

A Model of Allergic Nasal Congestion in Dogs Sensitized to Ragweed

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ABSTRACT

Background: Acoustic rhinometry is a noninvasive method that uses sound waves to measure dimensions of the nasal cavity.

Methods: In this study, nasal patency was measured by acoustic rhinometry in allergic Beagle dogs sensitized to ragweed allergen. Ragweed (0.03–0.3%) or vehicle were administered intranasally in isoflurane-anesthetized dogs.

Results: The instillation of ragweed caused a dose-related decrease in nasal cavity volume and minimal cross-sectional area (A_{min}) without adverse systemic effects. Nasal cavity volume and A_{min} decreased within 30 minutes after instillation of the highest ragweed dose by $35.1 \pm 6.0\%$ and $66.4 \pm 13.8\%$, respectively, and persisted for at least 90 minutes. Oral administration of α -adrenergic agonist, *d*-pseudoephedrine (3 mg/kg), or histamine H_1 antagonist chlorpheniramine (10 mg/kg) blocked the ragweed-induced nasal congestion.

Conclusion: These results suggest that the canine model may be used to study upper-airway diseases such as allergic rhinitis and to evaluate the pharmacologic activity of nasal decongestants. (American Journal of Rhinology 17, 227–232, 2003)

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Dogs immunized with ragweed as puppies develop an allergic immune phenotype.^{1,2} The dogs are the offspring of parents with high serum immunoglobulin E (IgE) levels and are sensitized to ragweed by repeated injections within 24 hours of birth through 22 weeks of age. These allergic dogs show elevated total and specific serum IgE^{3,4} and increased numbers of eosinophils^{5,6} in their blood and lungs, as well as an increase in airway resistance^{7–9} and a decrease in dynamic compliance after an inhalation challenge with ragweed.

Acoustic rhinometry (AcR) is a simple objective noninvasive method used to measure nasal cavity dimensions of the upper airways.^{10,11} The technique can be used to characterize nasal patency in humans and animal models. The purpose of this study was to test the hypothesis that the instillation of ragweed extract into the nasal passages of allergic dogs sensitized to ragweed will cause nasal congestion as evaluated by AcR. AcR has been used extensively in humans to study acute nasal airway responses after challenge with pharmacologic treatments such as histamine and after provocation with allergen in sensitized subjects (e.g., house-dust mites, pollen, and cats).^{12–14} The acoustic rhinometer used in humans can be modified to assess dimensions of nasal airway geometry in small experimental animals¹⁵ such as guinea pigs,¹⁶ cats,^{17–19} and large animals like dogs.^{20,21} In previous studies,^{17,20,21} a pharmacologic approach such as intranasal application of histamine or compound 48/80, a mast cell degranulator, was used in cats and dogs to elicit decreased nasal patency. These same models were used to test the pharmacologic activity of oral and topical decongestants. Presently, we studied changes in nasal patency caused by intranasal exposure to ragweed in sensitized dogs. In addition, the effect of oral administration of the α -adrenergic agonist *d*-pseudoephedrine (*d*-PSE) and

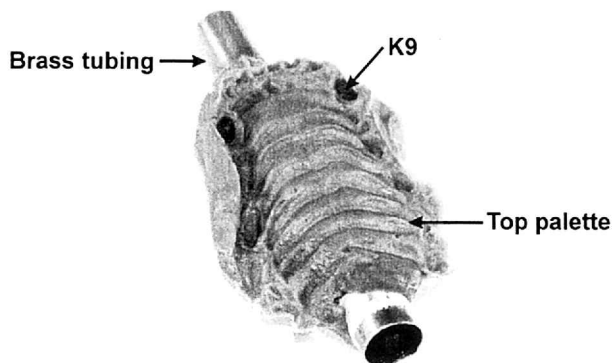


Figure 1. Canine custom-fit inhalation mouthpiece.

histamine H₁ receptor antagonist chlorpheniramine (CTM), given orally, on allergic nasal congestion was evaluated.

