Effects of Hyperoxia and Allergic Airway Inflammation on Cough Reflex Intensity in Guinea Pigs

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Summary
Toxic influence of high oxygen concentration on pulmonary function and structures has been known for many years. However, the influence of high oxygen concentration breathing on defensive respiratory reflexes is still not clear. In our previous experiments, we found an inhibitory effect of 100 % oxygen breathing on cough reflex intensity in healthy guinea pigs. The present study was designed to detect the effects of hyperoxia on cough reflex in guinea pigs with allergic airway inflammation. In the first phase of our experiment, the animals were sensitized with ovalbumin. Thirty-two sensitized animals were used in two separate experiments according to oxygen concentration breathing: 100 % or 50 % oxygen for 60 h continuously. In each experiment, one group of animals was exposed to hyperoxia, another to ambient air. The cough reflex was induced both by aerosol of citric acid before sensitization, then in sensitized animals at 24 h and 60 h of exposition to oxygen/air in awake animals, and by mechanical stimulation of airway mucosa in anesthetized animals just after the end of the experiment. In contrast to 50 % oxygen, 100 % oxygen breathing leads to significant decrease in chemically induced cough in guinea pigs with allergic inflammation. No significant changes were present in cough induced by mechanical stimulation of airways.

Key words
Hyperoxia • Allergic inflammation • Ovalbumin • Citric acid cough • Mechanically induced cough

Introduction
Inhalation of oxygen in higher concentration than that in normal ambient air is widely used in clinical practice. The concept of oxygen as a therapeutic agent was introduced in the 1920s by Alvin Barach (1922). It has been generally known that long term oxygen therapy is used in patients with chronic hypoxemia that can occur in several respiratory and cardiac disorders, including chronic obstructive pulmonary disease (COPD), chronic severe asthma and interstitial lung diseases. In patients with hypoxemia, oxygen supplementation improves survival, pulmonary hemodynamics, exercise capacity and improves the quality of life (Tarpy and Celli 1995).

On the other hand, most clinicians today are well aware of the toxic consequences to the lung of prolonged exposure to high oxygen tension. The basic mechanism underlying oxygen toxicity appears to be the generation of reactive oxygen species (ROS). ROS have the ability to react with and damage many important biomolecules, including enzymes, membrane lipids and nucleic acids (Jenkinson 1993).

There is increasing evidence that oxidative stress and ROS are involved in the pathogenesis of chronic
inflammatory airway diseases, including asthma (Barnes 2000). ROS have several effects on the airways, which could increase inflammation. The allergic airway inflammation is characterized by infiltrating inflammatory cells into the tracheobronchial mucosa and lumen of the airways. The influx of eosinophils and neutrophils is accompanied by marked and characteristic pathophysiological changes to the airways including thickening of the airway wall and the development of airway hyperresponsiveness (Underwood et al. 1995, Barnes 2000).

There is the unresolved question whether overproduction of ROS after inhalation of higher oxygen concentration is able to potentiate primary tissue damage originating from basic serious lung pathologic changes in men and animals.

The experimental evidence suggests that cough reflex intensity or cough reflex sensitivity became significantly changed in experimental animals with airway inflammation (Haniáček 1986) as well as in patients suffering from different airway and lung diseases (Pěcová et al. 1999, Doherty et al. 2000).

On the basis of the available information we can hypothesize that oxygen breathing by patients suffering from advanced airway and lung pathology may potentiate lung tissues damage arising from primarily pathological processes. Combination of lung tissue injury and lung exposition to high oxygen concentration, two damaging factors enhancing reactive oxygen species generation, should probably potentiate the damaging influence to lung tissues including nerve endings localized in the airway mucosa, which are responsible for production and modulation of cough reflex. We suppose that the effect of airway damage due to primary airway disease alone on cough reflex intensity will be different when compared with the effect caused by combination of primary airway damage and hyperoxia.

The purpose of this study was to test the mentioned supposition in guinea pigs with allergic airway inflammation and exposed to 100 % or 50 % oxygen and the animals of the second group (8 animals) breathed ambient air under the same conditions as the first group (control group).

