cause a delayed increase in nasal reactivity to antigens or other provocative stimuli. Our study excluded an acute change in nasal resistance and reactivity in most subjects with allergic rhinitis, but we did not examine whether people are more susceptible to antigenic provocation in the day to week after an exposure.

Effect of sulfur dioxide on nasal function in subjects with symptoms exacerbated by allergens or by chemical or physical irritants

Introduction

The purpose of this study as initially proposed in our contract was to examine whether sulfur dioxide (SO_2) causes significant nasal symptoms or dysfunction in healthy subjects and in subjects with chronic nonallergic rhinitis.After some preliminary studies, we felt we could provide a more conclusive study by studying a population with nasal symptoms distinctly exacerbated by allergens or chemical and physical irritants. If this well defined, well characterized, and perhaps more susceptible index population developed SO_2 -induced symptoms, we would then examine whether healthy subjects also developed nasal dysfunction after breathing SO2. We thus focused our studies on subjects who develop rhinorrhea, nasal congestion, or sneezing when exposed to allergens (pollens, grasses, dust, danders), chemical irritants such as perfumes and fumes, or physical irritants such as cold dry air or smoke.

Our reasons for studying the effects of SO2 on nasal function in these subjects derived from studies of SO2's effects on the lower airway. It is known that SO2 has important effects on the lower respiratory tree. In healthy people, acute exposure to 5 ppm of SO2 for 10 minutes can cause bronchoconstriction, as shown by an increase in airways resistance (1). Moreover, in work supported by the California Air Resources Board, we have shown that people with asthma develop symptomatic bronchoconstriction at rest with oral inhalation of 1 ppm of SO2 (2) , and during eucapnic hyperventilation or during moderate to heavy exercise with as little as 0.1 ppm (3,4).

It is reasonable to suspect that SO2 has effects in the nose and upper airway as well, for it is here that 99% of the highly soluble gas is adsorbed from the inspired air (5). Since some investigators have found that sneezing, nasal obstruction, and mucus secretion are all provoked by lower doses of methacholine, histamine, and ammonia in people with allergic rhinitis than in healthy subjects (6-8), we reasoned that there may be subpopulations whose nasal passages are especially sensitive to SO2, in much the same way that people with asthma have an increased bronchial sensitivity to SO_2. In particular, people with chronic allergic rhinitis contain in their nasal mucosa an increased number of basophils and mast cells (9,10). These cells have been implicated in the responses of the lower airway to SO2, for sulfur dioxide-induced bronchoconstriction can be attenuated by disodium cromoglycate, a drug thought to stabilize mast cells (11, 12, 13). The increased number of basophils and mast cells may thus predispose people with allergic rhinitis to respond more readily to SO2.

Another population that may be more susceptible to the effects of air pollution are people with chronic nonallergic (or vasomotor) rhinitis. The mechanisms and definitions of this entity are unclear, but the general impression is that these subjects develop distinct nasal reactions to environmental irritants and do not have clear manifestations of allergy (15). Whatever mechanisms may cause such subjects to react to smoke, cold dry air, or fumes may also predispose them to react to SO2.

Despite the prevalence of chronic rhinitis (16) and the ubiquity of SO2 pollution, few studies

have been done on the effects of SO_2 on nasal symptoms and obstruction in people with rhinitis (17, 18, 19), and these studies did not always exclude the effects of other physical and environmental factors on nasal function. We therefore examined whether brief exposure to moderately high concentrations of SO2 acutely provokes nasal symptoms and increases in nasal resistance in people with chronic or frequent sneezing, rhinorrhea, or nasal congestion induced by allergens or physical and chemical irritants.

Materials and Methods

Subjects

We studied 19 women and 3 men, 23 to 44 years old, who gave a history of chronic intermittent symptoms of nasal congestion, rhinorrhea, or sneezing after exposure to common allergens or irritants (such as perfume and smoke), unassociated with respiratory infections. Subjects were characterized according to their personal and family history of allergy or asthma, the precipitants and seasonality of their nasal symptoms, the serum IgE level, skin test responses to a panel of 5 common northern California antigens, current medications, and smoking habits (Table I). Only 3 subjects (subjects 14, 17, 18) had no manifestation of atopy. The remainder of our subjects had either a high serum IgE level, at least one positive skin test to an allergen, or a history of nasal symptoms clearly exacerbated by common allergens. Thirteen subjects with a positive skin test to an allergen and/or a history of nasal symptoms provoked by a common antigen were given a nasal challenge with that antigen (Table 2). All but 1 of these subjects developed sneezing, rhinorrhea, nasal congestion, or at least a doubling in nasal resistance after nasal antigen challenge. Eight other subjects were not tested with nasal antigen because they either did not give a clearcut history of allergic rhinitis or did not have a positive skin test.

Four subjects who took daily medications (oral contraceptives, thyroid hormone replacement, or niacin and colestin) continued their regimen through the study. Five of our subjects smoked fewer than 5 cigarettes per day; the remainder were non-smokers. We excluded from the study people with a history suggestive of a viral respiratory infection in the 4 weeks before the study, and we studied subjects with seasonal allergic rhinitis during a season when they were least symptomatic. No subject used nasal preparations of steroids or cromolyn chronically. All refrained from any medication for at least 24 hr, and from tea or coffee for at least 4 hr, before each study. All were informed of the risks of each procedure and signed consent forms approved by the Committee on Human Research of the University of California.

Experimental design

Subjects came to the laboratory at the same time of day on 2 study days at least 48 hr apart. After subjects described their nasal symptoms, the baseline nasal airway resistance was measured. The mean of 3 nasal resistance measurements taken 30 s apart was used in data analysis. (If subjects felt unusually congested and were unable to breathe freely through the nose, we had them return on a different day for the study.) Subjects then breathed either filtered humidified room air or 4 ppm of SO2 in filtered humidified room air for 10 min through a mask which covered only the nose. Subjects breathed tidally while sitting quietly, breathing in through the nose and out through the mouth. The gas mixtures were delivered in random order, so that neither the subject nor the

investigator making the resistance measurements knew which gas had been given on each day. Subjects were asked to describe any symptoms during and after the exposure. Nasal airway resistance was measured 2 min after the completion of the exposure.

To determine if breathing SO_2 caused a significant change in the cellular and histamine content of nasal lavage fluid, we obtained nasal lavage fluid from 4 of the subjects. We determined the subject's baseline nasal resistance, performed 4 10-ml lavages of the nose, and determined the nasal resistance once more. If nasal lavage changed nasal resistance then we waited until repeat measurements returned to within 20% of the initial reading or were stable for 10 min. Subjects then breathed air or 4 ppm SO_2 through the nasal mask for 10 min. Nasal resistance was measured 2 min after the completion of the exposure and at 5 min intervals for 15 min. A fifth lavage was performed 15 min after the end of the exposure.

Skin testing

At least 1 week before the actual experiment began, each subject had skin prick tests to saline and histamine controls (Center Lab, Port Washington, NY) and to extracts of mixed weeds, mixed grasses, cat hair, house dust, and house dust mite which contained 50% glycerin and 0.4% phenol as preservatives (Hollister-Stier, Spokane, WA). Skin reactions were read 15 minutes after the tests were placed. Subjects were considered to have had a positive response to a particular antigen if 1) they had no reaction to saline and 2) the wheal and flare caused by that antigen was greater than or equal to that caused by histamine alone.

Nasal airway resistance measurements

Nasal airway resistance was measured by posterior rhinomanometry (20, 21). Nasal air flow was measured through a Fleisch #1 pneumotachygraph inserted in the faceplate of an airtight diving mask which covered the eyes and nose. The pressure drop across the nose was measured as the difference between the pressure in the mask adjacent to the nares and the pressure in the oropharynx, sampled through a catheter placed appproximately 5 cm into the mouth and held between the lips. Using 2 differential pressure transducers (Validyne MP45-16-871, Northridge CA), the transnasal pressure was displayed on the x-axis and flow on the y-axis of a calibrated image-retentive oscilloscope (Tektronix 5115). We measured the transnasal pressure during inspiration at a reference flow of 0.15 L/s. Nasal resistance was computed as transnasal pressure ÷ 0.15 L/s. If a subject was unable to generate a nasal airflow rate of 0.15 L/s, then we measured the flow at a reference transnasal pressure of 0.25 cm H_2O. Nasal resistance was then computed as 0.25 cm H_20 ÷ flow. This occurred only rarely after nasal challenge with allergen, and not after challenge with air or SO_2.

Nasal allergen challenge

We measured the responses of 13 subjects to nasal antigen challenge. After subjects described their nasal and allergic symptoms at baseline, we measured the baseline nasal resistance and delivered nasal sprays first of diluent and then of increasing concentrations of antigen solution until subjects developed typical symptoms of an allergic attack or at least doubled the nasal airway

resistance or until the most concentrated dose of antigen was achieved, whichever occurred first. The antigen to be given was chosen on the basis of history and a positive skin test. Antigen extracts used in intranasal challenges were similar to the extracts used for skin prick tests, except that the extracts given nasally contained less than 2% glycerin (Hollister-Stier, Spokane, WA). Dilutions of 1:10000, 1:1000, 1:100, and 1:10 in phosphate-buffered saline were made once weekly and stored at 4°C. Antigen solutions were delivered to the nose with spray bottles which delivered 65 ± 7 µl per activation (Syntex Laboratories, Palo Alto, CA). There was no significant difference in the volume delivered between 8 bottles that we calibrated or with up to 50 repeated activations per bottle.

