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INHALED TOXICANTS AND AIRWAY HYPERRESPONSIVENESS

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In 1986 and 1987, approximately 136 metric tons of particulates, sulfur oxides, nitrogen oxides, volatile organic compounds, carbon monoxide, and lead were released per year into the atmosphere in the United States. Although the amounts of lead and particulate matter released into the atmosphere have substantially decreased since the 1940s, the amounts of nitrogen and sulfur oxides have remained largely unchanged (1) as illustrated in Figure 1A. It is only necessary to read the newspaper reports of smog alerts in Los Angeles and other cities to realize the potential hazards of breathing air pollutants. Some respiratory diseases are increasing in incidence. As shown in Figure 1B, the frequency of death from chronic obstructive pulmonary diseases as a class has increased (1, 2). Particularly disturbing has been the rise in the incidence of deaths due to asthma and pneumonia. The rise in the rate of death due to pulmonary diseases has occurred despite the advances made in the treatment of respiratory ailments. Whether there is a direct causal relationship between the continued presence of pollutants in the atmosphere and the increase in death due to respiratory disease is still a matter of debate. It has been clearly established, however, that air pollution has deleterious effects on respiratory function, particularly in a susceptible population, such as asthmatics. Many people by choice also inhale toxicants in large quantity in the form of tobacco smoke and some drugs of abuse (e.g. cocaine or crack).

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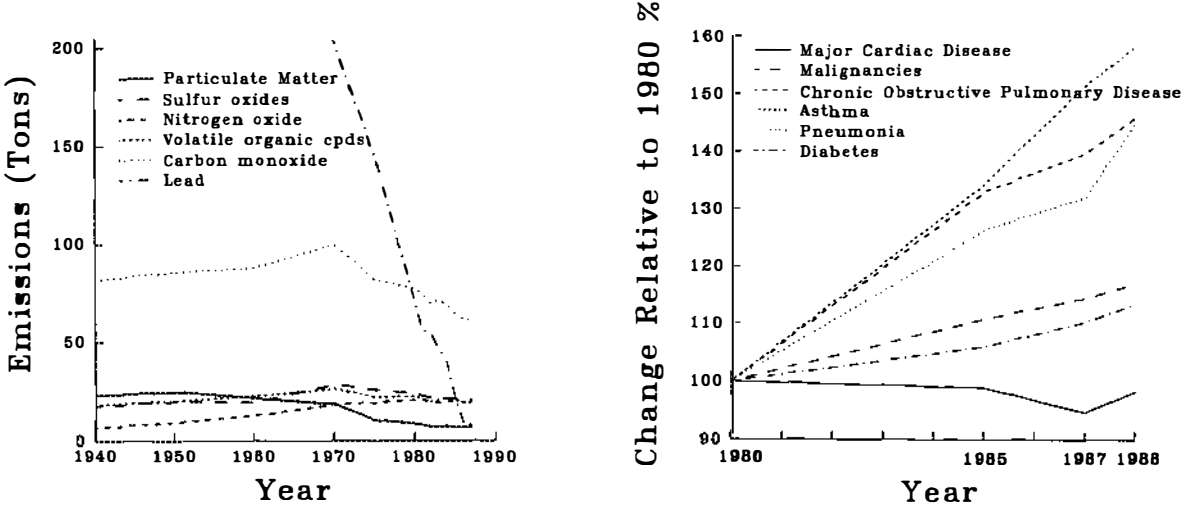

Figure 1 *A.* The number of tons of pollutants put into the atmosphere in the United States from 1940 to 1987.

B. Number of deaths per 100,000 for various diseases normalized to the number of deaths for each of those diseases in 1980. Note the rise in deaths due to pulmonary disease. The deaths due to pulmonary disease, however, represent only 2% of the deaths relative to cardiac disease and malignances. (The data in graph were extracted from Tables 350 and 115, respectively, in US Bureau of the Census, *Statistical Abstracts of the United States: 1990* (110th edition).

This review provides an overview of the effects of inhaled toxicants on the function of the airways. It examines both the acute and chronic actions of toxicants on several tissues within the airway. A brief review of the physiology of the airways is given first. Several excellent reviews and books are available on this subject (3–8). Since the various cell types within the airways affect each other (9), the interaction of the various cells and tissues is discussed. The discussion is limited to only a few toxicants: ozone, cocaine, sulfur oxides, and tobacco smoke. Other reviews deal with the short- and long-term biological effects associated with inhalation of air pollutants (10–18).

