Nasal cavity dimensions in guinea pig and rat measured by acoustic rhinometry and fluid-displacement method

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Straszek, Sune P., and Ole F. Pedersen. Nasal cavity dimensions in guinea pig and rat measured by acoustic rhinometry and fluid-displacement method. J Appl Physiol 96: 2109–2114, 2004. First published February 13, 2004; 10.1152/japplphysiol.00540.2003.— The purpose of the study was to measure nasal passageway dimensions in guinea pigs and rats by use of acoustic rhinometry (AR) and by a previously described fluid-displacement method (FDM) (Straszek SP, Taagehoj F, Graff S, and Pedersen OF. J Appl Physiol 95: 635–642, 2003) to investigate the potential of AR in pharmacological research with these animals. We measured the area-distance relationships by AR of nasal cavities postmortem in five guinea pigs (Duncan Hartley, 400 g) and five rats (Wistar, 250 g) by using custom-made equipment scaled for the purpose. Nosepieces were made from plastic pipette tips and either inserted into or glued onto the nostrils. We used liquid perfluorocarbon in the fluid-displacement study, and it was carried out subsequent to the acoustic measurements. We found for guinea pigs that AR measured a mean volume of 98 mm$^3$ (95–100 mm$^3$) (mean and 95% confidence interval) of the first 2 cm of the cavity. FDM measured a mean volume of 146 mm$^3$ (117–175 mm$^3$), meaning that AR only measured 70% (50–90) of the volume by FDM. For rats, the volume from 0 to 2 cm was 58 mm$^3$ (55–61 mm$^3$) by AR and 73 mm$^3$ (60–87 mm$^3$) by FDM, resulting in AR only measuring 83% (66–100%) of volume by FDM (see Table 2). We conclude that absolute nasal cavity dimensions are underestimated by AR in guinea pigs and rats. This does not preclude that relative changes may be correctly measured. In vivo trials with AR using rats have not yet been published. The FDM is possibly the most accurate alternative to AR for measurements of the nasal cavity geometry in small laboratory animals, but it can only be used postmortem.

no published studies applying AR in rats, we included this animal because it may be a potential study object.

MATERIALS AND METHODS

Care and Use of Animals

Research, investigations, and care of animals has been in accordance to American Physiological Society’s Guiding Principles in the Care and Use of Animals. The protocol was approved by the Danish Regulations for Use of Laboratory Animals.

AR

We used a system (GJ Elektronik, Skanderborg, Denmark) slightly modified from the one described by Pedersen et al. (15). A sound pulse propagates through a sound tube with an internal diameter of 4 mm, passes a microphone, and enters the nasal cavity via a funnel-shaped nosepiece. The reflected waves travel back through the tube and are recorded by the microphone connected to an amplifier (GJ Elektronik) and via an analog-to-digital converter (PC-Card Das16/12, Computer Boards, MA) sampled by a computer (700 MHz Armada, Compaq) at 100,000 Hz. Measurements are displayed as an area-distance curve, where area is a function of distance with 1.72 mm between sampling points. Before measurements in the animals, the system was calibrated by use of two rigid plastic tube models with step changes of cross-sectional areas and minimum areas of 0.02 cm$^2$, a worst-case model of small-animal nasal cavities. One acoustic measurement in this study always consisted of 5–10 curves of each side of the cavity, each curve made from averaging 10 consecutive sound pulse responses.

FDM

The nasal cavity is placed with the axis of the nasal cavity in a vertical position. It is filled from below with perfluorocarbon through the nostril by a constant flow from a Harvard pump. Changes in the slope of the pressure-time curve at the inlet is proportional to changes in the cross-sectional areas of the nasal cavity with distance. Pressure is measured consecutively at every 0.5 s. For a detailed description of the fluid-displacement-method, physical properties of perfluorocarbon, and potential errors, we refer to a previous study by Straszek et al. (17). A moving-average algorithm was applied to reduce noise in the measurements. We used two consecutive moving averages of five and three samples for guinea pigs, and a single moving average of three samples for rats.