MATERIALS AND METHODS

General

Four ragweed-sensitized dogs (9.5–12.5 kg and 2.5–3 years; LRR colony, Albuquerque, NM) were used for the experiments. Dogs were sensitized by repeated ragweed injections starting within 24 hours of birth until 22 weeks of age.¹

During induction of anesthesia, a custom-fit, mouth-only inhalation mouthpiece, and a rubber mask were used to avoid isoflurane (Abbott Laboratories, North Chicago, IL) entering the nasal passages. The mouthpieces were made by applying dental impression material to 1.27 × 10.16-cm sections of brass tubing and placing them in the anesthetized dogs' mouths. After a period of 2–3 minutes, the dental material solidified into the shape of the dog's palette, tongue, and bite pattern (Fig. 1). Custom-made facemasks, constructed out of gauze and latex mold rubber, were placed over the muzzles of the dogs. The facemasks had a hole cut in the end to allow the brass tubing from the inhalation mouthpiece to protrude ~1.27 cm while sealing around its outside. The facemasks also served to occlude the nares of the dogs, thus assuring mouth-only inhalation of the anesthesia. After inducing anesthesia, an endotracheal tube was placed in the trachea, and anesthesia was maintained with isoflurane throughout the experiment. An endotracheal tube is necessary to maintain anesthesia and to prevent aspiration of ragweed. Dogs were placed in a supine position throughout the experiment. Blood pressure, heart rate, body temperature, and oxygen saturation were monitored during each experiment.

Measurement of Nasal Cavity Geometry by Acoustic Rhinometry

Nasal cavity volume and minimum cross-sectional area ($A_{\min}$) were measured in the anesthetized dog using an EcoVision Acoustic Rhinometry System (Hood Laboratories, Inc., Pembroke, MA) according to the methods de-

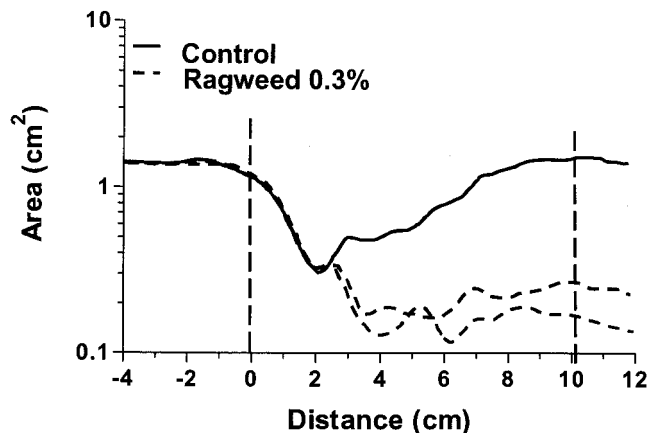


Figure 2. Typical area-distance curves generated using acoustic rhinometry. This figure shows the effects of nasal instillation of vehicle (control) and ragweed (0.3%) in sensitized dogs. Ragweed response was measured twice at the same time point. Dashed lines indicate the distance of the nasal cavity. Note the reduction in the nasal cross-sectional area elicited by ragweed exposure.

scribed earlier.^{15,18,20} Briefly, a wave tube containing a spark sound generator was connected to the nasal cavity using a plastic nosepiece. Based on nasal cast impressions and x-ray measurements from the dog nasal cavity, a distance from the nostril opening into the nasal cavity of 10 cm was used for all experiments. Acoustic reflections were converted to area-distance function curves and were used to determine nasal volume and cross-sectional area (Fig. 2).

Effect of Ragweed Instillation on Nasal Geometry

Baseline measurements were determined for both the left and the right nasal cavities. Responses to three doses of ragweed extract (Hollister-Stier Laboratories, Spokane, WA) were evaluated. Each dose (0.03, 0.1, and 0.3%) in 0.5-mL of phosphate buffered-saline (PBS) was instilled into the left nostril using a 1-mL syringe. The opposite nostril was instilled with 0.5 mL of PBS as control. Previous experiments suggest that repeated inhalation challenges with ragweed within a short time alter the airway responses (unpublished data). To eliminate the effect of repeated nasal exposure to ragweed on nasal congestion, each dog was exposed to the different doses in separate experiments with at least 2-week washout period. Quantitative measurement of the nasal cavities was made at baseline and every 15 minutes after instillation for a maximum of 90 minutes.

