

Measurement of Nasal Airway Resistance and Response in Mice

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To the Editor,

Measurements of nasal airway resistance (R_{NA}) have been reported in large animal models, such as guinea pigs [1], cats [2] and dogs [3]. Measurement of R_{NA} directly in mice is technically difficult since mice have anatomically smaller nasal cavities than those animals mentioned above. We would like to introduce a simple method to measure R_{NA} and responses in vivo using available and inexpensive equipment.

Female BALB/c mice (6–8 weeks old, 18–20 g body weight) were obtained from Guangdong Laboratory Animal Center (Guangzhou, China) and housed in a specific pathogen-free animal facility at the State Key Laboratory of Respiratory Diseases Guangzhou. The Animal Ethics Committee of the First Affiliated Hospital, Guangzhou Medical College, approved our experimental procedures. Mice were studied in 2 groups: allergic rhinitis (AR) and control groups. The AR group was actively sensitized by three intraperitoneal (i.p.) injections of 20 μ g ovalbumin (grade V, Sigma, USA) emulsified in 1.3 mg of aluminum hydroxide gel (Sigma, USA) in a total volume of 0.2 ml on days 1, 7 and 14. Intranasal (i.n.) challenges with 1% ovalbumin (20 μ l) were performed daily from days 28 to 32 without anesthesia. The control group was sensitized and challenged with normal saline.

On day 33, each mouse was anesthetized with pentobarbital sodium (90 mg/

kg i.p.) and fixed in the supine position. A 15-mm long midline cervical incision was made to expose the trachea. An 18-gauge cannula was inserted into the lower airway trachea directed towards the lung via a tracheostomy. After incision of the frontal wall of the pharynx, a blunt 19-gauge needle was carefully inserted into the nasopharynx through the pharynx (fig. 1). Small wet cotton was put into the oral cavity to prevent any air leak. At the end of the experiment, an autopsy was performed to confirm the position of the needle tip at the nasopharynx. The needle was connected to a polyethylene T tube (inner diameter 0.305 cm). One end of the T tube was connected to a syringe pump (LSP02-1B, Longer Precision Pump, China) which delivered airflow to the nasal passages. Operating in the infuse/withdraw mode, the flow rate was adjusted to 1.0 ml s^{-1} , mimicking the expiration/inspiration cycle of the mouse. Another end of the T tube was connected to a pressure transducer (TRD 4515, Buxco, USA) for measurements of posterior nares pressure. During this flow delivery, the transducer measured the pressure difference (ΔP) through the nasal passage (fig. 2). Data of ΔP were sampled at 1,000 times a second, and were divided by 1.0 ml s^{-1} to give values of total expiration/inspiration resistance. The resistance of the upper airway catheter (0.215 cm H_2O $ml^{-1} s^{-1}$) was then deducted from the total resistance to yield R_{NA} .

For measurements in the early-phase nasal reaction, 20 μ l of a 5-mg/ml solution of histamine in normal saline was nebulized into the nasal cavity by a nebulizer (REF AG-AL1000, Aerogen, Ireland). The nebulizer was placed in front of the nostril, particles were drawn into the nasal cavity by the syringe pump in the withdraw mode, the lower airways were shielded from inspiring histamine. R_{NA} was measured 3 min after histamine challenge.

Lung resistance (R_L) was measured using the RC system (Buxco, USA), as described previously [4]. The mice were then killed by an overdose of pentobarbital sodium, injected i.p., and histological examinations of the nose and lungs were performed. Data are shown as means $\pm$ SD. Statistical significance was measured by Student's t test.

