The Direct and Indirect Action of Inhaled Agents on the Lung and Its Circulation: Lessons for Clinical Science

Tim Higenbottam, Tom Siddons, and Eric Demoncheaux
Division of Clinical Sciences, Central Sheffield University Hospital Trust, Medical School, University of Sheffield, Sheffield, United Kingdom

Inhalation of particles, gases, and vapors from environmental pollution results in a number of localized and general responses by the lungs. In this article we report investigations performed in humans that have enabled the identification of these specific processes in response to inhaled materials. We also offer insights that could help generalize environmental inhaled pollutants and potential means of studying them in humans. Three specific areas are covered: impact of denervation of the lungs and airway inflammation on the acute defense mechanism of the lungs to inhaled "irritants," differential uptake of inhaled particles into separate regions of the lungs, and the effect of inhaled nitric oxide on pulmonary vasculature and gas exchange. The inhalation of nitric oxide reflects the potential of inhaled pollutants to influence gas exchange, especially in patients with established lung disease, such as chronic obstructive pulmonary disease. Key words: asthma, bronchial hyperresponsiveness, chronic obstructive pulmonary disease, gas exchange, heart-lung transplantation, MIGET, nitric oxide, pulmonary hypertension. — Environ Health Perspect 109(suppl 4):559-562 (2001).

http://ehpnet1.niehs.nih.gov/docs/2001/suppl-4/559-562higenbottam/abstract.html

Inhalation of particles, gases, and vapors from environmental pollution produces numerous localized and general responses by the lungs. These responses can involve distinct processes such as neural reflex responses and chemical interactions with lung structures, and may initiate alterations in the distribution of ventilation and perfusion. In this article we report investigations performed in humans that have enabled the identification of specific processes in response to inhaled materials. We offer insights that are generalizable to environmental inhaled pollutants and potential means of studying their effects in humans.

We cover three specific areas. The first describes the impact of denervation of the lungs and airway inflammation on the acute defense mechanism of the lungs to inhaled "irritants," namely cough and bronchoconstriction responses. Here we discuss physiologic studies of heart-lung transplantation (HLT) in humans. The second area illustrates differential uptake of inhaled particles into separate regions of the lungs with emphasis on the importance of physicochemical properties of the inhaled materials in determining their site of interaction and the manner of inhalation. Third, we assess the effect of inhaled nitric oxide (NO) on the pulmonary vasculature and gas exchange. The inhaled NO draws out the potential of inhaled pollutants to influence gas exchange, especially in patients with established lung disease, such as chronic obstructive pulmonary disease (COPD).

Importance of Lung Reflexes and the Interaction of Nerves and Inflammation in Determining Airway Responses: Lessons from Heart-Lung Transplantation

HLT was introduced in 1981 (1) for treatment of pulmonary hypertension. The operation involves an airway anastomosis at the lower end of the trachea. All nerves to the lungs below this level are lost. Studies on reinervation (2) have shown loss of the airway afferent nerves. Only postganglionic nerves are retained and these were found not to contain substance P. The central airways in humans are richly supplied by myelinated and nonmyelinated afferent nerves (3). Myelinated nerves extend to the spaces between the epithelial cells of the airway mucosa (4,5). M- and nonmyelinated afferent nerves are both mechanoreceptors, responsive to pressure, and chemoreceptors, responsive to irritants (3) and to changes in osmolarity and chloride concentration of airway surface liquid (6). The study of cough in HLT patients used aqueous, low-chloride concentrations, distilled water, nebulized by an ultrasonic device. Unlike normal individuals, these patients had lost the cough reflex elicited from the lungs (7). However, if distilled water was added to the larynx directly, during bronchoscopy, cough was still elicited. The results of this study, taken with evidence of afferent nerve loss after HLT, clearly indicated the role of the airway's rapidly adapting afferent receptors in initiating cough in humans, as distinct from the role of laryngeal receptors.

