Measurement of nasal patency in anesthetized and conscious dogs

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ABSTRACT

Experiments were undertaken to characterize a non-invasive, chronic, model of nasal congestion in which nasal patency is measured using acoustic rhinometry. Compound 48/80 was administered intra-nasally to elicit nasal congestion in five beagle dogs either by syringe (0.5 ml) in thiopental-anesthetized animals or as a mist (0.25 ml) in the same animals in the conscious state. Effects of mast cell degranulation on nasal cavity volume as well as on minimal cross sectional area ($A_{\text{min}}$) and intra-nasal distance to the minimal cross sectional area ($D_{\text{min}}$) were studied. Compound 48/80 caused a dose-related decrease in nasal cavity volume and minimal cross-sectional area ($A_{\text{min}}$) together with a variable increase in $D_{\text{min}}$. Maximal responses were seen at 90-120 minutes. Compound 48/80 was less effective in producing nasal congestion in conscious animals which also had significantly larger basal nasal cavity volumes. These results demonstrate the utility of using acoustic rhinometry to measure parameters of nasal patency in dogs, and suggest that this model may prove useful in studies of the actions of decongestant drugs.
Running Title: Acoustic Rhinometry in Dogs

Key Words:
Methods; Acoustic Rhinometry; Conscious Animals; Nasal Congestion; Compound 48/80; Nasal Airway Volume; $A_{\text{min}}$; $D_{\text{min}}$

Glossary of Terms:

**Nasal Airway Volume** is the total volume of the nasal cavity in the analysis segment (10 cm). It is calculated as an integral of the area-distance function over the analysis system (Shown in Fig. 1). Units are cm$^3$.

$A_{\text{min}}$ is the minimal cross-sectional area detected within the analysis system (0-10 cm area). Units are cm$^2$.

$D_{\text{min}}$ is the intra-nasal distance from the end of the nosepiece (0 cm) to the $A_{\text{min}}$ (Shown in Fig. 1). Units are cm.
INTRODUCTION

Most preclinical experimental studies concerning nasal congestion have utilized invasive nasal resistance techniques in experimental animals such as dogs (19), pigs (1, 14) and cats (2). Subsequently, a non-invasive technique, using acoustic reflection (acoustic rhinometry), was developed and characterized (7, 10). Acoustic rhinometry has been used to evaluate changes in the geometry of the human nasal cavity under a variety of conditions (5, 6, 12, 18), however, only very recently has this technology been applied to studies using experimental animals. To date, acoustic rhinometry studies have been performed in guinea pigs (11, 24, 25) and cats (4, 22, 23). Although non-invasive, the use of this technique in these species still requires general anesthesia.

The present experiments were designed to extend the acoustic rhinometric technique to investigate changes in nasal cavity geometry in a large animal model using dogs. As the technique is non-invasive, chronic, repeated measurements can be made in the same experimental subjects. In addition, it is possible to train dogs to remain quiet during simple experimental manipulations. To test this model, sequential experiments were undertaken in 5 beagle dogs in both anesthetized and non-anesthetized states. Nasal cavity geometry was assessed following topical application of the mast cell degranulator, compound 48/80, which has been shown to produce nasal congestion in cats (4, 22, 23).
MATERIALS AND METHODS

General Procedures

Two series of experiments were undertaken using 5 adult male, purpose bred beagle dogs (C and C Kennels, Wewoka, OK) weighing 9-11 kg. All studies were performed in an AAALAC-accredited facility and were undertaken in accordance with the "NIH Guide To The Care and Use of Laboratory Animals".

In one series of experiments, the animals were anesthetized with intravenous thiopental sodium (e.g., 25 mg/kg bolus plus 50 mg supplements at 15-30 minute intervals as needed). Following tracheal intubation with a cuffed endotracheal tube, blood pressure and heart rate were monitored using a V6004 monitor (Surgi Vet, Inc., Waukeha, WI). Body temperature was maintained at approximately 37 °C using a recirculating hot water system. An 8500V pulse oximeter (Nonin Medical, Plymouth, MN) was used to continuously monitor arterial PO₂.

