8

Environmental Airway Injury

Mucosal Changes and Airway Hyperreactivity

CHRISTOPHER GEORGE MURLAS

Rush University
Chicago, Illinois

I. Significance of the Problem

Environmental or occupational airway disorders are common worldwide and are most prevalent in overpopulated or heavily industrialized urban areas. Hundreds of different agents have been associated with these disorders which affect millions of people. Perhaps the most common type of these disorders is so-called occupational, or environmentally related asthma, which affects more than a million people in the United States (Salvaggio, 1982). The importance of this problem is further enhanced by the fact that it is a preventable and reversible disease. In certain situations, however, particularly those related to the isocyanates or to western red cedar, the disorder may persist for months or years after the last exposure.

Agents that have been linked to this type of asthma can be classified into three broad categories (Moller et al., 1986): large molecular weight biological substances, small molecular weight chemicals, and various fumes or gases. Biological agents include dusts from animal danders and secretions, insects and crustaceans, vegetable gums, and plant bacterial enzymes. The pathogenesis of asthma associated with these substances may or may not relate to

their stimulating IgE-mediated reactions when inhaled. Small molecular weight chemicals linked with asthma include isocyanates, acid anhydrides, platinum, and resin. The pathogenesis of environmentally related asthma caused by these agents and to various fumes and gases remains controversial in most instances. Because of increasing air pollution and steadily rising industrial use of a wide variety of potentially harmful agents, especially oxidants, these environmental health hazards represent serious public health concerns.

II. Acute Versus Chronic Airway Hyperreactivity

Environmentally related asthma is characterized by chronic increased irritability (hyperreactivity) of the airways to a wide variety of stimuli. The mechanisms causing this airway hyperreactivity are unknown. Learning about them may be fundamental to an improved understanding of (1) the pathogenesis and course of *chronic* airway diseases, such as asthma, whether allergic or nonallergic; and (2) the mechanisms by which *acute* airway injury, whether immunological or nonimmunological, leads to airway dysfunction. It is feasible that the mechanisms underlying airway hyperreactivity in these two settings are different. The mechanisms of the chronic hyperreactivity that occurs in asthmatic patients are poorly understood. Furthermore, an irreconcilable problem in studies concerning possible mechanisms of hyperreactivity in this chronic disorder is the inability to separate the causes from the consequences of the disease.

This review will concentrate on information that has provided insight into the mechanisms by which acute, nonimmunological airway injury leads to *acute* airway hyperreactivity. We hypothesized some time ago that acute hyperreactivity results from mediators generated from normal lung constituents after airway injury, especially from mucosal cells (including nonepithelial bodies, mast and epithelial cells), which augment neuromuscular responsiveness of the airways (Murlas and Roum, 1985b). It is quite possible that other mechanisms are responsible for the chronic hyperreactivity that occurs in asthma.

To study in more detail the cellular pathophysiology linked to the development of acute airway hyperreactivity, various groups of investigators have focused on acute experimental airway disorders, caused by gases or fumes, that are characterized, in part, by hyperreactivity. Two types of such disorders—those caused by oxidant exposure, as exemplified by ozone and sulfur dioxide, and those not associated with oxidants, as exemplified by toluene diisocyanate—have been intensively studied and are directly relevant to common, naturally occurring airway disorders in humans.

III. Acute Oxidant-Induced Airway Hyperreactivity

Air pollution by oxidants has been a cause of great concern, in part, because the acute respiratory injury that may result can exacerbate, or may even lead to chronic airway disorders. One of the most common gaseous oxidants is ozone. The potential importance of airway mucosal cell damage to the pathogenesis of oxidant-induced hyperreactivity was first evidenced by the relationship that was found to exist between the signs of conducting airway injury and the hyperreactivity that occurs after high-dose ozone exposure (Murlas and Roum, 1985b). In guinea pigs, the developmental time course of increased airway reactivity after ozone exposure is associated with the presence and progression of various pathological changes in the airway mucosa (Fig. 1). These studies were conducted in intact, unanesthetized, spontaneously breathing animals to obviate the effects that endotracheal intubation and anesthesia may have on airway tone, morphology, and hence, airway reactivity.

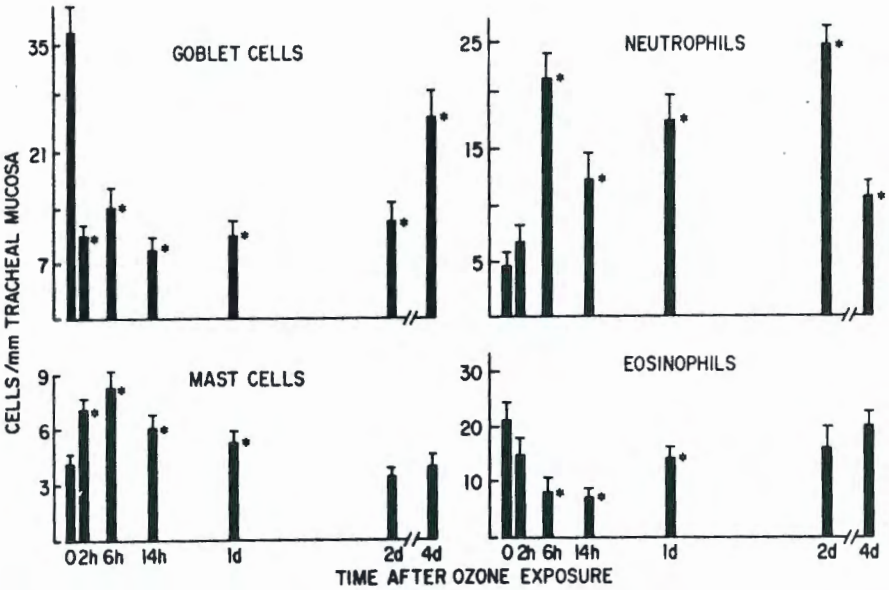


Figure 1 Effects of high-dose ozone exposure on guinea pig tracheal mucosal numbers of goblet cells, mast cells, eosinophils, and neutrophils. Values represent mean ± SE mean of cells per millimeter tracheal mucosa from four animals at each time point except at 2 h (7 animals). Asterisks denote significant differences from control values ($p<0.05$). (From Murlas and Roum, 1985b.)

Exposures as brief as 15 min to 3 ppm of ozone typically increase reactivity to muscarinic agonists 30 min after exposure (Lee and Murlas, 1985). These changes are as striking as those observed 2 h after a 2-h exposure to the same ozone concentration.

A. Mucosa-Linked Pathophysiology

In the guinea pig, reactivity to intravenous acetylcholine (ACh) invariably increases within 2 h after high-dose ozone exposure (Roum and Murlas, 1984). During the early phase of ozone-induced airway injury, there is a large decrease in airway mucosal goblet cells and an increase in mucosal mast cells associated with this hyperreactivity. Loss or matting of epithelial cilia also occurs during this time. In contrast, the number of airway mucosal neutrophils is not significantly increased 2 h after ozone, at which time hyperreactivity is marked. In other species that have been studied, within a few hours of high-dose ozone exposure, quantitative histopathological evidence of inflammatory cell infiltration of the central airways is also lacking (Scheel et al., 1959; Nasr et al., 1971; Evans et al., 1988). Similarly, cigarette smoke inhalation, which results in mucosal hyperpermeability in the guinea pig within 2 h of exposure, is not associated with airway cellular inflammation early on, although various signs of epithelial cell damage are present pathologically (Hulbert et al., 1981).

B. Airway Neutrophilia: A Consequence Not a Cause

Taken together, the foregoing studies suggest that instead of being a *cause*, airway wall inflammatory cell infiltration is an *effect* of the airway damage that results in acute airway hyperreactivity. This temporal dissociation between acute, environmentally induced bronchial hyperreactivity and airway neutrophilic infiltration in guinea pigs is similar to other investigators' findings in dogs with SO_2-induced respiratory disease (Jackson and Eady, 1988) and in guinea pigs with acrolein-induced airway hyperreactivity (Leikauf et al., 1989b). In the later phase of ozone-induced damage, however, neutrophilic infiltration of the mucosa is substantial. Whether components of ensuing airway inflammatory cell infiltration and exudation maintain the hyperreactive state after immune or nonimmune airway injury remains to be determined.

Before 1985, there was little information in the literature concerning possible acute, central airway damage after high-dose ozone exposure. It is possible that such changes were overlooked because of the damage that is found more peripherally postozone exposure. Conventionally, it has been thought that the major sites of ozone-induced pulmonary injury are the terminal bronchioles and adjacent centriacinar alveoli (Scheel et al., 1959; Plopper et al., 1973; Bils and Christie, 1980). The characteristics of ozone-induced lung injury may depend upon the animal species, as well as the dose and duration

of ozone delivered (Bils and Christie, 1980). Type I alveolar epithelial cells and capillary endothelial cells appear to be exquisitely sensitive to high-dose ozone (Plopper et al., 1973; Bils and Christie, 1980). Their damage results in alveolar edema soon after exposure. Neutrophilic exudation into the alveolar spaces may or may not occur at that time (Scheel et al., 1959). In monkeys, immediately after exposure to 3 ppm ozone for 3 h, terminal bronchiole mucosal necrosis and submucosal edema are present (Bils, 1974). In mice, polymorphonuclear leukocytes occur around the terminal airways 10 h after a 4-h exposure to 3 ppm O_3 (Scheel et al., 1959). In neither of these reports is there mention of possible central airway damage. In rats, immediately after a 1-h exposure to 33 ppm, no tracheal inflammation is present (Nasr et al., 1971). In cats, immediately after an approximately 5- to 7-h exposure to 1.0 ppm, prominent, but uneven, airway ciliated cell and goblet cell desquamation is present (Boatman et al., 1974). Acute mucosal inflammation in either large or small airways apparently does not occur during this time, although much more subtle ultrastructural abnormalities do. Thus, the available histopathological evidence suggests that there is some delay between lung injury after high-dose ozone exposure and airway tissue neutrophilic infiltration. Unfortunately, reports concerning bronchoalveolar lavage (BAL) cell findings after ozone exposure are not particularly useful, given the uncertain origin along the bronchoalveolar tree of lavage fluid cells. It is often tempting, although unjustifiable, to presume that changes in lung fluid cells derive from changes in the alveolar space, given its much greater cross-sectional area in relation to that of the bronchi and bronchioles.

In the guinea pig (Murlas and Roum, 1985b) and dog (Turner et al., 1987), there is a significant increase in mucosal mast cells during the early phase of ozone-induced injury. In sheep exposed to 0.5 ppm ozone for h 2, a significant increase in tracheal lavage mast cells occurs within 24 h thereafter (Sielczak et al., 1983). These findings suggest that an oxidant airway insult, such as ozone, may be chemotactic for mast cells. Whether ozone exposure results in the release of preformed mediators, such as histamine, from pulmonary mast cells is uncertain (Easton and Murphy, 1976; Shields and Gold, 1982). Other preformed mediators released by mast cells, such as neutrophil and eosinophil chemotactic factors, may be important later in maintaining the hyperreactivity state (Murlas and Roum, 1985b).

C. Dissociation from Circulating Granulocytes

In guinea pigs depleted of circulating and airway leukocytes, neither the occurrence nor the degree of acute, ozone-induced bronchial hyperreactivity is altered (Fig. 2). These results derived from studies in which guinea pigs were treated with a combination of cyclophosphamide and cortisone that causes substantial decreases in circulating and airway granulocyte[redacted]d in circulat-

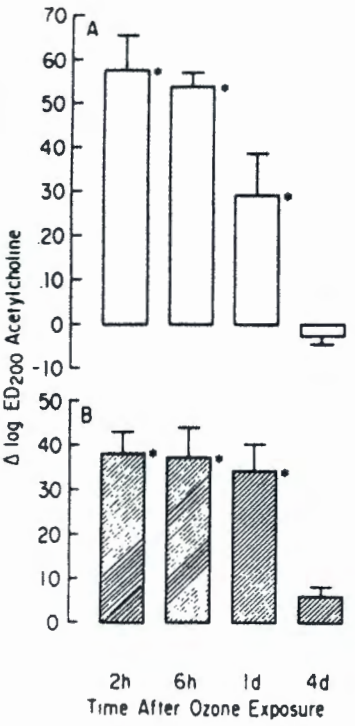


Figure 2 Comparison of the time course of O_3-induced muscarinic hyperreactivity in (A) normal animals and (B) in animals treated with a combination of cyclophosphamide and cortisone, to deplete circulating and airway granulocytes and circulating lymphocytes. The ED_{200} values were derived by interpolation from dose-response curves plotted before and at times specified after O_3 exposure. Values represent means ± SE mean of change in log ED_{200}ACh for 10 animals at all time points. Experimental values with asterisks are different from zero ($P < 0.02$). The degrees of hyperreactivity at each time point in normal and treated group were similar. (From Murlas and Roum, 1985a).

