Amrinone Attenuates Airway Constriction during Halothane Anesthesia

W. Casey Lenox, M.D.,* Carol A. Hirshman, M.D.†

Background: Phosphodiesterase (PDE) inhibitors should, in theory, be useful in patients with both cardiac and reactive airway disease who require anesthesia. The effects of the PDE inhibitor, amrinone, on the release of endogenous catecholamines and on airway reactivity to aerosol methacholine challenge were evaluated during thiopental/fentanyl and halothane anesthesia in five mongrel dogs.

Methods: Responses to methacholine aerosol challenge (0.03, 0.075, 0.15, 0.30, 0.75, and 3.0 mg/ml) were measured during four conditions: thiopental/fentanyl anesthesia, thiopental/fentanyl anesthesia with amrinone infusion, halothane anesthesia, and halothane anesthesia with amrinone infusion. Increase in pulmonary resistance (R50) and decreases in dynamic compliances (Cdyn) were calculated for each methacholine dose. Plasma epinephrine and norepinephrine concentrations were measured during thiopental/fentanyl anesthesia with amrinone infusion.

Results: Before aerosol challenge, baseline R50 and Cdyn did not differ in the four groups. Amrinone significantly attenuated the pulmonary response to methacholine during both thiopental/fentanyl and halothane anesthesia. Plasma catecholamine concentrations did not increase during amrinone infusion.

Conclusions: Amrinone attenuates methacholine-induced airway responses even during halothane anesthesia. These data indicate that isozyme-selective PDE inhibitors hold promise for the perioperative treatment of bronchospasm. (Key words: Lungs: asthma; bronchoconstriction. Pharmacology, phosphodiesterase inhibitors: amrinone. Sympathetic nervous system: catecholamines.)

Although the mechanisms controlling airway smooth muscle tone are not completely understood, the second messenger cyclic nucleotides, cAMP and cGMP, are thought to be important in regulating airway tone such that increases in either cyclic nucleotide decrease tone and relax the muscle.1-3 Cyclic nucleotide phosphodiesterases (PDE) are a family of enzymes that metabolize and inactivate cAMP and cGMP.4 Five isoenzymes of PDE have been identified in airway smooth muscle,5,6 and drugs are being developed that selectively block each of the isoenzymes in an attempt to decrease side effects and increase potency. Compounds that selectively inhibit cAMP PDE have been identified, and the amount of inhibition correlates well with the ability of the inhibitor of cAMP PDE to relax canine tracheal smooth muscle.7 Phosphodiesterase (PDE) inhibitors should, in theory, be useful in patients with both cardiac and reactive airway disease who require anesthesia. Aminophylline, a nonselective PDE inhibitor, however, is ineffective as a bronchodilator during inhalational anesthesia in dogs because the major effect of acutely administered aminophylline on airways is not caused by PDE inhibition. Rather, it is indirect, and is caused by release of aminophylline of endogenous catecholamines. The release is inhibited by inhalational anesthesia.8

Amrinone, a drug currently in clinical use as an inotropic agent and vasodilator in patients with heart failure,9 is a cGMP-inhibited cAMP PDE inhibitor (PDE III).5-7 This class of drugs could, in theory, have therapeutic effects on airways during inhalational anesthesia, as well as on the cardiovascular system during anesthesia, if amrinone acts directly on the airway rather than indirectly by releasing catecholamines. We, therefore, evaluated the effects of amrinone on pulmonary reactivity to methacholine and on endogenous catecholamine release during thiopental/fentanyl and during halothane (1.5 MAC) anesthesia, first, to determine whether amrinone has beneficial effects on airway reactivity; second, to delineate the role of endogenous catecholamine release in the mechanism of action of amrinone on airways in vivo; and, third, to determine if amrinone would inhibit airway reactivity during halothane anesthesia.

* Assistant Professor of Anesthesiology.
† Professor of Anesthesiology and Environmental Health Sciences and Medicine.