Model of experimental allergic inflammation of airways

In the first phase of our experiment, we induced experimental allergic inflammation in all animals using the method described by Underwood et al. (1995). All animals were sensitized with ovalbumin (10 µg) intraperitoneally administered together with aluminium hydroxide (100 mg) in saline (1 ml i.p.). Twenty-one days later, successful sensitization was confirmed by the intradermal injection of ovalbumin (25 µl of 200 µg.ml⁻¹) into the skin of the back. Animals that did not respond with marked erythema and edema (maximal 20-40 min after injection) were excluded from the study. Sensitized animals were used 7 days later for experiments aimed to ascertain if the airway allergic reaction is present. Guinea pigs were placed into a plastic chamber and were exposed to aerosol of ovalbumin (10 mg.ml⁻¹) generated by a nebulizer (Pari Inhalierboy, Pari-Werk, GmbH, mass median diameter 4.8 µm). The aerosol was introduced into the chamber by an air pump. To avoid an anaphylactic reaction of sensitized animals, we administered Diphenylhydramin (Sigma; 0.2 ml i.p. of 80 mg.ml⁻³) 30 min before the challenge. Exposure to ovalbumin lasted about 15 min until clear signs of difficult breathing were present. If present, airway allergic reaction was taken as confirmed.

Exposure to oxygen/air

Twenty-four hours after confirmation of the airway allergic reaction we continued with the second phase of the experiment. Animals of the experimental group were exposed to 100 % or 50 % oxygen for 60 h continuously and control sensitized animals were exposed to ambient air.

The exposures of animals to oxygen or air, were performed in individual glass metabolic cages. Biophysical parameters of the cage environment were maintained at the following level: temperature 23-25 °C, humidity 60-70 %, concentration of CO₂ ≅ 0.2 vol %, concentration of O₂ in hyperoxic cages ≅ 100 % or 50 %, concentration of O₂ in cages with room air ≅ 21 %.

Chemically-induced cough

Awake guinea pigs were placed individually into a plethysmograph box (type 855, Hugo Sachs Elektronic) and exposed to aerosol of citric acid (Lachema, 0.3 mol.l⁻¹) for 2 min to elicit coughing. The citric acid
aerosol (mass median diameter 1.2 µm) was generated by a jet nebulizer (Pariprovocation test I, Pari Starneberg) and delivered to the head part of the plethysmograph box. The cough was distinguished on the basis of plethysmograph airflow changes induced by chest movement and measured by a Fleish pneumotachograph head. The airflow was registered by Multiscriptor Hellige 21 and in PC using an analog-to-digital converter. We counted the number of coughs from the airflow trace on the basis of sudden enhancement of expiratory airflow and accompanied by typical cough sound present during 2 min of exposition and 1 min after the end of exposure. The cough reflex was elicited before sensitization and before exposure to oxygen, then at 24 h and 60 h of exposure to oxygen/air and the number of cough expiratory efforts was used to quantify the intensity of cough reaction.

Mechanically-induced cough
Just after the end of exposure to hyperoxia/air and the end of chemically induced cough the animals were anesthetized (Urethane, 1 g/kg, i.p.) and tracheostomy was performed. Cough was immediately induced by mechanical stimulation (nylon fiber) of laryngopharyngeal (LPh) and tracheobronchial (TBr) mucosa during 5 seconds (Korpáš 1970). The number of coughs was then counted from the trace of interpleural pressure registered by an electromanometer using a pleural cannula.

Other measured parameters
The respiratory rate in all animals was counted by visual observation of chest movements at two-hour intervals during the whole oxygen (air) exposition time with the exception of night hours.

After mechanically induced cough had been discontinued, the thorax cavity was opened in anesthetized animals and the reactivity of airway smooth muscles and pulmonary strips to histamine was investigated in vitro. The amplitude of contraction (in mN) on cumulative doses of histamine (10^-8 - 10^-3 mol/l.) was used for evaluation of smooth muscle reactivity (Strapková et al. 1995). At the end of the experiment, anesthetized animals were killed by sectioning of the aorta and samples of trachea, bronchi and lungs were removed, fixed in 10 % formalin solution and subsequently embedded in paraffin. A transverse section was cut and stained with hematoxylin and eosin. Histopathological assessment was performed by light microscopy.

Statistical analysis
The numbers of cough efforts are expressed as the median and interquartile range. The data of respiratory rate and in vitro reactivity are expressed as mean values ± S.E.M. Statistical analysis was performed using Mann-Whitney U test for non-parametric data, Student’s t-test for unpaired data and the p<0.05 value was considered as significant.