ing lymphocytes (Murlas and Roum, 1985a). Other investigators have shown that this drug regimen also impairs neutrophil and pulmonary macrophage function, as well as their influx into the lung after injury (Pennington, 1977; 1978; Pennington and Ehrie, 1978). Both neutrophils and macrophages are known to produce mediators, such as various arachidonic acid metabolites (Lewis, 1983), that could augment bronchial reactivity after ozone. In animals treated with cyclophosphamide and cortisone, the same early signs of airway mucosal injury as those seen in normal guinea pigs are present (Murlas and Roum, 1985a). Later (6h after exposure), when significant mucosal neutrophil infiltration and eosinophil depletion occur in normal animals, no signs of cellular inflammation are seen in the leukopenic animals (Fig. 3). Despite the absence of these signs, ozone-induced bronchial hyperreactivity occurs in these animals, and its degree and time course is similar to that occurring in normal guinea pigs. This disparity between the onset of ozone-induced hyperreactivity and airway cellular inflammation has led to the suspicion that mucosal neutrophilic infiltration is not a primary determinant of acute hyperreactivity, just as it may not be fundamental to the development of the so-called immediate asthmatic response (Thorpe et al., 1987;

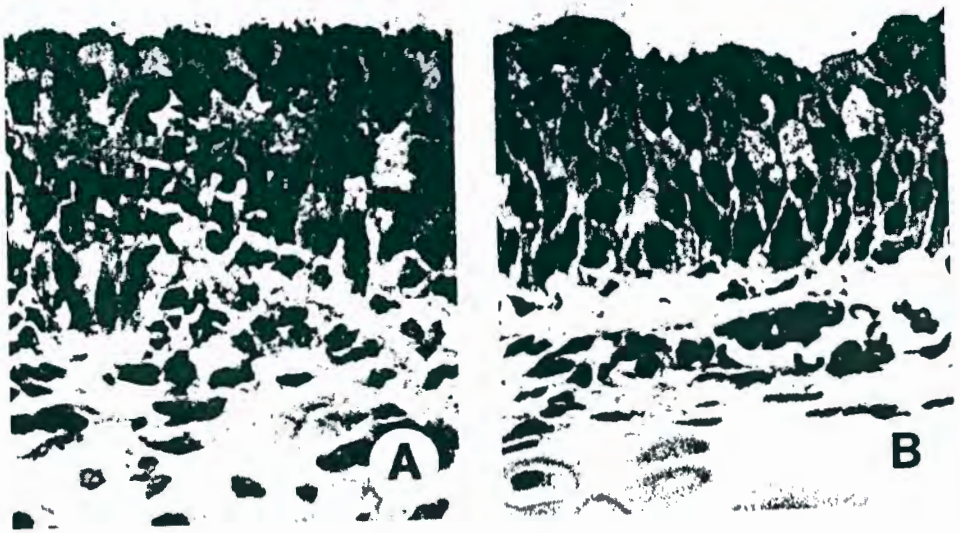


Figure 3 Light micrographs of tracheal mucosa from normal guinea pigs and 6 h after O_3 exposure from guinea pigs treated with a combination of cyclophosphamide and cortisone, to deplete circulating and airway granulocytes and circulating lymphocytes. (A) In control, O_3-treated animals, intraepithelial neutrophils (solid arrows) were numerous and mucosal disorganization was present. (B) In drug-treated animals, mucosal neutrophilic infiltration was not seen. Phenol chromotrope 2R; original magnification ×500. (From Murlas and Roum, 1985a).

Steinberg et al., 1989). However, in mongrel dogs made neutropenic by hydroxyurea treatment, other investigators reported that ozone-induced muscarinic hyperreactivity to inhaled ACh does not occur (O'Byrne et al., 1984b). It is especially difficult to compare results after cyclophosphamide administration with these studies that used hydroxyurea. First, hydroxyurea treatment may produce baseline airway hyporesponsiveness (Hinson et al., 1984). Second, it is known that platelet as well as leukocyte counts may be markedly decreased by hydroxyurea. Certain platelet-derived mediators, including so-called platelet-activating factor, possibly contribute to the pathogenesis of bronchial hyperreactivity in some settings.

IV. Toluene Diisocyanate Airway Hyperreactivity

Toluene diisocyanate (TDI) is a common and highly reactive industrial chemical, used in the production of polyurethane plastics, that has been reported to cause asthma in humans (Chester et al., 1979). Increased airway irritability is typical of patients with TDI-related asthma.

Four-hour exposures to high levels of TDI during 5 consecutive days typically produce airway hyperreactivity in guinea pigs, but not positive passive cutaneous anaphylaxis (PCA) tests when using a stable, low substituted antigen (Chen and Bernstein, 1982). The hyperreactivity lasts only 24 h and is associated with marked signs of airway injury (Cibulas et al., 1986), although airway obstruction persists for a week. These findings suggest that the pathogenesis of TDI-induced airway hyperreactivity is not immunologically mediated or linked to the development of TDI-specific antibodies. Indeed, it is possible that protracted, high-level exposure causes immunological tolerance, rather than sensitization.

Morphological findings in recent studies indicate that airway hyperreactivity after recurrent TDI exposure is associated with airway mucosal damage (Cibulas et al., 1986; 1988). It is doubtful that airway obstruction alone explains this disorder post-TDI since, although hyperreactivity remits, the specific airway conductance remains decreased. Even substantial changes in airway size may not, in fact, affect bronchial reactivity. After certain types of acute airway injury, Hulbert et al. (1981) suggested that the resulting mucosal hyperpermeability may be the cause of airway hyperreactivity. In addition, mediators, possibly derived from mast cells, may affect airway nerve endings and smooth-muscle cells (Cibulas et al., 1986).

To assess the possibility that TDI-induced bronchial hyperreactivity is linked to the presence of circulating granulocytes or their influx into the airways, the effect of cyclophosphamide-induced granulocyte depletion on reactivity post-TDI has been studied (Cibulas et al., 1988). The cyclophosphamide regimen employed was one previously shown to dramatically reduce cir-

culating leukocyte counts and function, as well as their influx into the lung after injury (Pennington, 1977; Pennington et al., 1985). It was found that despite profound cyclophosphamide-induced decreases in both circulating and airway granulocyte counts, guinea pigs develop a degree of bronchial hyperreactivity 2 h after a 10-minute exposure to TDI that is comparable with that which develops in guinea pigs with normal leukocyte counts (Fig. 4). This effect at 2 h is not associated with airway obstruction. These findings, which have been duplicated by other investigators (Thompson et al., 1988), suggest that the pathogenesis of acute airway hyperreactivity produced by a brief, high-level TDI exposure is not dependent on circulating leukocyte counts or on infiltration of airway tissue by granulocytes.

Alternatively, it is possible that TDI-induced airway hyperreactivity may be due, in part, to increased airway capillary permeability (Elwood et al., 1983). However, it is known that reactivity to aerosolized histamine is similarly affected by TDI exposure (Cibulas et al., 1986; Miller et al., 1986). One might expect that increased capillary permeability, if complicated by airway wall edema, could result in histamine hyporeactivity owing to drug dilution or to impaired diffusion because of the edema fluid present. Thus, it seems unlikely that airway capillary hyperpermeability is the sole or primary mechanism involved in TDI-induced bronchial hyperreactivity.

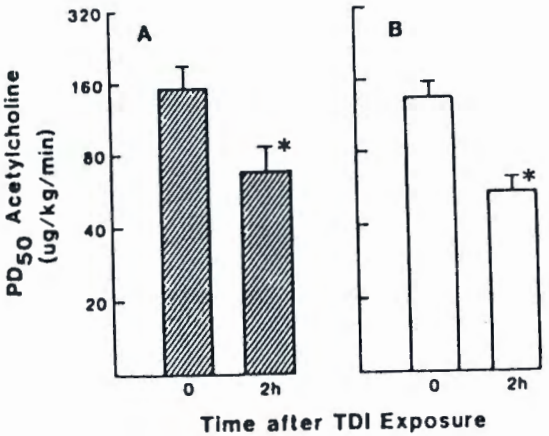


Figure 4 Comparison of the effect of 10-min toluene diisocyanate (TDI) exposure on muscarinic reactivity (A) in cyclophosphamide-treated guinea pigs and (B) in control animals. In neither group was there a significant change in baseline-specific airway conductance 2 h after TDI. Mean potentiating doses (PD_{50}) of ACh were derived by interpolation from dose-response curves plotted before and 2 h after TDI exposure. Values represent means ± SE mean for six animals. Asterisk indicates value different from value before TDI ($p < 0.05$). The degrees of muscarinic hyperreactivity at this time were similar in normal and treated groups. (From Cibulas [illegible], 1986).

These observations are analogous to studies concerning acute, ozone-induced bronchial hyperreactivity summarized in the foregoing. It is now unclear whether the pathogenesis of oxidant- and toluene diisocyanate-induced airway hyperreactivity is similar to, or distinctly different from, that associated with the so-called late asthmatic response in allergic humans. Although more information is clearly needed, many investigators believe that asthma is, in part, characterized pathologically by airway eosinophilia. Epithelial damage in various airway generations may also be a cardinal feature (Laitinen et al., 1985). In comparison, the acute bronchial hyperreactivity occurring in experimental animals after exposure to TDI ozone, sulfur dioxide (Jackson and Eady, 1988), or cigarette smoke (Hulbert et al., 1981) appears to be closely associated temporally with signs of airway mucosal injury, but not with airway eosinophilia.

V. Mucosa-Linked Airway Muscle Hyperresponsiveness in Environmentally Induced Hyperreactivity

From the work of many investigators, it appears that the airway mucosa in a variety of species may normally produce several factors that affect airway muscle tone (Flavahan et al., 1985; Farmer et al., 1986; Goldie et al., 1986; Hay et al., 1986; Murlas, 1986; Raeburn et al., 1986; Vanhoutte, 1987; Stuart-Smith and Vanhoutte, 1988). This conclusion derives from studies in which the physical removal of respiratory mucosa was shown to increase airway smooth-muscle responsiveness to a variety of bronchoconstrictors in vitro. Whether and how other respiratory disorders not associated with mucosal denudation, such as acute, ozone- or TDI-induced *airway hyperreactivity* derive from *airway muscle hyperresponsiveness* are subjects of considerable interest.

Recently, it has been discovered that smooth muscle from the airways of guinea pigs with ozone-induced hyperreactivity is hyperresponsive to agonist stimulation in vitro (Murlas et al., 1990a). Mucosa-intact tracheal tissue rings were obtained from ozone- and air-exposed animals and pretreated with indomethacin to inhibit effects of cyclooxygenase products that are generated in vitro (Orehek et al., 1973; Walters et al., 1986) may be derived from the respiratory mucosa (Butler et al., 1987). Responsiveness of those rings upon stimulation by ACh, KCl, or substance P were assessed (Fig. 5). This was done in the presence or absence of phosphoramidon, an inhibitor of neutral endopeptidase (NEP; EC 3.4.24.11), an enzyme that degrades substance P. Neutral endopeptidase appears to be present in certain respiratory mucosal cells (Johnson et al., 1985; Jacoby et al., 1988), and has been shown to affect guinea pig airway response to substance P in vivo (Thompson and Sheppard, 1988) and in vitro (Stimler-Gerard, 1987).

We have found that smooth muscle of mucosa-intact airways from guinea pigs with ozone-induced bronchial hyperreactivity is hyperresponsive in

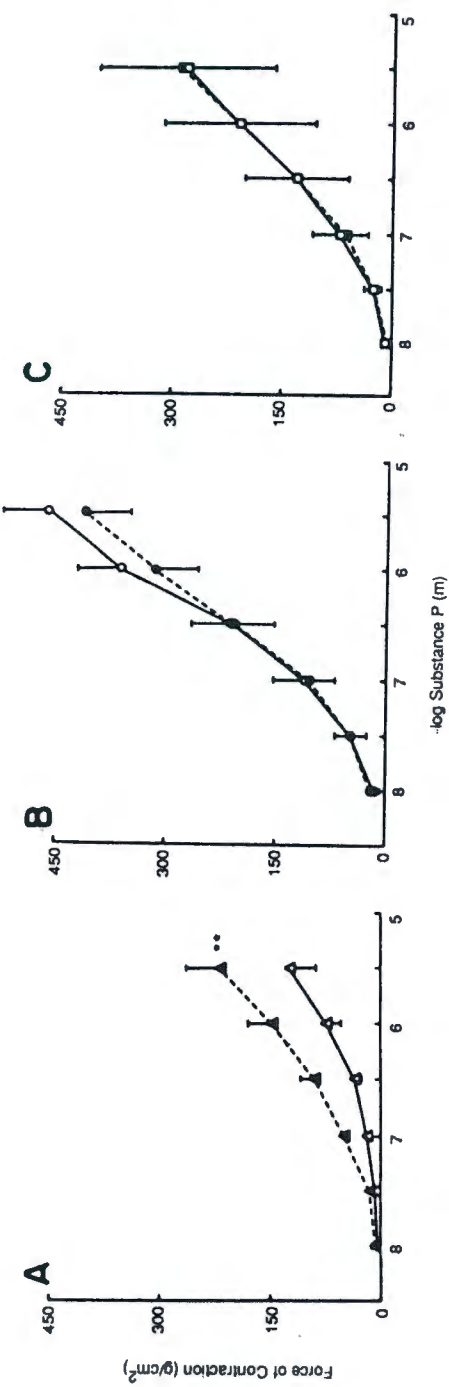
vitro to substance P and ACh, but not to KCl (Murlas et al., 1990a). Pretreatment with phosphoramidon abolishes the increased substance P responsiveness seen in the ozone-exposed compared with the air-exposed group, but it has no effect on the difference in muscarinic responsiveness between these groups. Furthermore, substance P responsiveness is not augmented in ozone-exposed airways in which the mucosa was removed before testing in vitro (see Fig. 5c). Likewise, muscarinic hyperresponsiveness is not present in ozone-exposed airways without mucosa. This data indicate that smooth-muscle responsiveness is increased in guinea pigs with ozone-induced bronchial hyperreactivity, and suggest that hyperresponsiveness may be linked to a mucosa-derived factors.