Gas delivery

We generated a stream of filtered air having a temperature of 24° C, a dew point of15°C, and an average relative humidity of 55% by passing air from a compressed air source through vapor filters, a bubble humidifier, and a high efficiency particle air filter. To deliver 4 ppm of SO2, we added air containing 500 ppm SO_2 at a metered rate into the stream of air as it passed through a 3L glass mixing chamber. The gas was delivered to the inlet port of a nasal mask at a flow rate greater than 0.5 L/s, the maximum nasal inspiratory flow rate generated by any of our subjects during tidal breathing. Gas exited the mask through a 45 cm length of Teflon tubing and emptied into a laminar flow hood. The temperature, dew point, and SO_2 concentration of samples taken from a port in the inspiratory limb of the airstream 30 cm from the mask were monitored continuously with a digital humidity analyzer (EG and G Model 911 Dew-All) and a pulsed fluorescent SO2 analyzer (Thermo Electron Corp. Series 43, Walnut CA). All tubing to be in contact with SO2 was constructed of Teflon, glass, or stainless steel. The nasal mask was constructed of rubber (Porter Instrument Co, Inc., Hatfield PA) and coated with a fluoropolymer (Fluoroglide, Norton Performance Plastics, New Jersey). We confirmed in preliminary trials that SO_2 concentrations in the mask reached 4 ppm within 1- 2 min of placing it over the subject's nose and remained at that level during 10 min of tidal breathing. Because expired water droplets interfered with our measurements of SO_2 , we chose to monitor SO_2 concentrations proximal to the mask during the experiments.

Nasal lavage

A soft rubber 12 French catheter fitted with an inflatable 5-ml balloon (Bardex Foley Catheter, CR Bard, Inc, Murray Hill, NJ) was inserted 1 to 1.5 cm into the vestibule of the nose so that the balloon, once inflated, rested firmly against the outside of the nostril. Five ml of warmed (37° C) calcium- and magnesium-free Hank's balanced salt solution (CMF HBSS) was instilled in the nose in increments of 1 to 2 ml while the subject, who sat upright with the neck extended and chin tilted slightly upward, whistled or exhaled slowly through pursed lips. After the fluid had remained in the nose for 10 s, the balloon was deflated, and the subject gently expelled the fluid into a polypropylene funnel and receptacle. Five ml of the solution was then instilled and expelled from the other nostril into the same receptacle, which was immediately placed in ice. This procedure was repeated for each of the five lavages on a given study day.

Cytological and biochemical studies

One ml of fluid was removed from the first, fourth, and fifth lavage specimens on each day for cell counts in a Neubauer hemacytometer. Lavage specimens were then centrifuged at 1000 rpm for 10 min at 4° C. Five hundred µl of supernatant from each sample was immediately placed in polypropylene tubes containing 500µl of 4% perchloric acid and kept at 4°C for at most 1 hr, when the samples were stored at -70°C until spectrofluorometric determinations of the histamine content could be done (22). Protein content of the supernatant was determined by the Bradford assay (23). For each subject, we were careful to include the samples from both the SO_2 and the control days in the same batch for histamine and protein determinations.

The cell pellet was resuspended in 200µl CMF HBSS. This cell suspension was then further diluted to yield approximately 200,000 cells per ml. Two hundred µl of this suspension (containing approximately 40,000 cells) was placed in a cytocentrifuge well, and centrifuged onto a glass slide at 800 rpm for 5 min (Cytospin II, Shandon, Selwickley PA). Slides were air-dried for 5 min, stained in DifQuik (American Scientific Products), and examined at 400 x. Differential cell counts were based on examination of at least 10 fields and 100 cells on each slide. To minimize bias, the subject, date, and stimulus associated with each slide was not disclosed to the microscopist. Spotchecks by a second examiner generally showed at least 85% agreement.

Data analysis

For each subject, the change in nasal resistance and the temperature and dew point of the inspired gas on the SO2 study day were compared to those on the air study day by paired T-tests. For the 4 subjects who had nasal lavage done, we also compared the change in cell, protein, and histamine content on the SO_2 day to that on the air day by paired T-tests. We considered a p value of ≤ 0.05 as significant and, because we specifically sought to determine if SO_2 caused a greater increase in nasal resistance and nasal lavage cell, protein, and histamine content, we used one-tailed tests for significance. Values are expressed as mean ± S.D.

Results

The increase in nasal resistance after subjects quietly breathed 4 ppm SO2 in filtered, conditioned room air was not greater than the increase in nasal resistance after subjects breathed only filtered conditioned room air (Table 3). For each subject, the temperature and dewpoint of the SO2 gas mixture (23.8 ± 1.2 and 14.5 ± 0.5 °C, respectively) were not significantly different from those of the air mixture (23.7 ± 1.1 and 14.7 ± 0.8 °C). Most subjects were unable to identify with certainty which gas they had breathed each day. Four subjects (subjects 9, 13, 18, 19) at least doubled their nasal resistance after breathing filtered conditioned air alone whereas only 2 subjects (subjects 6, 17) did so after breathing SO2. Seven subjects (subjects 3, 10, 13, 14, 15, 19, 20) felt slightly more congested after breathing SO_2 whereas five subjects (6, 11, 13, 16, and 22) felt more congested after breathing air. Five subjects described cough, chest discomfort, or an unusual taste while breathing SO2 (attesting to its delivery to the airways), unassociated with nasal symptoms. No one complained of those symptoms after breathing conditioned room air.

In the 4 subjects in whom nasal lavage was performed, we did not detect in the nasal fluid a

consistent change in the total number of cells, percentage of polymorphonuclear leukocytes, histamine or protein content after subjects breathed SO_2 than after subjects breathed room air (Table 4). We discontinued the lavage studies because of the lack of striking or consistent physiological changes in the nose and because of the large within-person variation in nasal lavage cell counts, cell differentials, protein, and histamine even in the control state. This large variability has been described by others (24, 25).

Discusssion

Neither subjects with demonstrated nasal responsiveness to antigen challenge nor those with a history of chronic or chronic intermittent nasal symptoms developed a significantly greater increase in nasal resistance after breathing 4 ppm of sulfur dioxide than they did after breathing conditioned air. Subjects who did develop an increase in resistance either had no associated nasal symptoms or had symptoms that were minimal compared to their typical hay fever symptoms.

By power analysis (26, 27), we are at least 90% certain that there was no acute, sustained, clinically important SO_2-induced physiologic change that we failed to detect. We took into account the small resistance changes (0.5 ± 1.1 cm H_2O/L/s) after subjects breathed filtered air and defined a clinically important change in nasal resistance to be at least a doubling of the baseline resistance (usually an increase of 1.5-2 cm H_2O/L/s). This is a reasonable definition of an important change, inasmuch as the subjects challenged with antigen did not usually note an increase in nasal symptoms unless nasal resistance at least doubled.

Summary and conclusions

We examined whether brief exposures to moderately high concentrations of sulfur dioxide causes acute increases in nasal symptoms and nasal resistance in subjects with chronic rhinitis. We studied 19 subjects with allergic rhinitis and 3 subjects with chronic intermittent rhinorrhea, nasal congestion, and sneezing without any other manifestation of allergy. We found that the change in nasal resistance and symptoms caused by nasal inhalation of 4 ppm of sulfur dioxide for 10 min was no greater than the changes caused by nasal inhalation of conditioned room air. We conclude that brief exposure to SO_2 at a concentration of 4ppm or less is unlikely by itself to cause significant nasal dysfunction in most subjects with chronic rhinitis and that the presence of the mechanisms of immediate hypersensitivity in the nasal mucosa (presumably mast cells) does not necessarily confer nasal sensitivity to sulfur dioxide. We also infer from our findings that healthy subjects without rhinitis are unlikely to suffer acute nasal effects after breathing similar or smaller concentrations of sulfur dioxide.

Effect of ozone on nasal resistance, nasal reactivity, and cellular content of nasal lavage fluid in people with allergic rhinitis

Introduction

The purpose of this study was to determine whether concentrations of ozone which provoke cough, substernal chest pain, and an increase in bronchial resistance and reactivity in healthy people also cause symptoms of nasal discomfort, increased nasal resistance, and increased nasal reactivity in subjects with allergic rhinitis. This study also sought to determine whether the clinical and physiological effects are associated with inflammation of the nasal mucosa, as inferred from analysis of the cellular content of nasal lavage fluid.

As in the first study, our strategy was to focus first on subjects who were likely to develop nasal symptoms after breathing ozone. If this population did develop symptoms, the study could then be expanded to include subjects without chronic nasal disease.

Materials and Methods

Subjects

We studied 13 women and 3 men, 23 to 44 years old, who gave a clear history of sneezing, rhinorrhea, and nasal congestion provoked by common allergens and unassociated with respiratory infections. Subject characteristics are presented in Table 6. All but 1 subject also had a high serum IgE level and/or at least 1 positive skin test. No subject took chronic medications. Subject 15 took nasal steroids on occasion, but did not take any for 1 week before each study day. All refrained from any medication for at least 24 hr, and from tea or coffee for at least 4 hr, before each study. We excluded from the study people with a history suggestive of a viral respiratory infection in the 4 weeks before the study, and studied subjects during a season when they were least symptomatic. All subjects were informed of the risks of each procedure and signed consent forms approved by the Committee on Human Research of the University of California.

Experimental Design

To determine if ozone causes nasal symptoms and increased nasal resistance, we measured symptoms and nasal resistance in 16 experiments on 14 subjects immediately before and after a 2-hr exposure to ozone and a 2-hr sham exposure. Subjects came to the laboratory at the same time of day on 2 study days at least a week apart. The baseline nasal resistance was measured after subjects answered a questionnaire regarding recent and current symptoms and medications. If subjects were able to breathe easily through the nose, they then entered a stainless steel exposure chamber filled with filtered air containing 0.5 ppm ozone on one day and less than 0.005 ppm ozone on the control day. The gas mixtures were given in random order and in single-blind fashion. Subjects breathed tidally through the nose while sitting quietly. They noted on their questionnaire any changes in symptoms during or immediately after the exposure. Nasal airway resistance was measured 2 min after the completion of the exposure.