Despite this evidence of denervation, patients after HLT still showed evidence of airway bronchial hyperresponsiveness. However, enhanced nonspecific bronchial hyperresponsiveness with methacholine, histamine, and ultrasonically nebulized distilled water is seen in patients only at times of acute lung rejection (8). It proved possible to demonstrate a relationship between the presence and degree of airway mucosal lymphocytic infiltration and the severity of nonspecific bronchial hyperresponsiveness. Detailed histologic studies of transbronchial biopsy during acute rejection and after treatment showed infiltrates of both airway and mucosa consisting predominantly of lymphocytes, neutrophils, and eosinophils. Steroids reduced these infiltrates within 3 weeks. Another measure of bronchial hyperresponsiveness is diurnal variation of peak flow (9). HLT patients at times of acute rejection showed marked diurnal variation in peak flow (10). The enhanced diurnal variation is lost with steroid treatment associated with the clearing of airway mucosal lymphocytic infiltration. The bronchoconstrictor response to inhaled irritants such as histamine, methacholine, and distilled water does not require innervation of the lungs. Instead it requires specific cellular infiltration of the airway mucosa. This difference has special importance in highlighting the susceptibility of asthmatic patients to inhaled environmental pollutants, which depends on an equivalent airway inflammation.

Inhaled capsaicin, which causes bronchoconstriction in asthmatics but not in normal volunteers, offers further insights into airway responses. In HLT patients, even when they are methacholine hyperresponsive, capsaicin fails to induce bronchoconstriction (11). This suggests that capsaicin represents a distinctive challenge to the airway in that it requires both an intact airway innervation and the presence of an airway-specific cellular infiltrate to cause bronchoconstriction. These observations could again be of special relevance to the behavior of asthmatic patients to environmental irritants such as sulphur dioxide and even nitrogen dioxide, which probably require the presence of capsaicin-sensitive nerves as well as airway inflammation (12). Therefore, the components of the acute responses of the lung to inhaled environmental irritants clearly are initiated through both neural and inflammatory processes.

This article is based on a presentation at the Workshop on Inhaled Environmental/Occupational Irritants and Allergens: Mechanisms of Cardiovascular and Systemic Responses held 31 March to 2 April 2000 in Scottsdale, Arizona, USA. Address correspondence to T. Higenbottam, Division of Clinical Sciences, Central Sheffield University Hospital Trust, F Floor, Medical School, University of Sheffield, Beech Hill Rd., Sheffield, S10 2RX, UK. Telephone: 44 (0)114 271 2196. Fax: 44 (0)114 271 1711. E-mail: t.higenbottam@shef.ac.uk

Received 2 February 2001; accepted 18 July 2001.

Environmental Health Perspectives • VOLUME 109 | SUPPLEMENT 4 | AUGUST 2001 559
Separate chemical interactions with the airway may elicit pure neural reflex coughing, whereas other types of reaction require the presence of inflammation in the airway mucosa to initiate bronchoconstriction. Those reactions, which act through the capsaicin nerves, require the enhancement of airway inflammation to induce bronchoconstriction (Figures 1, 2).

**The Impact of Inhalation Pattern and Chemistry on the Distribution of Inhaled Pollutant Effects: Lessons from Tobacco Smoking**