In conjunction with previous observations of the striking histopathological injury, but not denudation, of guinea pig airway mucosa after ozone exposure (Murlas and Roum, 1985b), these findings indicate that increased substance P responsiveness of guinea pig airway muscle occurs in airways with an intact, but functionally abnormal, mucosal cell layer. Physical absence of the airway mucosa has previously been shown by many investigators to increase airway muscle responsiveness in vitro to a variety of bronchoconstrictors (Flavahan et al, 1985; Farmer et al., 1986; Goldie et al., 1986; Hay et al., 1986; Murlas, 1986; Raeburn et al., 1986; Vanhoutte, 1987; Jacoby et al., 1988; Stuart-Smith and Vanhoutte, 1988).

A. Airway Neutral Endopeptidase Hypoactivity

These data also suggest that ozone-induced substance P hyperresponsiveness may be due to decreased NEP activity of the respiratory mucosal cell layer. This conclusion is supported by the observations that (1) denudation of air-exposed tissues causes these preparations to become as hyperresponsive to substance P as ozone-exposed intact tissues, and that (2) mucosal denudation of ozone-exposed airway tissues does not further increase substance P responsiveness. In other words, removal of ozone-damaged mucosa, lacking NEP activity, has no effect on the underlying smooth-muscle response to substance P, whereas removal of mucosa from air-exposed tissue, with normal NEP activity, increases substance P responsiveness (Murlas et al., 1990a). The fact that phosphoramidon pretreatment of either air- or ozone-exposed mucosa intact tissues causes greater maximal force generation to substance P than exists in either ozone-exposed intact or mucosa-denuded tissues, suggests that ozone only partially inactivates airway NEP. In other words, phosphoramidon treatment inactivates all airway endopeptidase activity (both mucosal and nonmucosal), whereas ozone exposure inactivates only mucosal NEP. Consistent with this hypothesis are previous observations by other investigators that NEP activity is present in both mucosal and nonmucosal lung cells (Johnson et al., 1985; Sekizawa et al., 1987).

Other potential mechanisms include ozone-induced changes in airway


A
B
C
450
300
150
0
8
7
6
5
-log Substance P (m)
Force of Contraction (g/cm²)

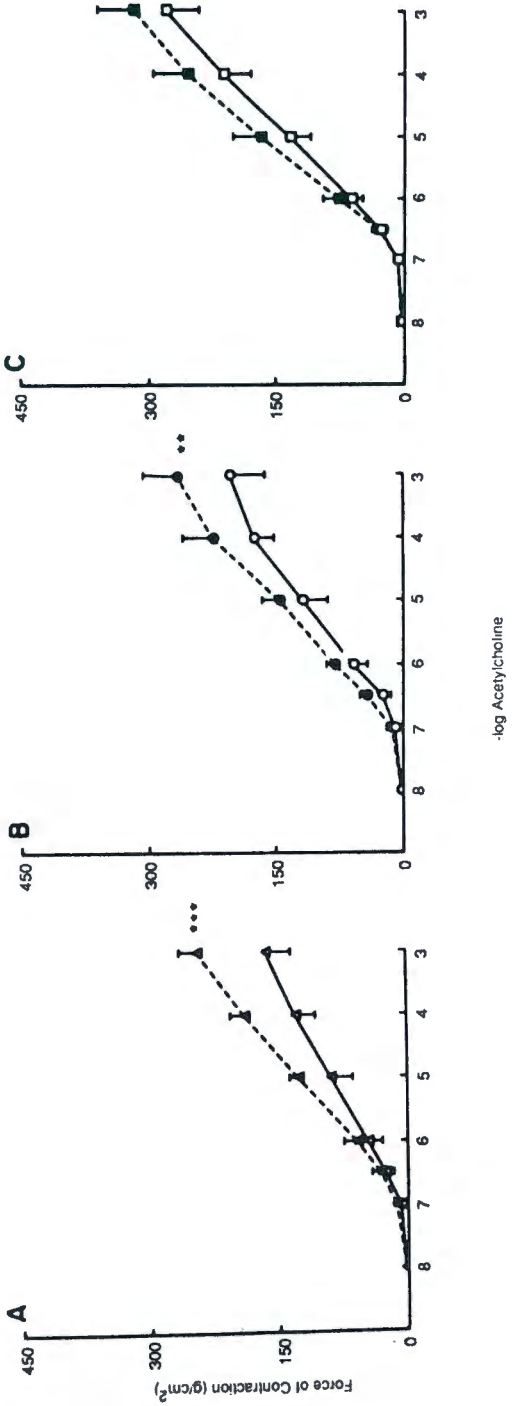
muscle muscarinic receptors or receptor-linked events, or the generation of a product that may inhibit NEP activity. The former seems unlikely, based upon the finding that maximal force generation increases after ozone exposure, suggesting nonreceptor mediation. The findings that (1) ozone exposure does not increase the KCl responsiveness of intact or denuded airways, and (2) that the substance P and the ACh responsiveness of denuded airways is unaffected by ozone exposure (Murlas et al., 1990a) suggest, but do not prove, that airway hyperresponsiveness after ozone exposure is not due to changes in smooth muscle alone.

B. Noncyclooxygenase, Epithelial-Derived Inhibitory Factor(s)

The finding that muscarinic responsiveness also increases in airway preparations with, but not without, mucosa (Fig. 6) indicates that ozone-induced airway muscle hyperresponsiveness is linked to more than one noncyclooxygenase, mucosa-derived factor. This conclusion is somewhat at variance with that made in a recent report in dogs by Jones et al. (1988a). These investigators concluded that loss of an epithelial-derived relaxant factor is not responsible for the development of ozone-induced airway hyperreactivity in dogs because they found increased muscarinic responsiveness in denuded tracheal preparations from both air- and ozone-exposed dogs. Published evidence, however, does not militate against the possibility that effects of ozone on respiratory mucosal cells is partial, rather than total (as is mucosal removal). It can be seen from our data that mucosal removal causes a much greater

---

Figure 5 Substance P concentration-response curves of tracheal rings from guinea pigs exposed either to ozone (solid symbols, dotted lines) or air (open symbols, solid lines) of mucosa-intact (A and B) and mucosa-denuded segments (C). Responses of ozone- and air-exposed airway tissues pretreated with 1 μm phosphoramidon are shown in (B). Responses are expressed in terms of force generation (in grams) per cross-sectional area of airway smooth muscle (in cm^2). Each symbol represents mean $\pm$ SE mean of six experiments in (A); and four in (B) and (C) (where error bars are not shown, they are smaller than the symbol). Double asterisk represents experimental valued significantly different from the control value, $p<0.01$. There was no difference in the substance P responsiveness of the ozone- and air-exposed airway tissue pretreated with phosphoramidon (B), or in preparations in which the mucosa was removed after exposure and prior to testing in vitro (C). Mucosal denudation of air-exposed tissue increased substance P responsiveness to a degree comparable with that caused by ozone exposure (A and C). However, mucosal denudation did not affect the substance P hyperresponsiveness of ozone-exposed tissues. The substance P responsiveness of ozone-exposed, denuded rings was no different from that of ozone-exposed airways that were intact. Phosphoramidon pretreatment significantly increased the substance P responsiveness of intact airway tissues ($p < 0.02$). (From Murlas et al., 1990a).


A
B
C
450
300
150
0
8
7
6
5
4
3
***
**
Force of Contraction (g/cm²)
-log Acetylcholine

change in airway muscarinic responsiveness than does mucosal ozone exposure alone (see Fig. 6). Thus, if assessed in combination (rather than individually when degrees of difference could be compared), the effect of the former (mucosal removal) may well obscure the effect of the latter (ozone exposure) on airway responsiveness.

Perhaps, the differences between these two recent studies (Jones et al., 1988a; Murlas et al., 1990a) also relate to an important difference in experimental design. In the latter study, animals were exposed to room air while awake and spontaneously breathing, rather than to "dry air" directly through an endotracheal tube while anesthetized as did Jones et al. (1988a). Barbet and colleagues (1988) have recently demonstrated that breathing dry room air causes substantial tracheal mucosal damage. The potential effect of this type of respiratory mucosal damage on airway responsiveness in vivo and in vitro is of considerable interest. It is conceivable that dry air exposure, through an endotracheal tube, may damage the respiratory mucosa sufficiently to increase muscarinic responsiveness of airways in vitro. Thus, these airways may not represent true control preparations. True control preparations may have otherwise shown a greater difference in response, compared with intact, ozone-exposed preparations, than that found by Jones and co-workers (1988a) between denuded, air-exposed, and denuded, ozone-exposed, airways. Employing a very similar protocol in the dog, Walters et al. (1986) found no difference in muscarinic responsiveness of air- and ozone-exposed airways devoid of mucosa. The findings in the guinea pig (Murlas et al., 1990a) are similar to those of Walters et al. (1986).

C. Acetylcholinesterase

The possibility that airway muscle hyperresponsiveness (to substance P or to ACh) is due to an ozone-induced decrease in airway acetylcholinesterase ac-

---

Figure 6 Acetylaholine concentration-response curves of tracheal rings from guinea pigs exposed either to ozone (solid symbols, dotted lines) or air (open symbols, solid lines). (A) and (B) mucosa-intact and (C) mucosa-denuded segments. Responses of ozone- and air-exposed airway tissues pretreated with 1 mm phosphoramidon are shown in (B). Each symbol represents mean ± SE mean of six experiments, except in (B) where n = 4. At the highest concentrations of ACh tested, the ozone-exposed, mucosa-intact airway tissue was substantially more responsive to stimulation in the absence (A) or presence (B) of phosphoramidon. There was no difference, however, in responsiveness of ozone- and air-exposed preparations in which the mucosa was removed after exposure and before testing in vitro (C). (From Murlas et al., 1990a).

tivity seems unlikely. This conclusion derives from the findings that ozone-exposed airways pretreated with phosphoramidon (compared with matched controls) are not hyperresponsive to substance P; and that there is no difference in responsiveness to ACh in denuded tissues from control and ozone-exposed animals (see Fig. 6), as others have reported in the dog (Walters et al., 1986). It should be mentioned, however, that another group of investigators found (in ozone-exposed dogs) that some indices of airway muscle muscarinic responsiveness increase in preparations without mucosa (Jones et al., 1988b). It is difficult to rationalize the different results from these two studies in dogs in which very similar methods were employed.

D. Respiratory Mucosal Neutral Endopeptidase

Given the foregoing evidence, it can be concluded that airway muscle responsiveness in acute, ozone-induced bronchial hyperreactivity is increased, and that this hyperresponsiveness may be linked to mucosa-derived factors in the guinea pig. This data also indicate that NEP may be one of those factors that link ozone exposure to airway hyperresponsiveness to substance P. Furthermore, these data suggest that ozone partially inactivates airway epithelial NEP.

Airway Hyperreactivity Mimicked by Neutral Endopeptidase Inhibition

To further evaluate the possibility that ozone-induced respiratory injury in guinea pigs may result in the inactivation of airway NEP and an increased reactivity to substance P, we recently examined the effects of the NEP inhibitor, phosphoramidon (Murlas et al., 1990c). Guinea pigs exposed to 3 ppm of ozone for 2 h were evaluated and compared with animals exposed to room air for the same time. In contrast with the substantial increase in substance P reactivity that phosphoramidon produces in control animals, drug treatment has no significant effect on substance P reactivity in ozone-exposed animals (Fig. 7). These findings are consistent with the hypothesis that ozone-induced airway injury is associated with decreased airway NEP. This possibility was corroborated by evidence confirming that NEP activity is significantly decreased in ozone-exposed airways (Fig. 8).