For studies without anesthesia, dogs were trained (daily over a period of about 1 month) to remain still during the measurement period of about 10-15 seconds required for 3 determinations. Animals were gradually acclimated to the procedure with positive reinforcement (dog treats) offered in response to the desired behavior. This initially included training the animals to sit quietly during presentation of the clicking sound produced by the acoustic rhinometer and gradually working up to acceptance of having the probe placed into the nasal cavity. The soft nosepiece used, together with the intranasal application of the
probe, allowed for an effective seal without need of sealant material (as is needed for similar measurements in humans).

For consistency, all determinations were made at the end of expiration. The average of 3 to 5 acoustic rhinometry readings was taken for each time period represented (30-minute intervals). In the majority of cases, 3 sequential readings were consistent and averaged. In a few trials, an obviously out-of-line measurement was obtained. When this occurred, two additional determinations were obtained with all, except for the questionable measurement, averaged and reported for that time point.

*Acoustic Rhinometry Measurement in Dogs*

Anesthetized dogs were placed in a supine position (on a heated thermal blanket) throughout the experiment. Conscious animals were trained to sit quietly on an operating table. Nasal cavity volumes, minimal cross-sectional areas ($A_{\text{min}}$) and the distance to the $A_{\text{min}}$ ($D_{\text{min}}$) were determined using an Eccovision Acoustic Rhinometry System (Hood Laboratories, Inc., Pembroke, MA) according to established methods (8, 22). In brief, a wave tube containing a spark sound generator was connected with the nasal cavity by means of a flexible plastic nosepiece. The distance measured from the nostril opening into the nasal cavity was 10 cm. This distance was chosen based on nasal cast impressions and X-ray determinations made of the dog nasal cavity. Acoustic reflections were recorded and amplified with a computer analysis made of the
local acoustic impedance changes, which, in turn, are used to provide estimates of volume and cross-sectional area of the nares.

After the experimental procedure, the anesthetized animals were placed in a recovery cage and closely monitored throughout the recovery from anesthesia under the close supervision of a member of the University of Oklahoma College of Medicine Veterinary staff. When the animals had completely recovered from the anesthesia, they were then returned to their home cage. Conscious animals did not require close post-experimental supervision. All of the animals tolerated repeated procedures well with no signs of distress and with no residual side effects.

Effects of Topical Application of Compound 48/80

Acoustic rhinometry was used to assess the effects of mast cell degranulation by compound 48/80 on nasal geometry in 5 anesthetized dogs. Each dog received 3 doses of compound 48/80 in a crossover design. In anesthetized preparations, the histamine releaser, compound 48/80 was administered ipsilaterally into the nasal cavity at 3 dosage levels (1.5, 5 and 15 mg) using a syringe. The volume was held constant at 0.5 ml. Conscious animals received compound 48/80 as a nasal mist using an atomizer Model IA-IB (Delong Distributors), also at 3 dosage levels (5, 15 and 45 mg), with a volume of 0.25 ml. In a preliminary study, with conscious animals, the mist and drops of 48/80 had similar effects on the ipsilateral nasal cavity. However, drugs seemed
to also enter into the contralateral side when applied in droplet form in the conscious state. This is likely due to reflex responses in the conscious dogs leading to some mixing of fluid containing compound 48/80 between the two sides of the nasal cavities. A comparable administration of phosphate buffered saline solution (PBS) was used for control experiments for both groups. All measurements were taken before, and for 3 hours after administration of compound 48/80 (at 30-minute intervals). A 2-week washout period was allotted between each experimental group receiving either PBS or compound 48/80.

Drugs and Statistics

Compound 48/80 was purchased from Sigma Chemical Co. (St. Louis, MO) and was dissolved in PBS. Control experiments were undertaken using PBS alone.