Effect of Oral Pretreatment with α -PSE and CTM on Ragweed-Induced Nasal Congestion

The effect of pretreatment with α -adrenergic agonist, α -PSE (3 mg/kg, orally) or the H₁ histamine receptor antagonist CTM (10 mg/kg, orally) on ragweed-induced nasal congestion was determined in separate experiments. Drugs were given orally 2 hours before ragweed (0.3%) or PBS instillation. Nasal volume was measured every 15

minutes before and after the instillation for a maximum of 90 minutes. The *d*-PSE was purchased from Sigma Chemical Co. (St. Louis, MO), and CTM was provided by Schering-Plough Research Institute (Kenilworth, NJ).

Data Analysis

The data for nasal cavity volume and $A_{\min}$ are expressed as the ratio between ragweed- and control-treated nares. Statistical significance ($p < 0.05$) was determined by two-way measure, analysis of variance, followed by Bonferroni *post hoc* analysis and unpaired two-tailed *t*-test (GraphPad Prism, San Diego, CA).

RESULTS

Effect of Ragweed Instillation on Nasal Geometry

The baseline left and right nasal cavity volumes for all treatment groups ranged from $4.45 \pm 0.24 \text{ cm}^3$ to $8.33 \pm 0.52 \text{ cm}^3$. The baseline left/right volume ratios before ragweed instillation (0.03, 0.1, and 0.3%) were 0.91 ± 0.14 , 0.98 ± 0.09 , and 1.07 ± 0.09 , respectively, and were not different. Nasal volume decreased within 15–30 minutes after instillation of ragweed (Fig. 3 A). The maximum nasal congestive effect of ragweed (0.3%) was observed at 60 minutes postchallenge, and the duration of this effect lasted for at least 90 minutes. Nasal volume, after instillation of 0.3% concentration, was significantly different compared with 0.1 and 0.03% concentrations; however, no difference was observed between the two lower doses. Left and right baseline $A_{\min}$ values were between $0.25 \pm 0.02 \text{ cm}^2$ and $0.29 \pm 0.01 \text{ cm}^2$. The effect of ragweed exposure on $A_{\min}$ expressed as the ratios of the treated/untreated is shown in Fig. 3 B. Ragweed produced a dose-dependent decrease in $A_{\min}$ ratios. Moreover, the 0.3% concentration of ragweed produced a maximum decrease in $A_{\min}$ of 34% at 30 minutes. However, because of considerable between-animal variability, a statistically significant effect of ragweed on $A_{\min}$ was not established.

Effect of Oral Pretreatment with *d*-PSE and CTM

The effects of oral *d*-PSE (3 mg/kg) and CTM (10 mg/kg) on the decrease in nasal volume and $A_{\min}$ elicited by ragweed are shown in Figs. 4 and 5. Both drugs attenuated the nasal congestion induced by ragweed instillation. At 60 minutes, the nasal cavity volume ratio for animals treated with *d*-PSE and CTM was 0.90 ± 0.13 and 0.91 ± 0.11 compared with 0.35 ± 0.06 for control animals (Figs. 4 A and 5 A). Similarly, both drugs attenuated the effect of ragweed instillation on $A_{\min}$ (Figs. 4 B and 5 B).

Oral treatment with *d*-PSE and CTM did not affect the heart rate, oxygen saturation, and body temperature (Table I). Blood pressure increased significantly at 60 (Table I) and 75 (not shown) minutes after ragweed instillation in the ephedrine group compared with controls. In contrast, treatment with CTM did not affect blood pressure.

