Changes in mucociliary activity may be used to investigate the airway-irritating potency of volatile anaesthetics

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Summary
We have examined the short-term effects of three volatile anaesthetics, halothane, isoflurane and desflurane, on mucociliary activity in the rabbit maxillary sinus in vivo. Mucociliary activity was recorded photoelectrically and the signal processed by fast Fourier transformation. Administration of 1.0 MAC of halothane, isoflurane or desflurane caused a temporary increase in mucociliary activity, with mean peak responses of 47.8 (SEM 13.0)%, 44.0 (9.6)% and 45.1 (23.7)% (n=6), respectively. The response to all three compounds was biphasic; an initial peak was observed within 2 min and a second peak at 3–6 min. The second response was not significant for halothane. In contrast, desflurane produced a significant second peak while the first was small and failed to reach significance. Halothane displayed an initial peak within 2 min which was blocked by atropine but not by the neurokinin 1 (NK1) receptor antagonist CP-99. The second peak at 3–5 min was less pronounced for halothane than for isoflurane or desflurane. The second peak was not affected by atropine pretreatment, but was blocked by pretreatment with CP-99. A combination of atropine and CP-99 pretreatment abolished the mucociliary response to halothane. Atropine pretreatment did not affect, whereas CP-99 significantly reduced, the response to desflurane. We conclude that the NK1-mediated response was most pronounced for desflurane which is considered the most airway-irritating compound of the three. It is likely that the size of the NK1-mediated response reflects the airway-irritating properties of the volatile anaesthetic used. (Br. J. Anaesth. 1998; 80: 475–480)

Keywords: anaesthetics volatile, halothane; anaesthetics volatile, isoflurane; anaesthetics volatile, desflurane; airway, mucosa; lung, trachea; rabbit

The mucociliary system is an important airway defence mechanism which removes inhaled debris and microorganisms. Impairment during anaesthesia may contribute to postoperative complications, such as pulmonary infection and atelectasis, which are particular problems in smokers.1 2 Previous studies have shown anaesthesia to impair mucociliary function in humans3 and other mammals,4 7 and also in vitro.8 12 Volatile anaesthetics such as halothane, enflurane and isoflurane, and i.v. anaesthetics such as thiopental, may be responsible for reduced mucociliary transport. In contrast with these studies, a recent investigation showed that halothane induced a short-term increase in mucociliary activity in the rabbit maxillary sinus followed by a reversible decrease in mucociliary activity.13 The acceleration was probably part of a mucociliary defence reflex involving release of substance P (SP) from unmyelinated sensory fibres and a cholinergic efferent reflex mechanism.14

Desflurane is a new volatile anaesthetic. It is especially noted for its rapid elimination, but also for its irritating effect on the airways, causing coughing, apnoea, laryngospasm and salivation, thus making it less attractive for induction of anaesthesia.15 Isoflurane is less irritating to the airways than desflurane, but more irritating than halothane.

The main aim of this study was to examine if different airway-irritating properties of anaesthetics are reflected in different responses in mucociliary activity, thus indicating whether or not this animal model would be useful as a tool for estimating the airway-irritating effect of future anaesthetic substances. Furthermore, by using pharmacological blockers, our aim was to study the mechanism responsible for the increase in mucociliary activity after exposure to halothane and desflurane.

Materials and methods
Experiments were performed in male and female adult New Zealand rabbits, weighing 2.2–3.1 kg (mean 2.6 kg). The animals were bred and housed in approved animal facilities, and the study was approved by the Committee of Animal Care and Use, Lund University. The animals were anaesthetized with urethane 2 g kg−1 i.m. as an initial dose, with an extra dose of 0.5 g kg−1 i.v. during operation. The facial artery was explored and a retrograde cannula (size 3 FG, Portex, UK) was inserted with its tip close to the maxillary artery. Details of the anaesthetic and surgical techniques have been published previously.16 An i.v. cannula was inserted into one of the ear veins and perfused with 0.9% saline or 5.5% glucose at approximately 6–12 ml h−1. The mucosa of the maxillary sinus was exposed via a trepanation of approximately 2 × 8 mm, which was covered immediately with a heated window, and sealed to the bone with bone wax (Ethicon, UK).

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Mucociliary activity (visible as flickering light reflections) was observed through a binocular microscope, the criterion of a functionally satisfactory preparation being visible transportation of small particles such as mucus clumps and cell debris. One of the eyepieces was switched to a phototransducer, and mucociliary activity recorded photoelectrically. The mucociliary wave pattern was monitored continuously on an oscilloscope and analysed by fast Fourier transformation (FFT) on a PC (Hewlett-Packard Vectra 486DX2, 66 MHz, France), equipped with an analogue–digital board (DAS 1401, Keithley, Taunton, MA, USA). The software used for FFT analysis was Viewdac (version 2.2.2, Keithley Data Acquisition Division, Taunton, MA, USA). The quality of the signal was assessed by its pattern on the oscilloscope combined with signal amplitude and frequency spectrum, as analysed and displayed by the computer program.