Results
Differences in cough reflex intensity in sensitized animals exposed to 100 % oxygen or ambient air
We have found that the intensity of the citric acid cough in sensitized animals breathing 100 % oxygen or air for 24 h did not significantly differ [6(4.5-9) vs 5(1.5-7.5), p=0.32; Fig. 1A]. The results obtained on 60 h of exposure to 100 % O₂ or air have shown that the intensity of citric acid cough in animals exposed to 100 %
$O_2$ was significantly lower compared to the control animals [8(6 – 10) vs 2(2-4), $p<0.05$; Fig. 1A].

![Graph A](image1)

**Fig. 1.** The effector response of afferent nerve terminals in an ovalbumin-sensitized guinea pig. The number of histamine-induced sneezes was significantly higher in the experimental group compared to the control group ($p<0.05$).

Using the same groups of animals we did not find significant differences in the number of coughs induced by mechanical stimulation of airways (LPh and TBr) between animals exposed to 100% $O_2$ or air (Fig. 2A).

Differences in respiratory rate were found in animals exposed to 100% $O_2$ or air. A significant decrease in the number of breaths/min in animals exposed to oxygen was present during two periods: between 18th and 24th hour of exposure ($p<0.05$) and between 36th and 60th hour ($p<0.01$) when compared with control animals breathing air (Fig. 3A).

The reactivity of airway smooth muscles to application of cumulative doses of histamine in sensitized animals exposed to 100% $O_2$ has been significantly changed compared with the controls. In animals exposed to 100% $O_2$ there was a significant gradual increase in the in vitro reactivity of tracheal smooth muscles at concentrations $10^{-5}$ to $10^{-3}$ mol.l$^{-1}$ of histamine (Fig. 4A). Conversely, we found a significant decrease in the reactivity of lung smooth muscles to cumulative doses of histamine in the same animals (Fig. 4B).

![Graph B](image2)

**Fig. 2.** The effect of 60 h exposure to 100% oxygen/air (A) or 50% oxygen/air on mechanically induced cough in sensitized guinea pigs [median and interquartile range]. OA – ovalbumin, LPh – laryngopharyngeal cough, TBr – tracheobronchial cough.

**Fig. 3.** Changes in respiratory rate (RR/min) in sensitized guinea pigs during exposure to 100% oxygen (A) or 50% oxygen (B) monitored in two-hours interval except night hours. Data are expressed as means.

Histological examination of samples taken from the trachea, bronchi and the lung revealed some pathological changes including hyperplasia of the tracheal epithelium, acute or chronic inflammation of tracheal mucosa with marks of exacerbation in both groups of animals. Acute inflammation of bronchial mucosa was only observed in some cases. Other pathological changes were found in the lung tissue, e.g. emphysema, atelectasis, inflammation with aggregates of lymphocytes and hyperplasia of vessels. We did not see any marked differences in the intensity and quality of morphological changes between animals of the control (sensitized animals, only) and experimental group (sensitized animals exposed to hyperoxia).
Fig. 4. The effect of exposure of sensitized guinea pigs to 100 % oxygen for 60 h on reactivity of tracheal (A) and lung strips (B) of smooth muscles to cumulative doses of histamine ($10^{-8}$-$10^{-3}$ mol.l$^{-1}$). Data are expressed as means ± S.E.M. (*p < 0.05, **p < 0.01, ***p < 0.001), OA – ovalbumin.

Fig. 5. The effect of exposure of sensitized guinea pigs to 50 % oxygen for 60 h on reactivity of tracheal (A) and lung strips (B) of smooth muscles to cumulative doses of histamine ($10^{-8}$-$10^{-3}$ mol.l$^{-1}$). Data are expressed as means ± S.E.M. OA – ovalbumin.

Differences in cough reflex intensity in sensitized animals exposed to 50 % O$_2$ or air

The experimental data revealed no significant differences in citric acid cough intensity between groups of animals with allergic airway inflammation exposed to 50 % oxygen or to ambient air for 24 h and 60 h (Fig. 1B).

