**Halothane and Isoflurane Decrease Alveolar Epithelial Fluid Clearance in Rats**

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**Background:** Active sodium transport is the primary mechanism that drives alveolar fluid clearance. In the current study, the effects of exposure to halothane and isoflurane on alveolar fluid clearance in rats were evaluated.

**Methods:** Rats were exposed to either halothane (0.4% for 6 h or 2% for 2 h) or isoflurane (0.6% for 6 h or 2.8% for 2 h). Reversibility of halothane effects was assessed after 2 h of exposure to 2% halothane. Alveolar and lung liquid clearance were measured by intratracheal instillation of a 5% albumin solution with 1.5 μCi of 125I-albumin, during mechanical ventilation with 100% FiO₂ and the halogenated agent. The effect of terbutaline (10⁻⁴ m) added to the albumin solution was tested after 2 h of exposure to 2% halothane. The increase in protein concentration in the airspaces over 1 h was used to evaluate alveolar liquid clearance. Lung liquid clearance was calculated gravimetrically.

**Results:** Alveolar liquid clearance rates were decreased by 24%, 30% and 40% compared with controls (P < 0.05) after 2 h of exposure to halothane, 6 h of exposure to halothane, and 6 h of exposure to isoflurane, respectively. After 2 h of exposure to isoflurane, alveolar liquid clearance did not change. In the 2-h halothane exposure group, alveolar liquid clearance returned to the control value 2 h after withdrawal of halothane. Terbutaline increased alveolar liquid clearance by 50% and 89% in the control and 2-h halothane exposure groups, respectively. In all experiments, the same results were obtained for alveolar and lung liquid clearance.

**Conclusions:** Halothane and isoflurane caused a reversible decrease in alveolar epithelial fluid clearance. Two hours of exposure to halothane did not alter the stimulatory effect of terbutaline on alveolar liquid clearance. (Key words: Halogenated agents; sodium transport.)

THE lung epithelium plays a critical role in maintaining the fluid-free airspaces required for normal gas exchange. The mechanisms that account for the maintenance of this "dry" alveolar state have been the subject of numerous studies. Evidence of active sodium transport was provided by several groups, including studies performed with human subjects; they demonstrated the reabsorption of isotonic fluid from the alveolar space into the pulmonary circulation against a rising protein osmotic pressure gradient and the inhibition of this process by amiloride or ouabain.¹⁻⁴

Recent studies have illustrated the pathophysiological importance of this active sodium transport process. For example, airspace instillation of an inhibitor of sodium uptake delays the clearance of fetal lung liquid at birth, resulting in hypoxemia and respiratory distress in otherwise healthy newborn guinea pigs.³ In humans, survival after pulmonary edema correlates with the ability of the epithelium to concentrate fluid within the airspace, presumably by actively transporting sodium out of the alveolar space.³ Moreover, tracheal instillation of either endotoxin⁵ or live bacteria in rats results in acute lung injury and is associated with an increased net removal of excess alveolar fluid in surviving animals. Therefore, this stimulated process is of major importance for the resolution of alveolar edema in the injured lung.

Many inhaled anesthetic agents are highly lipophilic
and have long been thought to affect the permeability of biologic membranes to water and electrolytes. It has also been suggested that volatile anesthetic agents may increase the severity of pulmonary edema. Moreover, halothane has been shown to affect transport and biosynthesis of lung epithelial cells in vitro. A reversible dose-dependent decrease in chloride and sodium transport in isolated canine tracheal epithelium has been reported. In addition, halothane decreases the biosynthesis of pulmonary surfactant by alveolar type II rat cells in primary culture and alters the high-energy phosphate metabolism of these cells.

We tested the effect of clinically relevant concentrations of inhaled halothane and isoflurane on alveolar epithelial barrier fluid transport and protein permeability in rats in vitro.