Experimental Protocol

Guinea pigs. We used five female Dunkin Hartley guinea pigs (weight 394–522 g), injected intraperitoneally with a lethal dose of 1 ml pentobarbital sodium (50 mg/ml) and decapitated before the experiments. We made a nosepiece, as described by Pedersen et al. (15), from two pipette tips so that a sleeve was formed to which

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suction is applied through an injection cannula. In guinea pigs, this opens the nasal passageway and secures a tight seal of the nostril. The head was placed with the nasal cavity in the horizontal plane with the back of the nose upward (Fig. 1). Five acoustic measurements were obtained with alternating measurements on each nostril. The sound waves travel through the cavity, reach the end of the nasal septum, and continue backward through the contralateral cavity as well as forward into a common conduit leading to the nasopharynx. AR beyond the septum thus measures the sum of the cross-sectional areas of the common conduit as well as the contralateral cavity.

The fluid-displacement study was carried out on all guinea pigs immediately after the acoustic measurements. Two conical pipette tips were cut at a suitable diameter and glued (Cyanoacrylate, NDO Superlim, Norden Olje, Hadsund, Denmark) around each nostril. They were glued to each nostril so that a fluid-tight transition from the narrow nostrils closed on suction. Instead we used the same technique as for fluid displacement: a few millimeters were cut off of two pipettes so they had an opening cross-sectional area of \( \approx 7.5 \, \text{mm}^2 \). They were glued to each nostril so that a fluid-tight transition from nosepiece to nostril was achieved. After decapitation, the head was placed in a supine position as described for the guinea pigs, and acoustic measurements were done. FDM was subsequently carried out as described above (Fig. 2).

RESULTS

Test Models

Figure 3 shows the results of the measurements on the test models. AR does not portray sudden area changes as accurately as FDM in model 1, but there is no systematic difference. For model 2, which is constricted over a longer distance, AR seems to underestimate the area behind the constriction slightly.

Guinea Pigs

An individual area-distance curve is shown in Fig. 4. The nasal cavity starts at 0 cm where the nosepiece depicted in Fig. 1 opens into the nostril. The high repeatability of both AR and FDM measurements applies to all animals used. At a depth of \( \approx 2 \, \text{cm} \), the nasal septum terminates, and fluid rises through the rhinopharynx common to both sides. In Fig. 5, data from all five guinea pigs (10 cavities) were pooled. Figure 5 shows mean area-distance curves for both acoustic measurements and fluid displacement along with 95% confidence intervals (CI) of the mean. The minimum cross-sectional area (95% CI) is a few millimeters inside the cavity, and it is measured to be 0.019 cm\(^2\) (0.017–0.022 cm\(^2\)) by AR, and 0.054 cm\(^2\) (0.037–0.070 cm\(^2\)) by FDM (Fig. 5). AR measures smaller cross-sectional areas compared with FDM in the distance interval from 0 to 2 cm but higher cross-sectional areas beyond 2 cm. CIs are also narrower for AR than FDM. At \( \approx 2 \, \text{cm} \), the nasal septum dividing the cavity comes to an end. This was confirmed by dissection that revealed an anatomic defect in the lower part of the nasal septum at this point. The mean cross-sectional area there was 0.094 cm\(^2\) measured by AR.

The mean nasal cavity volume (95% CI) from the end of the nosepiece and 2 cm into the cavity was calculated from FDM measurements and found to be 146 mm\(^3\) (117–175 mm\(^3\)). AR measured 98 mm\(^3\) (95–100 mm\(^3\)), which means that volume by AR measures 70% (50–90%) of the volume measured by FDM (Table 1). There was no significant difference between sides or correlation between individual minimum areas and corresponding cavity volume.
Fig. 3. Measurements by AR and FDM on 2 models consisting of joined plastic tubes with cross-sectional areas between 0.02 and 0.075 cm². The filter algorithm applied to FDM is the same as the one used in the guinea pig experiments (see text).

Fig. 4. For guinea pig 4, left side, cross-sectional area measured by AR ($n = 5$ measurements) and FDM ($n = 5$ measurements) as a function of distance is shown. The 95% confidence interval by AR is also shown as lines without symbols. The nasal cavity begins at distance $= 0$ cm. The nasal septum terminates at a distance of $= 2$ cm. The contralateral cavity is filled with fluid during FDM but open during AR.

