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Study of Air Pollution:

Effects of Ozone on Neuropeptide-Mediated Responses in Human Subjects

State of California
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Research Division

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Abstract

We examined the effects of exposure to 0.2, 0.4, and 0.6 ppm of ozone on the cough response to capsaicin aerosol in healthy human subjects to examine the hypothesis that ozone inactivates the enzyme, neutral endopeptidase, responsible for limiting the effects of neuropeptides released from afferent nerve endings. Cough response was measured by delivering single inhalations of serially increasing concentrations of capsaicin solution delivered from a nebulizer (10^{-8} to 10^{-4} M) at 2 min. intervals until two or more coughs were produced. Other endpoints measured included irritative symptoms as rated by the subjects on a nonparametric scale, spirometry, and specific airway resistance as determined by body plethysmography. The effects of each concentration of ozone were compared to those of filtered air in a single-blind randomized sequence. Our first study was of 4 subjects exposed to air or 0.6 ppm of ozone for 2 h. while performing intermittent exercise. Ozone caused a significant increase in specific airway resistance, an increase in symptoms of borderline significance, and a decrease in cough threshold (i.e. cough occurred on inhaling a lower concentration of capsaicin) in 2 subjects. Six subjects were then exposed to air and to 0.4 ppm of ozone in a similar protocol. Ozone caused significantly greater changes ($p<0.05$ by paired t-test) in cough threshold, symptoms, and specific airway resistance and a marginally greater ($0.05<p<0.10$) fall in FEV_1. Nine subjects were exposed to 0.2 ppm ozone and to air for two hours, and eight subjects were exposed to 0.2 ppm ozone and to air for three hours. Neither duration of exposure to this lower concentration of ozone had a significant effect on cough threshold, symptoms, or tests of pulmonary function.

These results indicate that a 2 h. exposure to 0.4 ppm of ozone with intermittent light exercise alters the sensitivity of airway nerves that mediate the cough response to inhaled materials. This dose of ozone also caused a change in FEV_1. A lower level of ozone, 0.2 ppm, caused a change in neither cough threshold nor FEV_1, even when the duration of exposure was extended to three hours. These findings are consistent with our hypothesis that ozone may sensitize nerve endings in the airways by inactivating neutral endopeptidase, an enzyme that regulates their activity, but they do not demonstrate that directly examining an effect directly mediated by airway nerves allows detection of effects of ozone at doses below those causing effects detected by standard tests of pulmonary function.

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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 sources or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

Glossary

Capsaicin - the active ingredient in hot (chili) peppers. A potent stimulus to nerve stimulation and substance P release; a known potent stimulus to cough when delivered by inhalation.

CT - cough threshold. The concentration of capsaicin aerosol causing distinct cough when delivered by a single inhalation of serially increasing concentrations.

FEV_1 - forced expiratory volume in one second. The amount of air exhaled during the first second of an FVC maneuver.

FVC - forced vital capacity. The amount of air that can be exhaled after a maximal inspiration.

NEP - neutral endopeptidase. A membrane-bound enzyme that rapidly and effectively inactivates substance P and other neuropeptides.

SP - substance P. A neuropeptide released from afferent nerves and that exerts several effects, including stimulation of cough, mucus secretion, airway smooth muscle contraction, vasodilation, and increased vascular permeability.

SRaw - specific airways resistance, the product of two measurements, airways resistance and thoracic gas volume, as measured in a body plethysmograph. The resulting units are liters x centimeters of water per liter per second.