Neutral Endopeptidase Hypoactivity Reversed by Neutral Endopeptidase Aerosol

We have recently found that acute, ozone-induced airway hyperreactivity can be reversed by inhalation of an aerosolized NEP preparation (Fig. 9), partially isolated and partially purified from guinea pig kidney tissue (Murlas et al., 1990c). Inhalation of phosphoramidon post-NEP inhibited this effect. Heat inactivated NEp had no influence on ozone-induced hyperreactivity. To our knowledge, this is the first study to demonstrate that an air-

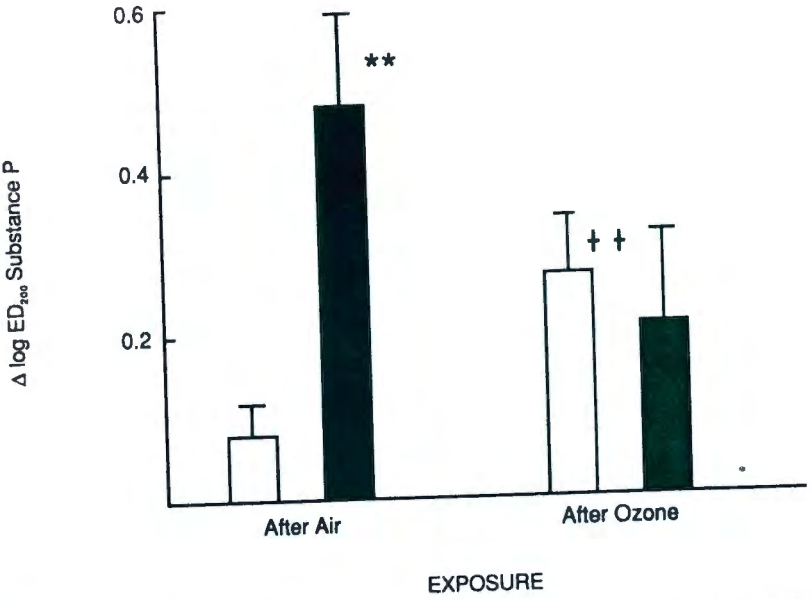


Figure 7 Comparison of mean (±SEM) values for the air- and ozone-exposed groups of changes in the $\log ED_{200}$ substance P after exposure and before (white bars) or after phosphoramidon administration (black bars). Double crossed value of ozone-exposed group before phosphoramidon was significantly greater than value for air-exposed group before phosphoramidon ($p<0.01$). Double asterisked value of air-exposed animals after phosphoramidon was significantly greater than value of air-exposed guinea pigs before phosphoramidon ($p<0.01$). (From Murlas et al., 1990c.)

way disorder associated with the loss of NEP activity can be reversed by inhalation of an aerosol containing that enzyme. Normally, this enzyme is present in certain respiratory mucosal cells (Johnson et al., 1985; Kohrogi et al., 1989; Lang et al., 1989), and can affect guinea pig airway response to substance P in vivo (Thompson and Sheppard, 1988) and in vitro (Stimler-Gerard, 1987). Inhaled NEP also has pharmacologic activity in the guinea pig (Kohrogi et al., 1989).

These findings support speculation that oxidant-induced increases in airway reactivity to substance P may be caused by oxidation of airway mucosal NEP. On the external surface of respiratory mucosal cell membranes, this enzyme may be quite vulnerable to inhaled environmental pollutants, as are the cilia of mucosal epithelial cells (Murlas and Roum, 1985b). In the airways of species thus far studied, NEP appears to be present in gland and smooth muscle cells, as well as in airway mucosal cells (Erdös and Skidgel, 1989). In addition to ozone, TDI also appears to produce an increase in airway reactivity to substance P and is associated with a decrease in airway NEP activity (Sheppard et al., 1988). In both of these experimental conditions, the

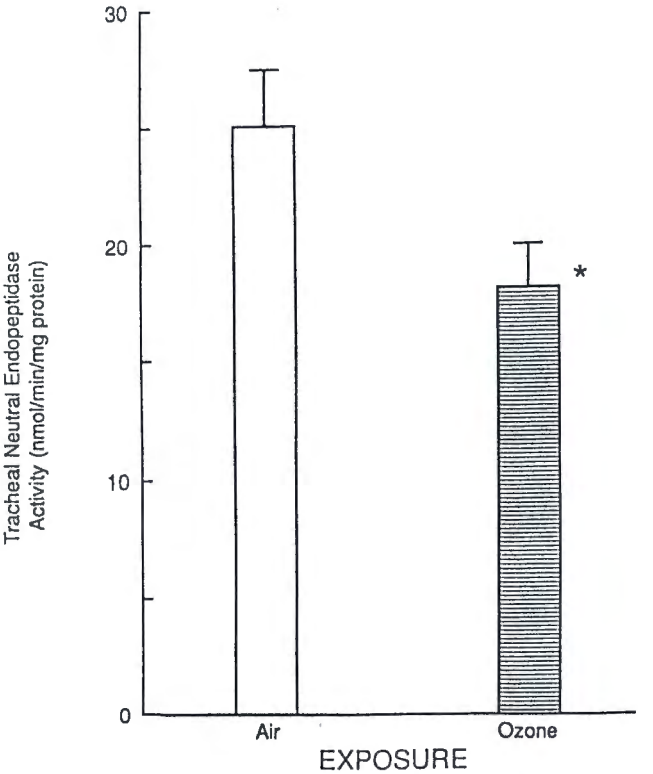


Figure 8 Effect of ozone exposure on guinea pig airway neutral endopeptidase activity assessed by HPLC. Values shown for control (air)- and ozone-exposed animals represent means ± SE mean each of five observations. Asterisk indicates ozone value significantly less than control value ($p < 0.05$). (From Murlas et al., 1990c).

cells of the respiratory mucosa (and, possibly, other airway cells) that may be affected by air pollutant injury have not been identified, nor have the mechanisms by which NEP is inactivated.

Neutral Endopeptidase Inactivation by Oxidizing Agents

Mucosal ozone exposure may generate oxygen-derived free radicals and oxidizing agents, like H_2O_2 and hypochlorous acid (HOCl), a product of chloride oxidation that is catalyzed by myeloperoxidase, an enzyme abundant in some leukocytes such as eosinophils. Interestingly, it has been found that exposure of guinea pig tracheal mucosa in vitro to 0.1 μm HOCl produces hyperresponsiveness to substance P that is abolished by phosphoramidon pretreatment, and that is not present in airways devoid of mucosa (Murlas

et al., 1990b). Airway hyperresponsiveness to substance P is associated with decreased airway NEP activity (Fig. 10). Hyperresponsiveness is also associated with a decreased disappearance of substance P in tracheal perfusates. Likewise, phosphoramidon exposure decreases substance P disappearance from tracheal perfusates, suggesting inhibition of substance P catabolism by tracheal tissue (Table 1). These results suggest that the increased smooth-muscle responsiveness to substance P produced by luminal exposure of airway tissue to HOCl is produced by HOCl inactivation of airway mucosal NEP.

Although there are changes in the guinea pig airway mucosa after HOCl injury, mucosal denudation does not occur (Murlas et al., 1990b). Nonetheless, NEP activity of whole tracheal homogenates is decreased and is clearly associated with airway muscle hyperresponsiveness to substance P. The significance of this decrease was corroborated in this study by a decrease in the disappearance of perfusate substance P from tracheal segments exposed to HOCl. Evidence from this study also suggests that NEP activity of airway cells, other than those of the respiratory mucosa, is substantial and, consequently, that whole tracheal NEP activity may obscure the effect of HOCl on mucosal NEP activity. Additional information also suggests that HOCl has a direct effect on NEP of airway mucosal cells (Lang et al., 1989). Enzymatic activity of cytosolic and membrane fractions from bovine tracheal mucosa homogenates was assessed by high-performance liquid chromatography (HPLC) of products cleaved from succinyl-$(Ala)_3$-p-nitroalanine (Kuwada and Katayma, 1984) in the presence of amastatin, an aminopeptidase inhibitor. It was found that the high specific enzymatic activity, inhibited by 1 μm phosphoramidon, is significantly reduced by a 10-min exposure to 0.1 nM HOCl. Taken together, these results support the speculation that HOCl-induced substance P hyperresponsiveness of airway muscle is caused by oxidation of airway mucosal NEP.

In contrast to HOCl, perfusing guinea pig airway segments with 10 mM H_2O_2 for as long as 10 min does not substantially change airway muscle responsiveness to substance P, ACh, or KCl (Murlas et al., 1990b). The effect of H_2O_2 perfusion on airway smooth-muscle responsiveness to bronchoconstrictors has not been previously studied, although the direct effect of immersion on H_2O_2 on airway muscle contractility has been investigated. The H_2O_2 immersion contracts bovine tracheal muscle, and this effect is enhanced under hypoxic conditions and blocked by cyclooxygenase inhibitors (Stewart et al., 1981). In two recent, and contradictory studies, the effects of H_2O_2 immersion on pulmonary arterial smooth muscle tone has also been evaluated. Exposure of bovine pulmonary artery preparations to H_2O_2 produces a concentration-dependent relaxation of precontracted rings by a mechanism that appears to be independent of the endothelium or PG mediators (Burke

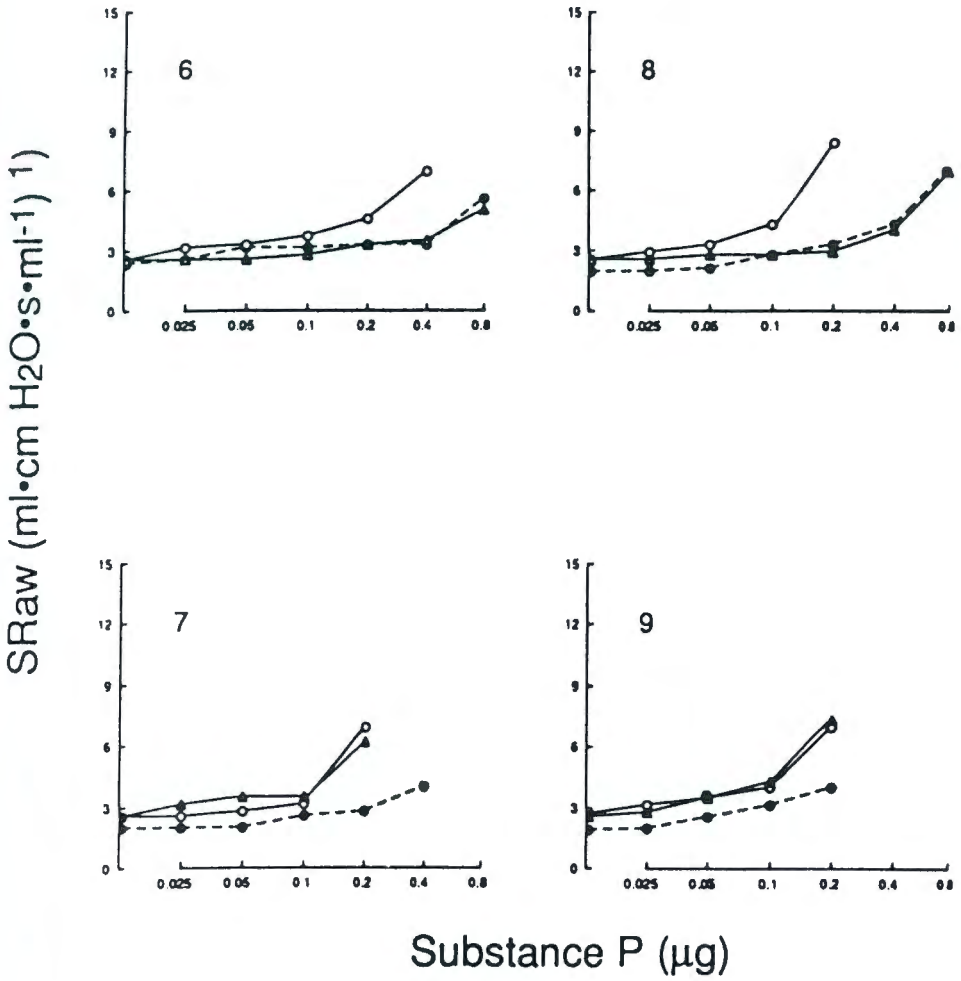
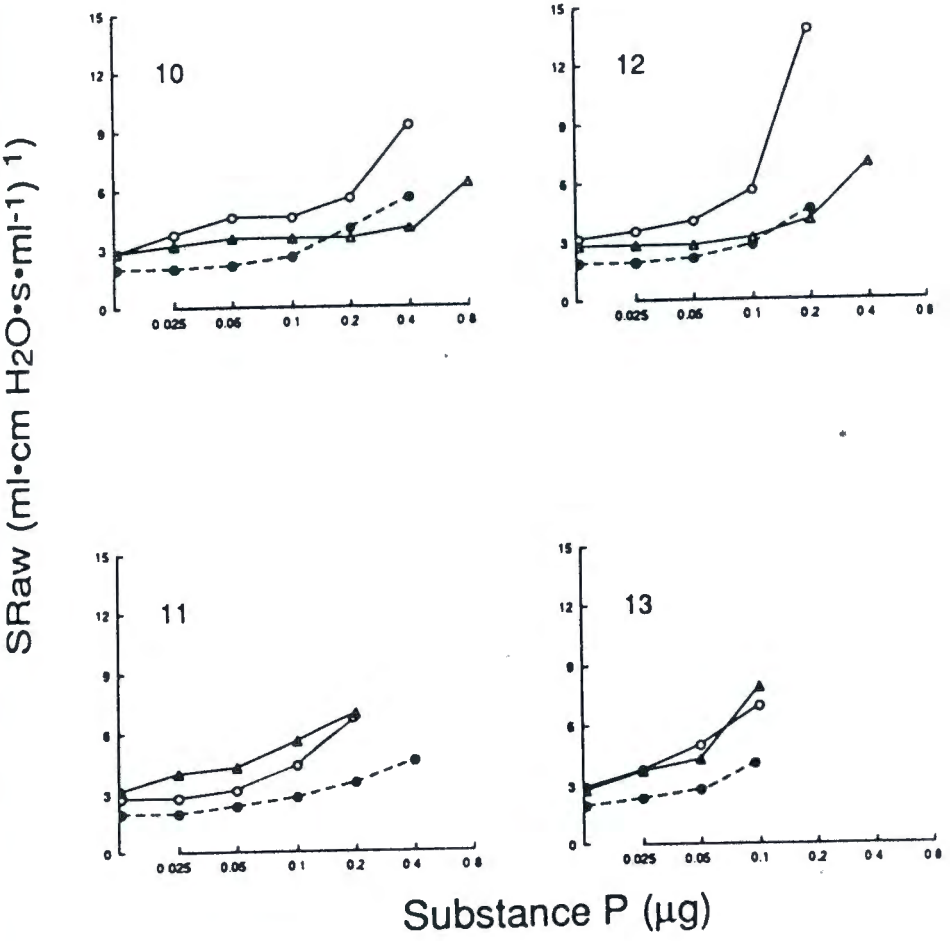