Mean R_{NA} baseline values for the control group were 1.715 ± 0.08 cm H_2O $ml^{-1} s^{-1}$ for expiration and 1.791 ± 0.08 cm H_2O $ml^{-1} s^{-1}$ for inspiration ($n = 8$ in each group). Mean R_{NA} baseline values for the AR group were 3.072 ± 0.29 cm H_2O $ml^{-1} s^{-1}$ for expiration and 3.659 ± 0.39 cm H_2O $ml^{-1} s^{-1}$ for inspiration ($n = 8$ in each group). The R_{NA} baseline values for the AR group were significantly higher than those of the control group (both $p < 0.001$, fig. 3). In both the AR and control groups, nasal challenge with histamine aerosol produced an increase in R_{NA} measured 3 min

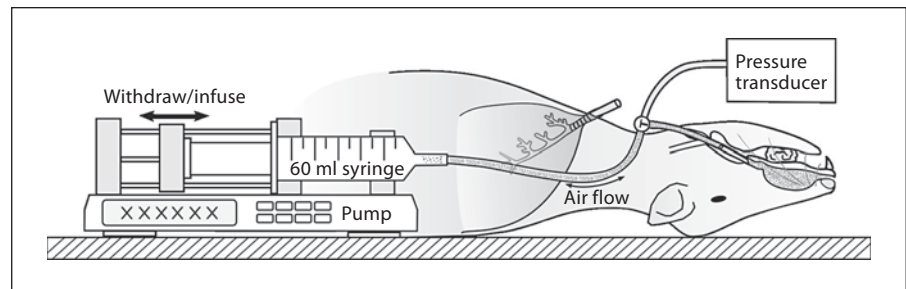


Fig. 1. Schematic for measuring nasal airway resistance in mouse.

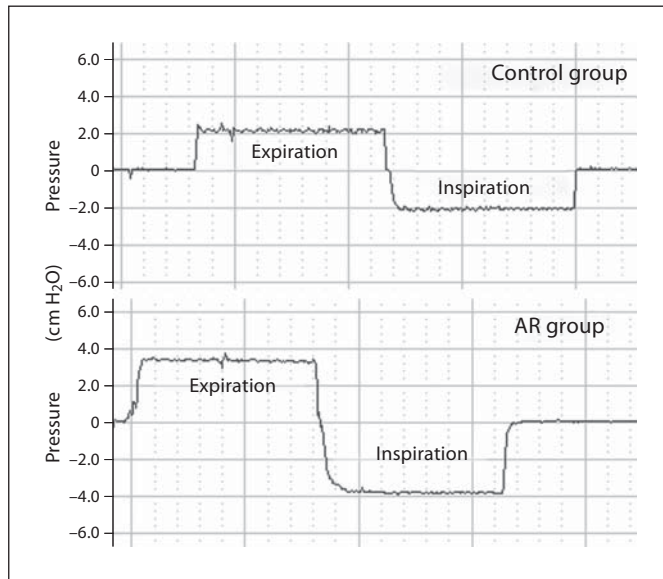


Fig. 2. Pressure tracings showing the pressure difference (ΔP) across isolated nasal airway before histamine challenge in the control and AR groups.

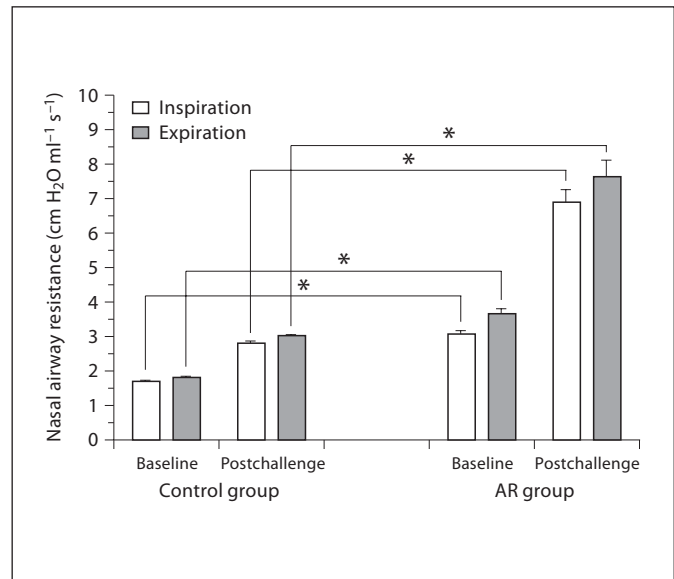


Fig. 3. Nasal airway resistance and response of the control and AR groups. Data are shown as means $\pm$ SD. Statistical significance was measured by Student's *t* test. * $p < 0.001$ vs. control group. $n = 8$ mice per group.