PHYSIOLOGY OF THE AIRWAY

Many different cells make up the airways. A schematic of a cross section through the airway wall is shown in Figure 2. The *dotted arrows* in the figure represent identified or proposed interactions between cell types. The tissue types will be broken down into several major groups. Many different types of nerves control the functions within the airways. The parasympathetic system innervates both the submucosal gland cells and the airway smooth muscle (19, 20). This system has ganglia imbedded within the airway wall (21). Sympathetic nonadrenergic, noncholinergic nerves also supply the mucous glands (4, 22). Airway smooth muscle is under the control of the parasympathetic autonomic nervous system and peptidergic nerves (23–27). The airways are lined with a mucosal layer consisting of many cell types, including epithelial and mucus cells, both of which are sources of the secretions that coat the airway lumen. The immune responsive cells, including lymphocytes, mast cells, etc, which can react to antigens and other stimulation, are important in the development of inflammation responses in the airways (28, 29). Obviously, there are many other cell types in the trachea/bronchioles, such as cartilage, blood vessels, and alveoli with Type I and Type II cells, which are not considered.

Innervation

Airway diameter and secretion from the mucosal layer are controlled largely via the autonomic nervous system. Airway smooth muscle in the airways is primarily controlled by parasympathetic nerves, which bring about contraction. Relaxation of the muscle occurs either via blood borne factors (i.e. epinephrine release from the adrenal medulla) or by nonadrenergic innervation (24–27). Airway smooth muscle is not innervated by sympathetic nerves to any great extent (30, 31). Nerve-evoked relaxation occurs via activation of NANC inhibitory nerves (27, 32). The identity of the neurotransmitter released from the NANC nerves is unknown, but vasoactive intestinal polypeptide (VIP) is a good candidate (33).

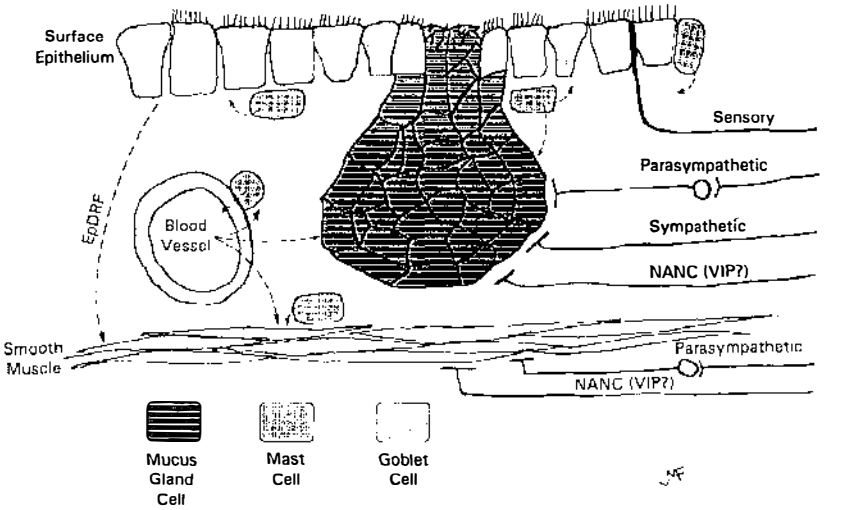

Figure 2 This schematic illustrates the interrelationships of the tissues in the airway. The *squares* at the bottom are shaded to match the types of cells in the diagram. *Broken arrows* indicate possible interactions between cells. Note that possible interactions between the sensory neurons are not shown and the neuroepithelial cells are not shown.

The surface epithelium forms a barrier between the air and underlying tissue and consists primarily of ciliated columnar epithelial cells and goblet cells. This is a tight epithelium with both absorptive and secretory properties (3). Secretion of water by the surface epithelium keeps a thin layer of solution on the airway surface (sol phase). The secretion of water is controlled by activation of β-adrenoceptors and other substances (3, 4). The surface gland cells are not innervated (34). The ciliated epithelial cells normally beat toward the mouth and provide a constant flow of sterile solution over the surface, washing it free of trapped substances.

Embedded within the airway lining are also myelinated and nonmyelinated sensory nerve endings (35) and neuroepithelial bodies (20). These respond to various stimuli and may initiate local (axon) as well as central reflexes, thereby inducing contraction of airway smooth muscle or mucus secretion. After epithelial damage, these nerve endings would be exposed to higher concentrations of airborne toxicants, which could initiate bronchoconstrictor and secretory reflexes. The location of the neuroepithelial bodies (neuroendocrine cells) within the surface epithelium would place them in a good location for exposure to inhaled toxicants (20).

Within the surface epithelial layer, primarily in the upper airways in healthy individuals, there are Clara and Goblet cells. The exact function and

control of these cells in unknown, but they can secrete glycoproteins (mucins, glycolipid, etc) into the lumen of the airways. Mucus forms a gel phase on top of the sol (liquid) layer covering the surface of the epithelium. The tips of the cilia are in contact with the mucus layer and propel it toward the mouth. Mucins are large ($> 10^6$ Daltons) glycoproteins that tangle and cross-link to form a network that can trap particulates, bacteria, etc (6). The trapped particulates can then be transported by the beating of the cilia and be swallowed or expectorated.