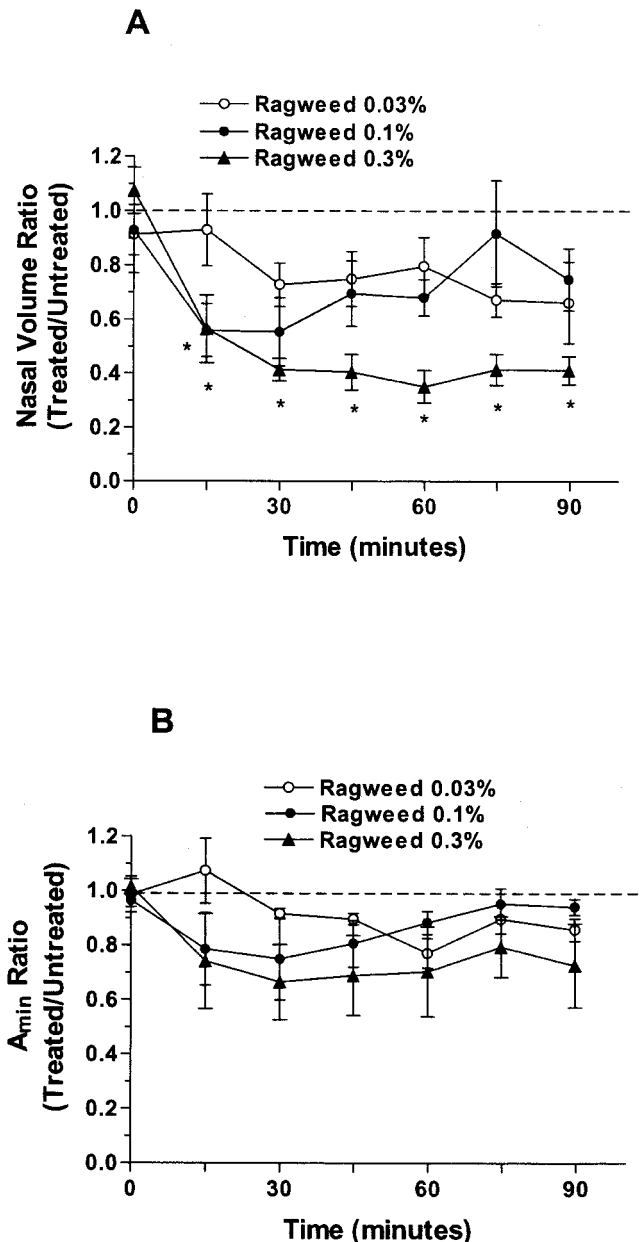


Figure 3. Dose-response characteristics of intranasal ragweed (0.03, 0.1, and 0.3%) exposure on (A) nasal cavity volume and (B) minimum cross-sectional area ($A_{\min}$). Data are expressed as the ratio between ragweed- and vehicle-treated nares. Each bar represents the mean $\pm$ SEM ($n = 4$; $*p < 0.05$ compared with baseline).

DISCUSSION

Our studies indicate that the allergic dog may be a useful model in which to study pathophysiological mechanisms of upper-airway diseases, including allergic rhinitis. Nasal congestion often is a prominent symptom associated with allergic rhinitis. In our canine experiments, we evaluated congestion (*i.e.*, decreases in nasal patency) elicited by nasal exposure to ragweed. We used a noninvasive AcR