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Address reprint requests to Dr. Hirshman: The Johns Hopkins School of Hygiene and Public Health, Division of Physiology/Room 7006, 615 North Wolfe Street, Baltimore, Maryland 21205.
Materials and Methods

These studies were approved by the Animal Research Committee of The Johns Hopkins University. Animals consisted of five mongral dogs ranging in age from 2–4 yr, and in weight from 19–24 kg. Each dog was studied during four separate conditions performed in random order and separated by at least a week. These four conditions included: 1) thiopental/fentanyl anesthesia, 2) thiopental/fentanyl anesthesia with amrinone infusion, 3) halothane (1.5 MAC) anesthesia, and 4) halothane anesthesia with amrinone infusion. Amrinone studies during thiopental/fentanyl anesthesia served as a positive control. Animals were fasted overnight, received no preanesthetic medication, and were anesthetized while standing supported in a sling. Anesthesia was induced in all conditions with 15 mg/kg intravenous thiopental and tracheal intubation was facilitated with 0.5 mg/kg succinylcholine. The dogs' tracheas were intubated with an 8.5-mm cuffed endotracheal tube, and the lungs were mechanically ventilated (Harvard Apparatus, Millis, MA) with 100% oxygen at a tidal volume of 15 ml/kg. Respiratory rate was 15–20 breaths/min, with an end-tidal carbon dioxide of 38–42 mmHg, and heart rate was monitored from the electrocardiogram (Tektronix 412, Bevron, OR). Blood pressure was measured with an automated blood pressure cuff (Datascop Accutor 1A, Paramus, NJ).

In studies involving intravenously administered anesthetics (conditions 1 and 2), anesthesia was maintained by a continuous infusion of thiopental (0.2 mg·kg⁻¹·min⁻¹) and fentanyl (1 μg/kg) every 20 min until completion of the study. No additional muscle relaxants were used. In studies involving inhalational anesthesia (conditions 3 and 4), halothane was started immediately after intubation and was administered until a steady state end-tidal anesthesia concentration of 1.5 MAC was established. The MAC value of halothane in the dog was assumed to be 0.87%.¹⁰ End-tidal halothane and carbon dioxide concentrations were sampled continuously using a Perkin Elmer 1100 mass spectrometer (Pomona, CA).

In studies involving amrinone, amrinone (2 mg/kg) was administered as a loading dose over 30 min followed by a continuous infusion of 10 μg·kg⁻¹·min⁻¹.

Measurement of Airway Mechanics

Airflow (V) was measured by a pneumotachograph (Fleisch type no. 1; OEM Medical, Richmond, VA) and a differential pressure transducer (Validyne DP45-16, Northridge, CA) that was connected to one channel of a pen recorder (Gould 2500S, Cleveland, OH). A balloon (Spectramed, Dayton, OH) was placed in the esophagus, filled with 0.8–1.2 ml of air and withdrawn to the point where end-expiratory pressure was most negative. A second catheter was placed alongside the balloon and connected to suction to keep the esophageal balloon free of air and secretions. Transpulmonary pressure was recorded by connecting one side of a differential pressure transducer (Valdyne MP 45-18, Northridge, CA) to the esophageal balloon and the other side to a needle in the airway. The output of the pressure transducer was recorded on the second channel of the pen recorder. Both records were electronically integrated by a dedicated pulmonary mechanics microprocessor (Buxco Model 6, Sharon, CT) to give values for lung resistance (Rₜ) and dynamic compliance (Cdyn), which were printed out and averaged over the six preceding breaths by a computer (Texas Instruments Model 703, Temple, TX). Apparatus resistance (2 cmH₂O·l⁻¹·s⁻¹), determined by ventilating a mechanical lung analog with known parameters, was subtracted from the results to give Rₜ.

Aerosol Challenges

After baseline stabilization (usually 30–60 min after induction), inhalation challenge with incremental doses of methacholine (0.03, 0.075, 0.15, 0.30, 0.75, and 3.0 mg/ml) were administered during the four different conditions. Aerosols were delivered by a Hudson 3000 nebulizer (Hudson, Temecula, CA) driven by compressed oxygen, which delivered aerosol particles with a mass median diameter of 5.7 μm. All solutions were dissolved in distilled water. Methacholine was administered for five standardized breaths using an Ayre's t-tube inserted between the nebulizer and the endotracheal tube. The expiratory port was occluded until an inflation pressure of 15 cm H₂O had been obtained.

Maximal change in Rₜ occurred within 3 min of challenge. Maximal Rₜ and Cdyn postchallenge was divided by Rₜ and Cdyn prechallenge (baseline Rₜ and Cdyn) to give fractional increase in Rₜ and decrease in Cdyn. Challenges with increasing methacholine concentrations were administered at 5-min intervals, even if Rₜ and Cdyn had not returned to baseline values.