Fig. 5. For all guinea pigs, mean cross-sectional areas from all cavities ($n = 10$ cavities) measured by AR and FDM as a function of distance are shown. The 95% confidence intervals for AR and FDM are shown as lines without symbols.
Rats

An individual area-distance curve is shown in Fig. 6. The cavity starts at 0 cm and ends at ~2.2 cm. Around 1.85 cm into the cavity, FDM rises sharply and falls again. This phenomenon is only seen in some of the cavities and to a varying degree. The measurements were continued until fluid reached the point where the airway was cut or until fluid ran out through the mouth. Data from all five rats (9 cavities) were pooled and presented in Fig. 7. One cavity was obstructed by glue and could not be reopened. Minimum cross-sectional area (95% CI) of all cavities was 0.017 cm² (0.016–0.018 cm²) by AR and 0.028 cm² (~0.05–0.11 cm²) by FDM. Between animals, variation in AR is minimal, but larger for FDM measurements, especially at the start and end of the nasal septum. FDM produces fairly consistent measurements in the middle part of the cavity, but fluid reaches the nasal septum after 1.9–2.1 cm depending on the animal, and the FDM 95% confidence intervals consequently widen considerably.

Mean volume (95% CI) in a 2.0-cm distance interval starting from the opening of the nostril was found to be 73 mm³ (59–86 mm³) for FDM and 58 mm³ (55–61 mm³) for AR (Table 2). Thus AR measures 83% (66–100%) of the volume measured by FDM. No correlation was found between individual minimum area and corresponding cavity volume.

**DISCUSSION**

**In General**

This study compared measurements obtained postmortem. There is reason to believe that some physiological changes take place shortly after death. However, the aims of this study were to validate how AR measures the complex geometry of nasal cavities in small laboratory animals and to investigate the methodological aspects for future in vivo comparative pharmacological studies. Studies in guinea pigs show that only 70% of the cross section is measured. We believe that AR can measure relative changes in rats, as has been shown to be the case in guinea pigs (5, 6, 12–16). When the curves obtained on the models and the individual animals are compared, it is seen (Figs. 4 and 6) that FDM has a higher resolution than AR, in terms of both distance (more points per distance unit) and cross-sectional area. Other explanations for the difference between AR and FDM in terms of cross-sectional areas and volumes have previously been discussed by Straszek et al. (17).

A number of assumptions concerning planar waves, symmetric structure, and minimal losses inside the cavity may be violated, and the errors increase with distance into the cavity. Other factors can theoretically contribute, including recesses only accessible to fluid, and deformation of the nasal passageways due to fluid pressure, which, however, is minimal. A constriction with a cross-sectional area below a certain threshold may result in severe underestimation by AR of the cross-sectional areas beyond the constriction (4). This is indicated in the narrower of the two models (Fig. 3). The exact value of the threshold area is not known, but it is most likely below 0.01 cm². Finally, a combination of errors, including capillary effects in the nostril and slight differences in position of the head in relation to the direction of gravity (18), may contribute to the differences but are considered of lesser importance (17).

**Table 1. Volume determined by acoustic rhinometry and fluid-displacement method in guinea pigs**

<table>
<thead>
<tr>
<th>Guinea Pig No.</th>
<th>Volume by AR, mm³</th>
<th>Volume by FDM, mm³</th>
<th>(AR/FDM) × 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>(Left + Right)</td>
</tr>
<tr>
<td>1</td>
<td>97</td>
<td>95</td>
<td>192</td>
</tr>
<tr>
<td>2</td>
<td>93</td>
<td>96</td>
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<td>3</td>
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<td>4</td>
<td>96</td>
<td>102</td>
<td>198</td>
</tr>
<tr>
<td>5</td>
<td>104</td>
<td>103</td>
<td>207</td>
</tr>
<tr>
<td>Mean ± 95% CI</td>
<td>98 ± 5</td>
<td>99 ± 2.4</td>
<td>196 ± 8</td>
</tr>
</tbody>
</table>

Values are left, right, and total (left + right) volumes of nasal cavities for both acoustic rhinometry (AR) and fluid-displacement method (FDM). Volumes are measured from the end of the nosepiece to 20 mm inside the cavity (see text). CI, confidence interval.