Figure 9 Individual dose-response curves demonstrating the effect of active (top panel (cases 6, 8, 10, 12), open triangles) and heat inactivated neutral endopeptidase inhalation (bottom panel (cases 7, 9, 11, 13), solid triangles) on the increase in airway reactivity to substance P produced by ozone (open circles) in guinea pigs in vivo. Substance P reactivity before ozone exposure is indicated by solid circles and dashed lines. Inhalation of aerosolized NEP reversed the ozone-induced increase in substance P reactivity. Inhalation of phosphoramidon post-NEP inhibited this effect. Heat inactivated NEp aerosol had no influence on ozone-induced hyperreactivity. (From Murlas et al., 1990c.)



and Wolin, 1987). In comparison, exposure of rat pulmonary artery rings to free radicals generated in vitro by xanthine-xanthine oxidase results in contractions that are endothelium- and cyclooxygenase product-independent, but that are completely blocked by catalase, suggesting that H_2O_2 is the major reactive species (Rhoades et al., 1987). These results conflict with studies concerning canine coronary smooth muscle in which H_2O_2 directly depresses smooth-muscle contraction and also initiates the release of endothelium-derived relaxing factor(s) (Rubanyi and Vanhoutte, 1986). Comparing all of these studies, it would appear that the effects of oxidant tissue injury on smooth-

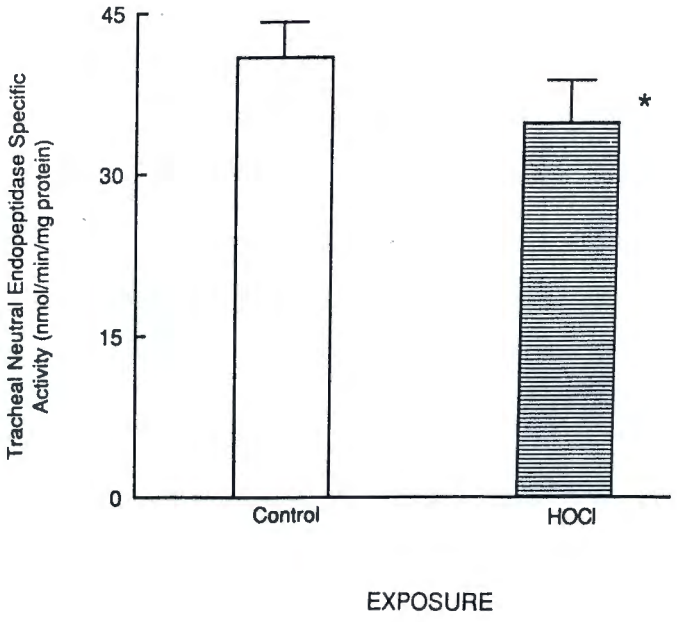


Figure 10 Effect of HOCl exposure on guinea pig airway NEP activity. Values shown for control (Kreb's solution)- and HOCl-exposed groups represent means ± SE mean of five experiments. Asterisk indicates value significantly different from control group, $p<0.05$. (From Murlas et al., 1990b.)

muscle responsiveness are dependent on a variety of factors, including the particular oxidant(s) generated, the route and degree of exposure, and the species and organ tissue studied, especially as they relate to products of arachidonic acid metabolism that may be elaborated.

E. Products of Lung Arachidonic Acid Metabolism

Products of lung arachidonic acid metabolism may also be linked to the early-onset increase in airway reactivity that has been observed after airway injury. Studies of bronchi and lung mast cells in vitro have demonstrated the capacity of these tissues, independently of factors that may circulate in vivo, to elaborate spasmogens that may augment airway reactivity (Adams and Lichtenstein, 1979; 1985; Schulman et al., 1980, 1982).

We have speculated that the acute bronchial hyperreactivity occurring during the early phase of ozone lung injury may be due, in part, to lipid me-

Table 1 Disappearance of Substance P from Guinea Pig Tracheal Segment Perfusates

Condition	% Disappearance
Control (no inhibitor present)	89.2 ± 0.7
HOCl (0.1 μM)	49.2 ± 8.2[a]
Phosphoramidon (1 μM)	66.9 ± 9.0[a]

100 mL aliquots from guinea pig tracheal segment perfusates containing 5 μM substance P (in 2 mL Kreb's solution) were analyzed. Values shown are means ± SE mean of four experiments.

[a]Value significantly different from control value ($p < 0.05$).

Source: Murlas et al., 1990b.

diators, derived from arachidonic acid metabolism, that are generated upon oxidant injury to normal lung cell constituents, including respiratory mucosa cells (Murlas and Roum, 1985b). It had been previously shown that arachidonic acid is substantially elevated in endobronchial washings after ozone exposure (Shimasaki et al., 1979). This arachidonic acid may be derived from cell membrane phospholipids and be rapidly oxygenated by both the cyclooxygenase and lipoxygenase pathways. Certain matabolites of arachidonic acid are potent bronchoconstrictors in humans (Smith et al., 1985) and in guinea pigs (Hamel et al., 1982), whereas arachidonic acid itself may only weakly affect airway smooth-muscle tone. In guinea pig airways, the effects of arachidonic acid metabolites in vivo (Leitch et al., 1983), as well as of histamine in vitro (Adcock et al., 1980), may be augmented by pretreatment with indomethacin, an inhibitor of the cyclooxygenase pathway of arachidonic acid metabolism. Potentiation of arachidonate-induced airway muscle contraction by indomethacin is abolished by BW 755C, a potent inhibitor of both the cyclooxygenase and lipoxygenase pathways (Mitchell, 1985). Indomethacin potentiates, rather than inhibits, bronchial reactivity in guinea pigs after an ozone exposure (1.5 ppm, 2h) that, by itself, does not affect reactivity to intravenous ACh (Murlas et al., 1986b). Interestingly, indomethacin is also linked to increased lung damage and mortality after hyperoxic lung injury (Smith et al., 1988). The mechanism of indomethacin's potentiation of these disorders is unclear. If its action is mediated by cyclooxygenase inhibition, either a decrease in PGE_2 or an increase in lipoxygenase product generation could result. In either event, airway muscle tone could be augmented.

Cyclooxygenase Products

Airway tissue from various species, including humans, releases several cyclooxygenase products of arachidonic acid metabolism that are bronchoactive. In the dog, it has been shown that the airway mucosa generates PGE_2 (Walters et al., 1984). Airway muscle preparations devoid of airway mucosa also produce prostaglandins, predominantly PGE_2 (Orehek et al., 1975; Gryglewski, et al., 1976; Yamaguchi et al., 1976; Adkinson et al., 1980; Anderson et al., 1980). The spontaneous release of PGE_2 is increased by contraction induced by a variety of different mediators.

Most studies concerning the effect of PGE_2 on airways have focused on its postjunctional, inhibitory action on the airways (Farmer et al., 1974; Grodzinska et al., 1975; Gardiner and Collier, 1980). However, PGE_2 also inhibits neuronal norepinephrine release (Brody and Kadowitz, 1974; Hedqvist, 1977; Malik, 1978). In addition, removal of the respiratory mucosa causes airway muscle hyperresponsiveness to ACh, histamine, and electrical field stimulation that is augmented by indomethacin pretreatment. Responses to the latter two stimuli are substantially mediated by presynaptic ACh release (Murlas, 1986). Indomethacin augmentation of histamine-induced and electrical field stimulation-induced responsiveness is greater in denuded airway preparations. The hyperresponsiveness to histamine in denuded airways is reduced by atropine (Fig. 11). Indomethacin augmentation of field stimulation-induced smooth muscle responsiveness relates to its inhibition of PGE_2 production.

Ozone increases airway mucosal cyclooxygenase product release in vitro. A primary product appears to be PGE_2 (Leikauf et al., 1988). It is conceivable, therefore, that oxidant-induced airway hyperreactivity may derive from ozone-induced depletion of PGE_2 derived from the epithelium. It is less certain whether ozone-induced airway hyperreactivity also relates to the production of thromboxane A_2 (TXA_2) (Aizawa et al., 1985) or to 6-keto-$PGF_{2\alpha}$. Data implicating TXA_2 derives from studies in dogs pretreated with OKY 046, a relatively nonspecific TXA_2 antagonist, which is reported to increase the production of PGE_2 and PGI_2 in vivo (Fitzgerald et al., 1983) and in vitro (O'Keefe et al., 1985; Nicosia and Patrono, 1989). Thus, the possible involvement of TXA_2 remains to be established by studies in which more specific agents or direct measurement of TXA_2 is included. Skepticism concerning 6-keto-$PGF_{1\alpha}$ relates, in part, to its uncertain pathophysiological role in a possibly related disorder, that produced by hyperoxia. Although indomethacin pretreatment lowers the increase in 6-keto-$PGF_{1\alpha}$ in bronchoalveolar lavage fluid after hyperoxia, both mortality and severity of lung damage induced by 100% oxygen increase dramatically after indomethacin administration (Smith et al., 1988).

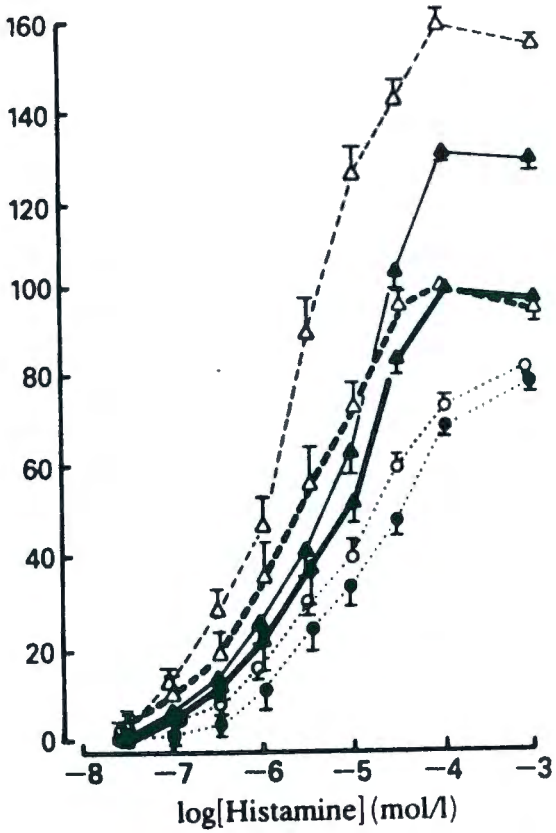


Figure 11 Histamine concentration-response curves of guinea pig denuded (open triangles, dotted line) or intact tracheal rings (solid triangles, solid line). Responses are given as percentage of maximal control response (i.e., maximal contraction developed by intact or denuded rings without indomethacin pretreatment). Each symbol represents the mean ± SE mean of 12 experiments. Maximal responses (nM/mm²) of intact and denuded preparations to histamine were 17.4 ± 2.0 and 16.2 ± 2.1, respectively. The effects of atropine pretreatment on histamine responsiveness of intact (solid circles) or denuded rings (open circles) are indicated by dotted curves. Also shown are effects of indomethacin pretreatment on sensitivity of denuded (open triangles, dotted line) or intact rings (solid triangles, solid line) to histamine. (From Murlas, 1986).

Lipoxygenase Products

To explore the possible role of lipoxygenase products on airway reactivity after injury, Lee and Murlas (1985) evaluated bronchomotor tone before and after ozone exposure in animals treated with indomethacin, BW 755C, or FPL 55712, an antagonist of slow-reacting substance of anaphylaxis (SRS-A). A 15-min exposure to 3.0 ppm of ozone produced a degree of hyperreactivity 30 min after ozone exposure that was comparable with the degree occurring 120 min after a 2-h exposure to the same concentration. Indomethacin treatment did not affect ozone-induced hyperreactivity, although specific airway resistance after exposure increased substantially more than it did in untreated animals. In contrast, animals treated with BW 755C or FPL 55712 did not develop hyperreactivity after ozone exposure (Fig. 12). Also, specific airway resistance was not affected. These results suggest that metabolites of arachidonic acid, other than cyclooxygenase products, may be involved in the pathogenesis of ozone-induced bronchal hyperreactivity in the guinea pig.

It is curious that in dogs, O'Byrne and colleagues (1984) found that orally administered indomethacin (1 mg/kg twice a day for 4 days before ozone exposure) inhibited ozone-induced bronchial hyperreactivity to inhaled ACh. Differences in the results may relate to differences in species or the presence of airway disease before and after ozone-exposure. Although much remains to be learned, it is becoming clear that different products of lung arachidonic acid metabolism may predominate in dogs in health or disease, compared with guinea pigs or humans. Such differences may be very important in relating experimental studies to clinical situations in humans. Similarly, the presence of air way disease may substantially influence the determination of reactivity status when challenge testing is done by aerosolization.

Both hypersecretion of mucus (Roum and Murlas, 1984) and its decreased tracheal velocity (Abraham et al., 1980) may occur within 2 h after ozone exposure. Indomethacin may augment this effect (Marom et al., 1981). Thus, it is possible that airway mucous hypersecretion and stasis could affect diffusion of an aerosolized bronchoconstrictor and result in apparent hyporeactivity after ozone exposure in indomethacin-treated subjects.