In humans one of the most extensively studied environmental pollutants is tobacco smoke from cigarettes. In the developed nations more than 25% of the adult population smokes (13). This form of self-injury produces both pulmonary and systemic disease. Tobacco smoke provides an example of how a complex aerosol can be absorbed and contribute to disease at sites distal from the lungs. The pattern of smoking varies enormously among individuals. There are two extremes, either “deep inhalation” or “mouth” smoking. Some individuals retain smoke in the mouth and upper airway; others inhale deeply. All still access smoke to their lungs (14). Those who inhale less are at a much higher risk of developing lung cancer than those who take deep inhalations of smoke (15). This phenomenon was attributed to the fact that the concentration of smoke in the upper airways, trachea, and major bronchi was higher in those who did not inhale fully. Depositing the smoke in these airways creates a higher concentration of smoke particles and little access to the phagocyte cells such as the alveolar macrophage and thus contributes to the development of cancer. Conversely, in the so-called inhalers there was a much higher mortality rate from coronary artery disease (15). Hence, higher alveolar uptake of smoke constituents exposes the body to the systemic effects of the tobacco smoke. The coronary artery risk diminishes within a year of quitting smoking, which has been attributed to the reversible effects when the specific constituent of the smoke is withdrawn. Continued exposure is necessary to maintain coronary heart disease risk.

Besides the inhalation pattern, the chemistry of the smoke also helps determine the site of action. The differential uptake of tobacco smoke depends on the physiochemical properties of its constituents and on how these characteristics influence lung retention. Table 1 shows the mouth and pulmonary retention of different constituents of smoke according to their solubility. The water-soluble acetaldehydes are retained in the aqueous linings of the mouth; the water-insoluble toluene is retained in those regions of the lungs where there is surfactant, e.g., the alveolar epithelium. The lipophilic nature of the surfactant-coated epithelial surface probably contributes to the retention of the lipophilic constituents of complex aerosols such as tobacco smoke. The particulates—the tar—are retained principally on deep inhalation because of the lipophilic nature of their hydrocarbon constituents. One constituent of tobacco smoke, nicotine, coexists in the volatile and particulate fractions of the smoke. It is taken up by both the airways and alveoli with almost equal facility due to its unique physiochemical properties allowing access to both the aqueous surface liquid of the airways and the lipophilic alveolar surfaces.

Another constituent of tobacco smoke selectively taken up by the alveolar pulmonary capillaries is CO. Again, this is a result of its unique chemical properties. Our original studies showed that NO-like carbon monoxide (CO) was not taken up by the airways.

**Table 1. Differential retention of tobacco smoke constituents in the mouth and in the lungs.**

| Constituent                  | Water soluble (%) | % Lung | % Mouth |
|-----------------------------|-------------------|--------|---------|
| Acetaldehyde                | Yes               | 21     | 99      | 60      |
| Isoprene                    | No                | 34     | 99      | 20      |
| Acetone                     | Yes               | 56     | 86      | 56      |
| Acetonitrile                | No                | 85     | 91      | 74      |
| Toluene                     | No                | 111    | 93      | 29      |
| Particles                   | No                | 96     | 96      | 16      |
| Carbon monoxide             | No                | 54     | 54      | 3       |

Data from Higenbottam (12).
However, on contact with the alveolar capillaries it was taken up 4.5 times faster than carbon monoxide (16). The solubility of NO in water is greater than oxygen (O2) or CO but it is still very low (Table 2). By contrast it has 400,000 times the affinity that oxygen has for hemoglobin (17). This high value for affinity reflects the ratio of the published rate constants for the forward and reverse reactions for NO and O2 with hemoglobin, as NO reacts with hemoglobin in the red corpuscles to form methemoglobin and nitrate (18). The abundant presence of methemoglobin reductase restores the methemoglobin to hemoglobin for further O2 carriage.

**Inhaled Pollutants That Alter Gas Exchange of the Lungs and Systemic Uptake: Lessons from Inhaled Nitric Oxide**

In 1987 we undertook the first studies of inhaled NO in patients with primary pulmonary hypertension (19). It appeared that 40 parts per million (ppm) NO was able to cause selective pulmonary vasodilatation with few or no effects on systemic vascular resistance (Figure 3). From these observations many studies were undertaken which culminated in the use of inhaled NO in the treatment of persistent pulmonary hypertension of the neonate (20). As a result, inhaled NO was registered as a treatment of persistent pulmonary hypertension of the neonate in December 1999.