Materials and Methods

Seventy-eight male Sprague-Dawley rats weighing 320–400 g were studied. The rats were housed in air-filtered, temperature-controlled units and were allowed food and water ad libitum. These studies were approved by the local Animal Care and Use Committee.

Inhaled anesthetic agents were studied over either 2 or 6 h. The short exposure (2 h) with 2% halothane and 2.8% isoflurane was performed with mechanical ventilation for 2 h. The long exposure (6 h) was done with spontaneous ventilation for 4 h followed by mechanical ventilation for 2 h. We used lower concentrations of halogenated agents during the long exposure (0.4% halothane and 0.6% isoflurane) to allow spontaneous ventilation during the first part of the experiments. In addition, because several in vivo studies have demonstrated the ability of exogenous β-adrenergic agonists to increase alveolar fluid clearance in several species, including humans, the effects of the β-adrenergic agonist, terbutaline, were tested in halothane-exposed rats.

The subsequent measurements were done in individual experiments. First, the net capacity of the alveolar epithelial barrier to remove excess alveolar fluid was assessed by calculating alveolar and lung liquid clearance in mechanically ventilated rats after intratracheal instillation of a 5% albumin solution. The change in protein concentration in the distal airspaces over 1 h was the index used to measure net alveolar fluid clearance. Lung liquid clearance (from both the alveolar and lung interstitial spaces) was calculated gravimetrically by measuring extravascular lung water. Second, the permeability of lung endothelial and alveolar barrier to protein was assessed using 125I-albumin as a vascular tracer and 125I-albumin as an alveolar tracer. In addition, excess lung water was measured in each experimental condition. Systemic arterial and airway pressures, arterial blood gases, and rectal temperature were monitored in all rats.

Exposure to Halothane and Isoflurane

The time frame of the experiments and exposure to halothane and isoflurane in each experimental condition are summarized in figure 1. All experiments were conducted in the following manner. All rats spontaneously breathed for 4 h in a 10-l chamber with inflow and outflow valves flushed with air (6 l/min). For the short-term exposures, rats ventilated air for only 4 h and then were anesthetized and ventilated for 2 h with either halothane or isoflurane (fig. 1). For exposure over 6 h to either 0.4% halothane (Halothane, Belamont, France) or 0.6% isoflurane (Forene, Abbott, France), volatile anesthetic agents were delivered by directing air (carrier gas) through a vaporizer (Fluotec 3; Fortec, Abbott, France) placed at the inlet of the chamber. The concentration of halogenated agent was controlled at the outlet of the chamber (capnomac, Datex, Antony 91, France). After this period, rats were taken out of the chamber, anesthetized and paralyzed (intraperitoneal pentobarbital sodium, 50 mg/kg, and pancuronium bromide, 0.5 mg/kg), and ventilated through an endotracheal tube (peak airway pressure, 8–10 cm H2O; rodent ventilator no. 683; Harvard Apparatus, Inc., South Natick, MA) with 100% inspired oxygen, a respiratory rate of 60 breaths/min, and a positive end-expiratory pressure of 3 cm H2O. Either halothane (0.4% in the 6-h halothane group and 2% in the 2-h halothane group) or isoflurane (0.6% in the 6-h isoflurane group and 2.8% in the 2-h isoflurane group) was administered through the endotracheal tube according to each experimental condition (fig. 1). A cannula was inserted into the carotid for blood sampling and to measure systemic arterial pressure. Delivered halogenated agent concentration was regularly controlled (capnomac Datex) through the inspiratory limb of the breathing circuit. Body temperature was kept constant at 38°C with a thermostatically controlled pad. The reversibility of the effects of exposure to halogenated agents was tested in six rats (in the 2-h halothane and 2-h
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Fig. 1. Exposure to the halogenated agents and time course of the experiment. All the rats were first spontaneously breathing for 4 h in a chamber, flushed with air (6 L/min). In some experimental conditions, volatile anesthetic agents were administered via a vaporizer, and the concentration was controlled (analyzer: Datex). After intraperitoneal administration of pentobarbital and pancuronium, tracheostomy was performed for mechanical ventilation. The albumin test solution was then intratracheally instilled (instillation) after 1 h (except for the 2-h halothane and 2-h 100% O₂ group, in which instillation occurred after 3 h of mechanical ventilation), and clearance was measured over the 1-h experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Spontaneous breathing (chamber)</th>
<th>Mechanical Ventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Tracheostomy</td>
<td>Instillation</td>
</tr>
<tr>
<td>2h Halo</td>
<td>Air only</td>
<td>Oxygen only</td>
</tr>
<tr>
<td>2h Iso</td>
<td>Air only</td>
<td>2.8% isoflurane</td>
</tr>
<tr>
<td>6h Halo</td>
<td>0.4% halothane</td>
<td>0.4% halothane</td>
</tr>
<tr>
<td>6h Iso</td>
<td>0.6% isoflurane</td>
<td>0.6% isoflurane</td>
</tr>
<tr>
<td>2h Halo +100%O₂</td>
<td>Air only</td>
<td>2% halothane</td>
</tr>
</tbody>
</table>