To determine if breathing ozone caused a significant change in the cellular and histamine content of nasal secretions, we obtained nasal lavage fluid during 8 of the experiments.

(To determine if breathing ozone caused an increase in nasal reactivity to allergens, we gave increasing doses of antigen to 10 of the subjects after ozone or air exposure was completed. This set of experiments is more fully described in Part III.)

Gas exposures

Subjects sat quietly in an independently ventilated stainless steel 8x8x8 foot exposure chamber supplied with filtered purified air. The air temperature and relative humidity was maintained at 22-25°C and 55-65%, respectively. The concentration of ozone on the control day was ≤ 0.005 ppm. On the ozone exposure day, ozone was generated by passing 100% oxygen through an ozonator (Wellsbach # T-408) to maintain an ozone concentration of 0.55 ppm as measured by an ultraviolet ozone analyzer (Dasibi 1003AH).

Symptom questionnaire

Subjects were asked to rate on a scale of 0 (no symptoms) to 4 (the worst ever) the following symptoms: runny nose, stuffy nose, urge to sneeze, actual sneezing, itchy or scratchy throat, urge to cough, chest tightness, wheezing, and shortness of breath. Other symptoms (for example, itchy eyes) could be entered on the form and rated by the subject.

All subjects rated symptoms before and immediately after the chamber exposure. The 8 subjects in whom nasal lavage was performed also rated their symptoms after the fourth lavage (before the exposure). As will be detailed in part III, the 8 subjects in whom an antigen dose-response curve was done also rated their symptoms after the antigen exposure, at the end of the experiment.

We generated 2 scores from the responses to this questionnaire. The total score was the mean of all symptoms (the sum of all scores ÷ 10). The nasal score was the mean of the first 4 symptoms (sum of the first 4 scores ÷ 4).

Skin testing, nasal airway resistance measurements, nasal lavage, cytological and biochemical processing were done as previously described.

Data analysis

For each subject, the changes in nasal resistance, nasal lavage fluid protein, histamine, cell count and differentials, temperature and relative humidity on the ozone exposure day were compared to those on the air day by paired T-tests. (If a significant difference was found, we went back to check that data for goodness of fit and, if appropriate, subjected that difference to nonparametric analysis.) Changes in nasal and total ysmptom scores were analyzed by the Wilcoxon signed rank test for nonparametric data. We considered a p value of ≤ 0.05 as signficant and because we specifically sought to determine if ozone caused a greater increase than did filtered

air, we used one-tailed tests for signficance. Values are expressed as mean±S.D.

Results

Conditions

The mean ozone concentration on the ozone exposure day was 0.55±0.02 ppm. The mean temperatures and relative humidity in the chamber during ozone and control exposures were not significantly different (ozone: 23.1±0.3 ^{o}C and 61±3.7 % , respectively. Control: 23.1±0.7^{o}C and 61.3±3.2%.)

Symptoms

The mean ozone-associated increase in nasal symptoms (+0.2±0.7) was not significantly greater than the change in nasal symptoms seen on the control day (-0.2±0.7, $p \leq 0.05$, Table 7). In the subjects in whom nasal lavage was done, nasal lavage caused a greater increase in the mean nasal symptom score than either air (lavage-associated increase of 0.3 compared to an air-associated increase of 0) or ozone (lavage-associated increase 0.2; ozone-associated increase of -0.2). Subjects 3,4,and 7 had particular difficulty with the lavage on at least one of their study days.

The mean ozone-associated increase in the total symptom score (+0.5±0.4) was significantly greater than the change in the total symptom score on the control day (+0.1±0.3, $p \leq 0.0005$, Table 8).

Nasal resistance

Nasal resistance did not increase significantly more after subjects breathed ozone (+0.3±1.1) than after subjects breathed conditioned air (+0.2±0.9, Table 9). Of the 8 subjects in whom nasal lavage was performed, subjects 3,5,7, and 8 developed at least a 50% increase in nasal resistance after lavages alone.

Nasal lavage volume, cells, protein, and histamine

We generally recovered at least 85% of the 10 ml that we instilled into the nose. The volumes collected before and after the control exposure (9.3±1.3 and 8.5±1.3) were not signficantly different from the volumes collected before and after ozone (9.9±0.8 and 8.1±2.1). We did not detect any significant differences between ozone and conditioned air in the changes in cell counts, percentages of neutrophils, eosinophils, and respiratory epithelial cells, total protein, and histamine.

Discussion

Subjects with a clearcut history of allergic rhinitis tended to develop more nasal symptoms after breathing ozone than they did after breathing conditioned air, but these changes were not statistically significant and were clinically minor when compared to symptoms caused by antigens. Subjects developed more lower airway symptoms (laryngeal and below) than nasal symptoms even though they had no recent history of asthma and did not have delivered to their lower airways as large a dose of ozone as former subjects have had in earlier ozone studies.

Ozone did not cause a significantly greater increase in nasal resistance than did filtered air alone. By power analysis of our resistance data, we are more than 90% certain that no acute clinically important increase in nasal resistance occurred that we failed to detect. We considered in this analysis the small resistance changes that occurred after subjects breathed filtered air (0.2±0.9 cm H_2O/L/s) and defined a clinically important change in nasal resistance to be at least a doubling of the baseline resistance (an increase of approximately 1.2 cm H_2O/L/s).

We cannot be as conclusive about our measurements of nasal lavage fluid. Both technical and physiologic factors contribute to variations in the amount of histamine, protein, or cells that we detected in the control state. Technical factors include the viscosity of nasal mucus, cell clumping, and poor or spotty cell adhesion to the slides (24). Numerous physiologic sources of variation in the nose have been described. To list but a few examples, the amount of measurable histamine in the nose is influenced by gender (25); the number of polymorphonuclear leukocytes can range 10-fold in the normal state, in the absence of clinically detectable infection or stimulation (24); and nasal secretion can change in response to posture, corneal irritation, or emotion (20,21). We made technical and design modifications to minimize these factors but there remains sufficient variation to markedly diminish the power of our statistical tests. Thus, an important change may have occurred at the cellular or biochemical level that we are unable to detect because of baseline "noise." We think this is unlikely because of the lack of a consistent ozone-induced clinical or physiological effect.

Ozone effects on nasal reactivity

We had proposed in our application to determine if ozone changes nasal reactivity. Histamine was proposed as the agonist, but we chose instead to use methacholine for several reasons. In our experience with methacholine and histamine in bronchial challenge, we find that methacholine has fewer side effects than does histamine. Other investigators (28) find that nasal challenge with methacholine increases nasal resistance fairly reproducibly and consistently. Finally, we planned to measure histamine release in nasal secretions as part of most of our studies and the instillation of exogenous histamine into the nose would have complicated our intended experiments.

We therefore performed 14 methacholine dose-response curves in 7 subjects with allergic rhinitis and 1 subject without rhinitis. After subjects described their symptoms, we measured their baseline nasal resistance and delivered nasal sprays (2 sprays per nostril per dose) first of phosphate-buffered saline and then of increasing concentrations of methacholine until subjects at least doubled the nasal resistance, until the highest concentration of methacholine was given, or until the onset of side effects, whichever occurred first. Methacholine solutions were made up no

more than 48 hr before the experiment in concentrations of 0.5,1,2,4,8,16, and 32 mg/ml. Solutions were sprayed into the nose with spray bottles which delivered 74± 4 µl per activation. Doses were given 5 min apart and nasal resistance was measured 2 min after each dose.

We found that increasing doses of methacholine did not reproducibly cause a sustained (>30 sec) increase in nasal resistance (Figure 1) or cause noticeably more nasal secretions during the time that we observed our subjects. More disconcerting, however, was that subjects often developed headache and/or facial flushing with doses that had no or little effect on the nose. It is possible that changing the agonist, the timing, or the delivery of the agonist may have permitted a successful study, but rather than spend several weeks attempting to perfect the timing and techniques of methacholine dose-response curves in the nose, we went on to study the effects of ozone on nasal reactivity to antigen. Such a study could potentially yield information not only about mechanisms of nasal reactivity but also about effects of an environmental interaction that is likely to occur.

Summary and conclusions

We examined whether concentrations of ozone which provoke cough, substernal chest pain, and an increase in bronchial resistance and reactivity in healthy people and in subjects with asthma also cause nasal discomfort, increased nasal resistance, and increased nasal reactivity to methacholine in subjects with allergic rhinitis. We also examined whether ozone causes the extravasation or secretion of cells, protein, or histamine into the nose. From 18 experiments in 16 subjects with allergic rhinitis we conclude that breathing 0.55 ppm of ozone for 2 hours causes a statistically insignificant and clinically minor increase in nasal symptoms. We infer from these findings that healthy subjects without rhinitis are unlikely to suffer acute nasal effects after breathing similar or smaller concentrations of ozone.

We did not detect any significant ozone-induced changes in nasal lavage fluid cells, total protein, or histamine in 8 subjects with allergic rhinitis. We were also unable to determine whether ozone changes nasal responses to methacholine. Doses of methacholine similar to those used by other investigators caused headache or facial flushing in our subjects, usually without provoking reproducible or sustained nasal symptoms or obstruction. Thus, technical difficulties and marked variation in the nose in the baseline state preclude firm conclusions about ozone-induced inflammation in the nose or ozone-induced nasal hyperreactivity to methacholine.