**Inhaled Nitric Oxide in Chronic Obstructive Pulmonary Disease**

It was hoped that inhaled NO could be used to treat a range of lung diseases. One such common disease was COPD, a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response to noxious particles and gases (21). We found that in patients with COPD and with forced expiratory volume in 1 sec (FEV1) values below 1.5 L, inhaled NO induced a fall in arterial oxygen partial pressure (PaO2) (22). We were able to demonstrate, using the multiple inert gas elimination technique (MIGET), that inhaled NO negatively affected the ventilation/perfusion (V/Q) matching (Figure 4). These data suggest that the distribution of NO to poorly ventilated alveoli reversed hypoxic vasoconstriction.

We suspect that variation in local NO production by the pulmonary endothelium contributes to local perfusion. It has been shown that endothelial NO release falls with acute hypoxia (23), and this could also contribute to the regional hypoxic vasoconstriction and matching between ventilation and perfusion.

The opportunity to test the idea has been made possible with the introduction of a “spiked” inhaled NO delivery system (24). This device was designed to provide a safe and practical way of delivering inhaled NO to the ambulatory patient. The spike device releases a bolus of NO at the onset of inspiration triggered by each breath (Figure 5). This not only reduces the amount of NO given in each breath, but also targets NO to the alveolar region of the most rapidly ventilated parts of the lungs. When COPD patients inhale spiked NO, the mismatch of V/Q is very much reduced and PaO2 does not fall as it does when NO is mixed in the whole inspired breath (25). We have demonstrated that although spiked inhaled NO prevents a drop in oxygenation, the reduction in pulmonary artery pressure is as effective as when NO is delivered in the whole inspired breath (26).

The lessons to learn from these observations are that gases diffuse to all regions of the lungs even in the presence of extensive COPD and emphysema. If vasodilatation occurs in poorly ventilated regions, PaO2 can fall as a consequence of a worsening of V/Q matching.

**Table 2. Nitric oxide, oxygen, and carbon monoxide solubility at 35°C in water.**

| Compound | Solubility coefficient (mL/mL) |
|----------|-------------------------------|
| NO       | 0.042                         |
| CO       | 0.021                         |
| O2       | 0.028                         |

Data from Wilhelm et al. (33).

**Figure 3.** Comparison of effects on (A) pulmonary (PVR) and (B) systemic (SVR) vascular resistance of an infusion of PG12 (0.5 mg in 250 mL) at rates of 4, 8, and 12 mL/hr and inhalation of NO (40 ppm in air) with baseline (BL) values in eight patients with pulmonary hypertension. Means ± SEM are shown. *p < 0.05, **p < 0.01.

**Figure 4.** Effect of inhaled nitric oxide on distribution of ventilation–perfusion ratios in a subject with severe chronic obstructive pulmonary disease (33).
inhaled nitric oxide. For example, myocardial systemic effect associated with the use of globin, and nitrate, there is some evidence of a reacting with hemoglobin to form methemoglobin. Systemic Uptake of Nitric Oxide inhaled nitric oxide in a manner analogous to oxygen. the release of a small bolus of nitric oxide at a fixed concentration on both temperature and pH: release NO by disproportionation of its conjugate acid, nitric acid, a process dependent on both temperature and pH:

\[ 3H N O_2 \rightarrow 2N O + N O_3^- + H^+ + H_2O \]

We have shown that nitrite causes respiratory alteration of the pulmonary artery partly by release of N O, which may be detected in the exhaled air. The circulating level of plasma nitrite is between 0.1 and 15 μM (31). Increase in circulating nitrite, which can release NO, causes systemic vasodilation. We can therefore see how a small but significant systemic effect can be produced by an inhaled gas such as NO as a consequence of its reaction with blood. Equivalent systemic uptake of inhaled pollutant might contribute to systemic disease. These observations in humans further emphasize the need for translating the concentration of an inhaled material to an estimated dose on the epithelial surface of the lungs. The need to achieve this dose has importance not only for toxicity but also for the rapidly expanding field of inhaled drug therapy.

