Prevention of Methacholine-induced Changes in Respiratory Mechanics in Piglets
A Comparison of Sevoflurane and Halothane

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Background: Sevoflurane is a new volatile anesthetic agent that may be a useful alternative to halothane for anesthesia in children. However, there is insufficient information about its effects on respiratory mechanics, particularly in the presence of constrictor stimuli.

Methods: Eighteen piglets had anesthesia induced and maintained with either pentobarbital (control: n = 8), 1 minimum alveolar concentration (MAC) sevoflurane (sevo: n = 5), or 1 MAC halothane (halo: n = 5). Pressure, flow, and volume were measured at the airway opening and used to calculate lung compliance (C\textsubscript{L}) and resistance (R\textsubscript{L}). Resistance was partitioned into airway (Raw) and parenchymal (Vti) components using alveolar pressure. Methacholine was infused intravenously in a dose sufficient (15 µg·kg\textsuperscript{-1}·h\textsuperscript{-1}) to approximately double R\textsubscript{L}.

Results: The increase in R\textsubscript{L} seen in the control group was almost entirely due to an increase in Vti. Sevoflurane and halothane prevented the increase in R\textsubscript{L} and Vti (both P < 0.02) and the decrease in C\textsubscript{L} (both P < 0.02).

Conclusions: Sevoflurane and halothane can prevent methacholine-induced changes in lung function. (Key words: Anesthetics, inhalation: halothane; sevoflurane. Animal models. Lung function.)

SEVOFLURANE is a new volatile anesthetic agent that may be a useful alternative to halothane for anesthesia in children. However, halothane is considered the best volatile anesthetic agent to maintain anesthesia in patients with asthma patients and to treat bronchospsasm. Although sevoflurane has been reported to attenuate bronchoconstriction associated with anaphylaxis in a canine model, there is insufficient information about its effects on lung mechanics in the presence of constrictor agonists.

Methacholine challenge is commonly used to mimic the acute changes in lung function seen clinically in persons with asthma and to assess bronchial responsiveness. Recent studies from Japan have shown that methacholine may alter lung function by acting on the central airways or on the peripheral airway and lung parenchyma. The pattern of response was associated with the degree of responsiveness, with the most responsive participants (those responding at the lowest doses) tending to respond in the lung periphery. Thus the ability of halothane, sevoflurane, or both to act on the airways and lung parenchyma is relevant to their use in persons with asthma.

The present study was designed to determine, in a piglet model, whether sevoflurane or halothane would protect against methacholine-induced changes in respiratory mechanics. We used methods previously developed to partition respiratory mechanics into components representing the airways and lung parenchyma (RL\textsuperscript{a}) to investigate the site of action of sevoflurane and halothane in the lungs.

Materials and Methods

Animal Preparation
After we received approval from our Institutional Animal Welfare Committee, we randomly assigned 18 pig-
lets weighing 4.5–8 kg to one of three groups: eight piglets in a control group, five piglets in the sevoflurane group, and five piglets in the halothane group. Piglets in the control group had anesthesia induced with pentobarbital (10 mg/kg given intravenously) before tracheal intubation and ventilation of the lungs and maintained with 5 mg/kg given intravenously every 45–60 min. Piglets in the sevoflurane and halothane groups received an inhalation induction with either sevoflurane or halothane, respectively, through a mask. Anesthesia was maintained with either sevoflurane or halothane at 1 minimum alveolar concentration (MAC). The MAC values of sevoflurane and halothane in piglets have been reported to be 2.12 ± 0.39% and 0.9 ± 0.12%, respectively.16 End-tidal anesthetic concentration was monitored continuously using a respiratory gas monitor (Artenua MM 207C, Sweden).

The trachea was intubated in all piglets with a 5-mm internal diameter cuffed tube. Fentanyl (2 μg/kg) was administered after tracheal intubation to ensure adequate analgesia, followed by a continuous intravenous infusion at 1 μg·kg⁻¹·h⁻¹. Mechanical ventilation was maintained with a volume-controlled ventilator (Harvard Apparatus model 708, Natick, MA). Arterial blood pressure was continuously monitored in the right femoral artery, and the internal jugular vein was cannulated to allow methacholine administration.