The epithelium also contains submucosal glands. The glands consist of acini, made up of serous and mucus gland cells connected to the surface by a network of ducts (3, 7). The gland system also contains myoepithelial cells that contract to aid in expulsion of the mucus into the lumen of the airway (36). The primary function of the glands is to secrete mucus glycoproteins; however, they may have other functions as well, such as the transport of water (both absorptive and secretory) (4, 37, 38). The glands are innervated by the parasympathetic system. Acetylcholine induces increases in mucus secretion (7, 39, 40) and ion transport by the glands (38, 41). Other transmitters may also be involved through activation of α and β adrenoceptors (39, 40, 42, 43) or nerves releasing substance P and neurokinins (32). The glands are also innervated by VIP-containing fibers (44). Although acetylcholine can induce mucus release from both serous and mucus gland cells, beta adrenoceptor activation appears to cause release from mucous cells and alpha adrenoceptor activation from serous cells (43). Mucus secretion is also induced by the release of local factors from mast cells, such as histamine and leukotrienes (7).

Cell-to-Cell Interactions

Other cells within the mucosal tissue release factors that induce changes in smooth muscle function. Mast cells present throughout the tissue degranulate in response to a wide variety of inflammatory stimuli. The products released upon degranulation lead to airway smooth muscle contraction. For example, the release of histamine, platelet-activating factor, leukotrienes (LTB_4, LTC_4, LTD_4, etc), and prostanoids can directly induce smooth muscle contraction and mucus release. Mast and other immune responsive cells release enzymes (1, 45) that can directly cause tissue damage. A recent review by Leff et al (29) elucidates in great detail the role of invading cells in epithelial damage and airway hypersensitivity. The release of chemotactic factors will attract neutrophils, eosinophils, and basophils (28). The invasion of the tissue by eosinophils, neutrophils, and basophils in turn causes further inflammation and releases prostanoids and leukotrienes. The products released during the invasion of the tissue by the various leukocytes appear to be the cause of the late phase of an asthmatic attack (28). Various tissue-damaging enzymes and

major basic protein (MBP) released from eosinophils are cytotoxic, causing death of epithelial cells (46).

Epithelial tissue also appears to have a direct function in control of airway tone through the release of relaxing factors (reviewed in Ref. 47). The exact nature of the factor is unknown; however, the factor may be analogous to the endothelial-derived relaxing factors (EDRF) of blood vessels and has been termed EpDRF (epithelial-derived relaxing factor). A relaxing factor would have the greatest physiological significance in the smaller airways, where the epithelium lies close to the smooth muscle. Recently, MBP has been shown to cause an epithelial-dependent contraction of airway smooth muscle, presumably by the direct release of a contracting factor (48).

Therefore, since the various tissues form an interactive system, alterations in function of one cell type through the action of toxicants may lead to alterations in function of one or more of the other parts of the system. The effects of toxicants on airway epithelium is considered first. Airway epithelium is the first tissue exposed to airborne pollutants and therefore is exposed to the highest concentrations of the compounds.

TOXICANTS AND THE MUCOSA

The surface epithelium forms the main barrier between the external environment and the underlying tissues in the airways. The secretion of water and ions by the surface and submucosal cells keeps the tissue surface hydrated. Mucus forms a gel layer on top of the liquid layer, which can trap particulates, bacteria, spores, etc. In addition to these active processes, the airway epithelium forms a physical barrier between noxious stimuli and the underlying nerve fibers and cells.

Ozone

Ozone is a very reactive molecule generated by photochemical/electrical oxidation that can react with proteins and lipids. Ozone has been proposed to act primarily by damaging the epithelium, thereby causing an inflammatory response. The inflammation brings about release of prostanoids, leukotrienes, and other factors from mast cells and leukocytes and causes bronchoconstriction and increased secretions (9). Short-term exposure to ozone causes changes in the epithelium, including cell death (15, 49–51), damage or loss of cilia (16, 49), loss of cell-to-cell contact, and increased permeability of the epithelium (15, 51–53). Exposure of underlying nerve fiber endings (9), invasion of the epithelium by neutrophils (9, 54), and changes in the transport properties of the epithelium (50) also occur. There is an increase in lipid peroxidation (51), which may explain the direct tissue damade due to ozone.

Longer term exposure (60–90 days) has been shown to cause changes in the

structure of the epithelium, such as increased cell division and cells with shorter cilia (49, 55), Clara cell hyperplasia (56–58), increase in nonciliated cells, and a thicker epithelium-interstitial cell layer with cells containing more protein synthetic machinery (59). Mariassy et al (60) have demonstrated that ozone caused a decrease in cell density and a change in the pattern of development of mucus gland cells in sheep exposed just after birth to ozone (four hours per day for five days). In animals exposed to ozone, the mucus gland cell types, as defined by lectin-binding profiles, were similar to those found at birth rather than those observed at five days of age. This suggests that ozone exposure during critical developmental periods could have lasting structural or functional effects even when shorter exposures are considered.