Fig. 6. For rat 3, right side, cross-sectional area measured by AR (n = 5 measurements) and FDM (n = 4 measurements) as a function of distance is shown. The 95% confidence interval by AR is also shown as lines without symbols. One FDM measurement was discarded due to air bubbles in the cavity. The nasal cavity starts at distance = 0 cm. Note that nosepieces for the rat were different in AR and FDM measurements. To demonstrate the abrupt termination of the septum, fluid was in this case allowed to leak through an empty contralateral cavity, which caused the abrupt rise in cross-sectional area as the nasal septum terminates at 2.23 cm. The origin of the sharp rise and fall in cross-sectional area at distance = 1.8 cm is discussed in the text.
Guinea Pigs

Both AR and FDM showed very good repeatability. However, the differences between animals were much greater for FDM than for AR (Fig. 5), probably due to higher spatial resolution of FDM in detecting individual differences. Our results correspond well with the findings of Kaise et al. (6), despite the fact that they used impression material to estimate the nasal cavity dimensions instead of FDM. The guinea pig appears suited for acoustic measurements because a volume of 146 mm$^3$ leaves enough space for intranasal application of compounds diluted in vehicle. This is supported by results from earlier in vivo experiments on guinea pigs (5, 12–16).

Rats

The same considerations expressed for the guinea pigs regarding comparability of AR and FDM also apply to the rat study. However, rats have a narrower but longer nasal cavity than guinea pigs, and their internal structures possibly reflect sound waves with greater loss. In Fig. 6, FDM measured a spike of $<0.5$ cm from the end of the septum, which was not seen by AR. This is likely because of lower spatial resolution of AR and a recess being filled through an orifice only detectable by FDM. This spike was observed in several cavities, but not all, and contributes to the large confidence interval at this distance (see Fig. 7).

The mean volumes measured by FDM, 72 mm$^3$ (58–86 mm$^3$), and by AR, 58 mm$^3$ (55–61 mm$^3$), contained in the first 2 cm of the nasal cavity are theoretically large enough for vehicle, e.g., 10–20 μl (mm$^3$). However, the cross-sectional area at 2-cm distance, 0.035 cm$^2$ (0.019–0.05 cm$^2$), is only 37% compared with the area in guinea pigs. Because the minimum cross-sectional areas by AR were similar in guinea pigs and rats, a difference here cannot explain the discrepancy between the measured volumes behind the narrowing. Future prospects include development of new algorithms, more sensitive microphones measuring higher frequencies, and optimal relationship between sound tube dimensions and the cavity measured (1). In that way, it may be possible to reduce the signal-to-noise ratio and make measurements of higher resolution. The suction device did not work in rats, but gluing a nosepiece to the nostril was a solution that produced consistent measurements and still allows substances to be applied through the nostrils with a pipette tip. Because of the type of glue, however, survival studies will hardly be possible before a new and better design of nosepiece has been developed. To examine even smaller animals, we tried to measure mice in our experiments. However, we discarded the measurements because of methodological difficulties, including a too-narrow nostril for satisfactory readings, both with AR and FDM. We estimated the volume of a nasal cavity to be 25 μl, hardly enough for intranasal application of pharmacological compounds, although it cannot be excluded with future technical development.

Conclusion

The main findings are an underestimation of cross-sectional areas by AR compared with FDM, both in guinea pigs

Table 2. Volume determined by AR and FDM in rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Volume by AR mm$^3$</th>
<th>Volume by FDM mm$^3$</th>
<th>(AR/FDM) $\times$ 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left (cm$^3$)</td>
<td>Right (cm$^3$)</td>
<td>Total (cm$^3$)</td>
</tr>
<tr>
<td>1</td>
<td>62</td>
<td>65</td>
<td>127</td>
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<tr>
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<td>55</td>
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<tr>
<td>4</td>
<td>58</td>
<td>53</td>
<td>111</td>
</tr>
<tr>
<td>5</td>
<td>*</td>
<td>55</td>
<td>110</td>
</tr>
<tr>
<td>Mean±95% CI</td>
<td>58±5</td>
<td>57±6</td>
<td>117±9</td>
</tr>
</tbody>
</table>

Values are left, right, and total (left + right) volumes of nasal cavities for both AR and FDM. Volumes are measured from the end of the nosepiece to 20 mm inside the cavity. *Measurements could not be obtained from 1 nostril.
and rats. In previous studies (5, 6, 12–16), it has been possible to measure relative changes in guinea pig nasal congestion. On the basis of these studies, we believe this is also possible in the rat. Both species have a nasal cavity volume large enough for intranasal application of mucosa active substances, making them interesting to pharmacological evaluation studies. Fluid displacement has a high repeatability, and it is possibly the closest we can get to estimate the true nasal cavity geometry in small laboratory animals.

ACKNOWLEDGMENTS

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REFERENCES