Effects of ozone on nasal sensitivity to allergen in subjects with allergic rhinitis

Introduction

The purpose of this study was to determine whether exposure to levels of ozone that have been shown to cause airway mucosal inflammation and reduction in airway caliber (0.5 ppm for 2 h) alters nasal sensitivity to inhaled allergens in people with allergic rhinitis.

Materials and Methods

Subjects

Subjects 1,4,9-16 (Table 5) participated in this third study. Subject characterization and exclusion criteria are described in Part 2. Subject 15 was the only subject who used nasal steroids, albeit sporadically. She refrained from using the medication for the 5 days preceding each study day.

Experimental design

Subjects came to the laboratory at the same time of day on 2 days at least 1 week aprt. They filled out a symptom questionnaire, had their baseline nasal resistance measure and, if able to breathe freely through the nose while at rest, entered an exposure chamber filled with filtered air containing ≤0.005 ppm ozone on the control day or 0.5 ppm ozone on another day. (Nasal lavages were not done as part of this study.) Gas mixtures were given in random order and in single-blind fashion. Subjects breathed tidally through the nose while sitting quietly. They noted on their questionnaire any changes in symptoms during or immediately after the exposure. Nasal resistance was measured 2 min after completion of the exposure.

If a subject's nasal resistance increased by more than 50%, we observed the subject and repeated the measurements until nasal resistance returned to within 20% of baseline, or was stable for 10 min. We then measured the changes in nasal resistance and nasal symptoms in response to increasing doses of antigen.

Antigen dose-response curves

The antigen with which each subject was nasally challenged was chosen on the basis of clinical history and skin tests; this test allergen is underlined in the "provoking allergen" column of Table 6. Antigen extracts used for intranasal challenges were similar to the extracts used for skin prick tests; 2% glycerin and 0.4% phenol were used as preservatives for the extracts (Hollister-Stier, Spokane, WA). Dilutions of 1:10000, 1:1000, 1:100, and 1:10 in phosphate-buffered saline were made once weekly and stored at 4°C. For the control challenge, 2% glycerin and 0.4% phenol (provided by Hollister-Stier) was diluted 1:10 in phosphate-buffered saline.

After completion of the chamber exposure and when nasal resistance was stable or had returned to within 20% of baseline, we measured a second baseline reading for the antigen dose-response curve. We then delivered 2 sprays per nostril first of diluent and then of increasing concentrations

of antigen solution until subjects developed typical symptoms of an attack of allergic rhinitis and at least a doubling of the nasal airway resistance, or until the highest concentration of allergen was given, whichever occured first. Only subject 9 received undiluted antigen extract. The highest concentration given to other subjects was 1:10. Challenges were given at 5-min intervals (preliminary experiments with single doses of allergen showed that allergic responses began within 5 min of nasal challenge in most allergic subjects). We measured nasal resistance 2 min after each challenge and repeated the measurements if symptoms occured. Nasal resistance and symptoms were recorded after each dose of antigen. Antigen solutions were delivered to the nose with spray bottles which delivered 65 ± 7 µl per activation (Syntex Laboratories, Palo Alto, CA).

Gas exposures and symptom questionnaires are described in Part 2.

Data analysis

We found that allergen-induced changes in nasal resistance and symptoms occur as threshold events. There was not a consistent or reproducible increase in nasal resistance with each increment of antigen concentration. We therefore used in analysis the actual concentration of antigen that caused symptoms and at least a doubling of nasal resistance (as compared to interpolating a concentration of antigen that caused a doubling of nasal resistance). In a few instances when nasal resistance did not double, we used for analysis the actual concentration of antigen which caused symptoms. We transformed the actual provocative concentration to its negative log (e.g., a 1:10000 dilution=10^{-4} concentration has a negative log of "4"). We compared the negative log of the provocative concentration after ozone exposure to the negative log of the provocative concentration after air exposure by the Wilcoxon signed rank test.

Results

Symptoms

Antigen caused a significantly higher nasal symptom score (2.0±0.1) than did air (0.3±0.5) or ozone (0.5±0.7).

Provocative concentration of allergen

The dose-response curves are presented in Figure 2. Arrows denote the concentration at which nasal symptoms occured. In 7 subjects (9, 10, 11, 13, 14, 15, 16) the provocative concentration was the same after ozone as that after air exposure. Subjects 1,4, and 12 responded to a 10-fold smaller concentration of antigen after breathing ozone than they did after breathing air. No subject responded to a smaller concentration of antigen after breathing air than after breathing ozone. Nonparametric tests indicate that this is a significant difference ($p \leq 0.05$).

Discussion

In this sample of 10 subjects with allergic rhinitis, 3 subjects were more sensitive to allergen after ozone exposure than they were after air exposure and no subject was less sensitive to allergen after their ozone exposure. The 3 subjects who appeared hyperresponsive to allergen had all been

randomized to receive ozone and antigen before receiving air and antigen. If anything,that order of exposure decreased the odds of finding ozone-induced hyperresponsiveness: the antigen given on the first study day may have primed the nose to respond to a smaller dose of antigen on the air study day a week later (29). That the reverse occurred suggests that ozone indeed increased the senstitivity of the nose to allergen. We had also decreased the odds of finding ozone-induced hyperresponsiveness by using 10-fold dilutions in our dose-response curve. Concerned about the nonstandardization of antigen extracts, the problems with storing, preserving, and delivering antigen to the nose (30), and the problems in detecting a response in the nose (20,21), we purposely chose to consider only a large change in sensitivity. That we found such a change after ozone in even few subjects, and never found a similar change after air exposure also suggests that ozone-induced hyperreactivity to allergen is a real finding, although the results in this small sample will have to be corroborrated in a larger study.

Summary and conclusions

We examined whether ozone alters nasal sensitivity to inhaled allergens in people with allergic rhinitis. We found that 3 of 10 subjects responded to 10-fold smaller concentrations of allergen after ozone exposure than they did after air exposure, suggesting that ozone increases nasal sensitivity to inhaled allergens.

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Table 1.
Sulfur Dioxide Study: Subject Characteristics

Subject	Age	Sex	History	Family History	Precipitants	# positive skin tests	IgE (U/ml)
1	44	M	hay fever	+	rats, rabbits, cats, grass, dust, trees	0	18
2	23	F	hay fever	-	cats, grasses, dust	3	33
3	30	F	hay fever/ asthma	+	rats, grasses, dust	1	22
4	44	F	hay fever	-	grasses, trees, dust	2	340
5	37	F	hay fever	+	cats, grasses, trees, dust	3	520
6	36	F	hay fever	+	grasses, trees, dust	1	82
7	25	F	hay fever	+	cats, rabbits, grasses,	5	297
8	35	F	hay fever	+	very allergic to grasses before hyposensitiza-tion, no symptoms now	0	6
9	30	F	hay fever/ asthma	+	cats, horses, rodents pollens, molds	4	155
10	38	F	hay fever	_	grasses, trees, dust	3	81
11	33	F	hay fever	-	grasses, trees, pollens	1	630

Table 1 (cont).
Subject Characteristics

Subject	Age	Sex	History	Family History	Precipitants	# positive skin tests	IgE
12	30	M	hay fever/ asthma	-	cats, grass, dust	5	600
13	23	M	hay fever	-	cats, dust	3	145
14	24	F	neither	-	perfume, smoke	0	37
15	26	F	hay fever	-	rabbits, pollens	0	102
16	28	F	asthma	+	cats, grasses, dust	0	11
17	37	F	neither	-	perfume, smoke	2	7
18	27	F	neither	+	cold air, smoke	0	5
19	28	F	hay fever	+	cats, dogs, dust	3	510
20	44	F	hay fever	+	cats, dust	2	27
21	31	F	hay fever	+	cats, grass	2	82
22	40	F	hay fever	_	house dust, mites	0	180

Table 2.
Responses to Intranasal Antigen in 13 Subjects

Subject	Test antigen	Provocative Dose	Nasal resistance before antigen	Nasal resistance after antigen	Symptoms
1	grass mix	1:1000	0.82	4.15	2 sneezes, congestion, rhinorrhea
2	grass mix	1:1000	1.65	2.7	3 sneezes, congestion, rhinorrhea
3	grass mix	1:100	2.37	5.96	2 sneezes, congestion, rhinorrhea
4	house dust	1:10	0.47	1.52	no nasal symptoms
5	grass mix	1:1000	2.65	5.26	8 sneezes, congestion, rhinorrhea
6	grass mix	1:10	2.51	5.44	1 sneeze, congestion, rhinorrhea

Table 2 (continued).
Responses to Intranasal Antigen in 13 Subjects

Subject	Test antigen	Provocative Dose	Nasal resistance before antigen	Nasal resistance after antigen	Symptoms
7	grass mix	1:1000	1.41	2.94	nasal itch, congestion, rhinorrhea
8	grass mix	undiluted	1.78	4.35	nasal congestion, rhinorrhea
9	grass mix	1:100	2.99	6.5	nasal itch, congestion, rhinorrhea
10	grass mix	1:10000	1.27	6.89	3 sneezes, congestion, rhinorrhea
11	grass mix	1:100	1.12	5.34	1 sneeze, congestion, rhinorrhea, itch
12	grass mix	1:10	0.75	1.89	10 sneezes, congestion, rhinorrhea
13	cat hair	1:10	0.74	.89	1 sneeze, congestion, rhinorrhea

Table 3.
Nasal resistance (cm H_2O/ L/s)