Therefore, although damage to the epithelium would increase the probability of reflex bronchoconstriction/secretion, the tissue damage would also provide freer access of mast cells to antigens (52). Mast cell products would then cause further increases in secretion and bronchoconstriction. The release of chemotactic factors would initiate the invasion of the tissue by eosinophils, neutrophils, and basophils. The resultant release of mediators would cause further secretion and bronchoconstriction. Cell damage would ensue from the release of enzymes and major basic protein and through direct tissue damage. More subtle forms of damage may also occur. For example, a loss, decrease in length, or change in beat frequency of the cilia would result in poor mucociliary clearance (16, 17), as would increases in mucus secretion (17). The communication between epithelia could also be damaged (16, 51), thus leading to alterations in mucociliary transport. The coordination of ciliary activity between cells is dependent on this coupling (16). In addition, it has been shown in guinea pig that short-term exposure to ozone increases net sodium absorption, a condition that could lead to a decreased sol layer on the airways (50), although Phipps et al (61) demonstrated that water secretion increased. Increased mucus secretion, changes in water movement, and other alterations in mucociliary transport could lead to inspissation of mucus and mucus plugging.

The increased permeability of the epithelium will permit greater penetration of antigenic compounds, which could result in mast cell degranulation. Osebold et al (52) demonstrated that ovalbumin-sensitized mice exposed to ozone were much more sensitive to the effects of inhaled ovalbumin. Roum & Murlas (62) suggested, however, that the increase in permeability of the airway could not totally explain the consistent hypersensitivity response (bronchospasm) they observed to a parenteral cholinergic challenge when compared to the inconsistent nature of the response to a nebulized methacholine challenge. Lipid peroxidation and the production of thromboxane (TxA_2) have also been suggested to be primary effects of ozone (14, 63–65). It is interesting that neutrophil depletion blocks the hypersensitivity response to

ozone (66). Thus, although changes in the permeability barrier to antigens and other noxious substances occur after ozone exposure, other changes in epithelial transport and structural properties are likely to be involved in the pathological actions of ozone.

Sulfur Dioxide

Sulfur dioxide is a common pollutant arising from the burning of high sulfur coal and other fossil fuels. High concentrations can be reached indoors from the use of kerosene heaters. In addition, it is a preservative added to many medicines (67) and to foods to improve crispness or freshness (e.g. on the lettuce at salad bars in restaurants). The use of sulfur dioxide (bisulfite or metabisulfate) is approved by the FDA and is on the generally recognized as safe (GRAS) list. A thorough review of the metabolism and chemistry of sulfites was made by Gunnison & Jacobson (68). Sulfur dioxide dissolves in water to form sulfurous acid and, depending on the pH, bisulfite and/or metabisulfite may form.

Sulfur dioxide has been known since the 1970s to cause airway hypersensitivity (see 67, 68). Imai et al (69) demonstrated a positive correlation between the sulfur oxide pollution levels and deaths due to asthma and bronchitis. The number of deaths attributed to asthma decreased quickly after decreases in the level of pollution, whereas the problems associated with chronic bronchitis took several years to abate. This suggests fundamental changes in the structure and function of the airways, probably including the epithelium. Chronic bronchitis is characterized by elevated levels of mucus secretion (70). The effects of sulfur dioxide on epithelial function have not been well defined. It has been argued that the main mechanism of the action of sulfur dioxide occurs through the generation of an acid and that the acid alters the function of the airway (68). Schlesinger (18) has reviewed the action of inhaled acids and their ability to reduce nonspecific clearance from the lungs. Gatzy & Stutts (71) demonstrated that acids caused a nonspecific increase in alveolar epithelial permeability. Fine (72) and Balmes et al (73) have suggested, however, that the sulfite or sulfur dioxide are active components and that changes in pH alone cannot explain the actions of sulfur dioxide. Also, the acid concentration that normally occurs in air pollution seems to have little effect on airway properties (74).

Short-term exposure to sulfur dioxide can increase the release of mucus (75, 76). Sulfur dioxide in environmentally significant concentrations generally does not cause great changes in tissue structure; however, there is an increased responsiveness to allergens (77). Exposure to high levels (300–500 ppm) of sulfur dioxide caused focal loss of cilia or exfoliation of ciliated cells (78). Sulfur dioxide also induced a dose-dependent increase in nonelectrolyte permeability of the epithelium and decreases in the potential difference across and electrical resistance of the epithelium (78).

The density of mucus-secreting cells is much lower in the smaller airways than in the trachea. Long-term exposure to sulfur dioxide, however, has been shown to increase mucus secretion in the lower airways and to change the nature of the glycoproteins secreted to be closer to that of the upper airways (70). This finding suggests that an increase in the number of mucus-secreting cells in the lower airways occurs after long-term exposure to sulfur dioxide. Increases in mucus cell mass in the smaller airways could increase the possibility of the occurrence of obstructive pulmonary disease. Epidemiological evidence suggests that there is a connection between sulfur dioxide and obstructive lung diseases (69, 79).