Similar results were found in mechanically induced cough from LPh and TBr mucosa (Fig. 2B). No significant differences were found between the two groups of animals in respiratory rate of both groups of guinea pigs during exposure to 50 % O$_2$ or air (Fig. 3B).

We did not find significant changes in reactivity of airway and lung tissue between sensitized animals exposed to 50 % O$_2$ or ambient air (Fig. 5A, B).

Light microscopy did not reveal any substantial quantitative or qualitative differences in pathological changes of the airways and lungs in sensitized animals exposed to 50 % O$_2$ or air.

Discussion

The aim of our experiment was to test the assumption that the effect of airway damage due to airway disease alone on the cough reflex will be different to that caused by combination of airway disease and hyperoxia. As a model of airway damage, we used allergic airway inflammation described by Underwood et al. (1995). We confirmed the high reliability of this method for the induction of allergic airway inflammation.
in guinea pigs. Both functional tests and histological examination showed clear signs of airway mucosa and/or lung tissue inflammation.

In our previous experiments (Hanáček et al. 1996a), we showed that long-term exposure of healthy cats to 100 % oxygen inhibits the cough provoked by mechanical stimulation of upper airway mucosa. Similar inhibitory effect of 100 % oxygen breathing for 60 h was observed on chemically induced cough in healthy guinea pigs in another experiment (Hanáček et al. 1996b). These results suggested that high oxygen concentration breathing inhibits the cough reflex intensity (CRI).

The experiments performed by Karlsson et al. (1992) and Tatár et al. (1994) have shown that CRI did not change in guinea pigs sensitized both by *Ascaris suum* or ovalbumin, compared with non-sensitized animals. We obtained confirmatory results when we compared CRI induced by citric acid in healthy (Hanáček et al. 1996b) and sensitized (present experiment) unanesthetized guinea pigs. These results are contradictory to our earlier results obtained in experiments on cats with experimental inflammation of the airway mucosa (Hanáček 1986). Under the above mentioned conditions, the CRI was significantly changed when compared with healthy animals. We suggest that the influence of damaged airway mucosa on CRI rather depends on its intensity, cause, duration and animal species. From this we can hypothesize that the intensity of allergic airway inflammation in guinea pigs used in our present experiments was not strong enough to influence significantly the nerve endings responsible for cough reflex induction. Histological findings are in accordance with this supposition, because we were not able to find signs of serious injury of airway mucosa in guinea pigs, whilst such signs were present in experiments on cats (Hanáček 1986).

It is generally known that hyperoxia has a number of adverse effects on the respiratory system (Clark and Lambertsen 1971, Frank and Massaro 1979, Crapo 1985, Klein 1990, Jenkinson 1993). These effects are ascribed to ROS, which are overproduced during hyperoxia. It has been shown that exposure to oxidants affects immunological lung defense mechanisms, as demonstrated both by a greater susceptibility to infection and by altered responses of the immune system (Chitano et al. 1995). With the exception of a few papers from our department, there is no information about the relation between hyperoxia and defensive respiratory reflexes. There is no available information related to function of defensive respiratory reflexes under the condition when airway mucosa is injured and high oxygen concentrations are inhaled simultaneously.

The present experiment has shown that a combination of allergic airway inflammation and hyperoxia lasting 24 h does not change the intensity of citric acid cough when compared with control animals. When the exposition to 100 % oxygen was extended to 60 h, the significant inhibition of CRI was present. These results are very similar to those obtained in nonsensitized guinea pigs exposed to hyperoxia (Hanáček et al. 1996b). It seems that hyperoxia plays a crucial role in the inhibitory effect on the cough reflex at different intensity of airway injury.

The results showed important differences between the influence of allergic airway inflammation plus hyperoxia on chemically and mechanically induced cough in guinea pigs. The basic difference in the cough response is affected by using different tussigenic substances – mechanical and chemical ones. While chemically induced cough was evoked in awake animals, mechanical stimulation was performed on anesthetized animals. The aim of the present paper was to find out if there are significant differences in cough reflex intensity (induced mechanically or chemically) between group of animals with allergic airway inflammation breathing room air and group of animals with allergic inflammation breathing 50 % O₂ or 100 % O₂. We did not compare the influence of these factors on cough reflex intensity induced mechanically or chemically, because of unsurpassed difference in the method employed. Our results indicate that while the citric acid cough was inhibited after 60 h exposure to 100 % oxygen, the mechanically induced cough was not. We can only hypothesize as to why this is so. In the classical paper devoted to airway receptors (Widdicombe 1954) it was stated that large airways predominantly react to mechanical stimuli, whilst small airway mucosa reacts rather to chemical stimuli. In our experiment the mechanical stimulus inducing cough was applied to the mucosa of trachea and large bronchi, whilst citric acid affected the whole surface of airways. Taken such information into account we can suppose that 100 % oxygen and/or allergic airway inflammation influenced more the “cough receptors” localized in small than in large airways.

