Effects of $\text{NO}_2$ on Chronic Bronchitics
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The acute influence of $\text{NO}_2$ on mechanics of breathing and respiratory gas exchange was investigated in a total of 111 subjects, aged 25 to 74 years, with chronic nonspecific lung disease (CNSLD). They breathed $\text{NO}_2$–air mixtures containing 0.5 to 8.0 ppm $\text{NO}_2$ for up to 15 to 60 min. Additionally in nine subjects the protective action of atropine, meclastine, and orciprenaline was investigated.

While the alveolar $\text{PO}_2$ remained nearly constant during inhalation of 5 and 4 ppm $\text{NO}_2$, a significant decrease of the arterial $\text{PO}_2$ and a corresponding increase of the arterial to alveolar $\text{PO}_2$ gradients occurred. Inhalation of 2 ppm $\text{NO}_2$ had not such an effect.

Inhalation of $\text{NO}_2$ at concentrations down to 1.5 ppm resulted in a significant increase of airway resistance. Lower concentrations had no significant effect.

Prolongation of the exposure period from 15 to 60 min at a $\text{NO}_2$ concentration of 5 ppm did not result in a more pronounced disturbance of the respiratory gas exchange for oxygen beyond the extent observed after exposure to 5 ppm $\text{NO}_2$ for 15 min.

Meclastine, in comparison with orciprenaline and atropine, showed a pronounced protective effect on the negative impact of $\text{NO}_2$ on respiratory gas exchange and airway resistance.

It is concluded that $\text{NO}_2$ may act by release of histamine, causing a bronchiolar, alveolar, and interstitial edema, thus differing from irritant air pollutants like $\text{SO}_2$, where reflex bronchoconstriction causes in some bronchitics dramatic increases of airway resistance at similar low concentrations.

Introduction

Chronic nonspecific lung diseases are an important cause of disability and untimely death in all industrialized countries; this seems to be related to occupational and environmental pollution.

Existing data from experimental animal studies with oxidizing air pollutants like $\text{NO}_2$ have shown that chronic inhalation leads to serious morphological lesions especially in the lung periphery (1) whereas $\text{SO}_2$, for example, does not seem to have such effects or only at higher concentrations (2, 3).

Human experimental exposure studies are restricted to short-term exposures in the range of MAK concentrations and below or of concentrations observed in ambient air because of ethical considerations, and it is discussed whether such experiments can be performed on the more susceptible subjects like asthmatics or bronchitics who need special protection by safe standards. [The German MAK value (maximal allowable concentration for occupational exposure) mentioned in this paper corresponds to the TLV, i.e., 5 ppm for $\text{NO}_2$.]

Although there is a quite general agreement that changes of lung function will occur when inhaling this pollutant at concentrations equal to or exceeding MAK values, some points request further examination, namely, determination of the concentration range where first changes of pulmonary function are to be observed ('no-effect level'); clarification whether changes are caused by reflex bronchoconstriction or effected by mediators like histamine; estimation from the results of these acute experiments the possibility of a risk of the development of chronic respiratory disease.

It has been the objective of the studies described here (4, 5) to support to the clarification of some of the aspects mentioned above. For this purpose, the acute influence of $\text{NO}_2$ on mechanics of breathing and respiratory gas exchange was investigated in subjects with chronic, nonspecific lung disease (CNSLD). They breathed $\text{NO}_2$–air mixtures from gas tight bags containing 0.5 to 8.0 ppm $\text{NO}_2$ for up to 5 and up to 60 min. Additionally in nine subjects the protective action of atropine, meclastine, and orciprenaline was investigated.

Methods and Subjects

A total of 116 patients, aged 25 to 74 years, suffering from chronic, nonspecific lung disease were
investigated. These patients were admitted to the hospital because of an exacerbation of their disease. During the time of investigation their status had bettered and they did not suffer from severe airway obstruction or hypoxemia.

Lung function parameters of interest were those for the respiratory gas exchange for oxygen and carbon dioxide (PAO₂, PAO₂, PaCO₂, PACO₂, and pHₐ), airway resistance (Rₑₑₑ) measured as total airway resistance from the extreme pressure points of the pressure/flow diagram (6) and thoracic gas volume (TGV) to indicate functional residual capacity (FRC) where Rₑₑₑ was measured. Phase shift caused by the Fleisch pneumotachograph when breathing at higher frequencies was avoided by having the subjects breathe at normal frequencies. Measurements of Rₑₑₑ has proved to be a sensitive indicator for changes in lung function associated with gaseous pollutants at low concentrations (7–10). (Dynamic manoeuvres like FEV₁ or V₃₀ probably are less suited for such investigations because they themselves—especially in bronchitics and asthmatics—may influence the results.)