	AIR			SO_2		
Subject	Before	After	Change	Before	After	Change
1	0.45	0.38	-0.07	1.36	0.82	-0.54
2	1.52	1.59	+0.07	2.47	1.65	-0.82
3	5.30	4.70	-0.60	1.42	2.37	+.95
4	0.53	0.84	+0.31	0.98	0.92	-.06
5	1.04	1.31	+0.27	1.39	1.74	+0.35
6	1.08	1.75	+0.67	0.65	1.34	+0.69
7	2.19	1.93	-0.26	2.23	3.72	+1.49
8	2.42	1.19	-1.23	1.61	2.94	+1.33
9	2.89	6.34	+3.45	2.67	3.77	+1.10
10	4.25	4.16	-0.09	0.53	0.65	+0.12
11	2.53	2.41	-0.12	2.65	2.66	+0.01
12	1.12	1.23	+0.11	1.19	1.11	-0.08
13	1.85	4.93	+3.08	2.11	3.82	+1.71
14	1.23	1.20	-0.03	1.13	1.12	-0.01
15	0.77	1.05	+0.28	1.26	0.90	-0.36
16	1.24	1.74	+0.50	0.92	1.22	+0.30
17	0.64	0.66	+0.02	0.46	1.05	+0.59
18	1.67	3.38	+1.71	2.61	2.63	+0.02
19	0.91	3.09	+2.18	1.67	3.38	+0.87
20	1.50	2.11	+0.61	2.68	2.12	-0.56
21	1.96	1.94	-0.02	3.43	2.23	-1.20
22	1.17	1.17	0.	0.89	0.91	+0.02
Mean.	1.8	2.3	+0.49	1.8	2.3	+0.27
(S.D.)	(1.2)	(1.6)	(1.14)	(1.2)	(2.1)	(0.76)

Table 4.

Nasal lavage fluid cells and percent polymorphonuclear leukocytes in four subjects

Subject	Before air	After air	Before SO_2	After SO_2
		Total number of cells (x 103)		
1	171	1040	225	405
2	374	24	198	477
3	63	368	90	57
4	18	36	90	64
Mean	157	367	151	251
S.D.	159	476	71	222
		% Polymorphonuclear leukocytes		
1	2	9	4	34
2	12	0	23	89
3	1	14	35	0
4	0	1	3	0
Mean	4	6	16	31
S.D.	6	7	16	42

Table 5.
Nasal lavage fluid histamine and total protein in four subjects

Subject	Before air	After air	Before SO_2	After SO_2
		Histamine (ng/ml)		
1	12.9	12.9	21.3	8.2
2	0	0	0	0
3	0.1	0.1	2.9	0.1
4	0	0	2.3	1.1
Mean	3.5	3.4	6.6	2.6
S.D.	6.3	6.4	9.9	3.8
		Total Protein (mg/100 ml)		
1	7	9	5	12
2	4	1	9	7
3	19	15	17	7
4	not done	4	2	5
Mean	10	7	8	8
S.D.	8	6	7	3

Table 6.
Ozone Studies: Subject Characteristics

Subject	Age	Sex	History	Family History	Provoking Allergen	# positive skin tests	IgE (U/ml)
1	25	F	hay fever	+	cats, rabbits, grasses	5	297
2	44	F	hay fever/ asthma	-	grasses, trees, dust	2	340
3	37	F	hay fever	+	cats, grasses, trees, dust	1	520
4	25	M	hay fever	+	cats	1	35
5	23	F	hay fever/ asthma	+	dust	1	152
6	33	F	hay fever/ asthma	+	pollens, grasses, dust	1	99
7	39	F	hay fever	-	cats, grasses, dust	0	11
8	24	M	hay fever	-	cats, dust	3	145

Subject	Age	Sex	History	Family History	Provoking Allergen	# positive skin tests	IgE (U/ml)
9	44	M	hay fever	+	rats, rabbits, cats, grass, dust, trees	0	18
10	25	F	hay fever	+	pollens, grasses, dust	1	73
11	22	F	hay fever	+	pollens, cats, dust; smoke, perfume, cold	3	22
12	29	F	hay fever	+	cats, dogs, dust	3	*510*
13	32	F	hay fever	+	cats, mold, grass, dust	1	10
14	33	F	hay fever	-	grasses, trees, pollens	1	630
15	28	F	hay fever	+	dust, grasses	2	99
16	33	F	hay fever	+	grasses, trees, weeds	2	150

Subjects 1-8 had nasal lavages before and after the chamber exposures.

Subjects 1,4,9-16 participated in a separate study to examine ozone's effects on nasal responses to antigen. The antigen with which each was challenged is underlined in the "provoking allergen" column.

Table 7.
Nasal symptom scores

	AIR EXPOSURE				OZONE EXPOSURE			
	before lavage	before air	after air	after antigen	before lavage	before ozone	after ozone	after antigen
Subjects in whom nasal lavage was performed (antigen not given)								
1	0	0	0		0	0.3	0	
2	1.5	0.3	0		0	0	0	
3	0.5	2.5	0		0.5	2.5	1	
4	0.5	1.3	0.5		0.3	0	0.3	
5	0	0	0		0	0	0	
6	0	0.3	0		0	0.3	0	
7	0.8	1.8	1.8		0.8	0.3	0.5	
8	0.5	0.5	1.3		0.5	0.3	0.8	
mean	0.5	0.8	0.8		0.3	0.5	0.3	
S.D.	0.5	0.9	0.9		0.3	0.8	0.4	
Subjects challenged with antigen (Nasal lavage not performed)								
1		0	0.3	1.8		0	0.3	1.8
4		0	0.3	0.8		0.5	2.8	1.8
9		0.3	0.3	2.5		0	0	0.3
10		0	0	2		0.3	0.5	1.5
11		0	0	1.6		0.3	0.5	1.8
12		0	0	3.5		0	0	3.3
13		0.5	0.8	3.5		0.5	1.8	4.0
14		0.3	0.3	3.0		0.3	0.3	2.0
15		0.3	0.3	1.3		0	0	0.8
16		0	0	2.8		0	0.3	2.5
mean		0.1	0.2	2.2		0.2	0.8	2.0
S.D.		0.2	0.1	0.9		0.3	1.0	1.0
All subjects								
mean		0.5	0.3			0.3	0.5	
S.D.		0.7	0.5			0.6	0.7	

Table 8.
Total symptom scores

	AIR EXPOSURE				OZONE EXPOSURE			
	before lavage	before air	after air	after antigen	before lavage	before ozone	after ozone	after antigen
	Subjects in whom nasal lavage was performed (antigen not given)							
1	0	0	0		0	0.1	0.1	
2	1.8	0.1	0		0	0	0.3	
3	0.2	2.0	1.1		0.2	0.2	0.6	
4	0.2	0.7	0.9		0.2	0.1	0.7	
5	0	0	0.1		0	0	0.2	
6	0.	0.1	0.1		0	0.1	0.3	
7	0.8	1.2	1.3		0.3	0.4	1.5	
8	0.2	0.2	0.5		0.2	0.1	0.3	
mean	0.4	0.6	0.5		0.1	0.3	0.5	
S.D.	0.6	0.8	0.6		0.1	0.6	0.5	
	Subjects challenged with antigen (nasal lavage not performed)							
1		0	0.1	0.7		0	1.0	0.8
4		0.1	0.2	0.3		0.2	1.6	1.3
9		0.1	0.1	1.2		0	0.1	0.2
10		0	0	1.5		0.2	0.7	1.1
11		0	0	0.1		0.1	0.6	1.2
12		0	0	1.6		0	0	2.0
13		0.4	0.7	2.1		0.5	1.3	3.0
14		0.1	0.1	1.8		0.2	1.1	1.9
15		0.2	0.2	0.8		0.1	0.1	0.4
16		0	0	1.1		0	0.3	1.3
mean		0.1	0.1	1.1		0.2	0.7	1.2
S.D.		0.1	0.1	0.5		0.2	0.6	0.8
	All subjects							
mean		0.3	0.3			0.1	0.6	
S.D.		0.5	0.4			0.1	0.5	

Table 9.
Nasal resistance (cm H_2O/L/s)

subject	AIR EXPOSURE before lavage	before air	after air	change	OZONE EXPOSURE before lavage	before ozone	after ozone	change
				Subjects in whom nasal lavage was performed (antigen not given)				
1	1.1	1.2	.9	-0.3	2.0	1.8	1.3	-0.5
2	0.7	0.7	1.2	+0.5	0.7	1.0	1.2	+0.2
3	1.3	2.1	1.3	-0.8	1.2	1.9	1.2	-0.7
4	1.3	1.2	2.1	+0.9	1.7	1.7	0.9	-0.8
5	1.3	1.9	1.5	-0.4	1.1	1.6	1.2	-0.4
6	0.9	1.0	0.9	-0.1	0.6	0.9	1.0	+0.1
7	0.9	1.2	4.1	+2.9	1.5	2.3	2.4	+0.1
8	1.7	3.1	1.8	-1.3	0.7	1.4	4.9	+3.5
mean	1.2	1.5	1.7	+0.2	1.2	1.6	1.8	+0.2
S.D.	0.3	0.8	1.0	1.3	0.5	0.5	1.4	1.4
				Subjects challenged with antigen (nasal lavage not performed)				
9	--	1.1	1.6	+0.5	--	0.9	1.0	+0.1
1	--	1.0	1.4	+0.4	--	1.5	1.9	+0.4
4	--	1.0	2.1	+1.1	--	2.0	2.2	+0.2
10	--	1.4	1.4	0.0	--	2.0	1.8	-0.2
11	--	1.5	1.9	+0.4	--	1.9	3.3	+1.4
12	--	1.3	1.2	-0.1	--	1.2	2.4	+1.2
13	--	1.1	1.5	+0.4	--	1.1	0.8	-0.3
14	--	1.1	1.2	+0.1	--	0.5	1.7	+1.2
15	--	1.3	2.6	+1.3	--	1.2	1.3	-0.1
16	--	1.7	2.0	+0.3	--	1.5	1.7	+0.2
mean		1.3	1.7	+0.4		1.4	1.8	+0.4
S.D.		0.2	0.4	0.4		0.5	0.8	0.6
				All subjects				
mean		1.4	1.7	+0.3		1.5	1.8	+0.3
S.D.		0.5	0.7	0.9		0.5	1.0	1.0

