Protective effects of volatile agents against acetylcholine-induced bronchoconstriction in isolated perfused rat lungs

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Background: Bronchoactive properties of volatile agents against lung constriction are well established. The purpose of this study was to investigate the ability of halothane (Hal), isoflurane (Iso), sevoflurane (Sev) and desflurane (Des) to alter the lung mechanics in the absence of an airway tone and during acetylcholine (Ach)-induced bronchoconstriction.

Methods: Low-frequency pulmonary impedance data ($Z_L$) were collected from isolated, normo-perfused rat lungs under baseline conditions and following the injection of Ach (0.1 mg/kg) into the pulmonary artery. Measurements were performed without the administration of any anaesthetic agent in the first phase of the experiments and during inhalation without any volatile agent (control group, $n = 6$) or during inhalation of Hal ($n = 6$), Iso ($n = 9$), Sev ($n = 6$) or Des ($n = 8$) at 1 minimum alveolar concentration (MAC). The airway resistance ($R_{aw}$) and parenchymal damping and elastance were estimated from the $Z_L$ data by model fitting.

Results: Under baseline conditions, the basic value of $R_{aw}$ was significantly decreased by Des ($–31.2 \pm 3.8\%$) and Sev ($–18.0 \pm 4.5\%$) administration, whereas Hal and Iso did not have a statistically significant effect on $R_{aw}$ ($–3.3 \pm 5.1\%$ and $–8.6 \pm 2.4\%$, respectively). Moreover, all four inhalation anaesthetics prevented the increase in $R_{aw}$ following Ach administration, the findings ranging between $–14.3 \pm 11.4\%$ for Hal and $–37.5 \pm 10.9\%$ for Sev.

Conclusions: Our results on a denervated isolated perfused lung model demonstrate the potential of Des and Sev to decrease the basal airway tone, whereas Iso and Hal are ineffective in this regard. All of these volatile agents markedly protect against Ach-induced bronchoconstriction.

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It is well established that volatile anaesthetic agents exert bronchoactive properties under clinical conditions and in experimental settings. There is a consensus in the literature that isoflurane and sevoflurane are at least as potent bronchodilators as halothane (1–4). In contrast, the data on desflurane are conflicting, with this agent being reported to exert a relaxing effect against bronchoconstriction (2, 5–8), to have no effect on the increased airway tone or to irritate the airways under clinical conditions (9, 10) and to worsen the resistance of the respiratory system (11).

The complex mechanisms involving multiple routes of action of volatile anaesthetic agents on lung mechanics may contribute to these controversial results. The relaxation effects of inhalation agents on the airway smooth muscle tone are attributed to hyperpolarization of the smooth muscle cell membrane, which results in a decreased transmembranous calcium inflow, and hence a lower intracellular calcium level (12, 13). In addition to this direct effect, airway tone is modulated by the autonomic nervous system, which is influenced by volatile agents (8, 14, 15). Finally, the mechanical properties of the lungs may also be affected by the altered pulmonary haemodynamics observed following the administration of inhalation agents (16). However, inhalation agents differ in their ability to express their bronchoactive properties via these pathways. Isoflurane and sevoflurane have been shown to exert the most potent effects in relaxation of the airway smooth muscle (17, 18). Conversely, desflurane stimulates the autonomic nervous system, resulting in the activation of bronchial smooth muscle, mainly via a reflex mechanism (18, 19).
In a previous study, we investigated the protective effects of all volatile anaesthetic agents against the bronchoconstriction induced by an intravenous infusion of methacholine to rats under in vivo conditions (2), in which all of the pathways described above are intact. The results of that study revealed that isoflurane, sevoflurane and desflurane were as effective as halothane in protecting against methacholine-induced airway constriction (2). Nevertheless, the respective contributions of the direct and indirect pathways to these results need to be clarified. Accordingly, we set out in the present study to characterize the bronchoactive properties of the volatile agents against airway constriction induced in the absence of neural control of the tracheobronchial tree. To exclude the neural and haemodynamic effects of the volatile agents, we designed our experiments on isolated perfused rat lungs. This setting allows the separate assessment of the airway and parenchymal mechanics is possible by the application of forced oscillations at low frequencies (2, 11, 20).