Alveolar Capsule Technique
After obtaining a steady-state level of anesthesia and analgesia, as evidenced by a stable heart rate and blood pressure, muscle relaxation was achieved with 0.2 mg/kg pancuronium bromide given intravenously. The chest was opened widely through a midline sternotomy to place alveolar capsules. Small plastic capsules were glued to the pleural surface with cyanoacrylate glue (Loktite Corporation, Ireland). The underlying alveoli were brought into communication with the capsule chamber by puncturing the pleura with a 25-gauge needle. Three capsules were glued to either right or left upper or middle lobes. A piezoresistive pressure transducer (Endevco 8507C-2, Endevco, San Juan Capistrano, CA) was lodged into each capsule to measure alveolar pressure (Pₐ).

Measurement of Respiratory Mechanics
The measurement equipment, consisting of a pressure port and transducer (Endevco 8510B-2) to measure airway opening pressure and a pneumotach (Hans Rudolph Inc., Kansas City, MO) to measure flow (V') was placed between the piglet’s tracheal tube and the ventilator circuit. The airway opening pressure and V' signals and Pₛ were amplified (PR signal conditioner, Endevco 4423), filtered with a low-pass device (902LPE, 8 pole Bessel filters; Frequency Devices, Haverhill, MA), and stored on computer using an acquisition and analysis package (Anadat & Labdat, RHT Infodat, Montreal, Canada).

Respiratory mechanics were calculated from measurements of airway opening pressure, Pₛ, and volume (V) recorded during mechanical ventilation, using a multilinear regression implementation of the equation:

\[ Pₛ = E₁ \cdot V + R₁ \cdot V' + Pₐ \]

where total pulmonary elastance (E₁) is represented by (E₁ + E₂·V'), with E₂·V representing any volume dependence of elastance and R₁ representing the pulmonary resistance. Previously we used the contribution of E₂ to total elastance (%E₂) as an index of lung overdistention.11 The constant term in equation 1 (Pₐ) reflects the end-expiratory alveolar pressure.12 Pulmonary compliance (Cₐ) is the reciprocal of E₁.

The contribution of the pulmonary tissue to energy dissipation (Vti) is calculated from the equation:

\[ Pₐ - Pₛ = Eₜi \cdot V + Vti \cdot V' + K \]

where Pₛ is the alveolar pressure and total tissue elastance (Eₜi) is represented by (E₁ + E₂·V'), with E₂·V representing any volume dependence of elastance. Airway resistance (Rₐ) during mechanical ventilation is calculated by subtracting Vti from R₁.

For both analyses, data were used only if the coefficient of determination (R²) of this fit was > 0.98.

Correction for Gas Physical Properties
Measurement of V' and V with a Fleish pneumotachograph is described by the Poiseuille equation and the pressure decrease across the Fleish head. However, the pressure decrease is related to the viscosity of the gas used during measurements. Johns et al.13 have shown that a Fleish pneumotachograph can be used to measure accurately the viscosity of a gas mixture. Applying this principle, we used a 100-ml glass syringe and a Fleish pneumotachograph to measure the viscosity of air and the mixture of 50% nitrous oxide in oxygen with and without I and 1.5 MAC halothane and sevoflurane. Correction factors were calculated and applied to the flow.
measured under experimental conditions when halothane or sevoflurane were administered before volume was calculated.

**Study Design**

Preliminary studies were conducted to determine the concentration of infused methacholine that would approximately double $R_I$. This dose was 15 $\mu$g·kg$^{-1}·h^{-1}$ (data not shown) and was used throughout the subsequent studies.

**Prevention of Methacholine-induced Changes in Respiratory Mechanics**

Methacholine-induced changes in respiratory mechanics in the control group were compared with the changes seen in the sevoflurane and halothane groups. Animals in the sevoflurane and halothane groups received 1 MAC of the relevant anesthetic agent. After measurement of baseline respiratory mechanics, the piglets received a continuous infusion of methacholine (15 $\mu$g·kg$^{-1}·h^{-1}$), and respiratory mechanics were measured every 2 min until a stable response was measured (at least 10 min). The methacholine infusion was stopped, and respiratory mechanics were allowed to return to baseline. The methacholine infusion was recommenced, and the response measured again, as described previously. This procedure was repeated twice, and then the concentration of the volatile agent was increased to 1.5 MAC, and further respiratory mechanics were collected.