Although the effect of BW 755C on ozone-induced bronchial hyperreactivity has not been otherwise studied in guinea pigs, this drug also inhibits airway hyperreactivity caused by ozone in dogs (Fabbri et al., 1983). This inhibitor also inhibits toluene diisocyanate-induced hyperreactivity in guinea pigs (Gordon et al., 1988). However, BW 755C may be of limited value in dissecting mechanisms, in that it inhibits both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism.

On the basis of these studies and the observation that U-60257, a lipoxygenase inhibitor, abolishes ozone-induced hyperreactivity in the guinea pig

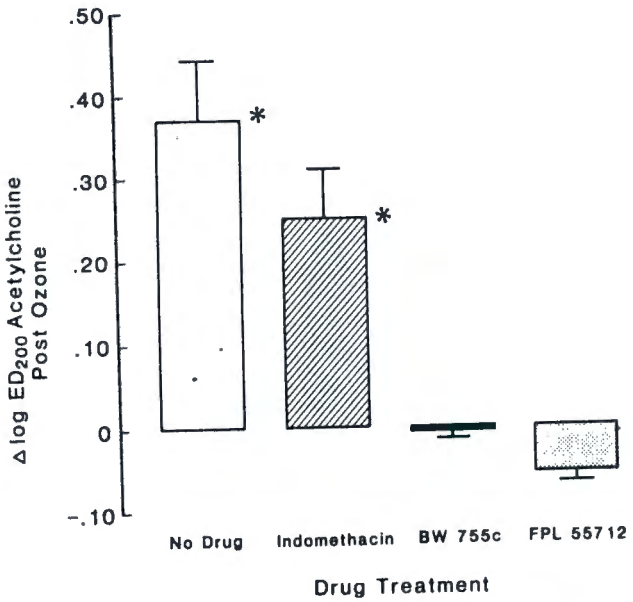


Figure 12 Comparison of magnitude of shift on the ED_{200} for ACh-induced bronchoconstriction after ozone in untreated guinea pigs (open bar) and in those treated with indomethacin, BW 755C, or FPL 55712. The ED_{200} ACh was derived by interpolation from dose-response curves. The differences (Δlog) in log values of ED_{200} ACh were calculated as (log ED_{200} ACh preozone − log ED_{200} ACh postozone). Values represent mean ± SE mean for each group (n = 5, except for untreated, where n = 10). Values with asterisk are different from the preozone values of that group ($p < 0.01$). Note that the degrees of ozone-induced hyperreactivity in the untreated and indomethacin-treated groups are similar. In contrast, BW 755C or FPL 55712 abolished the O_3-induced airway hyperreactivity. (From Lee and Murlas, 1985).

(Murlas and Lee, 1985), it seems reasonable to speculate that the bronchial hyperreactivity occurring within minutes of ozone exposure may be linked to the generation of lipoxygenase products. Arachidonic acid is substantially elevated in endobronchial washings after ozone exposures that are not associated with extensive cellular damage. Whereas arachidonic acid per se may only weakly affect airway smooth-muscle tone, the effects of some of its lipooxygenase products are considerable (Sirois et al., 1981). Thus, acute ozone-induced airway hyperreactivity may be mediated, in part, by lipoxygenase products, possibly leukotrienes (LT) (Murlas and Lee, 1985) derived from arachidonic acid by cells in the lung other than neutrophils. Having recently identified the relationship between an early and progressive increase in LTD_4 and the severity of hyperoxic lung damage, other investigators have

come to similar conclusions (Smith et al., 1988). After ozone injury, it is possible that lung cells may have a lower threshold for stimulation or may generate greater quantities of lipoxygenase products. The number of specific mediators involved in this process and their sources are currently unknown. However, evidence from studies of acrolein-induced airway disease (Leikauf et al., 1989a) corroborates the suspicion that LTC_4 and LTD_4 are key mediators in the pathogenesis of acute airway hyperreactivity. In guinea pigs, acrolein exposure leads to bronchial hyperreactivity and increased immunoreactive LTC_4 in bronchoalveolar lavage fluid.

VI. Cellular Mechanisms of Airway Muscle Hyperresponsiveness

It is likely that the airway cellular events leading to acute hyperreactivity after environmental injury involve an increase in airway neuromuscular responsiveness and, possibly, edema formation, but not tissue inflammatory cell infiltration, although this may, indeed, prolong the disorder. Initial environmental injury of the respiratory mucosa is likely to cause the release of certain mediators—including substance P from airway afferent nerve endings—that elicit bronchoconstriction, in part, by stimulating ACh release from airway postganglionic efferent fibers (Lundberg and Saria, 1983; Lundberg et al., 1983; 1984). The inactivation of airway mucosal NEP by such injury would amplify these effects.

In general, augmentation of airway neuromuscular responsiveness may occur in two ways: (1) prejunctionally, by modulating the degradation or release of (a) bronchoconstrictor mediators, such as PGD_2, or of endogenous neurotransmitters, such as ACh and substance P, and/or (b) of bronchodilator mediators, such as PGE_2; and (2) postjunctionally, by increasing the responsiveness of airway smooth muscle itself upon stimulation.

The effects of these mediators on the plasma membranes of excitable cells of the airways may be mediated by (1) voltage-dependent mechanisms and (2) voltage-independent events. Most, if not all, bronchoconstrictors elicit spasm, at least in part, by cell membrane depolarization. A primary mechanism by which extracellular K^+ elicits smooth-muscle spasm is by membrane depolarization. The effects of increasing extracellular K^+ on ferret airway muscle responsiveness to electrical field stimulation or exogenous ACh have been evaluated (Murlas et al., 1986a). In this study, changes in force and cell membrane potential were measured simultaneously in muscle preparations devoid of mucosa and submucosa (Fig. 13). This research may be of particular relevance to the hyperreactivity occurring after airway injury, in that damaged airway cells may leak K^+ into the microenvironment

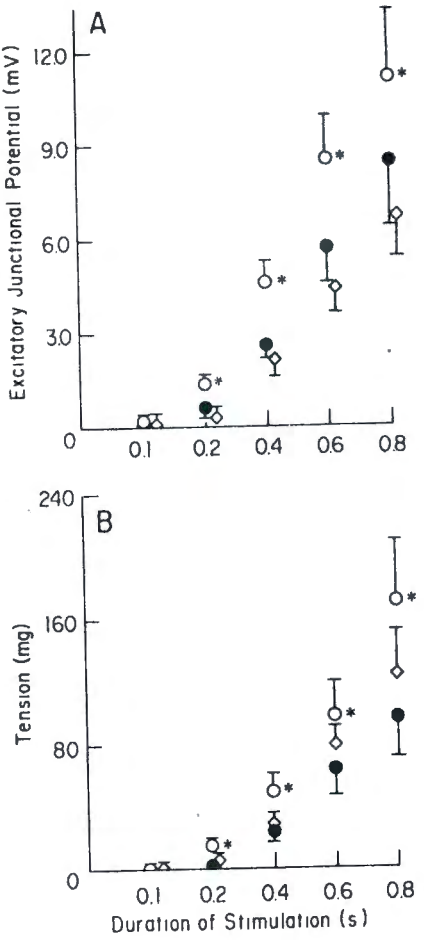


Figure 13 Effect of various extracellular K^+ concentrations on simultaneously recorded excitatory junctional potential and tension produced in ferret midtracheal smooth muscle by increasing durations of electrical field stimulation. Symbols represent 6 (solid circles), 12 (open circles), and 18 mM K^+ (open diamonds). Responses are given in absolute terms. Each symbol represents mean ± SE mean. Asterisk indicates value significantly different from response to same duration of stimulation in 6 mM K^+ ($p < 0.05$). K^+ at 12 mM clearly potentiated both electrical and mechanical responses to field stimulation. (From Lee and Murlas, 1985).

of airway nerve and smooth-muscle cells. These results, which were corroborated by parallel studies of airway preparations having [^{3}H]ACh-loaded nerve terminals, strongly suggest that elevations in extracellular K^+ from 6 to 12 mM augment responsiveness of the airways by increasing the release of endogenous ACh (Fig. 14).

In comparison with K^+ and PGE_2, LTD_4 appears to have prominent postsynaptic effects on the muscarinic sensitivity of airway smooth muscle (Lee and Murlas, 1989). Leukotriene-induced potentiation of the muscarinic responsiveness of airway muscle appears to involve both voltage-dependent and voltage-independent mechanisms (Fig. 15). The voltage-dependency is suggested by the potentiation of ACh-induced membrane de-

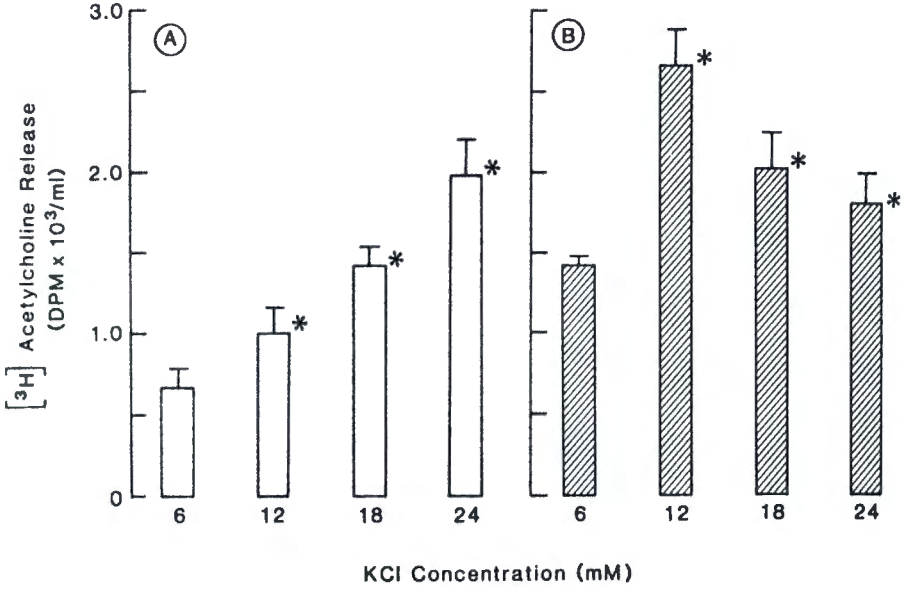


Figure 14 Effect of various extracellular K^+ concentrations on release of tritiated acetylocholine ([^{3}H]ACh) from ferret midtracheal preparations at rest (A, open bars) or during electrical field stimulation (EFS) for a 5-min period from a 20 V source at 15 Hz (B, hatched bars). Each bar represents mean ± SE mean of six experiments. Measurements of [^{3}H]ACh release were made both at rest and during EFS. Asterisk indicates value significantly different from release measured in 6 mM K^+ either at rest or during EFS. Despite there being a progressive increase in spontaneous [^{3}H]ACh release in higher extracellular K^+ concentrations, release evoked by EFS was maximal in 12 mM K^+. (From Murlas et al., 1986a.)

polarization caused by LTD_4. The LTD_4-induced depolarization may be sufficient to reduce the threshold for the opening of voltage-sensitive, slow Ca^{2+} channels, or to increase the number of channels available for activation upon stimulation by ACh. Alternatively, primary effects on cell membrane conductance(s) for Ca^{2+}, Na^+, or K^+ may be caused by LTD_4, independently of its effects on resting membrane potential. To resolve these questions, electrophysiological studies are currently underway. Leucotriene$_4$

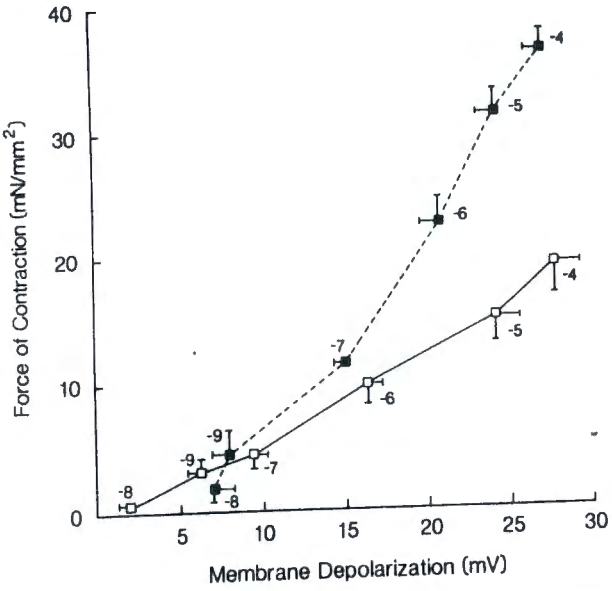


Figure 15 Effects of 10^{-10}FM LTD_4 pretreatment (solid squares, dashed line) on relationship between membrane depolarization and force generation caused by increasing acetylcholine concentrations (log molar) indicated by number over each symbol. Each symbol represents mean value ± SE mean from 12-16 cell impalements of six tissue preparations from six animals. Pretreatment with this subthreshold concentration for 20 min substantially augmented the electromechanical response to ACh, compared with values determined in the absence of LTD_4 (open squares, solid line). (From Lee and Murlas, 1989.)

also appears to have electromechanical effects on airway smooth muscle (Murlas and Doupnik, 1989). In addition, both LTE_4 (Lee et al., 1984) and LTB_4 (Thorpe and Murlas, 1986) appear to potentiate the sensitivity of guinea pig airway smooth muscle to exogenous bronchoconstrictors. The mechanisms by which LTE_4 or $LTCB_4$ potentiate the airway muscle responsiveness have yet to be elucidated.