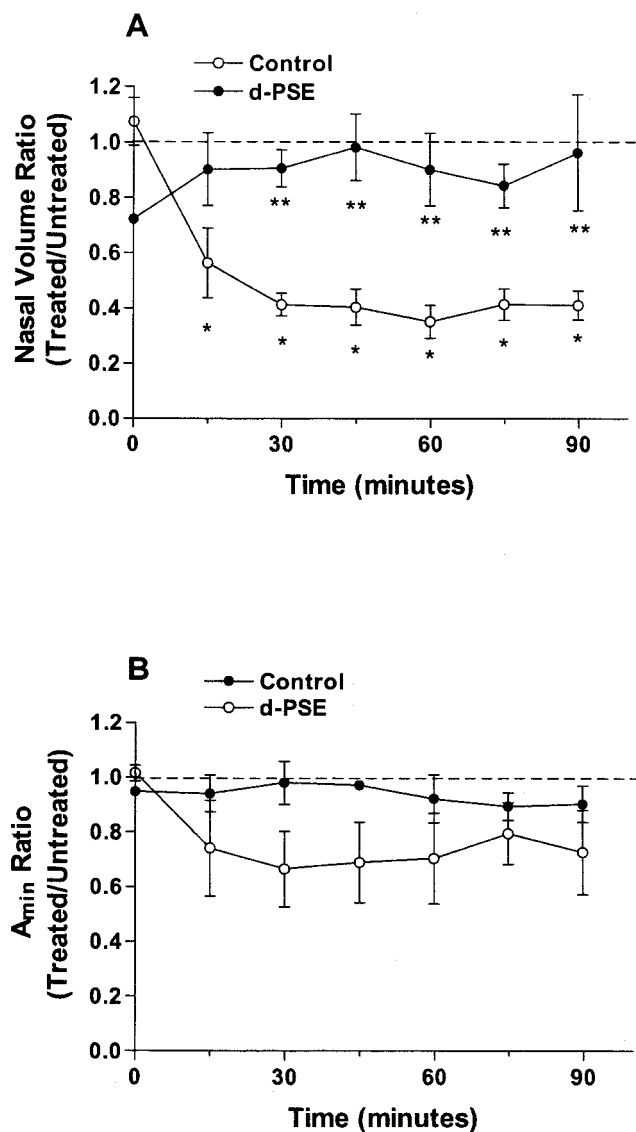


Figure 4. Effect of oral d-PSE on ragweed-induced decrease in nasal cavity volume and minimum cross-sectional area (A_{min}). (A) Shows the effect of d-PSE (3 mg/kg, orally) administered 2 hours before intranasal ragweed (0.3%) challenge on nasal cavity volume. (B) Shows the effect of d-PSE on A_{min} . Each bar represents the mean $\pm$ SEM ($n = 4$; * $p < 0.05$ compared with baseline and ** $p < 0.05$ compared with control animals).

technique that could repetitively determine changes in nasal cavity geometry without significant distress to the animals. Moreover, compared with other techniques, AcR has important advantages. Unlike plethysmography, which measures total pulmonary resistance, AcR measurements can be customized to determine nasal volume between any two points within the nasal cavity.²² Compared with magnetic resonance imaging techniques, AcR is less expensive and easy to learn.^{23,24} Notwithstanding, AcR has been used extensively in human clinical studies.¹²⁻¹⁴ The method has been used to study early and late-phase responses after nasal