**Methods**

*Preparation of animals for isolated lung harvesting*

After approval had been obtained from the Animal Care Committee of the Canton of Geneva, 35 adult male Sprague-Dawley rats [mean body weight ± standard error (SE), 373 ± 4.17 g] were anaesthetized with 50 mg/kg pentobarbital (Nembutal) intraperitoneally. The rats were then tracheotomized with a polyethylene cannula (14-gauge, Braun, Melsungen, Germany) using sterile techniques and normoventilated mechanically with a tidal volume of 7 ml/kg body weight. A positive end-expiratory pressure of 2.5 cmH₂O was applied, together with a respiratory rate of 70–80 breaths/min with a constant volume-cycled rodent ventilator (model 683, Harvard Apparatus Co. Inc., South Natick, MA, USA). After induction, anaesthesia was maintained with intravenous injections of pentobarbital when required (10 mg/kg). Analgesia was provided by the continuous infusion of fentanyl at 2 μg/kg/h. In one group of rats, the induction and maintenance of anaesthesia were achieved with Nembutal (50 mg/kg intraperitoneally for induction and 10 mg/kg intravenously for maintenance); these animals served as a control group without the application of volatile anaesthetics.

Respiratory gases were monitored continuously with a Datex monitor (Ultima™, Datex/Instrumentarium, Helsinki, Finland), and the airway pressure was also measured continuously using a calibrated pressure transducer (Validyne DP 45 and Validyne model 2D15 carrier demodulator, Northridge, CA, USA).

The femoral vessels were cannulated (28-gauge catheter, Portex, Hythe, Kent, UK) for blood sampling and continuous arterial blood pressure monitoring using a calibrated pressure transducer (Honeywell, model 156-PC 06-GW2, Zurich, Switzerland). The rats were fully anticoagulated with 1.5 IU/g body weight heparin injected intravenously. Twenty-five millilitres of blood was then gently withdrawn over 5 min via the arterial cannula, the collected blood being replaced continuously by the intravenous infusion of a 6% colloid solution of hydroxyethyl-starch at a constant rate in order to maintain a constant intravascular and systemic blood pressure above 50 mmHg, so as to minimize lung ischaemic lesions during this normovolaemic haemodilution procedure. The collected diluted blood was centrifuged (1800 g for 10 min), and 7 ml of plasma was extracted. The resulting concentrated blood, with a haematocrit of around 30%, served as priming volume for the isolated perfusion circuit. A midline sternotomy was performed next; the chest was widely retracted, and a heart–lung block was prepared, as described in detail previously (21).

*Procedure and continuous monitoring of the isolated lung model*

The heart–lung block was placed in a thermostatically controlled and humidified Plexiglas chamber, as detailed previously (21). Lung perfusion was performed from a perfusion reservoir initially at a pulmonary artery perfusion pressure (Pₚₐ) of 15 mmHg. The distal extremity of the left ventricular outflow cannula was placed at a sufficient height to obtain a left atrial pressure (Pₐₐ) of 7.5 ± 2 mmHg at the beginning of reperfusion, which resulted in West zone 3 conditions (Pₚₐ > Pₐₐ > mean Pₐᵥ). The blood dripping from this cannula was collected in a 5-ml collection cylinder, and aspirated from this reservoir with polyethylene tubing passing through a roller pump (Ismatec Pump, Glattburg, Zurich, Switzerland). The priming volume of the tubing and reservoirs was 18 ml. A transit-time flowmeter (T-201 CDS, Transonic Systems Inc., Ithaca, NY, USA) was placed between the perfusion reservoir and the catheter cannulating the main pulmonary artery for continuous monitoring of the pulmonary blood flow (Qₚ). The mean Pₚₐ and Pₐₐ values were measured continuously using calibrated pressure transducers (Honeywell, model 156-PC 06-GW2) zeroed at the
level of the lung hilus. The pulmonary vascular resistance \( R_L \) was calculated by dividing the pressure drop on the pulmonary vasculature by \( Q_p \).

The airflow, tidal volume, pressures and circuit flow were recorded and stored at a sampling rate of 50 Hz via an analogue-to-digital interface converter (Biopac, Santa Barbara, CA, USA). The perfusate temperature and pH were measured with a pH meter (691 pH meter, Metrohm, Herisau, Switzerland), or the pH was determined on repeated blood gas samples. It was maintained between pH 7.35 and 7.45 and, if necessary, was corrected with sodium bicarbonate or a change in inspired CO\(_2\) as required by the blood gas analysis (model 505, Acid Base Laboratory, Copenhagen, Denmark). Steady-state gas exchange was confirmed by stable \( P_{O_2} \), \( P_{CO_2} \) and haematocrit levels during the experiments.