Summary and Conclusions

Our results indicate that a 2 h. exposure to 0.4 ppm of ozone with intermittent light exercise alters the cough response to capsaicin aerosol, an effect consistent with our hypothesis that ozone alters the sensitivity of afferent nerves in the upper airway, possibly by oxidative inactivation of neutral endopeptidase on the airway mucosal surface. This level of ozone also caused a change in FEV_1. A lower level of ozone, 0.2 ppm, caused a change in neither cough threshold nor FEV_1 even when the duration of exposure was extended to 3 h. The similarity of the dose of ozone needed to cause changes in FEV_1 and in cough threshold is consistent with the hypothesis that both effects are mediated by sensitization of nerve endings: in the one case, "sensitized" nerve endings responsible for cough are stimulated by a lower dose of capsaicin, causing cough; in the other, "sensitized" nerve endings are stimulated by deep inspiration, causing discomfort and a limitation of the inspiratory effort. Analysis of individual responses to 0.4 ppm of ozone suggested that a change in cough threshold and a change in FEV_1, are indeed linked, but changes in the two functions did not appear to be linked in the responses of individuals to 0.2 ppm ozone.

Recommendations:

This study has not proved but has provided data consistent with the hypothesis that ozone increases the sensitivity of afferent nerves in the airways to a stimulus known to stimulate the release of neuropeptides. Based on previous work in animals showing the sensitivity to oxidation of neutral endopeptidase, the enzyme responsible for the breakdown of neuropeptides, a likely explanation for our findings is that ozone inactivates neutral endopeptidase in the airway epithelium, thus unmasking the effects of neuropeptides released from nerve endings in response to capsaicin stimulation.

We were surprised to find no evidence that ozone increased neural sensitivity to an exogenous stimulus at doses below those that cause changes in symptoms on standard tests of pulmonary function. Our findings thus do not suggest a need for modifying the existing AAQS for ozone.

Our findings do, however, suggest a possible basis for predicting interaction between ozone and other pollutants, especially the aldehydes. Non-myelinated nerve endings make up a nociceptive system in the mucous membranes of the conjunctivae, nose, throat, and tracheobronchial tree. Neutral endopeptidase is found in the same sites. The chemical structure of NEP suggests that it would be highly sensitive to oxidation. Because the sites where NEP is found are accessible to atmospheric ozone, ozone exposure would be expected to heighten sensitivity to any airborne agent that stimulates nerve endings. Aldehydes and acroleins, trace constituents of photochemical smog, are indeed known to stimulate nerve endings. Thus, the reported correlation of ozone levels with cough and eye redness may not reflect a direct effect of ozone but rather an indirect effect, altering neurally-mediated responses (cough and conjunctivitis) to other coincident pollutants.

Before undertaking studies of the interaction between ozone and other pollutants known or suspected to stimulate non-myelinated, nociceptive nerves, it would first be important to confirm the hypothesis supported--but not proven--by this study. This could be undertaken in parallel studies of isolated cells, laboratory animals, and human subjects. Transformed human airway epithelial cells, known to be rich in NEP, could be studied in vitro by measuring NEP activity after incubation in media into which air (control) or a range of concentrations of ozone have been added. The NEP activity of tracheal epithelium obtained from rats or guinea pigs after exposure to air and to ozone could also be compared to determine the effects of ozone in the activity of this enzyme in vivo. A pilot study of five human volunteers has already been undertaken in our laboratory, comparing the mean NEP activity of three bronchial mucosal biopsies obtained via a fiberoptic bronchoscope one hour after exposure to air or to 0.5 ppm of ozone. Results from this study are pending at the time of this writing.

Finally, it might be useful and interesting to examine the effects of ozone exposure on conjunctival responses, especially given the frequency of complaints of eye irritation on days of increased photochemical pollution. The phenomenon of "neurogenic inflammation" (i.e. vasodilation and edema caused by stimulation of sensory nerves) was first recognized in the eye, and NEP is found in the conjunctival epithelium. Inactivation of this enzyme at this site by ozone would heighten the sensitivity of the conjunctivae to the wide variety of irritants that stimulate afferent nerves. Formal examination of the role of NEP in modifying conjunctival responses to capsaicin and then of the effects of ozone on NEP activity and on conjunctival responses could be undertaken in rabbits, the species most often used in studies of conjunctival responses.