Table 10.
Nasal lavage fluid cells, percent polymorphonuclear and eosinophilic leukocytes, and respiratory epithelial cells in 8 subjects

	before lavage	before air	after air	before lavage	before ozone	after ozone
			Total cell count (in thousands)			
1	41	30	41	14	23	19
2	16	13	10	11	4	44
3	3	2	8	0	45	4
4	7	2	6	8	14	2
5	30	18	9	16	6	8
6	13	27	10	43	26	9
7	17	10	4	16	13	56
8	3	1	3	11	3	14
mean	16	13	11	15	17	20
S.D.	13	11	12	12	14	20
			percentage of polymorphonuclear leukocytes			
1	70	63	63	0	4	15
2	14	10	24	13	9	40
3	20	26	48	75	56	57
4	44	21	27	1	10	4
5	*	71	75	67	54	37
6	*	*	*	25	6	29
7	23	65	69	87	39	89
8	12	10	10	21	11	*
mean	31	38	45	36	24	39
S.D.	22	27	25	35	22	28

*not done because of technical difficulty (too few cells to count, clumping, poor cell adhesion to slide during cytocentrifugation, mucus too viscous to allow separation)

Table 10 (continued).
Nasal lavage fluid cells, percent polymorphonuclear and eosinophilic leukocytes, and respiratory epithelial cells in 8 subjects

	before lavage	before air	after air	before lavage	before ozone	after ozone
				percentage of eosinophils		
1	4	6	8	0	0	0
2	1	0	4	5	2	0
3	23	20	28	3	11	11
4	0	0	4	0	0	0
5	*	0	0	0	0	0
6	*	*	*	0	0	0
7	0	0	0	0	0	0
8	57	68	62	48	51	*
mean	14	13	15	7	8	2
S.D.	23	25	23	17	18	4
				percentage of respiratory epithelial cells		
1	8	1	4	20	7	8
2	10	20	14	8	15	6
3	34	20	10	11	20	12
4	10	2	3	16	17	3
5	*	30	24	3	4	5
6	*	*	*	6	9	4
7	6	1	0	1	13	0
8	8	2	5	5	6	*
mean	13	11	9	9	11	5
S.D.	11	12	8	7	6	4

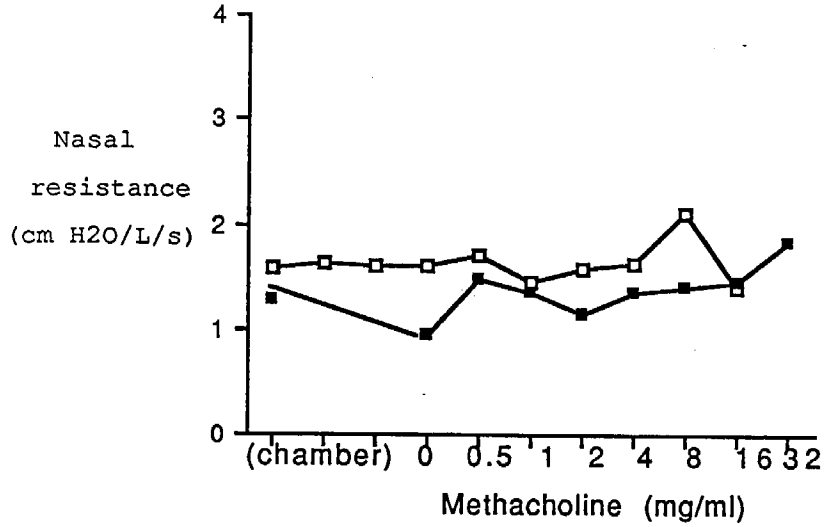
*not done because of technical difficulty (too few cells to count, clumping, poor cell adhesion to slide during cytocentrifugation, mucus too viscous to allow separation)

Table 11.
Nasal lavage fluid histamine and total protein in 8 subjects

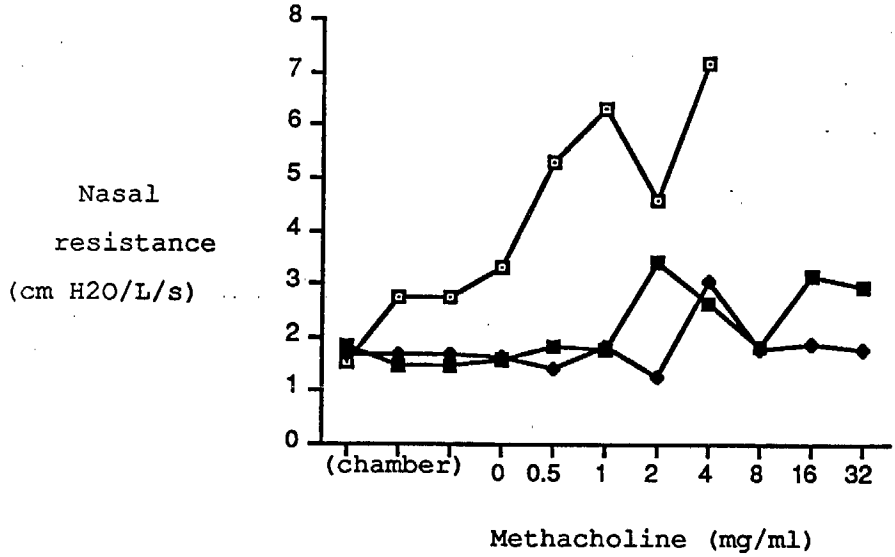
	before lavage	before air	after air	before lavage	before ozone	after ozone
				Total protein (mg/100ml)		
1	3.4	2.2	2.6	8.9	4.7	6.6
2	3.4	2.2	4.4	5.0	1.9	6.7
3	0	5.9	20.3	18.4	13.3	3.0
4	2.0	3.6	5.5	7.5	6.7	7.8
5	9.1	0	0	1.1	0	3.1
6	2.9	2.0	5.1	2.8	1.7	2.3
7	1.7	.5	1.2	2.2	0.9	2.4
8	26.0	7.3	3.9	6.3	4.4	4.0
mean	6.5	3.0	5.4	6.5	4.2	4.5
S.D.	8.5	2.6	6.3	5.5	4.3	2.2
				Histamine (ng/ml)		
1	0	0	0	16.6	5.6	3.9
2	0	0	0	1.8	0.9	0.6
3	2.4	1.5	0.9	0	0	0
4	5.4	2.4	1.4	14	4.2	2.0
5	37.2	4.4	5.9	17	3.3	8.5
6	1.0	0	0	1.0	0	19.0
7	1.0	0	0	0	0	0
8	1.1	1.1	7.6	0	0	0
mean	6.0	1.2	2.0	6.3	1.7	4.2
S.D.	13.0	1.6	3.0	8.0	2.3	6.6

Effect of ozone on nasal resistance, nasal reactivity, and cellular content of nasal lavage fluid in sujbects with allergic rhinitis.

Figure 1. Results of a pilot study on 7 subjects to determine the feasibility and reproducibility of nasal methacholine dose-response curves.



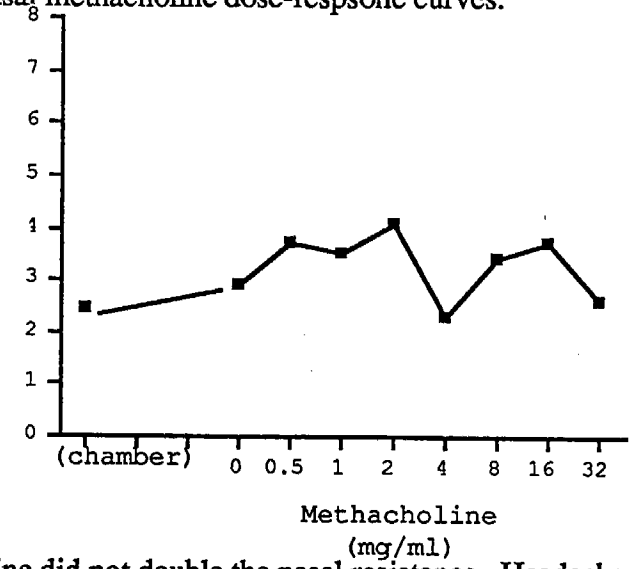
Subject 1. Methacholine did not double the nasal resistance on either day. The curves were dissimilar on different days. Facial flushing occurred at 16 mg/ml on day ■, and at 32 mg/ml on day□



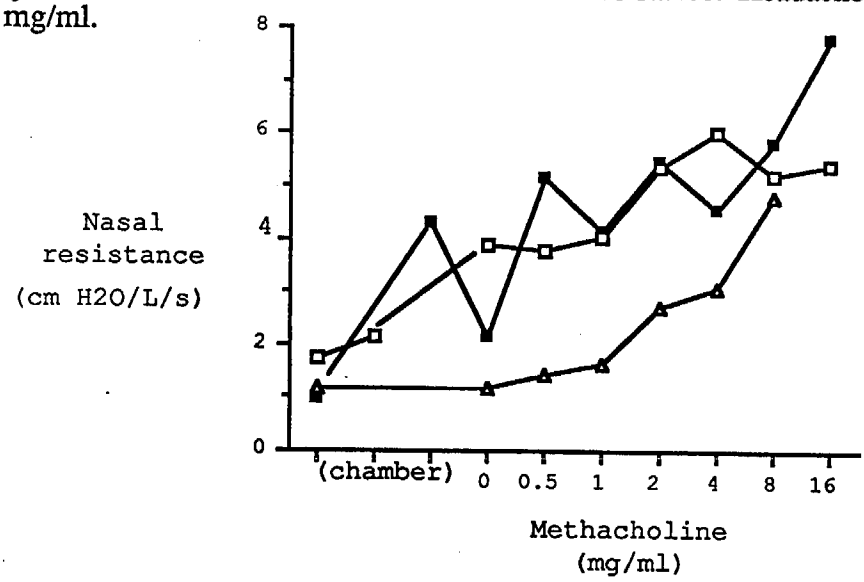
Subject 4. Nasal resistance increased after 1 mg/ml but decreased after the next higher dose on day□. Curves were not reproducible from day to day.