Nasal cavity volumes, minimal cross-sectional areas ($A_{\text{min}}$), and the distance to the $A_{\text{min}}$ ($D_{\text{min}}$) were derived directly from the computer calculations of the acoustic rhinometry apparatus. Statistical significance was determined, for values (means $\pm$ SEM) taken at 30-minute intervals, using analysis of variance (ANOVA) followed with Dunnett’s two-tailed t test. Cardiovascular parameters, before and after treatment, were evaluated using a paired two-tailed Student’s t test. Differences were considered statistically significant at $p<0.05$ levels.
RESULTS

**Effect of Compound 48/80 on Nasal Airway Patency in Anesthetized Dogs**

There were no differences between the baseline volumes obtained for the left and right nares or between the baseline values of the dogs prior to compound 48/80 administrations. Basal nasal volumes were $7.0 \pm 0.3 \text{ cm}^3$, $6.7 \pm 0.6 \text{ cm}^3$ and $7.2 \pm 0.5 \text{ cm}^3$ for the 1.5, 5 and 15 mg compound 48/80 trials respectively. Similarly, no significant differences were seen with regard to $A_{\text{min}}$ or to $D_{\text{min}}$ baseline values (see Tables 1 & 2).

Typical examples of area-distance curves taken before and after challenge with compound 48/80 are shown in Figure 1. In these, control values for nasal volume and $A_{\text{min}}$ were $8.52 \text{ cm}^3$ and $0.37 \text{ cm}^2$ in the anesthetized state, and $11.49 \text{ cm}^3$ and $0.45 \text{ cm}^2$ in the same dog without anesthesia. These parameters were reduced to $2.71 \text{ cm}^3$ and $0.12 \text{ cm}^2$, and to $5.63 \text{ cm}^3$ and $0.27 \text{ cm}^2$, 3 hours after topical application of compound 48/80 (15 and 45 mg) respectively. $D_{\text{min}}$ increased from $0.66 \text{ cm}$ to $3.06 \text{ cm}$ and from $0.42 \text{ cm}$ to $5.23 \text{ cm}$ after administration of compound 48/80.

Figure 2 shows composite nasal volume responses of all 5 dogs in response to topical application (0.5 ml) of 3 doses of compound 48/80 (1.5, 5 and 15 mg) in the anesthetized state. Effects of compound 48/80 on $A_{\text{min}}$ and $D_{\text{min}}$ values are shown in Tables 1 & 2.

Composite basal mean arterial blood pressure prior to compound 48/80 administration, under anesthesia, ($n=15$) was $124.8 \pm 4.6 \text{ mmHg}$ and heart rate
was 117.4 ± 7.2 beats/min. There were no significant alterations of these values following any of the doses of compound 48/80 in these anesthetized dogs.

**Effect of Compound 48/80 on Nasal Airway Patency in Conscious Dogs**

Acoustic rhinometry was used to assess the effects of mast cell degranulation by compound 48/80 on nasal geometry in these same 5 dogs, in this case, without anesthesia. As above, each dog received 3 doses of compound 48/80 in a crossover design with no differences between the baseline volumes obtained for the left and right nares or between the baseline values (means ± SEM) of the dogs prior to compound 48/80 administration. Basal nasal volumes were 13.5 ± 1.0 cm³, 12.1 ± 0.3 cm³, and 12.6 ± 0.3 cm³ for the 5, 15 and 45 mg compound 48/80 trials respectively. Similarly, no significant differences were seen with regard to $A_{\text{min}}$ or to $D_{\text{min}}$ baseline values (Tables 1 & 2).