Report of Project:

Purpose:

To determine whether 2 h. of exposure to 0.6, 0.4, and 0.2 parts per million of ozone while performing intermittent moderate exercise increases the cough response to inhalation of serially increasing doses of capsaicin delivered as an aerosolized solution.

Background:

Both acute exposure studies and epidemiologic studies suggest that ozone causes irritation of nociceptive nerve endings in the respiratory tract. The prototypic response to stimulation of an afferent nerve in the airways is cough (Karlsson, Sant'Ambrogio 1988). Many of the stimuli that cause cough (e.g. distilled water aerosol, sulfur dioxide, inert particles, citric acid aerosol) have been shown to stimulate discharge from afferent nerves in the airways of animals (Karlsson, Sant'Ambrogio 1988; Boushey, Richardson 1974). Aerosols of capsaicin, the active ingredient in hot peppers, have been shown to cause cough in people (Collier, Fuller 1984, Fuller, Dixon 1985) and stimulation of afferent nerve activity and release of

neuropeptides from afferent nerves in animals (Coleridge, Coleridge, Luck 1964; Lundberg, Saria 1983). The involvement of neuropeptide release in the cough response to capsaicin aerosol is suggested by the finding in guinea pigs that the cough provoked by capsaicin is potentiated or inhibited by drugs or agents that reduce or augment the activity of neutral endopeptidase, the enzyme principally responsible for metabolizing neuropeptides (Kohrogi, Graf 1988). Thus, it appears that the neuropeptides released from nerve endings themselves stimulate the nerve in a positive feed-back loop, with neutral endopeptidase regulating the system by degrading neuropeptides.

Neutral endopeptidase is a Zn-containing metalloendopeptidase that is active at the neutral to alkaline pH of extracellular fluids. It is an ectoenzyme expressed on the surface of a variety of cell types, and is particularly abundant on the surface of epithelial cells that line the airway lumen. It hydrolyzes a variety of bioactive peptides. In the airways, it is particularly active in destroying substance P, a sensory neuropeptide whose release is provoked by capsaicin and that has potent bronchoconstriction and secretagogue activity. In addition to its unique specificity for peptide substrates, neutral endopeptidase differs from other proteases in its resistance to inactivation by circulating protease inhibitors, its neutral pH profile, its dependence on a divalent metal for activity, and its expression on the cell surface as a transmembrane protein whose active site is extracellular.

Neutral endopeptidase is found on the airway epithelium and is readily inactivated by oxidation (Dusser, Djokic 1989). We therefore hypothesized that ozone, a potent oxidant, would increase the cough response to inhaled irritants. Of the potential irritants, capsaicin is the one that has most clearly been shown to trigger the release of neuropeptides from afferent nerves in animals and to provoke cough in both animals and people, and so best permitted examination of our hypothesis that ozone decreases neutral endopeptidase activity and best allowed correlation with animal studies on the importance of the neuropeptide-neutral endopeptidase system in regulating neuropeptide-mediated effects.

In this study, we proposed first to examine whether exposure to a concentration of ozone known to have effects on pulmonary function tests in most people, 0.6 ppm, also altered the cough response to capsaicin aerosol. Accordingly, we first studied the effects of this concentration of ozone on cough threshold in 4 people. Because we found that cough threshold was lowered, we then proceeded to examine the effects of 0.4 ppm in another 6 subjects, and found that this concentration also altered the cough response. We then examined in 17 subjects the effects of a concentration, 0.2 ppm, that is unlikely to affect standard tests of pulmonary function in most individuals under the conditions of exposure, to determine whether a change in cough sensitivity is a sensitive indicator of an effect of ozone on the lungs.

Materials and Methods

In brief, our study involved determining whether 2 hours of exposure to 0.6, 0.4 and 0.2 ppm of ozone while performing intermittent moderate exercise increases the cough response to inhalation of serial increasing concentration of capsaicin delivered in an aerosolized solution. Each ozone exposure was paired with an exposure to filtered air at least 4 days apart from the ozone exposure. The exposures were performed in random order and by a single-blind design, with the experimenter, but not the subjects, knowing the nature of the exposure. All exposures were carried out in our exposure facility, an independently ventilated 8 x 8 x 8 ft. stainless steel and glass chamber. Ozone was generated from oxygen by an electrical arc generator (Welsbach Model T-408) and was passed into the chamber's intake system. Concentrations in the chamber atmosphere were measured continuously by a Dasibi ozone analyzer.