Effect of ozone on nasal resistance, nasal reactivity, and cellular content of nasal lavage fluid in sujbects with allergic rhinitis.

Figure 1 (continued). Results of a pilot study on 7 subjects to determine the feasibility and reproducibility of nasal methacholine dose-respsone curves.



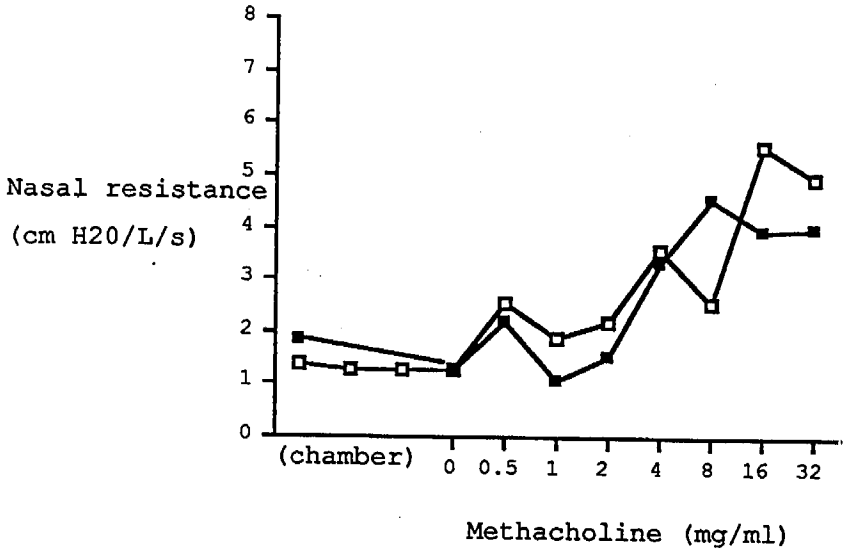
Subject 9. Methaholine did not double the nasal resistance. Headache and facial flushing occurred at 32 mg/ml.



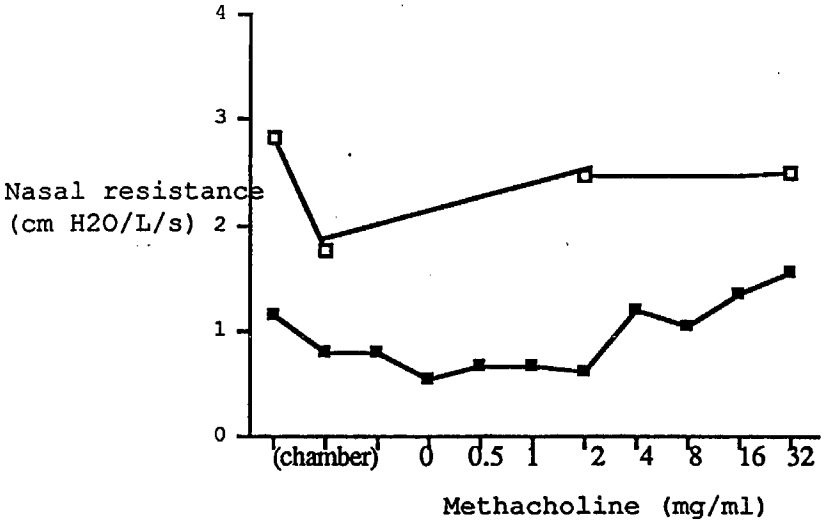
Subject 11. Methacholine doubled the nasal resistance in each of 3 dose-response curves in this subject, but the curves were not always the same. An increase in nasal resistance induced by one dose of methacholine may not be sustained or further increased by the next higher dose.

Effect of ozone on nasal resistance, nasal reactivity, and cellular content of nasal lavage fluid in sujbects with allergic rhinitis.

Figure 1. Results of a pilot study on 7 subjects to determine the feasibility and reproducibility of nasal methacholine dose-response curves.



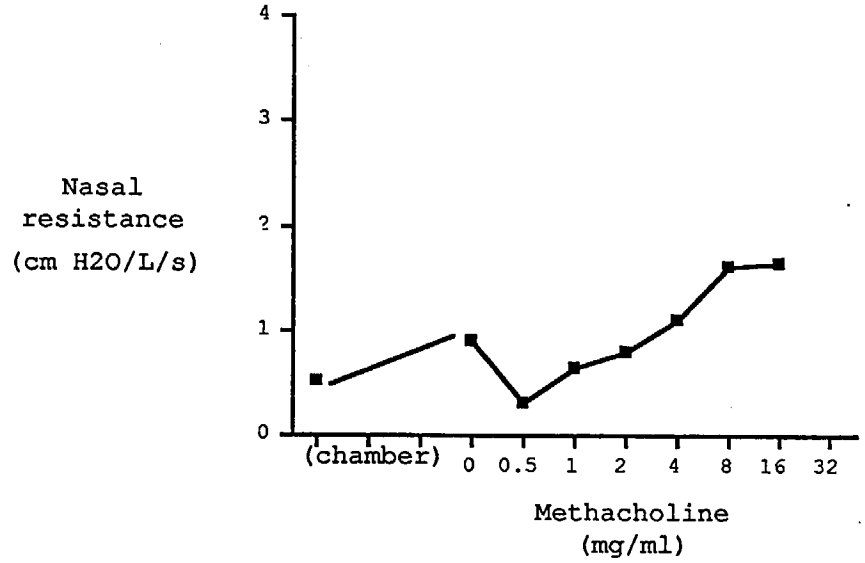
Subject 12. Methacholine doubled the nasal resistance with reproducible curves on 2 days in this subject. Facial flushing occurred on day ■ at 32 mg/ml.



Subject 14. Methacholine did not double the resistance on either day. Facial flushing and headache occurred at 16 mg/ml on day ■ and at 32 mg/ml on day □.

Effect of ozone on nasal resistance, nasal reactivity, and cellular content of nasal lavage fluid in sujbects with allergic rhinitis.

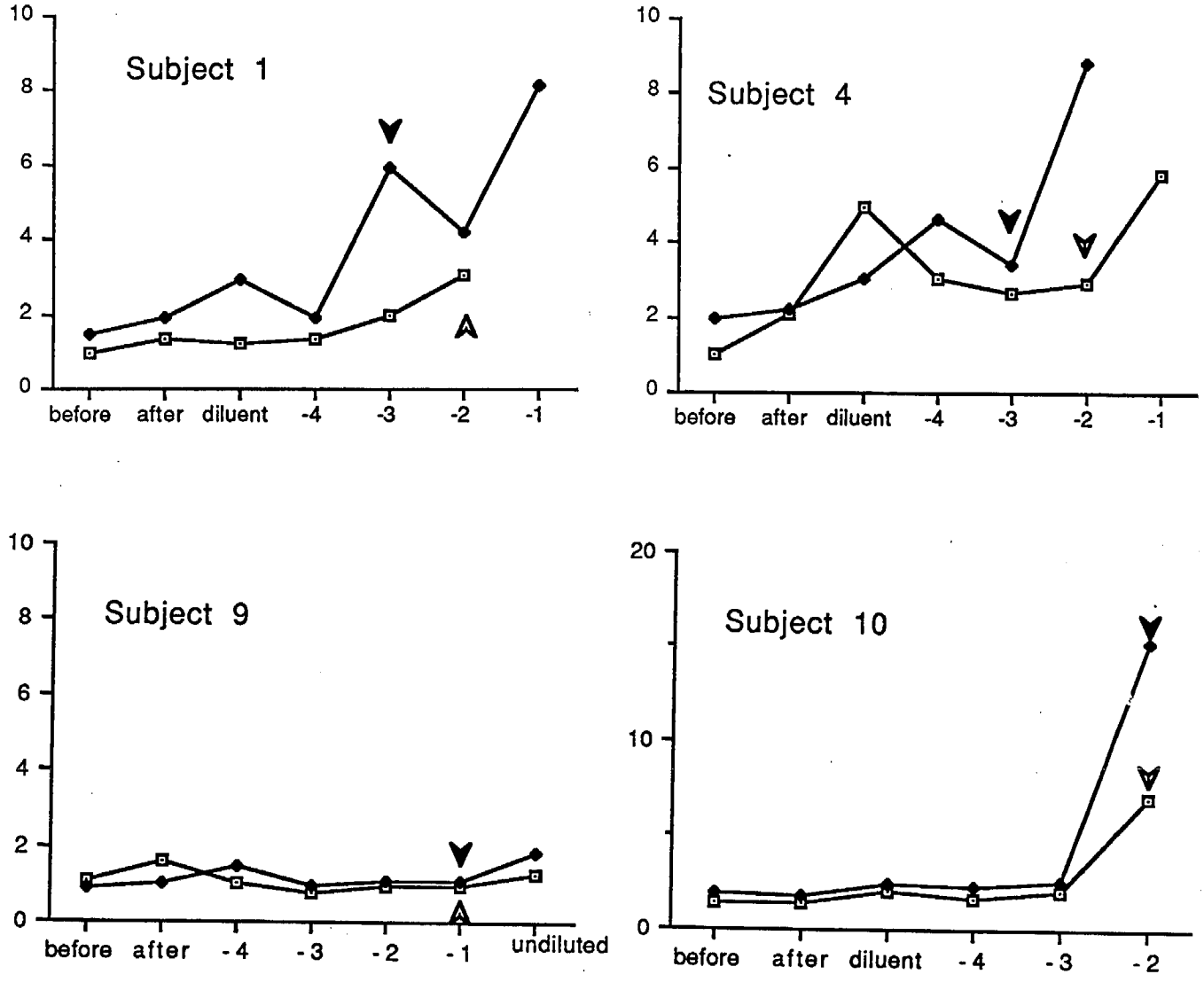
Figure 1(continued). Results of a pilot study on 7 subjects to determine the feasibility and reproducibility of nasal methacholine dose-response curves.