100% oxygen groups). Rats were mechanically ventilated, as described previously, with 2% halothane for 2 h and with 100% inspired oxygen for 2 h (fig. 1).

After 4 h of spontaneous breathing of air in the chamber, the control rats were removed, prepared in the same way as described previously, and mechanically ventilated during the experiments with 100% inspired oxygen without inhaled anesthetic agents (fig. 1, top).

During mechanical ventilation with 100% inspired oxygen without halogenated agents or with low concentrations, anesthesia was maintained by supplemental pentobarbital intraperitoneal injection (50 mg·kg⁻¹·h⁻¹).

Measurement of Extravascular Lung Water

To evaluate the effect of halothane and isoflurane on extravascular lung water, the rats were prepared as described previously, mechanically ventilated for 2 h, and exsanguinated. The lungs were then removed, homogenized, and processed for gravimetric determination of extravascular wet-to-dry weight ratio, taking into account the lung residual blood volume, as previously described.⁵,¹³ After preparation for mechanical ventilation as described previously, a 45-min baseline stable heart rate, blood pressure, and halogenated agent concentration were required to achieve steady-state conditions. Then, 1.5 μCi⁷¹I-labeled albumin was injected as a vascular protein tracer. Fifteen minutes later, a 5% albumin Ringer’s lactate solution with 1.5 μCi¹²⁵I-albumin (as an alveolar protein tracer) was instilled into the distal airspaces. Using a syringe and Silastic tubing, 6 ml/kg was instilled into the trachea over 1 min, so that the fluid could be distributed to both lungs. The effect of β-adrenergic stimulation on alveolar and lung liquid clearance was measured in a group of six rats exposed to 2% halothane (2-h halothane exposure and terbutaline), by adding terbutaline (10⁻⁴ M) to the instilled 5% albumin solution. For controls, a group exposed to air (control and terbutaline, n = 6) was similarly instilled with the albumin test solution mixed with terbutaline (10⁻⁴ M). Mechanical ventilation was continued, and 1 h after the tracheal instillation, rats were exsanguinated and a sample of alveolar fluid was aspirated. Then, the lungs were removed through a midline sternotomy. Blood samples were obtained at the beginning and at the end of the procedure. Systemic arterial and airway pressures were monitored continuously with pressure transducers (Baxter, Healthcare Corp., Deerfield, IL) and recorded with a computer system (Windo Graf, Gould, Longjumeau 91, France). Arterial blood gases were measured before and
30 min after the instillation of the 5% albumin solution. Osmolarities of the plasma and the 5% albumin solution were measured to verify their isosmolarity. Protein concentration and radioactivity were measured in plasma, in instilled albumin solution samples, and in final alveolar fluid samples. Hemoglobin was measured by the cyanmethemoglobin method in blood samples and in the supernatant obtained after centrifugation of the lung homogenate (15,000 g for 1 h).