Our protocol was similar for all exposures. All subjects were healthy, non-smoking adults with normal values for FEV1 and FVC on screening spirometry. After obtaining consent (as approved by the UCSF Committee on Human Research), we measured specific airway resistance by body plethysmography, forced vital capacity by spirometry, and cough threshold. Capsaicin aerosol challenge was performed in the following manner. Two cc. of serial increasing concentrations of capsaicin were delivered through an ultrasonic nebulizer (Pulmo-sonic model no. 25, De Vilbiss Co., Somerset, PA) to our seated volunteers. With a nose clip applied, each volunteer was instructed to simply take one continuous inhalation from the nebulizer over three seconds. The nebulizer was then emptied and air dried prior to being refilled with the next higher concentration of capsaicin solution. Each capsaicin aerosol challenge began with inhalation of the diluent (saline with 1% ethanol) and then of capsaicin at 10^{-8} m. In the pre- and post-0.6 and 0.4 ppm ozone/air exposed population, we used increasing whole log steps. To better assess the change in "cough threshold" after exposure to lower concentrations of ozone, we used half log steps in the pre- and post-0.2 ppm ozone/air population. The capsaicin aerosol challenge was stopped when the subject produced a sharp cough, sometimes occurring in volleys during the three seconds inhalation phase or within one second thereafter. The capsaicin concentration at which this occurs was considered the "cough threshold." Within two minutes after reaching this threshold, we remeasured specific airway resistance. This was done partly to distract the subject from cough as the endpoint of interest, for cough is a highly suggestible symptom.

On the days of air and ozone exposure we had subjects rate the severity of 10 lung, airway, and irritative symptoms on a scale of 0-10 in the 0.6 and 0.4 ppm exposure groups and a scale of 0-3 in the 0.2 ppm group. (See Tables for symptoms rating forms). We perform this rating and other studies in the following sequence:

1. Symptom rating
   Body Plethysmography
   Capsaicin challenge
   Body Plethysmography

2. 15 min. later:
   Body plethysmography
   Spirometry
   Body plethysmography

3. 2 h. exposure to air or ozone with
   intermittent exercise

4. Immediately after exposure:
   Symptom score
   Body plethysmography
   Spirometry
   Body plethysmography

5. 15 min. after exposure:
   Body plethysmography
   Capsaicin challenge
   Body plethysmography

6. 50 min. after exposure:
   Symptom score
   Body plethysmography
   Spirometry
   Body plethysmography

7. 60 min. after exposure:
   Capsaicin challenge
   Body plethysmography

Our focus was on the effects of exposure on cough threshold, so the tables of this report present the provocative concentration measured before and 15 minutes after exposure. Because we hypothesized that oxidation of neutral endopeptidase by ozone would increase the sensitivity of nerve endings, we examined whether changes in cough threshold correlated with changes in symptoms. Finally, we also hypothesized that the fall in FEV_1 and FVC caused by ozone is caused by discomfort--also mediated by sensitized nerves--so we examined whether changes in cough threshold correlated with changes in FEV_1 and FVC.

We first examined the effects of relatively high concentrations of ozone, 0.6 and 0.4 ppm, that predictably provoke symptoms and a reduction in FEV_1 and FVC in nearly all subjects, and then examined whether similar effects would occur with exposure to 0.2 ppm after exposures of 2 and 3 hours in separate groups of subjects. We anticipated finding that ozone's effects on cough would be a highly

sensitive index of ozone exposure. We report separately our results in subjects exposed to 0.6, 0.4, and 0.2 ppm of ozone.