Figure 3 shows composite nasal volume responses of all 5 dogs in response to topical application (0.25 ml mist) of 3 doses of compound 48/80 (5, 15 and 45 mg) in the non-anesthetized condition. Cardiovascular parameters were not measured in the freely moving conscious animals. Tables 1 & 2 document effects of compound 48/80 on $A_{\text{min}}$ and $D_{\text{min}}$ in these animals.
DISCUSSION

Allergic rhinitis is among the most common medical conditions worldwide and presents with a decrease in nasal patency resulting from inflammation of the nasal mucosa, congestion, and rhinorrhea. In the United States, it is estimated that 10-20% of all adults are affected (3). Preclinical studies designed to elucidate the pathophysiological mechanisms as well as drug discovery research in this area have used a variety of experimental animal models.

The "ideal" model for assessment of nasal congestion in animals would be non-invasive, reproducible, easily performed, and would focus on the nasal cavity as compared with the other airway components. The ability to use conscious animals also would eliminate potential confounding influences of general anesthetic agents.

Although a number of different techniques have been utilized to assess nasal patency in animals, none of these fulfills all of the above criteria. For example, although plethysmographic techniques (9) are non-invasive and can be undertaken in conscious animals, plethysmographic airway resistance measurements are not restricted to the nasal cavity. More direct measurements of nasal airway resistance give values restricted to the nasal cavity. However, most of these techniques are highly invasive and usually involve isolation of the nasal cavity from influences of the rest of the airway (14, 19, 29). Magnetic resonance imaging (8, 30) is non-invasive and provides accurate and
reproducible images of the nasopharynx, but requires expensive equipment and highly trained personnel.

Acoustic rhinometry is widely used to study nasal patency in humans (5, 6). Use of acoustic rhinometry has been well-validated using magnetic resonance scanning techniques in humans (8) as well as by comparison with cast impressions of nasal cavities of humans, guinea pigs, and cats (7, 11, 21, 22). Caveats concerning potential sources of artifact and possible misinterpretations of components of the acoustic rhinometry tracings have recently been addressed (31). Acoustic rhinometry is relatively inexpensive and repeated measurements can be made that are restricted to the nasal cavity. This technique is non-invasive, highly reproducible and correlates well with rhinometric (21, 27) and with magnetic resonance techniques (8). Initial preclinical applications of acoustic rhinometry were limited to studies in the guinea pig (25, 26) in which correlations with nasal resistance changes and directly measured nasal cavity volume have been established (11, 24).

More recently, an experimental model using acoustic rhinometry has been applied to studies on the anesthetized cat (4, 22, 23). Nasal congestion is produced by topical administration of histamine or by nasal application of a liberator of mast cell histamine, compound 48/80 (17). In these studies, both procedures significantly reduce the ipsilateral volume and the minimum cross-sectional area of the nasal cavity ($A_{\text{min}}$) while increasing the distance to $A_{\text{min}}$ (4, 22, 23). These findings indicate that this cat model may prove useful in investigations of the basic mechanisms of nasal congestion as well as for
elucidating the mechanisms of action of anti-allergic agents. Although this cat model fulfills many of the criteria listed above, it is unlikely that cats could be easily trained to allow the acoustic rhinometry procedure to be performed in the absence of anesthesia.

In comparison, dogs are more amenable to training for procedures using conscious animals. Use of dogs for studies of nasal congestion may provide several added benefits over other animal models. Not the least of these is the fact that dogs are widely used in the pharmaceutical industry as a model to profile pharmacokinetic and safety aspects of potential new drugs. More specifically, with regard to the upper airway, dogs have a total nasal cavity volume more comparable to that of humans and the nasal physiology and pharmacology is well characterized (19, 20, 28).