**Statistics**

Comparisons among three groups were made using one-way analysis of variance. Unpaired two-tailed $t$ tests were used for comparisons between the two groups. Paired two-tailed $t$ tests were used for comparisons in the control group between methacholine baseline with or without volatile agent, and for comparisons between 1 MAC and 1.5 MAC concentrations. Significance was accepted at the 5% level.

The study was powered to detect a 20% difference in the percentage change in $R_I$ between the sevoflurane and halothane groups. This effect size was chosen because we routinely use this level of response as indicating a clinically significant bronchodilator effect in children receiving ventilatory assistance. Based on the intra-subject variability of $R_I$ calculated using the multilinear regression technique in puppies, the five piglets in each group would have 80% power at the 5% level of significance to detect a 20% difference in $R_I$ between the groups. Throughout this article, data are presented, and group means are ± SEM.

**Results**

As shown in table 1, ventilation parameters were comparable between the groups. There were no differences in the mean weight between groups. Baseline respiratory mechanics were comparable in the three groups (table 2).

**Site of Action of Methacholine**

Methacholine infusion (15 $\mu$g·kg$^{-1}·h^{-1}$) resulted in a mean increase in $R_I$ of 100% (range, 31–197%) in the control group. This increase was caused almost entirely by a mean increase in $V_{ti}$ of 282% (range, 116–483%), with no change in $Raw$ (mean 10%; table 3). Lung compliance decreased by 49% after methacholine (table 3).

**Prevention of Methacholine-induced Changes in Respiratory Mechanics**

Sevoflurane and halothane markedly attenuated the methacholine-induced increase in $R_I$. The mean per-

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Table 1. Demographic and Ventilation Parameters

<table>
<thead>
<tr>
<th></th>
<th>Control $(n = 8)$</th>
<th>Halothane $(n = 5)$</th>
<th>Sevoflurane $(n = 5)$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>6.6 ± 0.32</td>
<td>6.2 ± 0.46</td>
<td>6.2 ± 0.44</td>
<td>0.73</td>
</tr>
<tr>
<td>Tidal volume (ml/kg)</td>
<td>10.3 ± 0.5</td>
<td>10.6 ± 0.7</td>
<td>11.2 ± 0.4</td>
<td>0.55</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>0.69 ± 0.0</td>
<td>0.69 ± 0.0</td>
<td>0.69 ± 0.0</td>
<td>0.89</td>
</tr>
<tr>
<td>Peak pressure (cmH$_2$O)</td>
<td>10 ± 0.4</td>
<td>10 ± 0.8</td>
<td>11 ± 0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Mean airway pressure (cmH$_2$O)</td>
<td>6.4 ± 0.1</td>
<td>6.4 ± 0.3</td>
<td>6.7 ± 0.1</td>
<td>0.44</td>
</tr>
<tr>
<td>End expiratory pressure (cmH$_2$O)</td>
<td>4.7 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Values are group mean ± SEM.
Table 2. Baseline Respiratory Mechanics in the Three Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Halothane (1 MAC)</th>
<th>Sevoflurane (1 MAC)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rₜ (×10⁻³ cmH₂O·ml⁻¹·s⁻¹)</td>
<td>12.04 ± 0.5</td>
<td>11.41 ± 0.6</td>
<td>12.9 ± 0.4</td>
<td>0.20</td>
</tr>
<tr>
<td>Vti (×10⁻³ cmH₂O·ml⁻¹·s⁻¹)</td>
<td>3.82 ± 0.3</td>
<td>3.8 ± 0.5</td>
<td>4.42 ± 0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>Raw (×10⁻³ cmH₂O·ml⁻¹·s⁻¹)</td>
<td>8.23 ± 0.9</td>
<td>7.61 ± 0.2</td>
<td>8.46 ± 0.5</td>
<td>0.23</td>
</tr>
<tr>
<td>Cₜ (ml/cmH₂O)</td>
<td>16.02 ± 1.2</td>
<td>16.98 ± 2.5</td>
<td>14.92 ± 1.2</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Values are group mean ± SEM.

Discussion

The results of this study show that sevoflurane is as effective as halothane in preventing the changes in respiratory mechanics that occur in response to intravenous administration of methacholine. Both drugs can act on lung tissue mechanics.