References

Abraham, W. M., Januszkiewicz, A. J., Mingle, M., Welker, M., Wanner, A., and Sackner, M. A. (1980). Sensitivity of bronchoprovocation and tracheal mucous velocity in detecting airway response to O_3. *J. Appl. Physiol.*, **48**:789-793.

Adams, G. K., and Lichtenstein, L. M. (1985). Indomethacin enhances response of human bronchi to antigen. *Am. Rev. Respir. Dis.* **131**:8-10.

Adams, G. K., and Lichtenstein, L. M. (1979). In vitro studies of antigen-induced bronchospasm: effect of antihistamine and SRS-A antagonist on response of sensitized guinea pig and human airways to antigen. *J. Immunol.* **122**:555-562.

Adcock, J. J., and Garland, L. G. (1980). A possible role of lipoxygenase products as regulators of airway smooth muscle reactivity. *Br. J. Pharmacol.* **69**:167-169.

Adkinson, N. F., Newball, H. H., Findley, S., Adams, K., and Lichtenstein, L. M. (1980). Anaphylactic release of prostaglandins from human lung in vitro. *Am. Rev. Respir. Dis.* **121**:911-920.

Aizawa, H., Chung, K. F., Leikauf, G. D., Ueki, I., Bethel, R. A., O'Byrne, P. M., Hirose, T., and Nadel, J. A. (1985). Significance of thromboxane generation in ozone-induced airway hyperresponsiveness in dogs. *J. Appl. Physiol.* **59**:1918-1923.

Anderson, W. H., Krzanowski, J. J., Polson, J. B. B., and Szentivanyi, A. (1980). Prostaglandins as mediators of tachyphylaxis to histamine in canine tracheal smooth muscle. *Adv. Prostaglandin Thromboxane Res.* **7**:995-1001.

Barbet, J. P., Chauveau, M., Labbe, S., and Lockhart, A. (1988). Breathing dry air causes acute epithelial damage and inflammation of the guinea pig trachea. *J. Appl. Physiol.* **64**:1851-1857.

Bils, R. F. (1974). Effects of NO_2 and ozone on monkey lung ultrastructure. *Pneumonologie* **150**:99-111.

Bils, R. F., and Christie, B. R. (1980). The experimental pathology of oxidant and air pollutant inhalation. *Int. Rev. Exp. Pathol.* **21**:195-293.

Boatman, E. S., Sato, S., and Frank, R. (1974). Acute effects of ozone on cat lungs. II. Structural. *Am. Rev. Respir. Dis.* **110**:157-169.

Brody, M. J., and Kadowitz, P. J. (1974). Prostaglandins as modulators of the autonomic nervous system. *Fed. Proc.* **33**:48-60.

Burke, T. M., and Wolin, M. S. (1987). Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. *Am. J. Physiol.* **252**:H721-732.

Butler, G. B., Adler, K. B., Evans, J. N., Morgan, D. W., and Szarek, J. L. (1987). Modulation of rabbit airway smooth muscle responsiveness by respiratory epithelium. *Am. Rev. Respir. Dis.* **135**:1099-1104.

Chen, S. E., and Bernstein, I. L. (1982). The guinea pig model of diisocyanate sensitization: immunologic studies. *J. Allergy Clin. Immunol.* **70**:383.

Chester, E. H., Martinez-Catinchi, F. L., Schwartz, H. J., Flemming, G. M., and McDonald, E. W. (1979). Patterns of airway reactivity to asthma produced by exposure to toluene diisocyanate. *Chest* **75**:229.

Cibulas, W., Murlas, C. G., Miller, M. L., McKay, R. T., and Brooks, S. (1988). Toluene diisocyanate-induced airway hyperreactivity occurs in guinea pigs depleted of granulocytes. *J. Appl. Physiol.* **64**:1773-1778.

Cibulas, W., Murlas, C. G., Miller, M., Vinegar, A., Schmidt, D., McKay, R. T., and Brooks, S. M. (1986). Toluene diisocyanate-induced airway hyperreactivity and pathology in the guinea pig. *J. Allergy Clin. Immunol.* **77**:828-834.

Dunnill, M. S., Massarella, G. R., and Anderson, J. (1969). A comparison of the quantitative anatomy of the bronchi in normal subjects, in status asthmaticus, in chronic bronchitis emphysema. *Thorax* **24**:176-179.

Easton, R. E., and Murphy, S. D. (1976). Experimental ozone preexposure and histamine: effect on the acute toxicity and respiratory function effects of histamine in guinea pigs. *Arch. Environ. Health* **15**:160-166.

Elwood, R. K., Kennedy, S., Belzberg, A., Hogg, J. C., and Pare, P. D (1983). Respiratory mucosal permeability in asthma. *Am. Rev. Respir Dis.* **128**:523-527.

Erdös, E. G., and Skidgel, R. A. (1989). Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *FASEB J.* **3**:145-151

Evans, T. W., Brokaw, J. J., Chung, K. F., Nadel, J. A., and McDonald D. M. (1988). Ozone-induced bronchial hyperresponsiveness in the ra is not accompanied by neutrophil influx or increased vascular permeability in the trachea. *Am. Rev. Respir. Dis.* **138**:140-144.

Fabbri, L. M., Aizawa, H., O'Byrne, P. M., Walters, E. H., Holtzman, M. J., and Nadel, J. A. (1983). BW 755C inhibits airway hyperresponsiveness induced by ozone in dogs. *Physiologist* **26**:A35.

Farmer, J. B., Farrar, D. G., and Wilson, J. (1974). Antagonism of tone and prostaglandin-mediated responses in a tracheal preparation by indomethacin and SC-19220. *Br. J. Pharmacol.* **52**:559-565.

Farmer, S. G., Fedan, J. S., Hay, D. W. P., and Raeburn, D. (1986). The effects of epithelium removal on the sensitivity of guinea-pig isolated trachealis to bronchodilator drugs. *Br. J. Pharmacol.* **89**:407-414.

Fitzgerald, G. A., Brash, A. R., Oates, J. A., and Pedersen, A. K. (1983). Endogenous prostacyclin biosynthesis and platelet function during selective inhibition of thromboxane synthetase in man. *J. Clin. Invest.* **71**:1336-1343.

Flavahan, N. A., Aarhus, L. L., Rimele, T. J., and Vanhoutte, P. M. (1985). Respiratory epithelium inhibits bronchial smooth muscle tone. *J. Appl. Physiol.* **58**:834-838.

Gardiner, P. J., and Collier, H. J. (1980). Specific receptors for prostaglandins in airways. *Prostaglandins* **19**:819-840.

Goldie, R. G., Papadimitriou, J. M., Paterson, J. W., Rigby, P. J., Self, H. M., and Spina, D. (1986). Influence of the epithelium on responsiveness of guinea-pig isolated trachea to contractile and relaxant agonists. *Br. J. Pharmacol.* **87**:5-14.

Gordon, T., Thompson, J. E., and Sheppard, D. (1988). Arachidonic acid metabolites do not mediate toluene diisocyanate-induced airway hyperresponsiveness in guinea pigs. *Prostaglandins* **35**:699-706.

Grodzinska, L., Panczenko, B., and Gryglewski, R. J. (1975). Generation of prostaglandin E-like material by the guinea pig trachea contracted by histamine. *J. Pharm. Pharmacol.* **27**:88-91.

Gryglewski, R. J., Dembinska-Kiec, A., Grodzinska, L., and Panczenko, B. (1976). Differential generation of substances with prostaglandin-like and thromboxane-like activities by guinea pig trachea and lung strips. In *Lung Cells in Disease*. Edited by A. Bouhuys. Elsevier/North Holland, Amsterdam, pp. 289-307.

Hamel, R., Masson, P., Ford-Hutchinson, A. W., Jones, T. R., Brunet, G., and Piechuta, H. (1982). Differing mechanisms for leukotriene D_4 induced bronchoconstriction in guinea pigs following intravenous and aerosol administration. *Prostaglandins* **24**:419-432.

Hay, D. W. P., Farmer, S. G., Raeburn, D., Robinson, V. A., Fleming, W. W., and Fedan, J. S. (1986). Airway epithelium modulates the reactivity of guinea-pig respiratory smooth muscle. *Eur. J. Pharmacol.* **129**:11-18.

Hedqvist, P. (1977). Basic mechanisms of prostaglandin action on autonomic neurotransmission. *Annu. Rev. Pharmacol. Toxicol.* **17**:259-279.

Hinson, J. M., Jr., Hutchison, A. A., Brigham, K. L., Meyrick, B. O., and Snapper, J. R. (1984). Effects of granulocyte depletion on pulmonary responsiveness to aerosol histamine. *Med. Clin. North Am.* **65**:411-417.

Hulbert, W. M., McLean, T., and Hogg, J. C. (1985). The effect of acute airway inflammation on bronchial reactivity in guinea pigs. *Am. Rev. Respir. Dis.* **132**:7-11.

Hulbert, W. C., Walker, D. C., Jackson, A., and Hogg, J. C. (1981). Airway permeability to horseradish peroxidase in guinea pigs: the repair phase after injury by cigarette smoke. *Am. Rev. Respir. Dis.* **123**:320-326.

Jackson, D. M., and Eady, R. P. (1988). Acute transient SO_2-induced airway hyperreactivity. *J. Appl. Physiol.* **65**:1119-1124.

Jacoby, D. B., Tamaoki, J., Borson, D. B., and Nadel, J. A. (1988). Influenza infection causes airway hyperresponsiveness by decreasing enkephalinase. *J. Appl. Physiol.* **64**:2653-2658.

James, A. L., Pare, P. D., and Hogg, J. C. (1989). The mechanics of airway narrowing in asthma. *Am. Rev. Respir. Dis.* **139**:242-246.

Johnson, A. R., Ashton, J., Schultz, W. W., and Erdös, E. G. (1985). Neutral metalloendopeptidase in human lung tissue and cultured cells. *Am. Rev. Respir. Dis.* **132**:564-568.

Jones, G. L., Lane, C. G., Daniel, E. E., and O'Byrne, P. M. (1988a). Release of epithelium-derived relaxing factor after ozone inhalation in dogs. *J. Appl. Physiol.* **65**:1238-1243.

Jones, G. L., O'Byrne, P. M., Pashley, M., Serio, R., Jury, J., Lane, C. G., and Daniel, E. E. (1988b). Airway smooth muscle hyperresponsiveness to acetylcholine in vitro from dogs with airway hyperresponsiveness in vivo. *J. Appl. Physiol.* **65**:57-64.

Kohrogi, H., Nadel, J. A., Malfroy, B., Gorman, C., Bridenbaugh, B., Patten, J. S., and Borson, D. B. (1989). Recombinant human enkephalinase prevents cough induced by tachykinins in awake guinea pigs. *J. Clin. Invest* **84**:781-786.

Kuwada, M., and Katayma, K. (1984). Differentiation of endopeptidases and aminopeptidases by high-performance liquid chromatography of reaction products from chromogenic peptide *p*-nitroanilines as substrate. *Anal. Biochem.* **139**:438-443.

Laitinen, L. A., Heino, M., Laitinen, A., Kava, T., and Haahtela, T. (1985). Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am. Rev. Respir. Dis.* **131**:599-606.

Lang, Z., Soskel, N., Williams, G., and Murlas, C. G. (1989). Identification

of metalloendopeptidase activity in bovine airway mucosal cells and its inhibition by hypochlorous acid. *Am. Rev. Respir. Dis.* **139**:A236.

Lee, H. K., and Murlas, C. G. (1985). Ozone-induced bronchial hyperreactivity is abolished by BW 755C or FPL 55712 but not indomethacin. *Am. Rev. Respir. Dis.* **132**:1005-1009.

Lee, H. K., and Murlas, C. G. (1989). Electromechanical effects of leukotriene D_4 on ferret tracheal muscle and its muscarinic responsiveness. *Lung* **167**:173-185.

Lee, T. H., Austen, K. F., Corey, E. J., and Drazen, J. M. (1984). Leukotriene E_4-induced airway hyperresponsiveness of guinea pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors. *Proc. Natl. Acad. Sci. USA* **81**:4922-4925.

Leikauf, G. D., Doupnik, C. A., Leming, L. M., and Wey, H. E. (1989a). Sulfopeptide leukotrienes mediate acrolein-induced bronchial hyperresponsiveness in guinea pigs. *J. Appl. Physiol.* **66**:1838-1845.

Leikauf, G. D., Driscoll, K. E., and Wey, H. E. (1988). Ozone-induced augmentation of eicosanoid metabolism in epithelial cells from bovine trachea. *Am. Rev. Respir. Dis.* **137**:435-442.

Leikauf, G. D., Leming, L. M., O'Donnell, J. R., and Doupnik, C. A. (1989b). Bronchial responsiveness and inflammation in guinea pigs exposed to acrolein. *J. Appl. Physiol.* **66**:171-178.

Leitch, A. G., Austen, K. F., and Corey, E. J. (1983). Effects of indomethacin on the guinea pig pulmonary response to intravenous leukotrienes C_4 and D_4. *Clin. Sci.* **65**:281-287.