Mucociliary wave frequency was calculated every 10 s during challenge. Induced frequency changes were expressed as percentages of baseline mucociliary wave frequency (zero frequency level) immediately preceding the challenge. Rectal temperature was monitored, and body temperature was maintained at 37–38.5°C using a heating pad. Ventilatory frequency was recorded from the thorax with a tocotransducer (152 78B, Hewlett-Packard, Germany). For pulse registration an ECG was monitored continuously and recorded on file, together with respiratory frequency and mucociliary wave frequency. An open Jackson–Rees anaesthetic system was used with vaporizers for halothane (Penlon Ltd, UK), isoflurane (Isotec 4, Cyprane Ltd, UK) and desflurane (Tec 6, BOC Ohmeda Ltd, UK). Halothane, isoflurane and oxygen concentrations were monitored continuously (Servo-Gas monitor 120, Siemens-Elema AB, Sweden). Desflurane concentration was monitored intermittently using a 5330 Agent Monitor (BOC Ohmeda Ltd, UK). The anaesthetic breathing system was connected to a 2-mm diameter syringe and introduced via the bone wax seal into the maxillary sinus. A heater–humidifier for anaesthetic gases (Dual Servo respiratory humidifier MR 600, Fischer and Paykel, New Zealand) was connected, and the temperature in the anaesthetic system was maintained at 31–35°C, which corresponds approximately to a nasal mucosal temperature of 34°C. Care was taken to direct the air stream directly towards the heated glass window and not towards the mucosa (fig. 1).

In blocking studies, bolus doses of halothane or desflurane 10 ml were administered into the nasal cavity via a small polyethylene catheter (3 FG, Portex Ltd, UK) which was inserted 20 mm into the nasal cavity via the ipsilateral nostril and secured. This method proved to be less technically demanding than that described above. The gas mixture was drawn from the anaesthetic system in airtight glass syringes and administered within 60 s. Boluses of air, halothane or desflurane delivered before the blockers were administered, and served as controls. All drugs were injected intra-arterially (i.a.) via a retrograde cannula in the maxillary artery.

Volatile anaesthetics were obtained from the following sources: halothane (ISC Chemicals Ltd, UK), isoflurane (Forene, Abbott Ltd, UK) and desflurane (Suprane, Kabi Pharmacia, Sweden). CP-99, 994–1 (CP-99), a non-peptide substance P antagonist, selective for the tachykinin neurokinin-1 (NK1) receptor, was a gift from Pfizer Inc. (CT, USA). CP-99 was dissolved in sterile water to a stock solution of 1 mg ml⁻¹. Atropine, a muscarinic receptor antagonist, contains atropine sulphate 2 mg ml⁻¹ in saline (ACO, Sweden). Substance P (SP, Peninsula Laboratories Europe Inc., St Helens, UK) was dissolved in 0.5% albumin to 100 µg ml⁻¹. Methacholine chloride 10 mg ml⁻¹ in saline was obtained from Apoteksbolaget (Sweden).

**EXPERIMENTAL PROCEDURES**

Six rabbits were used to investigate the effects of halothane, isoflurane and desflurane. All animals
Five rabbits were pretreated with atropine 0.2 mg kg\(^{-1}\) i.a. and within 5 min exposed intranasally to a 10-ml bolus dose of 1.1% halothane. Eight rabbits were used (five of which had been used in previous experiments but allowed a 60-min resting period). To confirm that cholinergic block had subsided, the preparation was challenged with the cholinergic agonist methacholine (0.1 \(\mu\)g/kg bodyweight i.a.). If an appropriate response occurred, the experiment was continued. The selective NK1 receptor antagonist CP-99 0.1 mg kg\(^{-1}\) was given i.v. Satisfactory block of the NK1 receptor was achieved when the response to SP 0.1 \(\mu\)g kg\(^{-1}\) i.a. was abolished. Halothane was then administered (at least 15 min after SP challenge) as a bolus dose of 10 ml into the ipsilateral nostril.

Six of the previously used animals were given another 60-min rest period and then re-tested with SP to confirm NK1 block. If this was found to be sufficient, atropine 0.2 mg kg\(^{-1}\) was administered i.a. and within 5 min halothane was administered as a bolus dose of 10 ml into the ipsilateral nostril.

In a separate series of eight rabbits, the blocking studies were repeated as described above using 9% desflurane, delivered as a bolus dose of 10 ml into the ipsilateral nasal cavity.