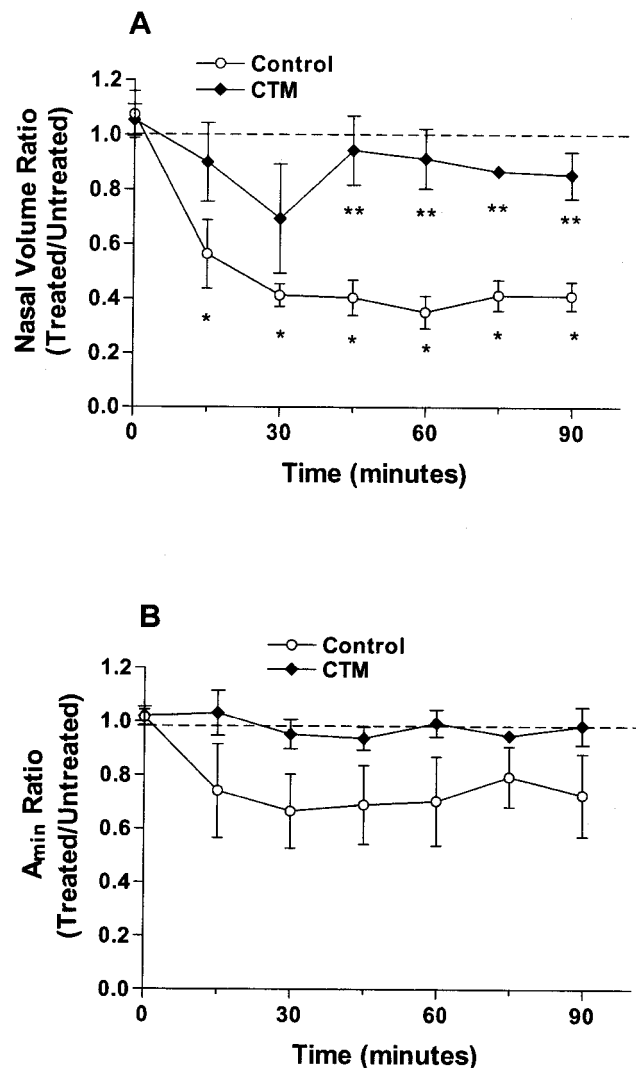


Figure 5. Effect of oral CTM on ragweed-induced decrease in nasal cavity volume and minimum cross-sectional area (A_{min}). (A) Shows the effect of CTM (3 mg/kg, orally) administered 2 hours before intranasal ragweed (0.3%) challenge on nasal cavity volume. (B) Displays the effect of CTM on A_{min} . Each bar represents the mean $\pm$ SEM ($n = 4$; * $p < 0.05$ compared with baseline and ** $p < 0.05$ compared with control animals).

challenges with allergen (e.g., house-dust mites, pollen, and cats) and histamine in asthmatic patients¹² and patients with allergic rhinitis.²⁵ AcR has been applied to a lesser degree in animal studies, viz., guinea pigs,^{16,24} cats,¹⁷⁻¹⁹ and dogs.^{20,21} However, with the exception of a limited number of guinea pig studies,¹⁶ all previous animal models used pharmacologic agents to mimic allergic upper-airway responses. For example, McLeod *et al.*¹⁸ measured decreases in nasal cross-sectional areas in the cat after nasal application of aerosolized compound 48/80.¹⁷ Recently, Koss *et al.* used the AcR technique to study compound 48/80-induced nasal congestion in anesthetized and conscious dogs.²⁰⁻²¹

This study is the first to describe changes in nasal cavity

TABLE I

Effect of *d*-PSE and CTM on Blood Pressure, Heart Rate, Oxygen Saturation, and Body Temperature
(**p* < 0.05 compared with control) in Dogs Sensitized to Ragweed

Treatment	BL	15 min	30 min	60 min	90 min
Mean Blood Pressure (mmHg)					
Controls (ragweed 0.3%)	67 ± 4	71 ± 8	78 ± 1	69 ± 2	78 ± 2
<i>d</i> -PSE (3 mg/kg, p.o.) + ragweed	79 ± 3	78 ± 1	86 ± 1	91 ± 5*	84 ± 3
CTM (10 mg/kg, p.o.) + ragweed	67 ± 6	72 ± 6	68 ± 8	72 ± 6	72 ± 7
Heart Rate (beats/min)					
Controls (ragweed 0.3%)	97 ± 7	88 ± 2	103 ± 9	87 ± 6	88 ± 8
<i>d</i> -PSE (3 mg/kg, p.o.) + ragweed	76 ± 2	71 ± 7	85 ± 18	113 ± 18	85 ± 14
CTM (10 mg/kg, p.o.) + ragweed	90 ± 3	81 ± 5	78 ± 6	76 ± 3	77 ± 5
Oxygen Saturation					
Controls (ragweed 0.3%)	96 ± 1	96 ± 2	97 ± 1	95 ± 1	95 ± 1
<i>d</i> -PSE (3 mg/kg, p.o.) + ragweed	96 ± 1	95 ± 1	97 ± 1	96 ± 1	96 ± 1
CTM (10 mg/kg, p.o.) + ragweed	96 ± 1	97 ± 1	96 ± 1	96 ± 1	96 ± 1
Body Temperature (°C)					
Controls (ragweed 0.3%)	37.3 ± 0.4	36.0 ± 0.0	37.1 ± 0.2	36.0 ± 0.9	36.0 ± 0.4
<i>d</i> -PSE (3 mg/kg, p.o.) + ragweed	37.3 ± 0.4	37.0 ± 0.3	36.5 ± 0.3	36.3 ± 0.3	36.3 ± 0.2
CTM (10 mg/kg, p.o.) + ragweed	37.2 ± 0.4	37.3 ± 0.3	37.0 ± 0.5	36.2 ± 0.6	37.0 ± 0.2

p.o. = orally.