In the present study, compound 48/80 produced a consistent dose-related decrease of nasal volume and minimal cross-sectional area ($A_{\text{min}}$) in the anesthetized dog. A more variable increase of the distance from the nosepiece to the $A_{\text{min}}$ ($D_{\text{min}}$) was found, consistent with previous studies in human and other species (4, 12, 26). When compared to results reported in the anesthetized cat, the peak responses were somewhat delayed in time, in that they occurred at about 2 hours after topical application of compound 48/80. Peak responses, in cats, were seen at approximately 1 hour post-treatment (4, 22). Dogs also appear to be somewhat less sensitive to the actions of compound 48/80, as the maximal dose of 15 mg was somewhat greater than the 5 mg dose needed for maximal responsiveness in the anesthetized cat (22).
Compound 48/80 also produced alterations of the nasal geometry in conscious dogs. As in the anesthetized animals, there was a dose-related decrease in nasal volume and $A_{\text{min}}$, as well as a variable increase of the $D_{\text{min}}$ at the highest dose (45mg). Overall, the conscious dogs appeared to be less sensitive to topical 48/80 than seen in the same animals when anesthetized. This observation could be due to the anesthetic drug, thiopental sodium, or to the fact that the nasal volume was much larger in the conscious state.

The pronounced difference in nasal volume between the anesthetized and non-anesthetized preparations is likely due to anesthesia-induced depression of sympathetic neural tone to the nasal vasculature. Nasal blood vessels appear to be highly innervated, as sympathetic nerve section, even in anesthetized animals, results in significantly increased nasal blood flow in rats (13), cats and dogs (15,16). In the anesthetized dog, sympathetic nerve section increases nasal blood flow by between 14 and 43% (15, 16).

In this study, we have characterized a chronic dog model of nasal congestion. Topical application of compound 48/80 was utilized to decrease nasal patency (due to local histamine release from mast cells) as measured by acoustic rhinometry. Mast cell degranulation resulted in a dose-related decrease in nasal cavity volume and minimal cross-sectional area ($A_{\text{min}}$) in both anesthetized and conscious dogs. Increased sympathetic nerve tone in the non-anesthetized preparations was reflected in a much larger basal nasal volume. Acoustic rhinometry in dogs may be a useful tool in investigating
pathophysiological mechanisms of allergic rhinitis, as well as for drug discovery oriented towards novel pharmacological treatments for nasal congestion.

ACKNOWLEDGEMENTS

The authors thank Ms. Linda Hess for her expert technical assistance in these studies.

REFERENCES


Table 1.

**Comparison (means ± SEM) of nasal cavity minimum cross-sectional areas [A\textsubscript{min} (cm\textsuperscript{2})] in anesthetized and conscious dogs after topical compound 48/80 administration (n=5)**

<table>
<thead>
<tr>
<th>Minutes</th>
<th>1.5 mg</th>
<th>5 mg</th>
<th>15 mg</th>
<th>5 mg</th>
<th>15 mg</th>
<th>45 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.36±0.02</td>
<td>0.36±0.01</td>
<td>0.37±0.01</td>
<td>0.40±0.01</td>
<td>0.41±0.01</td>
<td>0.42±0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.33±0.03</td>
<td>0.32±0.03</td>
<td>0.26±0.03*</td>
<td>0.40±0.04</td>
<td>0.41±0.01</td>
<td>0.34±0.03*</td>
</tr>
<tr>
<td>60</td>
<td>0.33±0.03</td>
<td>0.24±0.04*</td>
<td>0.24±0.05*</td>
<td>0.37±0.02</td>
<td>0.37±0.02</td>
<td>0.35±0.03*</td>
</tr>
<tr>
<td>90</td>
<td>0.33±0.02</td>
<td>0.23±0.02*</td>
<td>0.19±0.03**</td>
<td>0.35±0.02</td>
<td>0.38±0.02</td>
<td>0.33±0.03*</td>
</tr>
<tr>
<td>120</td>
<td>0.33±0.03</td>
<td>0.21±0.03**</td>
<td>0.15±0.02**</td>
<td>0.36±0.01*</td>
<td>0.36±0.01*</td>
<td>0.31±0.02**</td>
</tr>
<tr>
<td>180</td>
<td>0.33±0.03</td>
<td>0.23±0.03*</td>
<td>0.14±0.02**</td>
<td>0.34±0.02*</td>
<td>0.36±0.02*</td>
<td>0.35±0.03*</td>
</tr>
</tbody>
</table>

Compound 48/80 was administered as droplets (0.5 ml) to the anesthetized animals and as a mist (0.25 ml) to the conscious dogs.