**Assessment of Alveolar and Lung Liquid Clearance.** The capacity of the alveolar epithelium to remove edema fluid was evaluated using two different methods. First, the increase in alveolar protein concentration was calculated by comparing final (TPf) and initial (TPI) (instilled protein solution) alveolar protein concentration. The residual alveolar liquid volume was then calculated. This is given by the equation

\[ V_f = \frac{(V_i \times TPI \times Fr)}{TPf} \]

Vf is the final alveolar fluid volume after intracheal instillation of the 5% albumin test solution and 1 h of mechanical ventilation; Vi is the volume of the 5% albumin test solution instilled into the airspaces at the beginning of the experiment (initial alveolar fluid); and Fr is the fraction of \(^{125}\)I-albumin intratracheally instilled with the albumin test solution remaining in the lung at the end of the experiment. Then, alveolar liquid clearance (ALC) was estimated as the percent loss over 1 h of the volume of liquid instilled from the alveolar space, as in previous studies. \(^{1,6,13,14}\) Alveolar liquid clearance is given by the equation

\[ ALC = \frac{(V_i \times Fwi - Vf \times Fwi)}{(V_i \times Fwi) \times 100} \]

Fwi is the volume of water per volume of 5% albumin solution, measured gravimetrically, and Fwi is the fraction of water in the alveolar fluid sampled at the end of the experiment (final alveolar fluid).

Second, lung liquid clearance was determined by calculating the excess lung water (E), as previously described. \(^{13,14}\) The lungs (instilled with the albumin solution) were homogenized and extravascular lung water determined by calculating the wet-to-dry weight ratio (We/De). Excess lung water is the residual volume (ml) in the alveolar and interstitial spaces of the liquid instilled into the distal airspaces after a 1-h experiment. The amount of excess lung water was calculated as the difference between the wet-to-dry weight ratio of experimental rat lungs (We/De) and that of additional rat lungs without administration of fluid (Wc/Dc). The dry weight of the instilled experimental lung was corrected for the weight of the instilled protein remaining in the lungs at the end of the experiment. To determine the remaining mass of protein (P), the dry weight of the instillate was multiplied by the fraction of \(^{125}\)I-albumin remaining in the lungs. This value was then subtracted from the total dry weight of the experimental lungs and excess lung water was calculated as follows:

\[ E = \frac{(We/(De - P) - Wc/Dc)}{(De - P)} \times (De - P) \]

This equation does not allow for the possibility that some of the circulating plasma may enter the instilled experimental lung. To estimate the quantity of plasma that accumulates in the instilled lungs, we measured the amount of vascular protein tracer, \(^{151}\)I-albumin, that transferred into the airspaces. Lung liquid clearance (LLC; percent loss over 1 h from alveolar and interstitial spaces of the volume of liquid instilled) is then given by the equation

\[ LLC = \frac{[(V_i \times Fwi) - E]}{(V_i \times Fwi) \times 100} \]

**Assessment of Alveolar Barrier Protein Permeability.** The bidirectional flux of albumin across the alveolar barrier was calculated using two methods previously described. \(^{1,7,13,15}\) First, residual \(^{125}\)I-albumin (the alveolar protein tracer) in the lung and the accumulation of \(^{125}\)I-albumin in the plasma were calculated. Second, the clearance of \(^{131}\)I-albumin (the vascular protein tracer) into the extravascular spaces of the lung was calculated.