Effects of 0.6 ppm of Ozone:

Four healthy nonsmoking volunteers were exposed to a target concentration of ozone of 0.6 ppm [actual concentration = 0.60 ± 0.005 ppm; 20.3 ± 0.8°C; 51.6 ± 1.4% RH (mean ± SD)]. Control (filtered air) exposure caused only modest and clinically unimportant changes in specific airway resistance and symptom score and no change in cough threshold to capsaicin aerosol (Table 1). Ozone exposure caused a modest increase in symptom score and specific airway resistance in all subjects, and a decrease in cough threshold (i.e. an increase in the sensitivity of the cough reflex to capsaicin aerosol) in two subjects. In one of these subjects, the increase was a three-log shift, the largest seen after any exposure in our study so far. There appeared to be no correlation between the magnitude of change in symptoms or in specific airway resistance and the change in cough threshold. That is, the subjects with the change in cough threshold appeared to have no greater change in symptoms or in SRaw than did those without a change in cough threshold.

In summary, the 2 h. exposure to 0.6 ppm of ozone caused a significant increase in specific resistance, an increase in symptoms of borderline significance, and decrease in cough threshold in two subjects (but an insignificant change in the group as a whole.) Reasoning that this high level of ozone may have obscured effects seen with lower levels of exposure, we next analyzed the effects of a 2 h. exposure to 0.4 ppm.

Effects of 0.4 ppm of Ozone:

Six volunteers were exposed to a target concentration of ozone of 0.4 ppm [actual concentration = 0.40 ± 0.005 ppm; 19.9 ± 0.6° C, 52.9 ± 3.2% RH]. Two of these subjects (#2 and #4) had taken part in the study of the effects of 0.6 ppm. We modified the protocol to include measurement of FEV_1, and FVC before and after the exposures. We again found that air exposure caused little change in symptoms, specific airway resistance, FEV_1/FVC, and cough threshold (Table 2). Ozone exposure caused significantly greater changes ($p<0.5$ by paired t-test) in cough threshold, symptoms, and specific airway resistance and a fall in FEV_1 marginally greater ($0.05<p<0.10$) than that caused by air exposure. Again, there was no apparent correlation between the change in symptoms and the change in cough threshold. There was a possible association between the change in FEV_1 and the change in cough threshold. Of the three subjects with the smallest changes in FEV_1 (0.10, 0.00, and -0.10 Liter), only one had a fall in cough threshold, whereas of the other three subjects (with changes in FEV_1 of -0.60, -0.40, and -1.30 L.), all had a change in cough threshold.

We interpret these findings as demonstrating that a 2 h. exposure to 0.4 ppm ozone increase the cough reflex to inhalation of capsaicin aerosol and as suggesting that sensitization of nerve endings may account for both the change in cough threshold and the fall in FEV_1 provoked by ozone. We then examined

whether these effects would also be caused by exposure to a lower concentration of ozone, 0.2 ppm. We anticipated that a change in cough threshold would be a sensitive index of ozone effect, occurring with exposures lower than those needed to cause spontaneously reported airway symptoms but possibly coinciding with the exposures needed to reduce FEV_1 or vital capacity, reasoning that capsaicin inhalation and deep inspiration both stimulate nerve receptors in the airways. Thus, a "sensitization" of nerve endings by ozone would concurrently alter responses mediated by nerve endings.

Effects of 0.2 ppm of Ozone (2 h. exposure)

Nine healthy nonsmoking subjects were exposed to a target concentration of 0.2 ppm ozone [actual concentration = 0.20 ± 0.01 ppm; 20.5 ± 1.3°C; 50.2 ± 2.8% RH] and to filtered air, following the protocol described above. We modified the protocol, delivering aerosolized capsaicin in increasing half-log steps instead of log steps. One subject (#4) had previously participated in the study of 0.6 and 0.4 ppm and two others (#6 and #7) had taken part in the study of 0.4 ppm ozone. We found no significant differences in the effects of air and 0.2 ppm of ozone on any of the end-points measured: cough threshold, FEV_1, FVC, symptom score, or SRaw. Analyzing the responses of individual subjects revealed that air exposure resulted in a greater than 10% fall in FEV_1 in no subject, whereas ozone exposure caused such a change in one subject (No. 10). The change in FEV_1 in this subject was associated with small changes in symptom score, SRaw and cough threshold.