**Measurement of airway and parenchymal mechanics**

The respective contributions of airway and tissue mechanical properties to the total lung resistance were estimated by measuring the forced oscillatory pulmonary input impedance \( Z_L \) *in vitro* (isolated perfused rat lung), as described in detail previously (22). This technique was specially designed for the measurement of the forced oscillatory input impedance of small animals without a need to estimate the oscillatory flow (23). Briefly, a three-way tap was used to switch the tracheal cannula from the respirator to a loudspeaker-in-box system at end-expiration. Before each measurement, the pressure in the box chambers was adjusted to 2.5 cmH\(_2\)O to keep the transpulmonary pressure constant during the measurements. The loudspeaker delivered a computer-generated, small-amplitude pseudorandom signal with frequency components between 0.5 and 20.75 Hz through a polyethylene wave-tube with known geometry (polyethylene tube: length, 100 cm; inside diameter, 2 mm). The wave-tube was equipped with side-arms and miniature identical transducers (ICS model 33NA002D, ICSensors, Malpitas, CA, USA) to measure the lateral pressures at the loudspeaker end \( P_1 \) and the cannula end \( P_2 \).

The pressure transfer functions (i.e. \( P_1/P_2 \)) were computed by fast Fourier transformation from the 6-s recordings using 4-s time windows and 95% overlapping. According to transmission line theory, \( Z_L \) can be calculated from the \( P_1/P_2 \) spectra as the load impedance of the wave-tube:

\[
Z_L = Z_0 \frac{\sinh(\gamma L)}{[(P_1/P_2) - \cosh(\gamma L)]}
\]

where \( L \) is the length, \( Z_0 \) is the characteristic impedance and \( \gamma \) is the complex propagation wavenumber of the wave-tube. The last two parameters were determined by the geometrical data and the material constants of the tube wall and the air.

**Separation of airway and parenchymal parameters**

To separate the airway and lung tissue mechanics, a model containing a frequency-independent airway resistance \( R_{aw} \) and inductance \( L_{aw} \) in series with a constant-phase tissue model (20), including parenchymal damping \( G \) and elastance \( H \), was fitted to the \( Z_L \) spectra by minimizing the relative differences between the measured and modelled impedance values:

\[
Z_L = R_{aw} + j\omega L_{aw} + (G - jH/\omega)^2
\]

where \( j \) is the imaginary unit, \( \omega \) is the angular frequency \((2\pi f)\) and \( \alpha = 2/\pi \text{ arctan}(H/G) \).

In all groups, the measurement of lung mechanics was started after steady-state haemodynamic conditions had been established.

**Experimental protocol**

Rats were randomly assigned to one of the following five protocol groups: group C (control group, \( n = 6 \)), no volatile anaesthetic was administered; group H (\( n = 6 \)), halothane; group I (\( n = 9 \)), isoflurane; group S (\( n = 6 \)), sevoflurane; group D (\( n = 8 \)), desflurane.

After the start of perfusion of the isolated rat lung, a period of 20–30 min was allowed for the pulmonary and haemodynamic variables to reach steady-state conditions and for the preparation to become isogravimetric. Before administration of the constrictor agent, the lungs were hyperinflated by superimposing two inspiratory cycles to standardize the volume history. After four to six successive baseline \( Z_L \) recordings, the concentration of acetylcholine (Ach) in the blood container supporting the pulmonary artery was doubled from 100 to 200 \( \mu \)g/kg, and \( Z_L \) was then recorded at 15 s.

When the end-tidal concentration of the volatile agent reached 1 minimum alveolar concentration (MAC) [1% for halothane (24), 1.4% for isoflurane (24), 2.4% for sevoflurane (25) and 6.9% for desflurane (26)] and stable haemodynamic conditions had been established, \( Z_L \) measurements were performed to obtain new baseline data, and the Ach challenge was repeated. The concentrations of \( O_2 \), \( CO_2 \) and the volatile agent were monitored throughout the study (Ultima\™, Datex/Instrumentarium).
**Statistical evaluation**

Scatter in the parameters was expressed as SE values. The Kolmogorov–Smirnov test was used to test data for normality. One-way analysis of variance (ANOVA) was applied to compare the mechanical parameters between the independent protocol groups involving different volatile anaesthetics. Within the protocol groups, repeated measures of ANOVA was used to assess the effects of Ach responses. The Student–Newman–Keuls multiple comparison procedure based on the means was applied to compare the protocol groups (for independent groups) or the different conditions (control vs. Ach for repeated measures). Statistical tests were performed with a significance level of $P < 0.05$.