*\( p < 0.05 \) compared with initial values.

**\( p < 0.01 \) compared with initial values.
Table 2.

Comparison (means ± SEM) of distance to minimum cross-sectional areas \([D_{\text{min}} \text{(cm)}]\) in anesthetized and conscious dogs after topical compound 48/80 administration \((n=5)\)

<table>
<thead>
<tr>
<th>Minutes</th>
<th>1.5 mg</th>
<th>5 mg</th>
<th>15 mg</th>
<th>5 mg</th>
<th>15 mg</th>
<th>45 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.68±0.10</td>
<td>0.76±0.17</td>
<td>0.68±0.08</td>
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</tr>
<tr>
<td>30</td>
<td>0.76±0.22</td>
<td>2.68±0.99</td>
<td>2.92±0.68*</td>
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<td>0.56±0.05</td>
<td>2.93±0.92*</td>
</tr>
<tr>
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<td>3.86±0.46**</td>
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<td>1.43±0.55</td>
<td>3.78±0.70**</td>
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<tr>
<td>120</td>
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<td>4.44±0.48**</td>
<td>0.52±0.02</td>
<td>0.67±0.06</td>
<td>3.64±0.52**</td>
</tr>
<tr>
<td>180</td>
<td>2.64±0.68</td>
<td>4.15±0.87*</td>
<td>3.59±0.76**</td>
<td>0.56±0.04</td>
<td>0.61±0.05</td>
<td>3.03±0.98*</td>
</tr>
</tbody>
</table>

Compound 48/80 was administered as droplets \((0.5 \text{ ml})\) to the anesthetized animals and as a mist \((0.25 \text{ ml})\) to the conscious dogs.

* \(p < 0.05\) compared with initial values.

** \(p < 0.01\) compared with initial values.
Fig. 1. Typical area-distance curves determined using acoustic rhinometry in an anesthetized and conscious dog. Responses on the left (A) are taken from a thiopental-anesthetized dog. Those on the right (B) are from the same animal in the absence of anesthesia. Horizontal axis represents distance from nosepiece in centimeters. Dashed lines represent the dimensions from which the volume is calculated. Fine dots around curves are generated by the acoustic rhinometer and represent 3 standard deviations of 10 rapidly obtained determinations. Arrows indicate the minimal cross-sectional areas ($A_{\text{min}}$). Top curved lines represent the geometry of nasal cavity for the control and the bottom lines represent the geometry of nasal cavity after compound 48/80 taken 3 hours after topical administration of 15 mg of compound 48/80 (0.5 ml) in panel A, and 45 mg of compound 48/80 (0.25 ml) in panel B.
Fig. 2. Effects of three doses (1.5, 5 and 15 mg) of compound 48/80 on nasal cavity volume in 3 separate experiments in anesthetized beagle dogs. Solubilized compound 48/80 was administered unilaterally into the nasal passage (0.5 ml). Values were determined using acoustic rhinometry and were taken at 30-minute intervals for 180 minutes after intra-nasal administration of compound 48/80. Values represent means ± SEM for 5 dogs. The same animals were used for each set of determinations. *p<0.05; **p<0.01 compared with initial values.
Fig. 3. Effects of three doses (5, 15 and 45 mg) of compound 48/80 on nasal cavity volume in 3 separate experiments in conscious beagle dogs. These are the same experimental animals as shown in Figure 2. Compound 48/80 was administered unilaterally as a mist into the nasal passage (0.25 ml). Values were determined using acoustic rhinometry and were taken at 30-minute intervals for 180 minutes after intra-nasal administration of compound 48/80. Values represent means ± SEM for 5 dogs. The same animals were used for each set of determinations. *p<0.05; **p<0.01 compared with initial values.