Plasma Catecholamine Analysis

Venous blood was drawn for plasma epinephrine and norepinephrine concentrations from a site not involved

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in the continuous infusions in the amrinone study during thiopental/fentanyl anesthesia (condition 2). Blood was drawn immediately after induction of anesthesia (just before administration of the loading dose of amrinone), before the first methacholine challenge, and at the completion of the final methacholine challenge. Three milliliters of plasma and 50 μl dihydroxybenzylamine internal standard were shaken for 5 min with acid-washed alumina (50 mg/ml) and 1 ml of tris (hydroxymethyl) aminomethane (pH 8.7). The alumina was washed three times with 1 ml of water and transferred to a 2.5-ml microcentrifuge tube. Water was aspirated and replaced with 100 μl of 0.1 M perchloric acid. Tubes were then vortexed for 15 s and allowed to stand for 5 min, after which they were vortexed for an additional 15 s and centrifuged for 30 s at 13,000 g. Fifty microliters of acid eluate were injected onto a 4.5 mm × 22 cm 5-μm C18 column and eluted with a mobile phase consisting of 70 mm monobasic sodium phosphate, 2.6 mm sodium octyl sulfate, 0.1 mm EDTA, and 8% acetonitrile. Epinephrine and norepinephrine were oxidized at 650 mV (vs. Ag-AgCl) on a Bioanalytical Systems (Columbia, MD) vitreous carbon working electrode. An integrator quantified catecholamines by the method of internal standard sensitivity. The sensitivity of the assay is 20 pg/ml and the intrassay and interassay coefficients of variability are both 3%. 12,13

Statistical Analysis
All data were expressed as mean ± SEM of five dogs. Dose-response curves were analyzed using a two-way ANOVA and a Scheffé multiple comparison test. Baseline values with and without amrinone were compared using a paired t test. A P value < 0.05 was considered significant.

Results
Blood pressure and heart rate are shown in table 1. There was no significant difference in heart rate or blood pressure during thiopental/fentanyl or halothane anesthesia in the absence of amrinone. Blood pressure was not significantly different in dogs anesthetized with either thiopental/fentanyl or halothane anesthesia. However, heart rate decreased during amrinone infusion during both thiopental/fentanyl anesthesia and halothane anesthesia, although it only reached statistical significance during halothane anesthesia.

Before methacholine aerosol challenge, there were no significant differences in baseline Rl and Cdyn values among the four conditions (table 2). The addition of halothane or amrinone did not significantly decrease Rl or increase Cdyn from the baseline values. Methacholine produced dose-related increases in Rl and decreases in Cdyn that became statistically significant at concentrations of 0.075 mg/ml in dogs that received thiopental/fentanyl (fig. 1) and halothane (fig. 2)
halothane (table 3). Plasma epinephrine and plasma norepinephrine concentrations obtained in dogs receiving thiopental/fentanyl anesthesia and amrinone did not increase significantly during amrinone infusion (table 4).

Discussion

This study demonstrates that the airways of dogs react to methacholine in a dose-related manner, and that, during both thiopental/fentanyl and halothane anesthesia, amrinone, a cAMP-selective PDE inhibitor, attenuates the bronchoconstrictor response.

In this study, we measured $R_L$, which is the sum of tissue resistance (viscance) and airway resistance. Ludwig et al. 14 have shown that the percentage of pulmonary resistance caused by tissue resistance remains constant throughout the concentration-response curve. Therefore, a linear correlation exists between changes in $R_L$ and changes in caliber of the small airways. Moreover, Warner et al. 15 have shown similar inhibition of $R_L$, tissue resistance, and airway resistance by halothane. As previously reported, 16 we found no decrease in baseline $R_L$ and increase in $C_{dy}$n during halothane anesthesia and in the presence of amrinone. Therefore, changes in baseline airway tone could not have accounted for our results.

We specifically selected methacholine as our agonist to constrict airways in this study because methacholine constricts airway smooth muscle directly by stimulating muscarinic receptors on the smooth muscle with no reflex effects. 1 Therefore, halothane, which reverses airway constriction, in large part, by blocking airway reflexes, 17 should have less effect on methacholine-induced airway constriction than on constriction produced by agonists, which have both direct and indirect effects. Two previous studies 18, 19 found that halothane produced a small, but significant, attenuation of methacholine-induced airway responses. Our current study showed a trend toward halothane attenuation of methacholine-induced airway responses, but, in this group of animals, it was not statistically significant.