**Results**

Figure 1 shows the percentage changes in the basic airway and lung tissue mechanical parameters in all groups of isolated perfused rat lungs. The basic airway tone was markedly and statistically significantly decreased by desflurane (−31.2 ± 3.8% change in $R_{aw}$) and sevoflurane (−18.0 ± 4.5% change in $R_{aw}$) administration, whereas halothane and isoflurane did not have a statistically significant effect on $R_{aw}$ (−3.3 ± 5.1% and −8.6 ± 2.4%, respectively). None of the volatile agents exerted a significant effect on the baseline levels of the parenchymal mechanical parameters $G$ and $H$.

Figure 2 illustrates the results of airway and parenchymal mechanical parameters under the control conditions (C1 and C2) and following the administration of the two different concentrations of Ach. In all groups, Ach induced statistically significant increases in $R_{aw}$ and $G$ as compared with the control conditions. Further, comparison of the Ach-induced increases in $R_{aw}$ in the protocol groups revealed that sevoflurane and desflurane markedly and significantly inhibited the Ach-induced bronchoconstriction. Desflurane displayed the strongest effect; sevoflurane afforded less, but still significant, protection; the protective potentials of isoflurane and halothane did not reach the level of statistical significance. With regard to the lung parenchymal parameters, the Ach-induced elevations in $G$ were slightly attenuated by all of the volatile anaesthetics; these minor effects were not statistically significant. In agreement with previous findings following the intravenous administration of methacholine *in vivo* (2, 23), the values of $H$ remained at the baseline level throughout the study protocol.

**Discussion**

The relaxation properties of the volatile anaesthetic agents applied commonly in clinical practice were compared in the present study in an isolated perfused rat lung model. This experimental setting, combined with a low-frequency forced oscillation technique, allows a separate assessment of the airway and
Parenchymal mechanics in the absence of neural control of the lungs and without the confounding influence of the alterations in pulmonary hemodynamics induced by bronchoactive drug administration. The current experiments revealed the potential of desflurane and sevoflurane to decrease the basal airway tone, whereas isoflurane and halothane were ineffective in this regard. In the presence of an increased airway tone induced by the administration of Ach into the pulmonary circulation, all of the currently used volatile agents exerted a marked protective effect against bronchoconstriction. The efficacy of this bronchoprotective potential of the volatile agents was not affected by the magnitude of the constrictor stimuli. The Ach-induced increases in the lung tissue parameter related to parenchymal damping were not prevented by any of the volatile anaesthetics applied in the current investigations.

Methodological considerations

Many previous studies have focused on the cellular mechanisms of action of volatile anaesthetics on airway smooth muscle cell cultures (12, 13, 17, 27–29) or isolated tracheal and/or bronchial rings (5–8, 30, 31). The structural integrity of the lungs has been lost in such investigations, which makes it difficult to extrapolate these findings to an organ level. The results obtained under in vivo experimental conditions, however, are influenced by numerous confounding effects of systemic hormonal and neurogenic origin. The isolated ex vivo rat lung model, applied in the present study, provides an ideal system for the investigation of the effects of volatile anaesthetics on the airway tone under baseline conditions and in the presence of airway constriction at an organ level. Furthermore, excised lungs are an ideal model in which to investigate the direct effects of bronchoactive agonists in the absence of reflex mechanisms involving neural pathways and without the biasing effects of acutely released humoral mediators.

Many of the procedures used in anaesthetic practice may involve direct stimulation of the muscarinic receptors (via mechanical stimulation of the airways, i.e. tracheal intubation, bronchoscopy or bronchial suction), resulting in cholinergic-mediated bronchospasm. In previous experiments performed by our research group (2, 11, 23), lung constriction was induced with methacholine to mimic vagally induced changes in lung function. As Ach acts as a physiological ligand on the muscarinic receptors under these conditions, in the present study, adverse changes in lung function were induced by generating a spastic condition following a stimulation of the muscarinic receptors by Ach.