Clearance from the lung of \(^{125}\)I-albumin was determined by dividing the \(^{125}\)I-albumin remaining in the lungs (measured by counting the radioactivity of the lungs) by the instilled radioactivity and expressed as a percentage of instillate. The instilled radioactivity (Total \(^{125}\)cpm) was obtained by multiplying the total radioactivity of the \(^{125}\)I-albumin in the instillate by the volume that was instilled. Total \(^{125}\)I-albumin in plasma was estimated according to the formula

\[ (\frac{\text{Total}^{125}\text{cpm PI}_i + \text{Total}^{125}\text{cpm PI}_d}{2}) \times \text{Vol PI} / \text{Total}^{125}\text{cpm}_i \]

\(^{125}\text{cpm PI}_i\) is the count per minute per milliliter of initial plasma; \(^{125}\text{cpm PI}_d\) is the count per minute per milliliter of final plasma; and Vol PI is the plasma volume, calculated by multiplying the \(^{131}\)I count per minute injected by the \(^{131}\)I count per minute per milliliter of initial plasma.
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Table 1. Effect of Halothane and Isoflurane on Oxygenation, Blood Pressure, and Extravascular Lung Water after 2 and 6 h Exposure

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>$\frac{P_{a}}{F_{o}}$ (mmHg)</th>
<th>Mean Systemic Arterial Pressure (mmHg)</th>
<th>Extravascular Lung Water* (g H$_2$O/g dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>490 ± 45</td>
<td>120 ± 24</td>
<td>4.1 ± 0.2</td>
</tr>
<tr>
<td>2 h halothane</td>
<td>6</td>
<td>460 ± 45</td>
<td>67 ± 15</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>2 h isoflurane</td>
<td>6</td>
<td>438 ± 48</td>
<td>54 ± 12</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>6 h halothane</td>
<td>6</td>
<td>470 ± 50</td>
<td>126 ± 20</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>6 h isoflurane</td>
<td>6</td>
<td>457 ± 72</td>
<td>118 ± 18</td>
<td>4.3 ± 0.3</td>
</tr>
</tbody>
</table>

Data are mean ± SD.
* Wet to dry weight ratio of lungs without albumin solution instillation (g water per g dry weight).

The second method measures the vascular protein tracer, $^{131}$I-albumin, in the extravascular spaces of the lungs, as described previously. To calculate the amount of $^{131}$I-albumin present in the extravascular spaces, the blood counts in the lung were subtracted from the $^{131}$I-albumin counts in the entire lungs. Plasma clearance into the extravascular spaces of the lungs was estimated by the following equation, which we have used previously:

\[ ^{131} \text{cpm lung} - \left( ^{131} \text{cpm Pl} \times \frac{Q_{b}}{Q_{a}} \right) ^{131} \text{cpm Pl} \]

in which $^{131}$cpm lung is the total count per minute in the lung; $^{131}$cpm Pl is the count per minute per milliliter in final plasma; $^{131}$cpm Pl is the count per minute per milliliter of plasma averaged over the time of experiment; and $Q_{b}$ is the residual blood volume in the lung at the end of the experiment (after exsanguination), calculated by using the gravimetric determination of the water content of lung and the hemoglobin concentration measured in the supernatant of the lung homogenate and in blood.

Statistical Analysis
Data are summarized as means ± SD. The paired Student's $t$ test was used to compare hemodynamic and airway pressure data before and after instillation of the albumin solution. One-way analysis of variance and Fisher's exact test were used to compare the different groups. A probability value $<$0.05 was considered statistically significant.

Results
Oxygenation, Airway Pressure, and Blood Pressure
Oxygenation, peak airway pressure, and rectal temperature were similar in all groups. The mean systemic arterial pressure decreased by 50% in rats exposed to 2% halothane and 2.8% isoflurane for 2 h (table 1).

Extravascular Lung Water
There was no significant increase in the extravascular lung water in rats exposed to halogenated agents. The mean values were between 4.0 and 4.3 grams of water per gram of dry lung weight (table 1).