On the basis of these results, we could not conclude that a two hour exposure to ozone at 0.2 ppm with intermittent exercise altered the sensitivity of airway receptors responsible for cough.

Effects of 0.2 ppm of ozone (3 h. exposure)

Because published reports of the effects of ozone sometimes noted that spontaneous cough often occurred in the third hour of exposure, we reasoned that the effects of ozone on NEP may be time-dependent. We therefore undertook a study of a more prolonged, 3 h. exposure in 8 healthy, non-smoking adult volunteers. These 8 volunteers were exposed to a target concentration of ozone at a concentration of 0.2 ppm [actual concentration = 0.20 ± 0.01 ppm; 20.2 ± 0.009°C, 50.8 ± 3.7% RH] for 3 hours with intermittent cycling of 60 rpm and at a work rate of 80 (women) or 100 (men) watts to achieve oxygen consumptions of equal to or greater than 40 lpm. Except for the additional hour of exposure (30 additional minutes of cycling) these volunteers followed the same protocol as their predecessors. Three of these volunteers (#15, #16, and #17) were previously determined to be part of the subgroup with heightened sensitivity to the effects of 0.20 ppm ozone on FEV_1 in a study conducted by Drs. John Balmes and Rob Aris at San Francisco General Hospital. In this study, this subgroup did not have a significant change in their pulmonary functions following ozone exposure. We therefore made no distinction among the 3 hour exposure group for data analysis. In this group, we found no significant changes in cough threshold to aerosol capsaicin, or specific resistance (SRaw) following a 3 hour exposure to ozone at

0.2 ppm (Table 4). Although volunteer #17 expressed a 13-point change in symptom score, there were no correlations with cough threshold, FEV_1 or SRaw.

Based on these results we are not able to conclude that exposure to ozone at 0.2 ppm alters airway receptors responsible for cough.

Discussion:

The work performed in fulfillment of this contract was aimed at examining the effects of brief (2-3 h.) exposure to 0.2 - 0.6 parts per million of ozone on the sensitivity of the cough reflex in healthy non-smoking human subjects. The purpose of the study was not, however, simply to describe an effect of ozone but also to examine a possible mechanism by which ozone could alter responsiveness to other stimuli. This mechanism is the inactivation of neutral endopeptidase (NEP), an enzyme found in the airway epithelium that appears to be important in modulating the activity of nerves and of the chemicals released from nerves when they are stimulated.

The background to the rationale for our study is as follows: a type of sensory nerve ending found in the lining of the eyes, nose, throat, and upper airways ("nociceptive" nerves) has been found to release potent chemicals, called neuropeptides, when they are stimulated. The best described of these is Substance P, a chemical that itself stimulates nerves and also stimulates airway mucus secretion, dilation of blood vessels, edema, and contraction of the muscle found in the airway wall. Substance P is rapidly and effectively inactivated and degraded by neutral endopeptidase. Thus, neutral endopeptidase plays an important role in limiting the inflammation (vasodilation and edema), cough, and airway narrowing that could result form stimulation of airway nerves. Research on guinea pigs has confirmed this concept. When neutral endopeptidase is inactivated, guinea pigs become much more sensitive to the effects of inhaled irritants on cough and airway narrowing. Additional research on guinea pigs has shown that neutral endopeptidase is impaired or inactivated by cigarette smoke or chemical oxidants. The effects of ozone, a highly potent oxidant, on this system have not been directly examined.