after challenge. Mean R_{NA} post-challenge values for the control group were 2.835 ± 0.43 cm H₂O ml⁻¹ s⁻¹ for expiration and 3.037 ± 0.48 cm H₂O ml⁻¹ s⁻¹ for inspiration ($n = 8$ in each group). Mean R_{NA} post-challenge values for the AR group were 6.902 ± 1.05 for expiration and 7.643 ± 1.41 cm H₂O ml⁻¹ s⁻¹ for inspiration ($n = 8$ in each group). The R_{NA} post-challenge values for the AR group were significantly higher than those of the control group (both $p < 0.001$, fig. 3). The R_L values in the control group (1.681 ± 0.11 cm H₂O ml⁻¹ s⁻¹) and AR group (1.712 ± 0.17 cm H₂O ml⁻¹ s⁻¹) were not significantly different from each other ($p > 0.05$). The R_L values in the control group are comparable to

those R_L values reported by other investigators in this strain of mice [4]. Histological examinations of the nasal mucosa showed obvious infiltration of eosinophils in the AR group, and showed few eosinophils in the control group.

The method is similar to passive posterior rhinomanometry in humans. The syringe pump has an infusion/withdraw mode so that it can mimic the expiration/inspiration flow cycle in mouse. A pressure transducer measures the ΔP across the nasal passage. Hamelmann et al. [5] and Irvin and Bates [6] reported that R_{NA} comprises 50% of the total airway resistance in mice. Using this approach, we showed similar results; R_{NA} comprises

one-half or more of the total airway resistance. R_{NA} baseline values for the AR group were significantly higher than those of the control group. Hellings et al. [7] revealed thickening of nasal mucosa 24 h after repeated allergen challenge of sensitized mice by magnetic resonance imaging scans. The mucosal swelling in the AR group may contribute to the higher baseline R_{NA} in the current study.

Two techniques [8] emerged in recent years to determine R_{NA} or response in mice. Both of them have disadvantages. The first method reported by Nakaya et al. [9] used enhanced pause (Penh) as a non-invasive indicator for evaluating allergen-induced nasal hypersensitivity in a murine

AR model. $Penh$ is a dimensionless marker based on pressure changes within a flow whole body plethysmography. Lower airway responses could affect the results. Moreover, we have found that the baseline values of $Penh$ for the AR mice were very similar to the control mice. Nakaya et al. [9] reported the same results. So $Penh$ is not a sensitive indicator to reflect the change of R_{NA} in mice. A change in respiratory frequency as measured in whole body plethysmography has recently been used to reflect nasal obstruction [10]. However, respiratory frequency is an indirect and insensitive indicator. In a work, only if $R_{NA} \gg R_L$ then changes in $Penh$ or

respiratory frequency can be interpreted as changes in R_{NA} .

Miyahara et al. [10] measured R_{NA} and responses in mice by using custom-designed ventilation equipment (FlexiVent; Scireq, Canada), which was used for both regular ventilation and delivery of the oscillatory signal and can measure the resistance of an open canal by means of piston volume displacement and cylinder pressure. However, the custom-designed ventilation equipment is expensive.

Mouse allergic airway models have been employed extensively, because in these animals various kinds of antibodies and experimental interventions are avail-

able. Simple and reliable methods for evaluating R_{NA} in mice are important for pre-clinical research and development of novel therapies. Here we provide a relatively simple method for measuring physiologic change in the nasal airway in mice.

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