Continuous measurement of PAO₂ and PACO₂ (as measured in bypass at the mouthpiece) was used for control of the steady state necessary for arterial PO₂ and PCO₂ analysis and for calculation of the alveolar-to-arterial PO₂ and PCO₂ gradients AaDO₂ and aADCO₂. The blood samples were taken from hyperemized ear lobe blood in sitting position. Hyperemized blood from the ear lobe was considered to be representative for arterial blood according to preparatory studies comparing PO₂ in blood simultaneously obtained by arterial puncture and in micro samples from the hyperemized ear lobe (11). The usability of this method was further approved by more recent investigations of PO₂ measurement via the skin by means of a continuously measuring platinum electrode, where the PO₂ follows immediately the changes in alveolar PO₂ thus giving exact the features of respiratory gas exchange (16).

The instruments used were a respiratory mass spectrometer (Varian MAT), platinum and glass electrodes (Eschweiler), and a constant volume body plethysmograph (own construction analogous to the Siemens body box with electronic compensation of differences in temperature and water vapor between inhaled and expired air allowing breathing at normal frequencies (12).

Measurement of lung function parameters was

![Figure 1. Mean values and standard deviations of PAO₂ and PaO₂ (A) before, (B) at the end, and (C) after inhalation of 5, 4, and 2 ppm NO₂ for 15 min.](image)
made at the end of the prephase, after exposure to the different NO₂-air mixtures (1.0–8.0 ppm) for different times (up to 5 and up to 60 min) and within 1 hr after termination of exposure. The protective action of atropine (0.75 mg SC), meclastine (2.68 mg IV) and orciprenaline (1.5 mg by inhalation) was tested as described previously (5).

Analysis of NO₂ was made by using the colorimetric Saltzman method (13).

The statistical evaluation was made with the aid of Wilcoxon’s ranking method of pair differences (14, 15) where the null hypothesis (= no effect) was subjected to a one-sided test.

Results

While the alveolar PO₂ remained nearly constant during inhalation of 5 and 4 ppm NO₂ (15 min), a significant decrease of the arterial PO₂ and a corresponding increase of the arterial to alveolar PO₂ gradients occurred. Inhalation of 2 ppm NO₂ had no such effect (Fig. 1).

A prolongation of the exposure period from 15 to 60 min at a NO₂ concentration of 5 ppm did not result in a more pronounced disturbance of the respiratory gas exchange for oxygen beyond the extent observed after exposure to 5 ppm NO₂ for 15 min (Fig. 2).

Inhalation of NO₂ concentrations down to 1.5 ppm (30 breaths ~ 5 min) resulted in a significant increase of airway resistance. Lower concentra-
FIGURE 5. Mean values and standard deviations of total airway resistance $R_t$ in 14 subjects with chronic bronchitis (left in each pair of columns) before and (right) after inhalation of 5 ppm NO$_2$ for up to 5 min without and with atropine, meclastine, orciprenaline, and NaCl as control.

The results indicate that short-term exposures to NO$_2$ may have discrete effects on human lung function. At NO$_2$ concentrations in the MAK range, the effect on respiratory gas exchange is relatively uniform: the consistent PaO$_2$ decrease of about 8 mm Hg indicates that irritant gases like NO$_2$ in low concentrations cause a uniform reaction in the lung. This is supported by a similarly uniform increase of airway resistance even in previously compromised subjects, where, corresponding to healthy subjects (9, 10), $R_{nt}$ increases between 0.5 and 2.0 cm H$_2$O/ (liter sec) may generally be observed.

After acute exposure to NO$_2$ airway resistance has shown to increase at concentrations above 1.5 ppm. In healthy subjects a similar increase of $R_{nt}$ at NO$_2$ concentrations of 2.5 ppm was shown (7).

There is some indication that histamine release might play a role in triggering the effects on lung function observed after inhalation of NO$_2$. Thomas (17) has shown degranulation of mast cells in rat lung tissue after acute exposure to low NO$_2$ concentrations (0.5–1.0 ppm). In human subjects exposed to 5–8 ppm NO$_2$ for up to 5 min, a marked protective action of a histamine-suppressing agent (meclastine) was demonstrated; atropine or $\beta$-stimulating agents did not have such an effect (5). However, in preliminary studies with quantitative estimation of the histamine content of plasma before, during, and after inhalation of a combination of NO$_2$, O$_3$, and SO$_2$ for 2 hr at MAK concentrations, a well-defined histamine release was demonstrated only in one half of the subjects (18).