In the current study, methacholine infusion increased pulmonary resistance by an action on the pulmonary parenchyma, with no significant effect on airway resistance. This is a somewhat surprising result. Methacholine, a nonspecific muscarinic receptor agonist, may have been expected to increase airway and tissue resistance, as has been shown in other species.¹⁵-¹⁷ It is unlikely that the lack of increase in Raw in our piglets was due to an immaturity of muscarinic receptor function, as has been shown in piglets younger than 1 week old.¹⁸,¹⁹ All the piglets in the current study were aged 4-6 weeks, and methacholine was administered through the internal jugular vein with the phrenic nerve intact. The vascular effects of methacholine were obvious, and lung function was altered by the methacholine infusion, but the response was not located in the airways. The lack of airway response may be due to a difference in the relative sensitivities of airways and parenchyma to methacholine, as has been reported in puppies.²⁰ The increase in Rₜ induced by the methacholine infusion was relatively modest. This was done deliberately to simulate a realistic clinical situation. This modest dose may have been responsible for the pattern of our results. If the parenchyma is more responsive to methacholine in this species than the airways, as is the case in puppies,²⁰ a higher dose of methacholine may produce significant increases in Raw.

One difficulty in inducing marked changes in lung function is that they are frequently associated with ventilation inhomogeneity. Under these circumstances, the utility of alveolar capsules for partitioning lung mechanics into airway and parenchymal components is questionable.

The attenuation of the increase in Rₜ caused by the administration of methacholine was comparable under 1 MAC sevoflurane and 1 MAC halothane. These results differ from those of a recent study in which sevoflurane was found to be less effective than halothane in preventing the increase in Rₜ in response to histamine-induced bronchospasm in dogs.²¹ Whether this differ-

Table 3. Changes in Respiratory Mechanics Following Methacholine Infusion (15 µg·kg⁻¹·h⁻¹) in the Three Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Halothane</th>
<th>Sevoflurane</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rₜ</td>
<td>100 ± 20</td>
<td>36 ± 3</td>
<td>38 ± 9</td>
<td>0.019</td>
</tr>
<tr>
<td>Vti</td>
<td>283 ± 56</td>
<td>111 ± 7</td>
<td>105 ± 20</td>
<td>0.016</td>
</tr>
<tr>
<td>Raw</td>
<td>10 ± 5</td>
<td>5 ± 5</td>
<td>4 ± 2</td>
<td>0.6</td>
</tr>
<tr>
<td>Cₜ</td>
<td>-49 ± 6</td>
<td>-28 ± 1</td>
<td>-29 ± 4</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Values are group mean ± SEM (% of baseline).
* Compared with Mch-induced changes in the control group using ANOVA.
ence is due to the species used, the age of the animals, the constrictor mediator, or the method of stimulation is unknown. Katoh and Ikeda did not partition \( R_p \) so we cannot compare the site of action of histamine. Sly and Lanteri reported a predominantly peripheral action of histamine in puppies, whereas Ludwig et al. found central and peripheral effects using similar techniques in adult dogs. Thus the age of the animal may also contribute to the pattern of results seen. We cannot preclude the possibility that sevoflurane and halothane may have truly different actions on airway smooth muscle, leading to differences between the results reported here and previous reports.

In the present study, sevoflurane also significantly attenuated the methacholine-induced decrease in \( C_l \). These results also differ from a recent study that failed to demonstrate any attenuation of changes in \( C_l \), in a canine anaphylaxis model, with sevoflurane or isoflurane. These differences are likely to be explained on technical grounds, including use of different techniques to calculate compliance and failure to correct for gas viscosity.

In the present study, halothane and sevoflurane prevented the methacholine-induced increase in \( R_b \) by preventing an increase in \( V_l \). These data are compatible with those of a previous report that found an effect of halothane on pulmonary parenchymal mechanics in canine lungs. Theoretically, the peripheral elements of the bronchial tree contribute largely to \( V_l \). Halothane has been shown to decrease \( V_l \) mainly by reducing the tone of contractile elements present in the parenchyma or respiratory bronchioles. Sevoflurane and halothane appear to have similar effects on \( V_l \). There was some evidence of dose-response behavior, with 1.5 MAC sevoflurane and halothane producing a stronger inhibition of the methacholine-induced constrictor response.

In conclusion, sevoflurane and halothane were effective in preventing methacholine-induced changes in lung function in piglets. Both drugs seemed to exhibit similar actions on the mechanical properties of the lung tissues.

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