It is possible that the beneficial effect of amrinone on airway reactivity seen in our study was caused by some property of the drug other than PDE inhibition. Mechanisms proposed to account for the bronchodilatory properties include adenosine antagonism 20 and release of endogenous catecholamines. 8 It is unlikely that the beneficial effects of amrinone on airway responses to methacholine in our study were caused by

Figure 1. Increase in pulmonary resistance (*top*) and decrease in dynamic compliance (*bottom*) in thiopental/fentanyl anesthetized dogs in the presence (O—O) and absence (■—■) of amrinone. Each point represents the mean ± SE of five dogs.

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Table 3. Changes in Pulmonary Resistance and Dynamic Compliance with Increasing Methacholine during Thiopental/Fentanyl and Halothane Anesthesia

<table>
<thead>
<tr>
<th>Methacholine Dose (mg·ml⁻¹)</th>
<th>Pulmonary Resistance (cmH₂O·l⁻¹·s⁻¹)</th>
<th>Dynamic Compliance (ml·cmH₂O⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thiopental/ Fentanyl</td>
<td>Halothane</td>
</tr>
<tr>
<td>0.03</td>
<td>5.7 ± 0.8</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>0.075</td>
<td>6.6 ± 0.7</td>
<td>4.7 ± 0.4</td>
</tr>
<tr>
<td>0.15</td>
<td>8.5 ± 0.5</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>0.30</td>
<td>9.8 ± 0.5</td>
<td>6.9 ± 0.4</td>
</tr>
<tr>
<td>0.75</td>
<td>10.7 ± 0.1</td>
<td>9.9 ± 0.4</td>
</tr>
<tr>
<td>3.00</td>
<td>11.0 ± 0.1</td>
<td>10.8 ± 0.2</td>
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Fig. 2. Increase in pulmonary resistance (top) and decrease in dynamic compliance (bottom) in halothane anesthetized dogs in the presence (O-----O) and absence (■-■) of amrinone. Each point represents the mean ± SE of five dogs.

Adenosine antagonism, because milrinone, a related drug, showed no adenosine antagonism. Three lines of evidence indicate that amrinone’s beneficial effects on airways were unrelated to the release of endogenous catecholamines. First, plasma catecholamine concentrations did not increase during amrinone infusion in this study. Second, amrinone attenuated airway responses to methacholine during halothane anesthesia at concentrations of halothane that have been shown to inhibit catecholamine release. Third, cAMP-selective PDE inhibitors dilated airways in dogs that were β-adrenergically blocked.

It is more likely that the beneficial effect of amrinone seen in our study was related to PDE inhibition. Although theophylline, like amrinone, is also a PDE inhibitor, theophylline inhibits cAMP PDE only at concentrations that would be toxic in vivo. Because amrinone is 11 times more potent than aminophylline at relaxing intrinsic tone of the guinea pig trachea, the concentration that produces relaxation of airway smooth muscle should be far lower than that of theophylline, and should be in the therapeutic range. The dose of amrinone used in our study was the same as that used by Makela and Kapur in dogs, which resulted in plasma amrinone levels of approximately 1.8 μg/ml. This is in the concentration range (2 μg/ml) that produced half-maximal relaxation of guinea pig airway smooth muscle. Moreover, the dose of amrinone used in this study, and which had significant effects on airways, is well within the range of plasma levels obtained after administration of therapeutic doses of amrinone in humans. These results are consistent with a study of Heaslip et al., who showed that four selective PDE III inhibitors were effective at reversing serotonin-

Table 4. Plasma Catecholamine Concentrations

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (μg/ml)</th>
<th>Epinephrine (μg/ml)</th>
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<tbody>
<tr>
<td>After induction</td>
<td>63 ± 13</td>
<td>21 ± 10</td>
</tr>
<tr>
<td>Before aerosol challenge</td>
<td>70 ± 25</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>After aerosol challenge</td>
<td>50 ± 9</td>
<td>12 ± 3</td>
</tr>
</tbody>
</table>
duced airway constriction in β-adrenergically blocked dogs, and provide further evidence that the beneficial effect of amrinone on airways is a general property of specific cAMP PDE inhibitors.

In summary, clinically relevant doses of amrinone attenuated methacholine-induced airway responses, even during halothane anesthesia. This attenuation was not caused by the effects of released catecholamines on airway smooth muscle, but, rather, by a direct effect of amrinone on the airway. These data indicate that isozyme-selective PDE inhibitors hold promise for the perioperative treatment of bronchospasm during inhalational anesthesia.

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References