Conclusion

These three areas of human physiopathology introduce several important concepts. Sensory innervation of the lungs is necessary for coughing. Conversely, bronchial hyperresponsiveness to nonspecific challenges—e.g., methacholine, histamine, and distilled water—depends on specific types of cellular infiltrates of the airway mucosa. Capsinic, however, requires the airways to be innervated to cause bronchostimulation. Uptake of constituents of tobacco smoke and their capacity to cause disease depend on their chemical properties and their distribution when inhaled into the lungs. Nitric oxide, through its unique capacity to combine with hemoglobin when inhaled, acts as a selective vasodilator. However, its capacity to override hypoxic vasodistortions in patients with COPD worsens the matching between distribution of ventilation and perfusion and improved gas-impaired gas exchanges. Chemical pollutants, such as nitric oxide, may exert a physiologic effect. Indeed, mining accidents in the early part of the 20th century recorded the hemodynamic effects of inhalation of the gas. We must be aware of the potential vascular effects and of the possibilities of alterations in gas exchange, particularly when individuals with disease are involved.

REFERENCES AND NOTES

1. Reitz BA, Wallwork JL, Hunt SA, Pennock JL, Billingham ME, Springall DR, Polak JM, Howard L, Power RF, Krausz T, Widdicombe J. Upper airway reflexes. Curr Opin Pulm Med 140:52–57 (1989).
2. Springall DR, Polak JM, Howard L, Power RF, Krausz T, Widdicombe J. Upper airway reflexes. Curr Opin Pulm Med 140:58–61 (1989).
3. Widdicombe J. Lung rejection and bronchial hyperresponsiveness. Annu Rev Physiol 37:131–137 (1975).
4. Widdicombe J. Upper airway reflexes. Curr Opin Pulm Med 140:52–57 (1989).
5. Das RM, Jeffrey PK, Widdicombe J. The epithelial innervation of the lower respiratory tract of the cat. J Anat 126:123–131 (1978).
6. Godden DJ, Bolrand C, Lowry R, Higenbottam TW. Chemical specificity of coughing in man. Clin Sci (Lond) 70:301–306 (1986).
7. Higenbottam T, Jackson M, Wollman P, Lowry R, Wallwork J. The cough response to ultrasonically nebulized distilled water in heat-lung transplantation patients. Am Rev Respir Dis 149:52–57 (1989).
8. Higenbottam T, Jackson M, Rashid T, Stewart S, Coutts C, Wallwork J. Lung rejection and bronchial hyperresponsiveness to methacholine and ultrasonically nebulized distilled water in heart-lung transplantation patients. Am Rev Respir Dis 150:52–57 (1989).
9. Lewinsohn H, Capel S, Smart J. Changes in forced expiratory volume throughout the day. Br Med J 1:482–484 (1969).
10. Otalana BA, Higenbottam T, Scott J, Clayland C, Igboala G, Wallwork J. Lung function associated with histologically diagnosed acute lung rejection and pulmonary infection in heart-lung transplant patients. Am Rev Respir Dis 142:329–332 (1990).
11. Hathaway TJ, Higenbottam TW, Morrison JF, Clelland CA, Wallwork J. Effects of inhaled capsaicin in heart-lung transplant patients and asthmatic subjects. Am Rev Respir Dis 148:1223–1227 (1993).
12. Bannenberg G, Atzori L, Jue E, Auberson S, Kimland M, Ryffeldt A, Lundberg J, M, Oudizius P. Sulfur dioxide and sodium metabisulfite reduce bronchconstriction in the isolated-perfused and ventilated guinea pig lung via stimulation of capsaicin-sensitive sensory nerves. Respir Res 61:130–137 (1994).