Results are expressed as mean (SEM), with the exception of baseline mucociliary wave frequency (zero frequency level) which is expressed as mean (SD). Peak response values and area under the curve (AUC) were used for statistical evaluation. All rabbits served as their own control. The results were analysed using two-way ANOVA repeated measurements and, when appropriate, Student’s \(t\) test for paired data. \(P < 0.05\) was considered significant. \(P\) values refer to both peak responses and AUC, unless otherwise stated. The software used for statistical analysis was Statview version 4.1 and 4.51 (Abacus Concepts, Berkeley, CA, USA).

**Results**

An airflow of 0.5 litre min\(^{-1}\) or bolus injections of 10 ml of air into the nose did not influence mucociliary activity compared with spontaneous activity (data not shown). There was no effect on heart rate for any of the anaesthetics. All three anaesthetics increased mucociliary activity. The mean peak response was 47.8.3 (SEM 13.0)% (\(n=6\), \(P < 0.05\)) for halothane, 44.0 (9.6)% (\(n=6\), \(P < 0.05\)) for isoflurane and 45.1 (23.7)% (\(n=6\)) for desflurane. The peak response for desflurane was not significant, but AUC was significant (\(P < 0.05\)) (fig. 2). There were no differences between the three compounds regarding the maximum effect or AUC. However, the peak effect of halothane was achieved in 60 s whereas the isoflurane response was biphasic, peaking at 120 s and 240 s.

The peak response to desflurane was also delayed, and appeared at approximately 240 s. Comparing responses during the first 2 min, the halothane response was significantly greater than that for desflurane (\(P < 0.05, n=6\)), while no significant differences were found between isoflurane and desflurane or halothane and isoflurane. The response to isoflurane and desflurane displayed a biphasic time course which was not observed for halothane. In some cases the initial response was associated with a
There were no differences in duration or incidence of apnoea between the anaesthetics.

In previous studies in the same animal model, atropine did not effect mucociliary activity. In our study the effect of the selective NK1 receptor antagonist, CP-99, was evaluated and shown to have no effect on mucociliary activity compared with saline but was effective in blocking the response to SP \( \frac{n}{p} 58, P < 0.05 \) (fig. 3). There were no significant differences in peak response between nasal and maxillary administration of halothane and desflurane, although there was a tendency towards a higher peak response when desflurane was administered intranasally.

In the halothane blocking experiments, pretreatment with atropine reduced the peak response from 44.8 (9.2) to 23.3 (6.4)% \( (P=0.11 \) for peak response

short period of apnoea. There were no differences in duration or incidence of apnoea between the anaesthetics.

In previous studies in the same animal model, atropine did not effect mucociliary activity. In our study the effect of the selective NK1 receptor antagonist, CP-99, was evaluated and shown to have no effect on mucociliary activity compared with saline but was effective in blocking the response to SP \( (n=8, P<0.05 \) (fig. 3). There were no significant differences in peak response between nasal and maxillary administration of halothane and desflurane, although there was a tendency towards a higher peak response when desflurane was administered intranasally.

In the halothane blocking experiments, pretreatment with atropine reduced the peak response from 44.8 (9.2) to 23.3 (6.4)% \( (P=0.11 \) for peak response
and $P = 0.13$ for AUC, $n = 5$) with the response at 3–5 min unaffected (fig. 4). Pretreatment with CP-99 altered the response to halothane, such that the initial peak response was unaffected by CP-99 while the response at 3–5 min was reduced (peak response $P = 0.8$, and for AUC $P = 0.09, n = 8$) (fig. 5). A combination of atropine and CP-99 significantly reduced the response to halothane ($P < 0.01, n = 6$) for both peak response and AUC (fig. 5).

Desflurane 9% injected into the nasal cavity increased mucociliary activity by 71.9 (10.3)% ($P < 0.001, n = 8$) compared with air controls (fig. 5). Pretreatment with atropine had no effect on the response to desflurane; peak response was 69.3 (20.3)% ($ns, n = 8$) compared with desflurane alone. Pretreatment with CP-99 significantly reduced the response to desflurane; peak response was 26.6 (10.7)% ($P < 0.05$ for peak response and $P < 0.01$ for AUC) compared with desflurane alone. The peak response after combined pretreatment with atropine and CP-99 was significantly reduced to 32.3 (7.0)% ($P < 0.05$), however it did not differ from block with CP-99 alone. The remaining peak response, but not AUC, after combined block was significantly increased compared with controls exposed to air ($P < 0.01$ for peak response and $P = 0.2$ for AUC).

**Discussion**

Previous investigations, both *in vivo* and *in vitro*, have focused on the slowing effect of halothane and other volatile anaesthetics on ciliary beat frequency. Physiological studies, with the possibility of recording rapid changes in mucociliary activity, have been lacking. It has been shown recently in the same animal model that short exposure to halothane stimulates mucociliary activity. Our study focused on the mechanisms involved in short-term exposure to volatile anaesthetics.