Alveolar and Lung Liquid Clearance
Effect of Halothane. Alveolar and lung liquid clearance values were significantly reduced by 24% after

![Graph showing alveolar and lung liquid clearance](image_url)

Fig. 2. Alveolar liquid clearance, as percent loss of liquid instilled after halothane and isoflurane exposure (mean ± SD). The same results were obtained for lung liquid clearance. The reversibility of the halothane effect was demonstrated in the 2 h halothane and 2 h 100% $O_2$ group. In each group, $n = 6$. * $P < 0.05$ compared with control group; † $P < 0.05$ compared with 2% halothane group.

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exposure to 2% halothane for 2 h and by 30% after exposure to 0.4% halothane for 6 h (fig. 2).

Reversibility of the Halothane Effect. In rats ventilated with 2% halothane for 2 h, alveolar and lung liquid clearance values returned to control values 2 h after withdrawal of halothane (fig. 2).

Effect of Isoflurane. Exposure to 2.8% isoflurane for 2 h did not reduce alveolar and lung liquid clearance values. In contrast, 0.6% isoflurane given for 6 h decreased alveolar and lung liquid clearance values by 40% ($P < 0.05$; fig. 2).

Effect of $\beta$-Adrenergic Agonist Treatment. When terbutaline was added to the instilled albumin solution in control rats, alveolar and lung liquid clearance values increased significantly by 50% compared with those of untreated control rats. Alveolar and lung liquid clearance values dramatically increased by 89% in rats exposed to 2% halothane for 2 h with instilled terbutaline compared with the 2% halothane group exposed for 2 h and not treated with terbutaline (fig. 3). Terbutaline enhanced alveolar and lung liquid clearance values to levels similar to control values (fig. 3).

Bidirectional Protein Permeability

No increased accumulation of the plasma protein tracer, $^{131}$I-albumin, in the extravascular spaces of the lung occurred in any of the study groups. The protein tracer that entered the lung in the halothane and isoflurane groups varied between 0.07 ± 0.03 ml and 0.11 ± 0.04 ml (control value = 0.10 ± 0.04 ml; there is no statistical difference between the different groups). Similarly, there was no significant change in the flux of the alveolar protein tracer ($^{125}$I-albumin) across the alveolar barrier. The mean percent recovery of $^{125}$I-albumin was between 95% and 98% in all groups (table 2).

Discussion

The distal lung epithelium plays an important role in preventing fluid accumulation within the airspace by maintaining the transport of sodium (and consequently water) out of the airspace. Until recently, very little was known about the effects of volatile anesthetic agents on alveolar epithelial barrier function, although in vitro studies have shown that metabolic function of epithelial type II cells and ion transport function of tracheal epithelium are altered by these lipophilic agents. The current study demonstrates that net alveolar fluid clearance is decreased after exposure to halothane for 2 h, halothane for 6 h, or isoflurane for 6 h but remained normal after 2 h of exposure to isoflurane. The data indicate that the halothane-decreased clearance, mainly consistent with an inhibition of active sodium transport, is reversible. In addition, when terbutaline was added to the instilled solution, alveolar and lung liquid clearance values were similar in both halothane-exposed rats and controls.

Methodologic Issues

The methods for measurement of epithelial liquid clearance and protein permeability have been described previously and evaluated carefully in normal and pathologic conditions. The concentrations of the native protein in liquid sampled by a catheter wedged into the distal airway were similar to that of an alveolar micropuncture sample obtained directly. The correlation was good between alveolar liquid clearance calculated according to albumin concentration and that calculated according to total protein concentration. In previous studies, the $^{125}$I and $^{131}$I labels remain attached to albumin during experiments in rat lung. The duration of our experimental procedure for measuring epithelial liquid clearance was short (1 h), thus minimizing bidirectional protein movements; the same values were
observed in all groups (table 2). In these conditions, protein concentration can be used to calculate alveolar liquid clearance. In addition, the same clearance values were found with each experimental approach (alveolar liquid clearance and lung liquid clearance), indicating that the results are internally consistent.