The increased mucus secretion is induced through reflex stimulation of the glands (75, 76). The mechanism by which the stimulation of these fibers occurs is unclear. Sulfur dioxide is a reactive molecule and it can react with disulfide bonds (68). For example, bisulfite acts on the nicotinic receptor at the neuromuscular junction, thereby inducing changes in the activity of the ion channel associated with the receptor (80). The activity of the receptor is potentiated. Whether this action occurs at the epithelial, mucus, or nerve cells is unknown. In addition, sulfur dioxide could have effects on immune cells within the epithelium either directly or through reflex release of neuropeptides (see Ref. 77).

Tobacco Smoke

Tobacco represents both a social and a biological insult. There are numerous reports linking tobacco use with cancer of the lung and other organs not considered here. Smoking of tobacco exposes the lung to high concentrations of particulates, organic compounds of various types (e.g. tars), nicotine, cyanide, carbon monoxide, and many other compounds. There is a correlation between smoking and the occurrence of chronic obstructive pulmonary disease (2, 81, 82). The mortality rates due to chronic obstructive pulmonary disease for heavy smokers are 2–25 times that for nonsmokers. In recent years, passive smoking has been associated with increased respiratory health risks, particularly to children (12, 81). Children exposed passively to smoking experience an increase in respiratory illnesses and a decrease in growth of lung functions (12, 81).

The large number of tobacco smoke products makes isolation of individual mechanisms of action on the epithelium difficult. The effects of in vitro exposure of the epithelium to tobacco smoke were studied by Welsh (83), who showed that the particulate phase of the smoke inhibited chloride secretion, thus causing an increase in transepithelial resistance and a decrease in short-circuit current. The decrease in chloride secretion would lead to a decrease in water secretion. These actions were not prevented by the use of oxygen radical scavengers or indomethacin. Thus the effects appear to be due to a direct action on the epithelium rather than through stimulation of the

release of cyclooxygenase or lipid peroxidation. By contrast, an oxygen radical scavenger, desferoxamine, blocked the increase in asbestos fiber penetration induced by cigarette smoke (53), and nonsteroidal anti-inflammatory drugs decreased the hyperplasia of epithelial cells (84). Cigarette smoke increases the permeability of the epithelium to penetration by large molecules [e.g. horseradish peroxidase (85), asbestos (53, 86)]. This increased permeability has been attributed to the decrease in coupling at the tight junctions (87, 88), although some workers have suggested that this is not the case (89). Gap junctions between cells in culture were shown to be reduced in number (87). The increased permeability to larger molecules would permit penetration of the epithelium by antigens or other factors and lead to stimulation of sensory fibers and antigen-responsive cells. Acute effects of cigarette smoke include an increase in the rate of mucus secretion, which is caused partly by reflex stimulation of glands but also by local stimulation of the mucosa through direct stimulation of the ganglia (7). There is a variable response of mucociliary clearance to acute exposure to tobacco smoke (16).

Longer exposures to tobacco smoke induce changes in the morphology of the airway. Tobacco smoke increases mitotic activity in the epithelium transiently after even a single exposure (84). With continued exposure, there is an increase in the number of cells within the epithelium. In culture, hyperplasia of the cells and atypical cells are observed after exposure of the cells to cigarette smoke condensate (90, 91). Mucus gland cells are increased in number 2–7 times (92–95) over controls, and the number of secretory cells in the lower airways increases significantly (93). The acute effects of stimulation of mucus secretion, increased epithelial permeability to larger molecules, and decreased chloride secretion will give rise to thicker secretions. After continued exposure to tobacco smoke, the additional problem of increased mucus cell mass will lead to the increased probability of mucus accumulation in the airways. Decreased mucociliary clearance is a common finding after prolonged exposure to tobacco smoke (17).

The initial mechanisms by which tobacco smoke brings about these changes are complex. An oxygen radical scavenger, desferoxamine, can decrease asbestos fiber penetration into the epithelium (53). This suggests that free radical production is involved in the effect of tobacco. Rogers & Jeffrey (95) demonstrated that *N*-acetylcysteine, a mucolytic drug, when given prophylactically was able to prevent many of the changes in gland cell number normally observed after exposure to tobacco smoke. They suggested that this agent was acting as a nonsteroidal anti-inflammatory drug. *N*-Acetylcysteine also acts as an antioxidant. Therefore, free-radical-induced damage could be one mechanism of induction of cellular hyperplasia and possibly also of mucus release. The free radicals could be supplied directly through the smoke

or indirectly through metabolic activation of foreign substances by epithelial cells. Epithelial cells have the capacity to metabolize xenobiotics (96, 97). Epithelial damage would then induce inflammatory responses, thus leading to release of mast cell and other mediators (thromboxanes, prostaglandins, etc), as discussed above. Induction of reflexes also could occur. The direct actions of the tobacco smoke on epithelial properties (83), in addition to increased mucus secretion, increased mucus cell mass, and decreased mucociliary clearance, would lead to accumulation of mucus in the lower airways and obstruction of the smaller airways.