In this study, we examined in healthy non-smoking human subjects the effects of ozone on the single effect of a neuropeptide that we could easily measure: cough. We chose capsaicin -- the active ingredient in chili pepper -- as the stimulus to cough, because capsaicin has been shown to have a highly specific effect on non-myelinated nociceptive nerves, provoking the release of substance P (and thereby causing the pain, swelling, and redness observed after applying capsaicin to the eyes, lips, tongue, or nasal mucosa). We predicted that if ozone oxidizes and inactivates neutral endopeptidase, it would increase the sensitivity of the cough response to inhaled capsaicin aerosol.

Summary of Study:

In a series of studies, we examined the effects of exposure to 0.2, 0.4, and 0.6 ppm of ozone on the cough response to capsaicin aerosol in healthy adults. Cough response was measured by delivering single inhalations of serially increasing concentrations of capsaicin solution delivered from a nebulizer (10^{-8} to 10^{-4} M) at 2 min. intervals until two or more coughs were produced. Other endpoints measured included irritative symptoms as rated by the subjects, airway caliber (from standard pulmonary function tests such as spirometry, and specific airway resistance as determined by body plethysmography). The effects of each concentration of ozone were compared to those of filtered air in a single-blind randomized sequence. Our first study was of 4 subjects exposed to air or 0.6 ppm of ozone for 2 h. while performing intermittent exercise. Ozone caused significant narrowing of the airways (increase in specific airway resistance), an increase in symptoms of borderline significance, and a decrease in cough threshold (i.e. cough occurred on inhaling a lower concentration of capsaicin) in 2 subjects. Six subjects were then exposed to air and to 0.4 ppm of ozone in a similar protocol. Compared to air, ozone caused significantly greater changes ($p<0.05$ by paired t-test) in cough threshold, symptoms, and airway caliber. Nine subjects were exposed to 0.2 ppm ozone and to air for two hours, and eight subjects were exposed to 0.2 ppm ozone and to air for three hours. Neither duration of exposure to this lower concentration of ozone had a significant effect on cough threshold, symptoms, or tests of pulmonary function.

Conclusions:

Our results indicate that a 2 h. exposure to 0.4 ppm of ozone with intermittent light exercise alters the sensitivity of airway nerves that mediate the cough response to inhaled materials. This dose of ozone also caused a change in FEV_1. A lower level of ozone, 0.2 ppm, caused a change in neither cough threshold nor FEV_1, even when the duration of exposure was extended to three hours. These findings are consistent with our hypothesis that ozone may inactivate neutral endopeptidase and thus sensitize nerve endings in the airways, but they do not demonstrate that directly examining an effect mediated by airway nerves allows detection of effects of ozone at doses below those causing effects detected by standard tests of pulmonary function.

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H.A. Boushey, Jr., M.D.

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Cardiovascular Research Institute and the Department of Medicine
University of California, San Francisco
San Francisco, CA 94143

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13. ABSTRACT *(Maximum 200 words)*

The study examined the hypothesis that ozone inactivates the enzyme, neutral endopeptidase, responsible for limiting the effects of neuropeptides released from afferent nerve endings. Cough response of capsaicin solution delivered from a nebulizer (10^{-8} to 10^{-4}) at 2 min. intervals until two or more coughs were produced. Other endpoints measured included irritative symptoms as rated by the subjects on a nonparametric scale, spirometry, of each concentration of ozone were compared to those of filtered air in a single-blind randomized sequence.

The results indicate that a 2 h. exposure to 0.4 ppm of ozone with intermittent light exercise alters the sensitivity of airway nerves that mediate the cough response to inhaled materials. This dose of ozone also caused a change in FEV_1. A lower level of ozone, 0.02 ppm, caused a change in neither cough threshold nor FEV_1, even when the duration of exposure was extended to three hours. These findings are consistent with our hypothesis that ozone may sensitize nerve endings in the airways by inactivating neutral endopeptidase, an enzyme that regulates their activity, but they do not demonstrate that directly examining an effect directly mediated by airway nerves allows detection of effects of ozone at doses below those causing effects detected by standard tests of pulmonary function.

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