An important result of our experiment concerns the fact that exposure of animals with allergic airway inflammation to 50 % oxygen for up to 60 h did not significantly change CRI induced chemically or mechanically. We obtained similar results in our previous
experiment performed on healthy guinea pigs (Brozmanová et al. 1996). From these data we can suggest that exposure of guinea pigs to 50 % oxygen does not influence the airway “cough receptors” irrespective of whether the airways are damaged by inflammation or not. One possible explanation for such a result may be the low intensity of airway mucosa injury caused by allergic airway inflammation and hyperoxia so that the mucosal antioxidant capacity need not be significantly damaged. Airway antioxidant mechanisms were thus able to cope with increased production of ROS due to 50 % hyperoxia.

One can suppose that inhibition of citric acid cough in guinea pigs exposed to 100 % O₂ for 60 h may arise from a direct inhibitory effect of hyperoxia on the respiratory center. The changes in respiratory rate support this view, but we do not propose that direct inhibition of the respiratory center is responsible for the mentioned phenomenon. Why? Firstly, during induction of cough, the animals were not in hyperoxic atmosphere and they breathed ambient air. Secondly, the respiratory rate of animals during induction of cough was quite similar to pre-experimental values. Thirdly, the mechanically induced cough was not changed. We also think that there is no reason to assume that citric acid cough can be influenced by tachyphylaxis because the experimental protocol ruled out this possibility.

A decrease in respiratory rate (RR) in guinea pigs exposed to oxygen at partial pressures 1 atm or less for a prolonged period was described by Binet and Bochet (1951). Labored breathing was also associated with change in RR in the above experiment. In our experiment, we observed a significant decrease of RR in animals exposed to 100 % oxygen during the period between 18th and 24th hour of exposure (p<0.05). RR decreased more progressively during oxygen exposure period between 36th and 60th hour of exposure time (p<0.01) from the control value of about 86 breaths/min to as low as 59 breaths/min at the end of exposure time. In contrast, we observed no differences in RR between sensitized animals exposed to 50 % oxygen and sensitized animals breathing air. The inhibitory effect of 100 % oxygen breathing on RR can partly be ascribed to decreased chemoreceptor activity and decreased afferent stimulating influence on respiratory center. The other mechanisms (e.g. changes in lung compliance, obstruction of small airways) could also participate.

In contrast to 50 % O₂, we have found the interesting differences in the reactivity of airways and lung tissue to histamine in sensitized animals exposed to 100 % O₂. We confirmed the expected increase in reactivity of tracheal smooth muscles in agreement with the data obtained by Karlsson et al. (1992) and Underwood et al. (1995). It was surprising for us that the reactivity of lung tissue was significantly lower in animals with allergic airway inflammation and breathing 100 % O₂. We suppose that differences between the reactivity of lung and tracheal strips of treated and control animals can be ascribed to different level of antioxidants in tracheal mucosa and mucosa of more peripheral airways. This assumption is based on the finding of Putman et al. (1997) that epithelial fluid in the upper airways containing surfactant and antioxidants is thick and contains high levels of antioxidants. Therefore oxidant injury is more common in the lower airways where the epithelial lining fluid is thin and contains fewer antioxidants.

In conclusion, we have found that combination of allergic airway inflammation and exposition to 100 % O₂ for 60 h lead to a significant decrease in citric acid-induced cough and of the respiratory rate. Many questions concerning the mechanism involved in oxygen pulmonary toxicity and influence of hyperoxia on the cough reflex remain to be elucidated. A provocative question arising from our observations concerns the problems whether patients suffering from COPD treated with long-term oxygen therapy do have a suppressed cough reflex similarly to laboratory animals.

References


Reprint requests
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