A nonallergic subject not studied in our formal projects. The nasal resistance increased from approximately 0.4 to 0.9 cm H_2O/L/s with saline alone, decreased with the smallest dose of methacholine and doulbed after several doses of methacholine. Facial flushing occurred at 16 mg/ml.

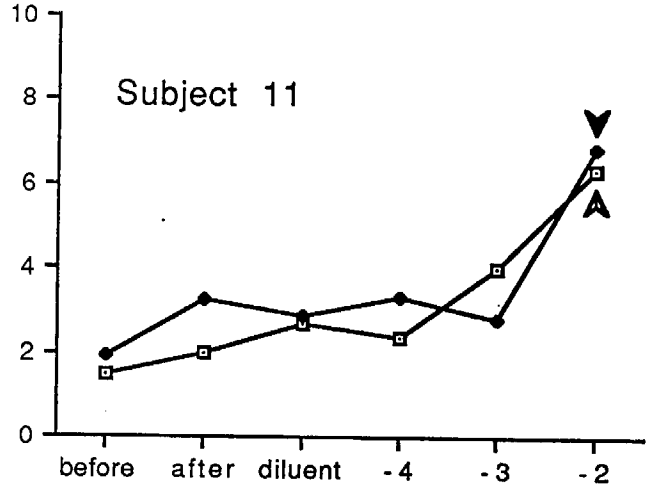
Effect of ozone on nasal sensitivity to allergen in subjects with allergic rhinitis

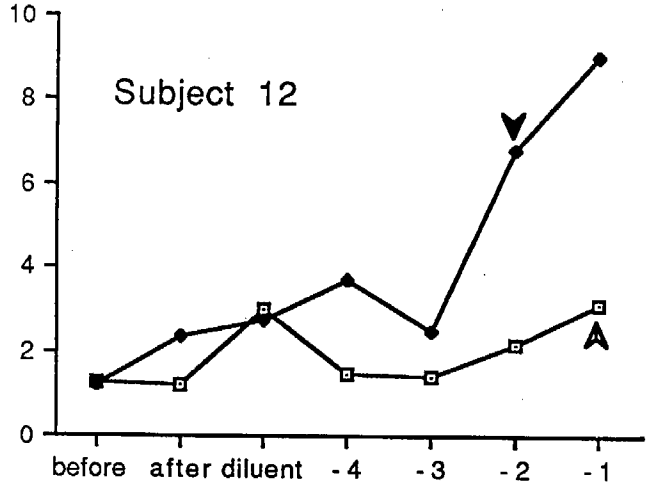
Figure 2. Antigen dose-response curves after subjects breathed conditioned room air (▫) or 0.5 ppm ozone (▪) for 2 hours. Changes in nasal resistance are depicted before and after exposure to air or ozone, after nasal challenge with diluent alone, and then after a 1:10,000 (-4), a 1:1000 dilution (-3), a 1:100 (-2), and a 1:10 dilution of antigen solution . Only subject 9 received a challenge with undiluted antigen solution. Arrows denote the onset of symptoms.

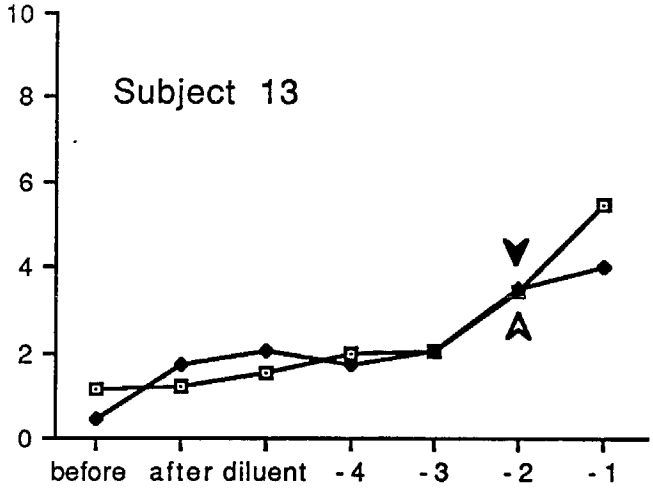


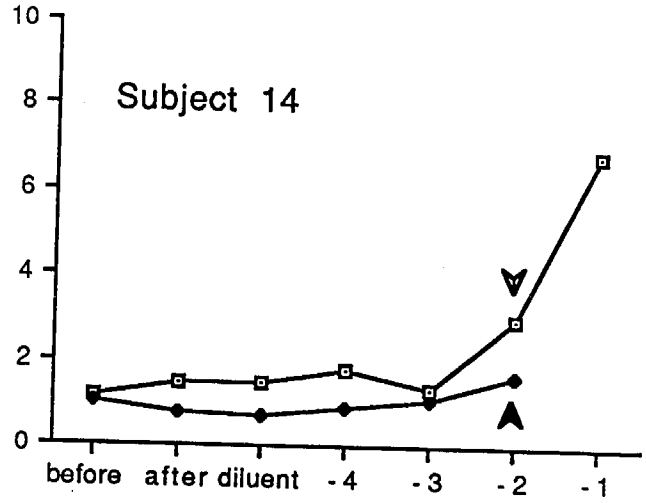
Effect of ozone on nasal sensitivity to allergen in subjects with allergic rhinitis

Figure 2 (continued). Antigen dose-response curves after subjects breathed conditioned room air (▫) or 0.5 ppm ozone (▪) for 2 hours. Changes in nasal resistance are depicted before and after exposure to air or ozone, after nasal challenge with diluent alone, and then after a 1:10,000 (-4), a 1:1000 dilution (-3), a 1:100 (-2), and a 1:10 dilution of antigen solution . Only subject 9 received a challenge with undiluted antigen solution. Arrows denote the onset of symptoms.









Effect of ozone on nasal sensitivity to allergen in subjects with allergic rhinitis

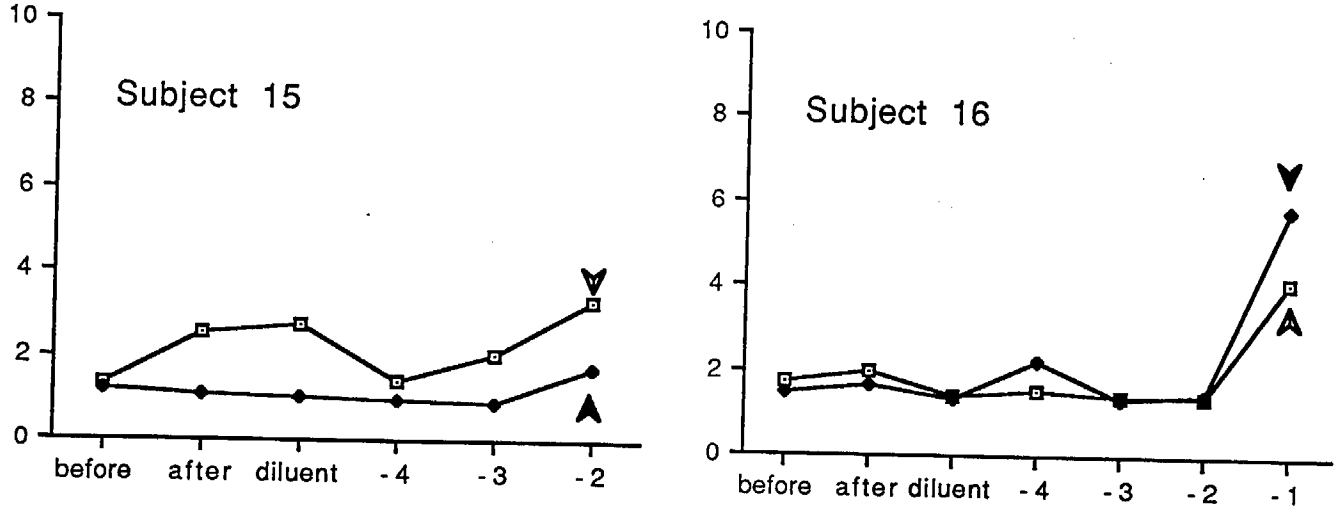



Figure 2 (continued). Antigen dose-response curves after subjects breathed conditioned room air (□) or 0.5 ppm ozone (■) for 2 hours. Changes in nasal resistance are depicted before and after exposure to air or ozone, after nasal challenge with diluent alone, and then after a 1:10,000 (-4), a 1:1000 dilution (-3), a 1:100 (-2), and a 1:10 dilution of antigen solution . Only subject 9 received a challenge with undiluted antigen solution. Arrows denoted the onset of symptoms.