13. Surgeon General. Reducing Tobacco Use: A Report of the Surgeon General. Washington, DC:U.S. Public Health Service, 2000.
14. Wald NJ, Idle M, Berehm J, Bailey A. Serum cotinine levels in pipe smokers: evidence against nicotine as cause of coronary heart disease. Lancet 2:775–777 (1981).
15. Higenbottam T, Shipley M, Rose G, Cigarettes, lung cancer, and coronary heart disease: the effects of inhalation and tar yield. J Epidemiol Community Health 36:113–117 (1982).
16. Balford CDR, Higenbottam TW. A simultaneous single breath measurement of pulmonary diffusing capacity with nitric oxide and carbon monoxide. Eur Respir J 5:256–63 (1990).
17. Carlsen E, Comroe J. The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. J Gen Physiol 42:83–107 (1958).
18. Widdicombe J. Upper airway reflexes. Curr Opin Pulm Med 140:58–61 (1989).
19. Carlsen E, Comroe J. The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes. J Gen Physiol 42:83–107 (1958).
20. Clark RH, Kuster TJ, Walker MW, Southgate WM, Hickby J, Perez JA, Roy BJ, Kessler M, Kinseila J. Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. N Engl J Med 342:449–474 (2000).
21. Katafaniy, N, Higenbottam TW, Cremona G, Akamine S, Demonceaux E, Smith, APL, Siddons TE. Minimizing the inhaled dose of NO with breath-by-breath delivery of spikes of concentrated gas. Circulation 98:2429–2432 (1998).
22. Siddons T, Asif M, Higenbottam T. Selective delivery of inhaled nitric oxide (iNO): effect on gas exchange in severe COPD. [Abstract] Am J Respir Crit Care Med 161:A48 (2000).
23. Siddons T, Asif M, M, Cormack K, Locke T, Higenbottam T. Spiked inhaled nitric oxide: an alternative therapy to conventionally delivered nitric oxide [Abstract]. Am J Respir Crit Care Med 159:1561–1562 (1999).
24. Auler JC, Carmona M, C, Bocchi EA, Bacil, F, Fiorelli AI, Stolf NAG, Jatene AD. Low doses of inhaled nitric oxide in heart transplant recipients. Heart Lung Transplant 15:443–450 (1996).
25. Siddons T, Asif M, M, Cormack K, Locke T, Higenbottam T. Spiked inhaled nitric oxide: an alternative therapy to conventionally delivered nitric oxide [Abstract]. Am J Respir Crit Care Med 159:1561–1562 (1999).
26. Jatene AD, Low doses of inhaled nitric oxide in heart transplant recipients. Heart Lung Transplant 15:443–450 (1996).
27. Demonceaux E, Smith A, Davies M, Higenbottam T. Is nitric oxide an important nitric oxide donor [Abstract]? J Physiol 491:101P (1996).
28. Yoshida K, Kasama K, Kitabatake M, Imai, B. Transformation of nitric oxide, nitrite and nitrate. Int Arch Occup Environ Health 62:103–115 (1993).
29. Demonceaux E, Higenbottam T, Akamine S, Smith A, M, M, Davies M. Exhaled nitric oxide from circulating nitric oxide anions [Abstract]. Am J Respir Crit Care Med 155:A18 (1997).
30. Monaghan JM, Cook K, Gara D, Crowther C. Determination of nitrite and nitrate in human serum. J Chromatogr A 707:143–149 (1993).
31. Bieker S, Classen HG, Loeffler K, Schumacher E, Thoni H. Antithrombotic effect of oral nitrate uptake in the spontaneously hypertensive rat. Arzneimittel-Forsch/Drug Res 45:258–261 (1995).
32. Higenbottam T. Pulmonary surfactant and chronic lung disease. Bronchol Res. Sectional Update 138:1:4501–1:4507 (1998).
33. Wilhelm E, Battino R, Wilcock R. Low-pressure solubility of gases in liquid water. Rev Chem Soc 7:219–262 (1977).
34. Unpublished data.