We studied the effects on alveolar epithelial barrier function for short (2 h) and longer exposure (6 h) to inhaled anesthetic agents using different concentrations of halothane and isoflurane. It has previously been assumed that the minimum alveolar concentrations (MAC) for isoflurane and halothane, capable of preventing purposeful movement to supramaximal noxious stimulation in 50% of Sprague-Dawley rats, are 1.4% and 1.0%, respectively. Therefore, during the short exposure, a concentration of ≈2 MAC of halogenated agents in our experimental conditions was associated with a decrease in alveolar fluid clearance with halothane and normal clearance with isoflurane. In long exposure experiments, both halogenated agents induced decreased alveolar fluid clearance, although the concentrations were lower (≈0.5 MAC). This low concentration allowed spontaneous breathing, and alveolar fluid clearance is similar in spontaneous or controlled ventilation.

Mechanisms
This reversible decrease in alveolar and lung liquid clearance with halogenated anesthetic agents is consistent with an inhibition of active sodium transport. Directional transport of fluid is a process driven by trans-epithelial ion transport. Fluid absorption (movement from the apical to the basolateral side) usually follows sodium transport, an active energy-dependent process. Sodium enters alveolar epithelial type II cells through channels in the apical membrane and is extruded on the basolateral side by the Na-K-ATPase pump. Among the apical transport systems, amiloride-inhibitable transport plays a major role in sodium absorption; however, additional pathways have been described in rat alveolar type II cells for sodium entry across the apical membrane of the alveolar epithelium, with a possible role in fluid absorption. These transport mechanisms include active sodium-coupled transporters and include sodium-neutrophic amino acid cotransport, sodium-phosphate cotransport, and sodium-glucose cotransport.

To prove that volatile anesthetic agents directly impair alveolar liquid clearance, several issues need to be clarified. First, the inhibition of fluid clearance by halogenated agents is not due to a decrease in arterial systemic pressure and pulmonary blood flow, as suggested by the studies using 0.4% halothane, in which the liquid clearance was decreased, although the mean arterial pressure was normal (fig. 2 and table 1). This observation further validated the results of previous in vivo studies, which demonstrated that the process of alveolar fluid reabsorption over 4 h is not dependent on blood flow. Supporting these data, in a previous study, we found that alveolar fluid clearance is normal, and further, is normally stimulated by bacteria-induced lung injury in exsanguinated rats.

Second, the effect of halogenated agents could not be ascribed to impaired release of endogenous catecholamines. Several studies have demonstrated that β-adrenergic agonists are potent stimulants of sodium transport and fluid absorption. Terbutaline increases the rate of alveolar liquid clearance in the human excised lung more than 100% higher than basal levels.
finding that β-adrenergic agonists exert a significant effect on alveolar liquid clearance is also indirectly supported by a structural autoradiographic study, in which large numbers of β-adrenergic receptors were identified in the alveolar walls of different species, including rats and humans.20,27

It is noteworthy that that endogenous catecholamines have been shown to increase alveolar liquid clearance. Pittet et al.14,28 in recent studies in rats measured an increase in alveolar epithelial clearance in septic shock11 and in hypovolemic shock.29 In both studies, the increase in alveolar liquid clearance was associated with an increase in plasma levels of epinephrine. In contrast, in the control rats, there was no change in the plasma levels of epinephrine over the entire study period, and moreover, propranolol, a β-adrenergic antagonist, did not affect the fluid transport across the lung epithelial barrier, as also shown in our previous studies.6,13 These results are in accordance with findings in cultured alveolar cells.35 Therefore, the normal basal level of alveolar fluid clearance may not be impaired by exogenous adrenergic antagonists or by a decrease in endogenous catecholamines.

Third, the effect of volatile anesthetic agents could not be due to a toxic effect on alveolar type II cells. It is noteworthy that Molliex et al.12 reported that the halothane-induced decrease in the biosynthesis of pulmonary surfactant by rat alveolar type II cells is reversible. In our study, the concentrations of halogenated agent were probably not cytotoxic because epithelial permeability remained normal. In addition, a direct cytotoxic effect is unlikely because the depressant effect of halothane on alveolar liquid clearance was completely reversible (fig. 2).