As mentioned above, the experimental lung function studies in humans demonstrate that the functional response to the inhalation of NO$_2$ is quite similar in all subjects. Additionally, these very uni-
form and discrete changes, especially those of the airway resistance, suggest that the observed effects are probably due to formation of microedema of the bronchial or interstitial epithelium by histamine liberation and not by reflectory bronchoconstriction. However, histamine could also directly increase the tonus of the peripheral muscles, not being influenced by the nervus vagus, with disturbance of the ventilation/perfusion ratios and consecutive decrease of $P_{a_o_2}$ (19).

Reflectory bronchoconstriction after vagal stimulation probably would lead to a more inhomogeneous reaction of the bronchial tree with less uniform reactions of airway resistance in the different subjects. Reflex bronchoconstriction may be true for $SO_2$ inhalation, where the possibility of a vagal stimulated bronchoconstriction is discussed (8) and where at similar low concentrations in patients with obstructive lung disease dramatic increases of $R_{aw}$ are likely to occur (20). The more pronounced increase of airway resistance after inhalation of NO, alone or NO, + $SO_2$ + $O_2$ in combination with a bronchoconstricting agent like carbachol or acetylcholine is probably due to an increased reactivity of sensory receptors caused by air pollutants and thus a vagal reaction (10, 21).

An estimation of the risk of the development of chronic respiratory disease may be drawn from experimental animal studies: e.g., after short-term exposure to 5 ppm NO, a configuration change occurs in lung collagen and elastin. This denaturation of collagen and elastin was shown to be reversible when the animals were sacrificed 24 hr after termination of exposure (22). An exposure of rabbits to low concentrations of NO, (0.255 ppm) for 4 hr/day for 6 days, however, showed irreversible structural changes in lung collagen as determined by electron microscopy (23). Possibly owing to increased collagen and elastin catabolism in the lung, the hydroxyproline excretion was elevated in workers chronically exposed to NO, levels of 0.4-2.7 ppm (24) and in experimental animals after long-term inhalation of concentrations slightly above 2.5 ppm (25).

A crosslinking of collagen and elastin fibers observed in animals after short-term exposure may be an important fact in estimating part of the risk of development of chronic lung disease: this resembles an accelerated aging process, as normally collagen and elastin become macromolecules by crosslinking in aging processes. Equally important is the observation of a delayed maturation of the rat lung in an environment containing nitrogen dioxide (26). More recent findings indicate that exposures to NO, at low concentrations will cause a significant increase in the mortality of mice challenged with streptococ-

Cus aerosol after exposure to the pollutant. The same effect was seen at even lower pollutant levels when mixtures of NO, and $O_3$ at concentrations frequently found in ambient air were used (27, 28).

Chronic exposure to $NO_2$ leads to morphological changes in rats such as terminal bronchiolar hypertrophy, loss of cilia, desquamation of alveolar cells and thickening of alveolar septa at $NO_2$ concentrations of 2.0 ppm this being an index for preemphsematous changes in the lung (1). Additionally, long-term as well as short-term exposure to $NO_2$ leads to changes in the alveolar cell populations; after chronic exposure to concentrations of 2 ppm NO, pneumocyte I type cells were gradually replaced by the more cuboidal type II pneumocytes (29-31).

Although the carcinogenic or cocarcinogenic effects of NO, have not been proved to date, nitratesamine formation is also a potential hazard (32, 33).

Conclusions

In considering the concentrations of NO, at which adverse effects in humans first appear with ambient air concentrations, there is relatively little room for a sufficiently great "safety factor" when considering ambient air standards for NO,.

A number of studies based on the use of lung function parameters as indicators for short-term effects have demonstrated that the MAK value for NO, is clearly above the level at which significant changes in lung function occur. It is concluded that NO, may act primarily by release of histamine, causing bronchial, alveolar, and interstitial edema, thus differing from irritant air pollutants like $SO_2$, where reflex bronchoconstriction is considered to cause dramatic increases of airway resistance in some bronchitics at similar low concentrations (20).

Increased susceptibility to infectious agents of bronchitics and asthmatics, demonstrated in animals (26), combination with an increased irritability of the bronchial system after short-term exposures to NO, as indicated by the study of Orehek et al. (20), and adverse effects on lung function, as discussed in this paper, may be a potential hazard; this has to be taken into consideration, when setting a short-term standard for NO,.

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