As most previous studies have applied global parameters to express the lung responses to volatile anaesthetic agents (1, 3, 4, 32, 33), the relative contributions of the airways and the lung parenchyma to the lung response to Ach-induced bronchospasm cannot be distinguished. In the present study, the low-frequency forced oscillatory technique and the model-based evaluation of the impedance data were applied to separate the airway and the lung parenchymal mechanical properties. This technique has been validated (23, 34) and used successfully in small rodents (2, 22, 23) and in isolated perfused rat lungs.

Fig. 2. Parameters of resistance ($R_{aw}$), tissue damping coefficient ($G$) and tissue elastance ($H$) under baseline conditions (C1 and C2) and following the administration of two different concentrations of acetylcholine (Ach) (Ach1, acetylcholine dose of 100 μg/kg; Ach2, acetylcholine dose of 200 μg/kg) in all experimental groups. C, control group; D, desflurane group; H, halothane group; I, isoflurane group; S, sevoflurane group. *P < 0.05 compared with control group.
(21, 22), and has been shown to supply selective parameters for the airway and parenchymal mechanics, and to allow reliable quantification of the changes in these compartments. The airway and parenchymal mechanical parameters under baseline conditions are in excellent agreement with those obtained previously (21, 22).

Effects of volatile anaesthetics
A number of previous in vivo experimental and clinical studies have described the influence of the commonly used volatile anaesthetics on the bronchial smooth muscle tone. Conflicting data have been reported concerning the ability of desflurane to affect the airway tone. Most of the clinical studies have indicated a high incidence of airway irritation during the administration of desflurane (coughing, breath-holding, excessive secretion and laryngospasm) (10, 14, 35), and a similar potency was observed under experimental conditions (11). In contrast, in a few clinical (36) and experimental (2) studies, desflurane exhibited a marked bronchodilator potential. The reasons for these controversial findings have not been fully elucidated. It is noteworthy, however, that in vitro studies have consistently demonstrated the bronchodilator effect of desflurane (5–8, 31), as for other volatile anaesthetics (5–8, 30, 31, 33). Analogous to our findings, desflurane and sevoflurane have been shown to exert more potent bronchodilator effects than those of halothane in isolated airway smooth muscle (5, 8) and in isolated tracheal rings (31). In contrast, other ex vivo studies have indicated that halothane has a greater relaxing effect on the airway smooth muscle than sevoflurane, isoflurane or desflurane (6, 28). Although the reasons for these conflicting results are not totally clear, all of these previous in vitro studies consistently demonstrated the bronchodilator potential of desflurane. The observations in the present study confirm the marked protective properties of desflurane against the airway constriction induced by cholinergic stimulation of the muscarinic receptors. Our observation that desflurane prevents Ach-induced bronchoconstriction may suggest that the humoral or neural pathways present in vivo are responsible for the lower protective potential of desflurane against airway constriction.

In the present study, Ach induced marked increases in $R_{aw}$ and $G$, but had no significant effects on $H$. This pattern of change in the lung mechanical parameters indicates that Ach induces heterogeneous airway constriction with marked ventilation heterogeneities, this phenomenon giving rise to changes in $G$ rather than altered intrinsic parenchymal mechanics (23, 34). We observed the potential of the volatile anaesthetics to prevent increases in $R_{aw}$ whereas they were all ineffective in inhibiting the Ach-induced increases in $G$. The inability of the volatile agents to prevent the increases in $G$ is in contrast with previous findings in vivo (2, 32). The fundamental difference in the site of action of the cholinergic stimulation may explain this controversy. Although the muscarinic receptors are stimulated in the whole lung during intravenous challenges under in vivo conditions, Ach administered into the pulmonary circulation in isolated perfused lungs reaches primarily the receptors located in the lung periphery, leading to a heterogeneous constriction of more distal airways. Under these conditions, the volatile agents are able to prevent the overall airway constriction, but the stability of $G$ indicates that they are not effective in reducing the ventilation heterogeneities.

Conclusions
Our results reveal the potential of desflurane and sevoflurane to decrease the basal airway smooth muscle tone, whereas isoflurane and halothane are ineffective in this regard. All the currently used volatile agents exert a marked protective effect against Ach-induced bronchoconstriction, desflurane and sevoflurane having the most potent inhibitory effect. These findings provide evidence that desflurane exerts relaxation activity on the airway smooth muscle that is similar to or even stronger than the activities of other common volatile anaesthetics at an organ level under in vitro conditions.

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