Measurement of nasal patency in anesthetized and conscious dogs

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Koss, Michael C., Yongxin Yu, John A. Hey, and Robbie L. McLeod. Measurement of nasal patency in anesthetized and conscious dogs. J Appl Physiol 92: 617–621, 2002. First published October 5, 2001; 10.1152/japplphysiol.00891.2001.—Experiments were undertaken to characterize a noninvasive chronic, model of nasal congestion in which nasal patency is measured using acoustic rhinometry. Compound 48/80 was administered intranasally to elicit nasal congestion in five beagle dogs either by syringe (0.5 ml) in thiopental sodium-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 ($A_{min}$) and intranasal distance to $A_{min}$ ($D_{min}$) were studied. Compound 48/80 caused a dose-related decrease in nasal cavity volume and $A_{min}$ together with a variable increase in $D_{min}$. Maximal responses were seen at 90–120 min. 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.

MATERIALS AND METHODS

General procedures. Two series of experiments were undertaken using five adult male, purpose-bred beagle dogs (C and C Kennels, Wewoka, OK) weighing 9–11 kg. All studies were performed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility and were undertaken in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 85-23, revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).

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- to 30-min intervals as needed). After tracheal intubation with a cuffed endotracheal tube, blood pressure and heart rate were monitored by using a V6004 monitor (Surgi Vet, Waukeha, WI). Body temperature was maintained at $37^\circ$C by using a recirculating hot-water system. An 8,500-V pulse oximeter (Nonin Medical, Plymouth, MN) was used to continuously monitor arterial $PO_2$.

For studies without anesthesia, dogs were trained (daily over a period of ~1 mo) to remain still during the measurement period of ~10–15 s required for three 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 changes in nasal cavity geometry in a large-animal model using dogs. Because the technique is noninvasive, 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 five beagle dogs in both anesthetized and nonanesthetized states. Nasal cavity geometry was assessed after topical application of the mast cell degranulator Compound 48/80, which has been shown to produce nasal congestion in cats (4, 22, 23).

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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 three to five acoustic rhinometry readings was taken for each time period represented (30-min intervals). In the majority of cases, three 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_min), and the distance to the A_min (D_min) were determined by using an Ecosvision Acoustic Rhinometry System (Hood Laboratories, 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 on the basis of 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 own cage. Conscious animals did not require close postexperimental 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 five anesthetized dogs. Each dog received three 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 three 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 by using an atomizer (model IA-IB, Delong Distributors), also at three 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 Compound 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 with the dogs 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 PBS was used for control experiments for both groups. All measurements were taken before, and for 3 h after, administration of Compound 48/80 (at 30-min intervals). A 2-wk 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 (St. Louis, MO) and was dissolved in PBS. Control experiments were undertaken by using PBS alone. Nasal cavity volumes, A_min, and D_min were derived directly from the computer calculations of the acoustic rhinometry apparatus. Statistical significance was determined, for values (means  ± SE) taken at 30-min intervals, by using ANOVA followed with Dunnett’s two-tailed t-test. Cardiovascular parameters, before and after treatment, were evaluated by 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 before Compound 48/80 administrations. Basal nasal volumes were 7.0 ± 0.3, 6.7 ± 0.6, and 7.2 ± 0.5 cm³ for the 1.5-, 5-, and 15-mg Compound 48/80 trials, respectively. Similarly, no significant differences were seen with regard to A_min or to D_min baseline values (see Tables 1 and 2).

Typical examples of area-distance curves taken before and after challenge with Compound 48/80 are shown in Fig. 1. In these, control values for nasal volume and A_min were, respectively, 8.52 cm³ and 0.37 cm² in the anesthetized state and 11.49 cm³ and 0.45 cm² in the same dog without anesthesia. These parameters were reduced to 2.71 cm³ and 0.12 cm², and to 5.63 cm³ and 0.27 cm², 3 h after topical application of Compound 48/80 (15 and 45 mg), respectively. D_min increased from 0.66 cm to 3.06 cm and from 0.42 to 5.23 cm after administration of Compound 48/80.

Figure 2 shows composite nasal volume responses of all five 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 and 2.

Composite basal mean arterial blood pressure before
Compound 48/80 administration, under anesthesia
($n = 15$), was 124.8 ± 4.6 mmHg and heart rate was
117.4 ± 7.2 beats/min. There were no significant altera-
tions of these values after any of the doses of Com-
 pound 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 dogs, in this
case, without anesthesia. As described in Effects of
topical application of Compound 48/80, each dog re-
ceived three doses of Compound 48/80 in a crossover
design with no differences between the baseline vol-
umes obtained for the left and right nares or between
the baseline values (means ± SE) of the dogs before
Compound 48/80 administration. Basal nasal volumes
were 13.5 ± 1.0, 12.1 ± 0.3, and 12.6 ± 0.3 cm$^3$ 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
and 2).