Cocaine

Little is known about the effects of cocaine on mucous gland or epithelial cell function. Cocaine is taken either by "snorting" the crystalline form or smoking "crack." Although absorption of cocaine through the mucosa occurs readily, cocaine can be detected on the mucosa for hours after exposure (98). We have calculated that the concentration of cocaine that can be reached on the mucosa could be > 1 M after inhaling 25 milligrams (99). Thus, not only will the cells be exposed to high concentrations of cocaine, but exposure can occur for extended periods, even after a single application. Cocaine paralyzes the cilia of the surface epithelial cells, presumably by the local anesthetic action of cocaine (100), and decreases the rate of mucus clearance from the airways, further slowing the clearance of the drug from the surface epithelium. We have recently shown that cocaine applied in vitro can increase at low concentrations (< 1 mM) and decrease at higher concentrations the short-circuit current developed across the epithelium (99). Cocaine also inhibited the increase in short-circuit current induced by both acetylcholine and isoproterenol. The inhibition was presumably due to effects on ion channels. These actions would alter the water transport across the cells. Effects on the respiratory epithelium can be deduced from the signs of cocaine exposure such as a runny nose and a chronic cough in cocaine users (101). The runny nose is at least consistent with the hyperemia known to occur in the nasal mucosa (102) and the stimulation we have observed after in vitro application of concentrations of cocaine < 1 mM. Mucus and water secretion would be expected to increase after acute exposure due to the sympathomimetic action of cocaine (103) and the known sympathetic innervation of the glands. The inhibition of epithelial function due to the local anesthetic action of cocaine as noted above would decrease secretions. The chronic cough of users of cocaine also suggests that changes in secretion occur.

A recent review by Ettinger & Albin (104) also noted the association of many different airway problems with cocaine: foreign body granulomas, a type of pneumonia, pulmonary edema, pulmonary hemorrhage, etc. Slavin & Goldwyn (105) discuss the nasal septal defects that occur with chronic

cocaine use and that result in loss of the epithelium and even perforation of the septum. Thus cocaine has both short- and long-term actions on the epithelium that cause dysfunction and decreased responsiveness.

TOXICANTS AND AIRWAY SMOOTH MUSCLE

The contraction of airway smooth muscle is the primary problem during an asthmatic attack. Airborne toxicants, such as ozone and sulfur dioxide that can induce airway hyperreactivity, have been used to develop models for asthma or as challenges in the study of airway hypersensitivity. A thorough review of the agents involved in induction of asthma in the workplace has been made by Chan-yeung & Lam (11).

Ozone

Asthma, and the response to ozone, have been related to inflammation of the epithelium (9, 54). Inflammation does not always cause hyperresponsiveness of the airways (13), however, thus suggesting that the responses to inflammation are complex. Ozone is a very reactive compound that causes an increase in response to inhaled bronchoconstrictors in both asthmatics and nonasthmatics (62, 106, 107).

The increased responses to inhaled challenges (cholinergic or histaminergic) after ozone exposure are not inhibited by blockade of autonomic reflex mechanisms (108), although earlier work had suggested a role for parasympathetic reflex mechanisms (109, 110). The effects of ozone can be eliminated by nonsteroidal anti-inflammatory drugs, presumably through inhibition of arachidonic acid metabolism (64, 111). It has been proposed that arachidonic acid metabolites are important in the hypersensitivity induced by ozone (9, 63, 66, 108, 112). Ozone is thought to produce bronchoconstriction primarily through inflammation and the release of mediators from the epithelium (e.g. LTB_4) that act as chemotactic factors and cause invasion of the airways by other inflammatory cells, particularly neutrophils (9, 13, 54, 113). Neutrophils also have been implicated as important in the response to ozone because their depletion results in a loss of the response (66). Mast cells (113–115) obtainable by bronchial alveolar lavage also increase in number. Jones et al (116) have also demonstrated that ozone exposure in vivo does not alter the release of EpDRF in vitro, thus suggesting that the loss of EpDRF after exposure to cocaine cannot explain the effects of ozone. Another possible explanation for the effects of ozone is that neutral endopeptidase, an enzyme responsible for the degradation of a number of neuropeptides (substance P for example), might be inhibited. Borson (117) discusses this possibility in a recent review and suggests that any chemical that can reduce neutral endopeptidases may cause symptoms of airway disease.

Airway smooth muscles retain their hyperresponsiveness in vitro after in vivo exposure to ozone. Walters et al (118) found that field-stimulation-evoked (i.e. nerve evoked) contraction of airway smooth muscle from dogs exposed to ozone occurred at lower stimulation frequencies than for muscle from control dogs. They found no effect on the maximal tension developed by the muscle strips or on the concentration-tension curves obtained for exogenously applied acetylcholine. The effects of ozone on nerve stimulation reversed in vitro after 6 hr of intermittent washing in vitro. Daniel et al (63) have suggested that thromboxane A_2 both directly contracts smooth muscle and permits increased release of acetylcholine from parasympathetic nerves. The actions of arachidonic acid metabolites are consistent with the effects observed by Walters et al (118). Jones et al (119), however, have found that ozone exposure in vivo caused increased contractile response of the tracheal muscle in vitro to field stimulation and to acetylcholine, but not to elevated potassium. They found no effect of ozone on the amplitude of excitatory junction potentials. They concluded that ozone has a potential-independent action on the muscle to increase its responsiveness to stimulation. Clearly, more work on the identification of the mediators of these responses needs to be done.