Several lines of evidence favor a direct and reversible effect of volatile anesthetic agents on alveolar type II cell function. First, Molliex et al.12 demonstrated that halothane decreases the biosynthesis of pulmonary surfactant, a metabolic function of rat alveolar type II cells in primary culture. Second, Pizov et al.11 reported that halothane decreases sodium transport in isolated canine tracheal epithelium. Third, we have now found that volatile anesthetic agents decrease basal alveolar liquid clearance.

Because sodium transport in upper (trachea) and lower (alveoli) airways involves different transporters, the impaired clearance does not seem to occur because of a direct effect on a specific transporter and could be due to indirect pathways, as sodium transport can be upregulated by terbutaline. In our study, terbutaline increased alveolar liquid clearance in the presence of halothane exposure, indicating that the alveolar epithelium responded normally to β-adrenergic stimulation. These results agree well with data previously reported for the tracheal epithelium exposed to halothane because pretreatment with isoproterenol enhanced chloride and sodium transport to levels similar to control values.11

Volatile anesthetic agents may act via indirect pathways by alternating intracellular calcium metabolism and functional G protein coupling. Anesthetic-induced alterations of calcium regulation can result in a variety of altered responses.29 Differential responses may be observed with different volatile anesthetic agents. For instance, halothane, but not isoflurane, inhibited transient calcium stimulation by bradykinin in isolated endothelial cells.30 This might explain the differential response observed in the current study between halothane and isoflurane during the 2 h of exposure. This discrepancy could also be attributed to the metabolites of halogenated agents. Isoflurane is one of the least metabolized of the volatile halogenated anesthetic agents and has a number of different characteristics compared with halothane. First, fluoride metabolite levels after halothane exposure are approximately 20-fold greater than those of isoflurane.31,32 Second, isoflurane metabolism does not produce free radicals and does not undergo reductive metabolism.32,33 Therefore, a greater level of metabolites with halothane compared with isoflurane could plausibly induce decreased clearance. In addition, long exposure to volatile anesthetic agents could result in metabolite accumulation. That may be associated with altered alveolar liquid clearance.

Clinical Implications and Significance
Despite a significant decrease in alveolar liquid clearance (50% and 40% for 6 h of exposure to halothane and isoflurane, respectively), volatile anesthetic agents did not affect extravascular accumulation in the lung of the plasma protein tracer nor the wet-to-dry lung weight ratio. Is this phenomenon of clinical significance? The lung epithelium plays an important role in maintaining fluid-free airspace by active sodium transport as demonstrated by several investigators.1,4,13,15 Moreover, recent studies have found stimulated sodium transport process in pathologic conditions, thus indicating that clearance may be of major importance for the
resolution of edema in injured lung. The intact α-adrenergic effect under volatile anesthetic exposure is an important issue, partly because of its potential therapeutic value: α-Adrenergic agonist therapy has been proposed as a possible treatment to accelerate the resolution of pulmonary edema in patients with acute pulmonary edema.

We did not study the effects of volatile agents in injured lungs, and extravascular lung water remained unchanged in the noninflamed lungs. During pathologic conditions, exposure to halogenated agents could result in more pulmonary edema. In a noteworthy study, Shayeitiz et al. reported increased sensitivity to oxidant injury of perfused rabbit lungs exposed to halothane, with increased permeability of the capillary wall and an increase in the quantity of pulmonary edema. In addition, halothane combined with a high concentration of oxygen may increase the permeability of the alveolar-capillary barrier.

We found that halothane and isoflurane cause a reversible decrease in alveolar epithelial fluid clearance in normal rats in vitro. These inhaled anesthetic agents may lower the threshold for alveolar edema in patients with heart failure or acute lung injury.

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