Figure 3 shows composite nasal volume responses of
all five dogs in response to topical application (0.25-ml
mist) of three doses of Compound 48/80 (5, 15, and 45
mg) in the nonanesthetized condition. Cardiovascular
parameters were not measured in the freely moving
conscious animals. Tables 1 and 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 pathophysiologic 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 noninvasive, reproducible, easily
performed, and focus on the nasal cavity compared
with the other airway components. The ability to use
conscious animals also would eliminate potential con-
 founding 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 nonin-
vasive and can be undertaken in conscious animals,
plethysmographic airway resistance measurements

![Fig. 1. Typical area-distance curves determined by using acoustic rhinometry in an anesthetized and a conscious dog. A: responses from a thiopental-anesthetized dog. B: responses from the same animal in the absence of anesthesia. x-Axis represents distance from nospiece. Dashed lines represent dimensions from which the volume is calculated. Fine dots around curves are generated by the acoustic rhinometer and represent 3 SDs of 10 rapidly obtained determinations. Arrows indicate the minimal cross-sectional areas. Curved lines at top represent the geometry of nasal cavity for the control, and curved lines at bottom represent the geometry of nasal cavity after Compound 48/80 taken 3 h after topical administration of 15 mg of Compound 48/80 (0.5 ml) in A and 45 mg of Compound 48/80 (0.25 ml) in B.](http://jap.physiology.org/)

**Table 2. Comparison of distance to minimal cross-sectional areas in anesthetized and conscious dogs after topical Compound 48/80 administration**

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Anesthetized Dogs</th>
<th>Conscious Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg</td>
<td>5 mg</td>
<td>15 mg</td>
</tr>
<tr>
<td>0</td>
<td>0.68 ± 0.10</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>30</td>
<td>0.76 ± 0.22</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>60</td>
<td>1.81 ± 0.76</td>
<td>0.56 ± 0.05</td>
</tr>
<tr>
<td>90</td>
<td>2.01 ± 0.74</td>
<td>0.55 ± 0.05</td>
</tr>
<tr>
<td>120</td>
<td>3.09 ± 0.81</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>150</td>
<td>2.64 ± 0.68</td>
<td>0.56 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE given in cm for 5 dogs. 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.
dogs. The same animals were used for each set of determinations. Techniques are highly invasive and usually involve measurements of nasal airway resistance giving values not restricted to the nasal cavity. However, most of these are not restricted to the nasal cavity. More direct administration of Compound 48/80. Values are means and were taken at 30-min intervals for 180 min after intranasal administration of Compound 48/80. Values were determined by using acoustic rhinometry and were limited to studies in the guinea pig (25, 26). 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 repeat measurements can be made that are restricted to the nasal cavity. This technique is noninvasive, highly reproducible, and correlates well with rhinometric (21, 27) and 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 $A_{\text{min}}$ while increasing $D_{\text{min}}$ (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 antiallergic 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 the present study, Compound 48/80 produced a consistent dose-related decrease of nasal volume and $A_{\text{min}}$ in the anesthetized dog. A more variable increase of the $D_{\text{min}}$ was found, consistent with previous studies in human and other species (4, 12, 26). Compared with results reported in the anesthetized cat, the peak responses were somewhat delayed in time, in that they occurred at ~2 h after topical application of Compound 48/80. Peak responses, in cats, were seen at ~1 h posttreatment (4, 22). Dogs also appear to be somewhat less sensitive to the actions of Compound 48/80, because 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 (45 mg). Overall, the same dogs appeared to be less sensitive to topical Compound.

**Fig. 2.** Effects of 3 doses 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 by using acoustic rhinometry and were taken at 30-min intervals for 180 min after intranasal administration of Compound 48/80. Values are means ± SE 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 3 doses 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 Fig. 2. Compound 48/80 was administered unilaterally as a mist into the nasal passage (0.25 ml). Values were determined by using acoustic rhinometry and were taken at 30-min intervals for 180 min after intranasal administration of Compound 48/80. Values are means ± SE for 5 dogs. The same animals were used for each set of determinations. *$P < 0.05$; ** $P < 0.01$ compared with initial values.
48/80 when conscious than when anesthetized. This observation could be due to the anesthetic drug, thiopental sodium, or the fact that the nasal volume was much larger in the conscious state.

The pronounced difference in nasal volume between the anesthetized and nonanesthetized preparations is likely due to anesthesia-induced depression of sympathetic neural tone to the nasal vasculature. Nasal blood vessels appear to be highly innervated, because 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 $A_{\text{min}}$ in both anesthetized and conscious dogs. Increased sympathetic nerve tone in the nonanesthetized 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 toward novel pharmacological treatments for nasal congestion.

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REFERENCES