As noted above, ozone induces an increase in mast cells and other cells in bronchial alveolar lavage solutions. One would presume that this would increase the responsiveness of the airways to release of mediators of inflammation and exacerbate the effect of an inhaled toxicant. Turner et al (113) have demonstrated, however, that acute pretreatment with ozone blocks the antigen-induced late asthmatic response, but does not affect the early response. The late-phase response has been attributed to the invasion of the tissues by eosinophils, neutrophils, and basophils (28). Thus ozone may not only initiate an increase in sensitivity to inhaled challenge agents, but it may also acutely blunt the immune responsiveness of the airways. Longer term treatment with ozone concurrent with hexachloroplatinate (a potent pulmonary-sensitizing agent) enhanced the development of sensitivity to the platinum compound (120). Ozone could therefore have a major long-term action of enhancing sensitivity to allergens.

Long-term exposure to ozone (90 days) leads not only to changes in the epithelium, as mentioned above, but also to increases in the amount of smooth muscle present (59). Thus most of the effects that ozone causes lead to an increase in airway sensitivity to challenge or to the potential for greater constriction in response to challenge.

Sulfur Dioxide

Sulfur dioxide has been known to induce bronchoconstriction and trigger asthmatic attacks since the early 1970s (see 67, 68). Asthmatics are much

more sensitive to the bronchoconstrictor effects of sulfur dioxide than nonasthmatics (67). The mechanism of action of sulfur dioxide in the induction of bronchoconstriction is unknown. As noted above, there is little change in the structure of the epithelium after exposure to sulfur dioxide and thus it seems unlikely that a change occurs in epithelial permeability (62).

The response to sulfur dioxide occurs rapidly, within 2 min after exposure (121). This is a fairly fast response for an inhaled substance and suggests possible neural involvement and reflex-induced bronchoconstriction. Nadel et al (122) found that reflex bronchoconstriction occurred in response to sulfur dioxide exposure, which was inhibited by atropine. In asthmatic subjects, however, it has been shown that atropine or ipratropium do not inhibit the bronchoconstriction induced by sulfur dioxide (123, 124). Similarly, inhibition of transmitter release from parasympathetic nerves by activation of presynaptic autoreceptors inhibited sulfur-dioxide-induced bronchoconstriction in nonasthmatics but not in asthmatics (125). These differences were attributed to defective presynaptic autoreceptors in asthmatics; however, at least in asthmatics, parasympathetic reflex stimulation does not explain bronchoconstriction induced by sulfur dioxide.

Exposure of guinea pigs to low concentrations of sulfur dioxide can facilitate local allergic reactions to ovalbumin in sensitized animals (126). Inhibitors of mast cell degranulation, nedocromil sodium and sodium cromoglycate, are effective in preventing the effects of sulfur dioxide (124, 127). Whether sulfur dioxide has a direct effect on mast cell degranulation or other immune responsive cells in the epithelium is unknown. Mast cell degranulation would release histamine, chemotactic factors, and cyclooxygenase products, thereby leading to bronchoconstriction. Sulfur dioxide does not seem to cause mast cell degranulation, however, (128) although this conclusion may be dependent on the mast cell type considered. Pearce (28) has demonstrated that superficial mast cells in the lung obtained by bronchial alveolar lavage, which would be exposed directly to inhaled substances, are significantly more sensitive to the inhibitory actions of sodium cromoglycate and nedocromyl sodium. Sulfur dioxide has been shown to increase the release of mediators from leukocytes of asthmatic patients in some studies but not in others (see 68).

Snashall & Baldwin (124) suggested that noncholinergic reflex mechanisms might be involved in the action of sulfur dioxide and that these might be inhibited by sodium cromoglycate. Afferent fiber stimulation has been proposed as a mechanism for the action of sulfur dioxide by Dixon et al (127). Sheppard (77) has suggested that the effects of sulfur dioxide could be mediated via actions on sensory neurons containing neuropeptides [for example, those containing substance P or calcitonin-gene-related peptide (CGRP)]. Boushey et al (129) have shown that sulfur dioxide induces afferent