architecture because of ragweed exposure in a large animal model of allergic disease. Our dogs were sensitized to ragweed early in life and developed allergic responses similar to humans as indicated by elevated total serum IgE^{3,4} and increased numbers of eosinophils^{5,6} in their blood and lungs. Furthermore, local instillation of allergen in the lung or whole lung exposure¹ by inhalation induces infiltration of eosinophils and production of total IgE and ragweed-specific IgE and IgG. In addition, these animals display increased pulmonary airway resistance after a challenge with ragweed.^{1,7,9} We found that instillation of ragweed in the naris of ragweed-sensitized dogs produced dose-dependent nasal congestion as indicated by reduction of nasal cavity volume and $A_{\min}$. The nasal congestion started within 15 minutes after instillation of ragweed in these sensitized dogs and was present for at least 90 minutes. Interestingly, the maximum nasal congestion elicited by ragweed instillation was equivalent to the maximum congestion observed by Koss *et al.*^{20,21} in the canine compound 48/80-induced nasal congestion model and by McLeod *et al.*¹⁷ in cats. Similar to the guinea pig model,¹⁶ the onset of nasal obstruction after allergen challenge is more rapid (within 10–15 minutes) compared with nonspecific stimuli such as compound 48/80 (within 60–90 minutes).^{17,20–21}

Congestion is characterized by an engorgement of erectile tissue within the nasal mucosa and is mediated by vasodilation of nasal blood vessels. Currently, clinically used nasal decongestants are α -adrenergic receptor agonists such as PSE. These drugs improve nasal patency by constricting nasal blood vessels. In our studies, *d*-PSE, given

orally 2 hours before ragweed challenge significantly blocked the decrease in nasal volume and $A_{\min}$ produced by intranasal ragweed exposure. Similar nasal decongestant effects of *d*-PSE have been reported in the compound 48/80-induced canine congestion model.²⁰ Additionally, extensive pharmacologic characterization in cats has found that compound 48/80-evoked nasal congestion is responsive to other α -adrenergic receptor agonists such as oral phenylpropanolamine and topically administered oxymetazoline or phenylephrine, all of which have been used to treat nasal congestion clinically. On the other hand, antihistamines such as CTM are not effective nasal decongestants in humans.²⁶ Interestingly, we observed that ragweed-induced nasal congestion was blocked completely by CTM, indicating that histamine plays a prominent role in the present acute allergic congestion model. This observation differs from the results of previous anesthetized canine or feline studies in which CTM did not block compound 48/80-induced nasal congestion.^{18,20} The apparent differences in nasal response to CTM between our results in the allergic dog and previous studies using compound 48/80 to elicit nasal congestion merits additional investigation. However, we cannot rule out the possibility that histamine-elicited mucus secretions partially contributed to the decreased nasal patency produced by ragweed. In support, we observed that the ragweed-treated animals displayed an increase in nasal secretions. However, the evaluation of nasal mucus concentrations and content was beyond the focus of the present studies. It also should be noted that we only evaluated acute nasal responses to ragweed challenge after a

single exposure. We did not study the delayed nasal responses that can occur several hours after nasal antigen exposure. Therefore, it is possible that although acute canine nasal ragweed congestion is sensitive to CTM treatment, delayed or secondary responses may not be as prominently mediated by H_1 receptors.

In conclusion, allergic dogs sensitized to ragweed can be used as a model of nasal congestion using topical application of the specific allergen. Furthermore, AcR can be a useful tool for the pharmaceutical industry to test potential drugs to treat nasal congestion. This model also can be used to achieve a better understanding of the mechanism of allergic rhinitis.

ACKNOWLEDGMENTS

All animal procedures for this study were reviewed and approved by the LRRRI Institutional Animal Care and Use Committee. The Institute is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

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