Lewis, G. P. (1983). Immunoregulatory activity of metabolites of arachidonic acid and their role in inflammation. *Br. Med. Bull.* **39**:243-248.

Lundberg, J. M., Lundblad, L., Saria, A., and Anggard, A. (1984). Inhibition of cigarette smoke-induced edema in the nasal mucosa by capsaicin pretreatment and a substance P antagonist. *Naunyn Schmiedebergs Arch. Pharmacol.* **326**:181-185.

Lundberg, J. M., Martling, C. R., Saria, A., Folkers, K., and Rosell, S. (1983). Cigarette smoke-induced airway edema due to activation of capsaicin-sensitive vagal afferents and substance P release. *Neuroscience* **10**: 1361-1368.

Lundberg, J. M., and Saria, A. (1983). Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature* **302**:251-253.

Malik, K. U. (1978). Prostaglandins-modulations of adrenergic nervous system. *Fed. Proc.* **37**:204-207.

Marom, Z., Shelhamer, J. H., and Kaliner, M. (1981). Effects of arachidonic

acid and prostaglandins on the release of mucous glycoproteins from human airways in vitro. *J. Clin. Invest.* **67**:1695-1702.

Metzger, W. J., Zavala, D., Richerson, H. B., Moseley, P., Iwamota, P., Monick, M., Sjoerdsma, K., and Hunninghake, G. W. (1987). Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. *Am. Rev. Respir. Dis.* **135**:433-440.

Miller, M. A., Andringa, A., Vinegar, A., Adams, W., Cibulas, W., and Brooks, S. M. (1986). Morphology of tracheal and bronchial epithelium and type II cells of the peripheral lung of the guinea pig after inhalation of toluene diisocyanate. *Exp. Lung Res.* **11**:145-163.

Mitchell, H. W. (1982). Effect of ETYA and BW 755C on arachidonate-induced contractions in the guinea pig isolated trachea. *Br. J. Pharmacol.* **76**:527-529.

Moller, D. R., Baughman, R. P., Murlas, C. G., and Brooks, S. M. (1986). New directions in occupational asthma to small molecular weight compounds. *Semin. Respir. Med.* **7**:225-240.

Murlas, C. G. (1986). Effects of mucosal removal on guinea pig airway smooth muscle responsiveness. *Clin. Sci.* **70**:571-575.

Murlas, C. G., and Doupnik, C. A. (1989). Electromechanical coupling of ferret airway smooth muscle in response to leukotriene C_4. *J. Appl. Physiol.* **66**: 2533-2538.

Murlas, C. G., Ehring, G. E., Suszkiw, J., and Sperelakis, N. (1986a). K^+-induced alterations in airway muscle responsiveness to electrical field stimulation. *J. Appl. Physiol.* **61**:61-67.

Murlas, C. G., and Lee, H. K. (1985). U-60257 prevents ozone-induced bronchial hyperreactivity in the guinea pig. *Prostaglandins* **30**:563-572.

Murlas, C. G., Lee, H. K., and Roum, J. H. (1986b). Indomethacin increases muscarinic bronchial reactivity after exposure to subthreshold ozone levels. *Prostaglandins Leukotrienes Med.* **21**259-268.

Murlas, C. G., Murphy, T., and Chodimella, V. (1990a). O_3-induced, mucosa-linked airway muscle hyperresponsiveness in the guinea pig. *J. Appl. Physiol.* **69**:7-13.

Murlas, C. G., Murphy, T. P., and Lang, Z. (1990b). HOCl causes airway substance P hyperresponsiveness and neutral endopeptidase hypoactivity. *Am. J. Physiol.* **258**:L361-368.

Murlas, C. G., and Roum, J. R. (1985a). Bronchial hyperreactivity occurs in steroid-treated guinea pigs depleted of leukocytes by cyclophosphamide. *J. Appl. Physiol.* **58**:1630-1637.

Murlas, C. G., and Roum, J. R. (1985b). Sequence of pathologic changes in

the airway mucosa of guinea pigs during ozone-induced bronchial hyperreactivity. *Am. Rev. Respir. Dis.* **131**:314-320.

Murlas, C. G., Williams, G., Lang, Z., and Chodimella, V. (1990c). Aerosolized neutral endopeptidase reverses the increased airway reactivity to substance P caused by ozone. *Am. Rev. Respir. Dis.* **141**:A734.

Nasr, A. N., Dinman, B. D., and Bernstein, I. A. (1971). An experimental approach to study the toxicity of nonparticulate air pollutants. *Arch. Environ. Health* **22**:538-544.

Nicosia, S., and Patrono, C. (1989). Eicosanoid biosynthesis and action: novel opportunities for pharmacological intervention. *FASEB J.* **3**:1941-1948.

O'Byrne, P., Walters, E. H., Gold, B. D., et al. (1984a). Indomethacin inhibits airway responsiveness but not the neutrophil influx induced by ozone in dogs. *Am. Rev. Respir. Dis.* **130**:220-224.

O'Byrne, P., Walters, E. H., Gold, B. D., Aizawa, H. A., Fabbri, L. M., Alpert, S. E., Nadel, J. A., and Holtzman, M. J. (1984b). Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. *Am. Rev. Respir. Dis.* **130**:214-219.

O'Keefe, E. H., Liu, E. C. K., Greenberg, R., and Ogletree, M. L. (1985). Effects of a thromboxane synthetase inhibitor and a thromboxane antagonist on release and activity of thromboxane A_2 and prostacyclin in vitro. *Prostaglandins* **25**:785-797.

Orehek, J., Douglas, J. S., nd Bouhuys, A. (1975). Contractile response of the guinea pig trachea in vitro: modification by prostaglandin synthesis-inhibiting drugs. *J. Pharmacol. Exp. Ther.* **194**:554-564.

Orehek, J., Douglas, J. S., Lewis, A. J., and Bouhuys, A. (1973). Prostaglandin regulation of airway smooth muscle tone. *Nature* **245**:84-85.

Pennington, J. E. (1977). Bronchoalveolar cell response to bacterial challenge in the immunosuppressed lung. *Am. Rev. Respir. Dis.* **116**:885-893.

Pennington, J. E. (1978). Differential effects of cyclophosphamide and cortisone acetate on bronchoalveolar phagocytic cell populations. *Am. Rev. Respir. Dis.* **118**:319-324.

Pennington, J. E., Cole, F. S., and Boerth, L. W. (1985). Intrapulmonary chemotoxins in the normal and in the cyclophosphamide-treated host. *Am. Rev. Respir. Dis.* **131**:845-849.

Pennington, J. E., and Ehrie, M. G. (1978). Pathogenesis of *Pseudomonas aeruginosa* pneumonia during immunosuppression. *J. Infect. Dis.* **137**: 764-774.

Plopper, C. G., Dungworth, D. L., and Tyler, W. S. (1973). Pulmonary lesions in rats exposed to ozone: a correlated light and electron microscope study. *Am. J. Pathol.* **71**:375-394.

Raeburn, D. Hay, D. W. P., Farmer, S. G., and Fedan, J. S. (1986). Epithelium removal increases the reactivity of human isolated tracheal muscle to methacholine and reduces the effect of verapamil. *Eur. J. Pharmacol.* **123**:451-453.

Rhoades, R., Packer, C. S., and Meiss, R. A. (1987). Pulmonary vascular smooth muscle contractility—effect of free radicals. *Chest* **91**:531-532.

Roum, J. R., and Murlas, C. G. (1984). Ozone-induced changes in muscarinic bronchial hyperreactivity by different testing methods. *J. Appl. Physiol.* **57**:1783-1789.

Rubanyi, G. M., and Vanhoutte, P. M. (1986). Oxygen-derived free radicals, endothelium, and responsiveness of vascular smooth muscle. *Am. J. Physiol.* **250**:H815-821.

Salvaggio, J. E. (1982). Overview of occupational immunologic lung disease. *J. Allergy Clin. Immunol.* **70**:5-16.

Scheel, L. D., Dobrogorski, O. J., Mountain, J. R., Svirbely, J. L., and Stockinger, H. E. (1959). Physiologic, biochemical, immunologic and pathologic changes following ozone exposure. *J. Appl. Physiol.* **14**:67-80.

Schulman, E. S., Adkinson, N. F., Adams, G. K., Lichtenstein, L. M., and Newball, H. H. (1980). Anaphylactic release of thromboxane A_2 from human bronchi and parenchyma. *J. Allergy Clin. Immunol.* **65**:235.

Schulman, E. S., Adkinson, N. F., Jr., and Newball, H. H. (1982). Cyclooxygenase metabolites in human lung anaphylaxis: airway vs parenchyma. *J. Appl. Physiol.* **53**:589-595.

Sekizawa, K., Tamoiki, J., Graf, P. D., Basbaum, C. B., Borson, D. B., and Nadel, J. A. (1977). Enkephalinase inhibitor potentiates mammalian tachykinin-induced contraction in ferret trachea. *J. Pharmacol. Exp. Ther.* **243**:1211-1217.

Sheppard, D., Thompson, J. E., Scypinski, L., Dusser, D., Nadel, J. A., and Borson, D. B. (1988). Toluene diisocyanate increases airway responsiveness to substance P and decreases airway neutral endopeptidase. *J. Clin. Invest.* **81**:111-115.

Shields, R. L., and Gold, W. M. (1982). Effects of acute ozone exposure on lung histamine concentration. *Am. Rev. Respir. Dis.* **125**(suppl):148.

Shimasaki, H., Takatori, T., Anderson, W. R., Horten, H. L., and Privett, O. S. (1979). Alteration of lung lipids in ozone exposed rats. *Biochem. Biophys. Res. Commun.* **68**:1256-1262.

Sielczak, M. W., Denas, S. M., and Abraham, W. M. (1983). Airway cell changes in tracheal lavage of sheep after ozone exposure. *J. Toxicol. Environ. Health* **11**:545-553.

Smith, L. J., Greengerger, P. A., Patterson, R., Krell, R. D., and Bernstein, P. R. (1985). The effect of inhaled leukotriene D_4 in humans. *Am. Rev. Respir. Dis.* **131**:368-372.

Smith, L. J., Shamsuddin, M., Anderson, J., and Hsueh, W. (1988). Hyperoxic lung damage in mice: appearance and bioconversion of peptide leukotrienes. *J. Appl. Physiol.* **64**:944-951.

Steinberg, D. R., Bernstein, D. I., Bernstein, I. L., and Murlas, C. G. (1989). Prednisone pretreatment leads to histamine airway hyperreactivity soon after resolution of the immediate allergic response. *Chest* **95**:314-319.

Stewart, R. M., Weir, E. K., Montgomery, M. R., and Niewoehner, D. E. (1981). Hydrogen peroxide contracts airway smooth muscle: a possible endogenous mechanism. *Respir. Physiol.* **45**:333-341.

Stimler-Gerard, N. P. (1987). Neutral endopeptidase-like enzyme controls contractile activity of substance P in the guinea pig lung. *J. Clin. Invest.* **79**:1819-1825.

Stuart-Smith, K., and Vanhoutte, P. M. (1988). Airway epithelium modulates the responsiveness of porcine bronchial smooth muscle. *J. Appl. Physiol.* **65**:721-727.

Thompson, J. E., Scypinski, L. A., Gordon, T., and Sheppard, D. (1988). Hydroxyurea inhibits airway hyperresponsiveness in guinea pigs by a granulocyte-independent mechanism. *Am. Rev. Respir. Dis.* **134**:1213-1218.

Thompson, J. E., and Sheppard, D. (1988). Phosphoramidon potentiates the increase in lung resistance mediated by tachykinins in guinea pigs. *Am. Rev. Respir. Dis.* **137**:337-340.

Thorpe, J. E., and Murlas, C. G. (1986). Leukotriene B_4 potentiates airway muscle responsiveness in vivo and in vitro. *Prostaglandins* **31**:899-908.

Thorpe, J. E., Steinberg, D. A., Bernstein, I. L., and Murlas, C. G. (1987). Bronchial reactivity increases soon after the immediate response in dual responding asthmatics. *Chest* **91**:21-25.

Turner, C., Kleeberger, S. R., and Spannhake, E. W. (1987). Acute increase in mast cell counts are observed after short-term ozone exposure in the canine peripheral lung. *Am. Rev. Respir. Dis.* **135**:A93.

Vanhoutte, P. M. (1987). Airway epithelium and bronchial reactivity. *Can. J. Physiol. Pharmacol.* **65**:448-450.

Walters, E. H., O'Byrne, P. M., Fabbri, L., Graf, P., Holtzman, M. J., and Nadel, J. A. (1984). Control of neurotransmission by prostaglandin in canine tracheal smooth muscle. *J. Appl. Physiol.* **57**:129-134.

Walters, E. H., O'Byrne, P. M., Graf, P. D., Fabbri, L. M., and Nadel, J.

A. (1986). The responsiveness of airway smooth muscle in vitro from dogs with airway hyperresponsiveness in vivo. *Clin. Sci.* **71**:605-611.

Yamaguchi, T., Hitzig, B., and Coburn, R. F. (1976). Endogenous prostaglandins and mechanical tension in canine trachealis muscle. *Am. J. Physiol.* **230**:1737-1743.

Lung Biology in Health and Disease Volume 55

Executive Editor: Claude Lenfant

The Airway Epithelium

Physiology, Pathophysiology, and Pharmacology

edited by

Stephen G. Farmer

Douglas W. P. Hay