discharge. Nerves containing substance P (both afferent and efferent) and CGRP (afferent) are present in most of the tissues within the epithelium, mucus glands, and the smooth muscle in cats and rats, respectively (24, 130). Capsaicin induced increases in CGRP in the lumen of guinea pig airways. Therefore, the neuropeptide-containing fibers are in an excellent location for stimulation during exposure to a toxicant such as sulfur dioxide. In addition, the neuroepithelial bodies (neuroendocrine cells) could also be affected (131). Sensory fibers containing substance P or CGRP located within the airway epithelium could be stimulated by sulfur dioxide or other toxicants, thereby initiating axon reflexes within the airway and inducing both secretion and bronchoconstriction (32). It has been proposed that, in addition to the known direct stimulatory effects of neuropeptides on airway epithelial and smooth muscle cells, neuropeptides may also be immunomodulatory (132). In this case, the neuropeptides released from the sensory fibers would initiate mast cell degranulation, leading to an inflammatory response within the epithelium, bronchoconstriction, and mucus secretion. It is interesting that the response to sulfur dioxide shows tachyphylaxis (124, 127) that takes approximately 24 hr to recover (124). A speculative explanation would be that the tachyphylaxis is due to the depletion of neuropeptides, which must be synthesized and transported to the terminals from the nerve cell body.

Tobacco Smoke

Even though inhalation of tobacco smoke is inflammatory to the airways, can induce mucus release and cell hyperplasia, and lead to chronic obstructive airway disease, it is not generally recognized to induce bronchial responsiveness (13). Acute increases in sensitivity to inhaled acetylcholine were observed in guinea pigs after exposure to the smoke from six cigarettes (133, 134). This was attributed to an increase in the permeability of the airway epithelium, since heart rate decreased in response to inhaled acetylcholine after tobacco smoke exposure but not before. One would hypothesize that the initial exposure to tobacco smoke should lead to bronchoconstriction from the inhaled nicotine. Tobacco smoke does not generally increase the ability of other agents to sensitize the animal (11). Recently it has been shown that tobacco smoke can increase the sensitivity to substance P challenge due to a decrease in neutral endopeptidase activity (135). In general, however, there is a lack of effect of tobacco smoke on the sensitivity of the airways. The reason is unknown.

Cocaine

The actions of cocaine on the epithelium are described above. Less is known about the actions of cocaine on airway smooth muscle. Several recent reports associate the incidence of severe cases of asthma with the snorting or smoking

of cocaine (136, 137). As noted above, cocaine can reach very high concentrations on the epithelium and therefore might be directly cytotoxic, causing epithelial cell damage and inflammation. Cocaine has been proposed to alter the immune system, probably indirectly via neuroendocrine mechanisms (138). The changes effected by cocaine in lymphoid cells have been variable. Thus, although long-term usage of cocaine can induce airway hypersensitivity, the mechanism is currently unknown but may involve immunomodulation.

CONCLUSION

The airway epithelium is very important in the activity of the airway. Several investigators have stated that asthma is a disease related to inflammation of the airways, particularly the epithelium. Although the epithelium functions as a secretory organ, secreting water and mucus, it also is a source for many other compounds that influence the reactivity of the airway. Changes in the modulatory action of the epithelium may form a common site for the action of the toxicants discussed in this review. Do all of the toxicants discussed here work in the same way? This question has not yet been answered completely, but let me speculate.

Ozone is a very reactive substance. It seems most likely that much of its action would be limited to the surface epithelial layer or the cells within the lumen of the airway. The initial step in the action of ozone may be the oxidation of proteins or lipids in the epithelial cells, which might bring about the release of prostanoids and lead to chemotaxis of neutrophils, eosinophils, etc. Other cells in the surface epithelium may also be directly affected, however, notably the neuroendocrine cells or sensory nerve fibers. The activation of these cells would induce the release of peptide mediators (e.g. substance P) that could stimulate mast cell degranulation, smooth muscle contraction, mucus release, etc. If the enzyme for the degradation of these substances is also inhibited by ozone, the peptides could reach higher, more persistent concentrations.

Sulfur dioxide reacts with disulfide bridges, although this generally is not suggested in its mechanism of action. Sulfur dioxide may work in a manner similar to ozone, inducing the release of peptides via stimulation of sensory fibers and initiation of axon reflexes or through the stimulation of the neuroepithelial cells.

Cocaine would alter the properties of the airway, reducing blood flow to the mucosa and causing damage to the epithelia through this mechanism, local anesthetic actions, or osmotic shock. During chronic use, these changes may bring about inflammation of the epithelium and an increased responsiveness due to invasion of the tissue by cells of the immune system. In addition, cocaine may alter immune function.

Cigarette smoke clearly alters the structure and function of the epithelium, yet even in the face of large changes in epithelial structure, there does not appear to be an induction of smooth muscle hyperreactivity. The inhibition of neutral endopeptidase by free radicals in the smoke suggests some commonality of action with the other toxicants. The epithelium is hyperresponsive; mucus gland cell hypertrophy and hypersecretion lead to airway obstruction.

Clearly, a lot of work still must be done before the site of action of even one of the compounds is fully elucidated. Examining in detail changes in the function of the surface epithelial cells in response to toxicants may provide the key to that initial step in the induction of hyperresponsiveness that will lead to a greater understanding of asthma and other diseases of the airway. A full understanding of the molecular action of toxicants will also provide better methods for the treatment of airway diseases such as asthma.

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