Regulation of mucociliary function in the upper airways is complex and mediated partly by neuronal mechanisms. Previous investigations have shown airway-irritating substances such as cigarette smoke and ammonia vapour to induce an increase in mucociliary activity. This response to noxious stimuli was partly blocked by atropine and partly blocked by a substance P antagonist. Further investigations on which of the different neurokinin receptors were involved in the response revealed that tachykinin-evoked acceleration of mucociliary activity was mediated by NK1 receptors, providing the rationale for choosing the NK1 receptor antagonist CP-99 in this study.

Exposure of the maxillary sinus to halothane, isoflurane and desflurane resulted in a transient increase in mucociliary activity. There were no significant differences in maximal responses to the anaesthetics. In the halothane studies, the initial peak (within 2 min) was the maximal response, in contrast with the desflurane response where the immediate response was small compared with the later response at 2–5 min. In blocking studies, nasal administration was used and a biphasic response was not observed. However, comparing figures 5 and 4, it is evident that atropine, in contrast with the NK1 antagonist, was not effective in blocking the immediate response to desflurane. On the contrary, in the halothane experiments, atropine was effective in blocking the initial response whereas the NK1 antagonist had little effect. These data suggest that the effect of the more airway irritating compound was mediated mainly via the NK1 receptor in contrast with the halothane experiments where the response was mediated mainly via cholinergic mechanisms. The mechanism for the increased sensitivity of airway sensory C-fibres to desflurane compared with halothane is not known but may be related to possible differences in the effects of Ca superscript +2 homoeostasis, as it has been shown recently that the capsaicin receptor is a non-selective ion channel. Another speculation is that tissue pH may be reduced by desflurane leading to increased sensitivity of the vanilloid receptors on sensory C-fibres as the receptor is sensitive to decreased tissue pH.

Desflurane is a new inhalation anaesthetic with physical properties which distinguish it from isoflurane and halothane. Data from the literature support the view that desflurane stimulates the sympathetic nervous system, and hypertension and tachycardia have been observed, in addition to coughing, apnoea, laryngospasm and salivation at concentrations of 6–8%. Desflurane is thus less suitable for induction of anaesthesia than halothane, which has little or no effect on the sympathetic nervous system. In blocking studies with desflurane, a biphasic response was not as evident as in previous experiments, which may be because the challenge was via the nose instead of the maxillary sinus mucosa. It was also noted that there was a tendency towards a higher maximal response to desflurane during nasal exposure than after administration into the maxillary sinus, although this was not significant. A denser distribution of sensory C fibres in the nose than in the maxillary sinus could provide an explanation of the increased response to desflurane, although to our knowledge there are no histological studies supporting this hypothesis.

In this animal model, sympathetic nerve stimulation has been found to increase mucociliary activity. The influence of the sympathetic nervous system was not evaluated in this study. It is possible that exposure to volatile anaesthetics initiates a sympathetic reflex, especially exposure to desflurane. There were no significant changes in heart rate and the combination of cholinergic and NK1 block was sufficient to abolish the response to halothane. However, the remaining response to desflurane after pretreatment with atropine and CP-99 could be explained by the sympathetic reflex mechanism, although there was no effect on heart rate. Future studies with a $\beta$-antagonist are warranted.

Anaesthesia and analgesia are prerequisites for this experimental model. Urethane provides both, with a minimum effect on respiration and circulation. A possible interaction with the anaesthetics used in the different challenges cannot be excluded. However, in a previous study using this animal model, urethane was compared with pentobarbital. Both drugs were given i.v. and the latter had a marked effect on mucociliary activity whereas urethane had little or no effect. Other investigators have shown urethane to have no effect on respiratory fluids.

Administration of gases directly into the maxillary sinus where the signal from the mucociliary system is...
picked up can easily disturb the delicate interaction between the beating cilia and mucus and the periciliary layer (the medium in which the cilia beat). In a previous study it was shown that airflow rates exceeding 0.5 litre min\(^{-1}\) caused a reduction in mucociliary activity.\(^{13}\) Therefore, the flow rate used in the experiments comparing the effects of halothane, isoflurane and desflurane was not allowed to exceed 0.5 litre min\(^{-1}\).

In summary, in this animal model, the mucociliary system responded readily to the different anesthetic gases. The response consisted of both a cholinergic pathway and an NK1 receptor-mediated pathway, probably by stimulation of afferent unmyelinated C fibres and via cholinergic effector neurones, combined with the release of SP from the peripheral endings of C fibres. The NK1-mediated response, which was greater after desflurane administration than halothane, may reflect the airway-irritating effects of this volatile anaesthetic. Our experimental model provides a potential tool for investigating the airway-irritating effects of volatile anaesthetics.

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