Measurement of Airway Responsiveness in the Anesthetized Mouse

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[Abstract] Airway hyperresponsiveness to methacholine is an important characteristic of asthma. Many devices can be used to measure airway responsiveness in the mouse but it is well established that the invasiveness of a measurement technique is correlated with its precision and reproducibility. This protocol describes how to measure airway responses to aerosolized methacholine in anesthetized, tracheotomized, and mechanically ventilated mice using the forced oscillation technique with FlexiVent® (SCIREQ). This is a computer-controlled precision piston pump that can intersperse mechanical ventilation with volume and pressure controlled manoeuvres to obtain accurate, reproducible measurement of respiratory mechanics.

Materials and Reagents

1. Sterile saline (0.9% NaCl) (B. Braun)
2. Acetyl-β-methylcholine chloride, powder (Sigma-Aldrich, catalog number: A-2251)
3. Drierite (Sigma-Aldrich, catalog number: 238988)
4. Sodium Pentobarbital (5.47%) (CEVA)
5. Xylazine (2%) (Rompun®, Bayer)
6. Cotton wire n50 (DMC, catalog number: 334A/50)

Equipment

1. FlexiVent® system (SCIREQ Technologies) composed of the following modules: FV-M1 (1.8 ml), FV-PP-M1, FV-XC, FV-EC, FV-BU and Aeroneb® Lab nebuliser system associated with Aeroneb® Pro (AG-AL-1000)
2. "1 ml" sterile syringes (Terumo, catalog number: SS+01T1)
3. "25-gauge 0.5 mm" sterile needles (Terumo, catalog number: NN-2516R)
4. 18-gauge metal needle (SCIREQ Technologies, catalog number: 73-0019)

Procedure

Summary of the protocol to measure airway responsiveness to methacholine:

- Mice are weighed and anesthetised i.p. with xylasine and pentobarbital.
- Mice are tracheotomised and a 18-gauge metal needle is inserted into the trachea and connected to the FlexiVent® computer-controlled small animal ventilator.
- Baseline values are measured.
- Saline and then methacholine are aerosolized in the ventilator circuit.
- Airway measurements are performed with the FlexiVent® protocol.
- Mice are disconnected at the end to ensure that they breathe normally again without the aid of the ventilator.

1. Anaesthesia
    a. Bring xylasine and pentobarbital solutions to room temperature (18-25 °C), and place methacholine and saline solutions on ice.
    b. Weigh the mouse.
    c. Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers.
    d. Use "1 ml" syringes and "25-gauge" needles to inject solution and administrate 6 ml/kg of the xylasine solution intraperitoneally (i.p.).
    e. Isolate the mouse in another cage. Wait for 10 min.
    f. Hold the mouse in your hand by the dorsal skin so that its head is up and its rear legs are down. Maintain its tail with fingers.
    g. Use another "1 ml" syringe and a "25-gauge" needle to inject 6 ml/kg of pentobarbital i.p.
    h. Replace the mouse in the cage. Wait for 10 min.
2. Tracheotomy
    a. Check that the mouse is profoundly anesthetised.
    b. Dissect and expose the trachea, place a cotton wire under the trachea, insert the 18-gauge metal needle into the trachea and tie a knot around the trachea with the cotton wire in order to hold the needle in place.
    c. Connect the needle (*i.e.* connect the mouse) to the computer-controlled small animal ventilator (FlexiVent®).
    d. Follow the “Mouse_AN_v5.2.ext” standard protocol supplied with the FlexiVent®. This protocol consists of 12 cycles of 2 perturbations (snapshot and Prime-8) after 10 seconds of nebulization, and allows to measure the resistance and compliance data.
3. Measurement processes and methacholine nebulization
    a. Start ventilation when the mouse is connected.

b. Start the protocol.
c. Add saline in the nebuliser for the first nebulization step (10 sec) and proceed to the 12 perturbations cycles.
d. Rinse the nebuliser 3 times with deionized water (eliminate solution with a syringe and replace it with water, repeat 3 times).
e. Add methacholine in the nebuliser for the second nebulisation step (10 sec) and proceed to the 12 perturbations cycles.
f. Stop the experiment and stop the ventilation.
g. Disconnect immediately the 18-gauge metal needle from the apparatus. The mouse should breathe again normally, indicating it was alive during the measures. Then, euthanize the mouse. If the mouse is dead, data should be eliminated.
h. Rinse the nebulizer 3 times with deionized water.
i. Begin a new analysis session with the following mouse.

4. Data analysis
   a. Export data from the Scireq FlexiVent® V5.2 program to an Excel file.
   b. For each saline or methacholine nebulization, the peak response will be calculated as the mean of the three maximal values (in red on the Figure 1 here below), and used for calculation of airway resistance and compliance. Airway resistance is expressed as cm $H_2O.s.ml^{-1}$ and airway compliance as ml.cm H_2O^{-1}.

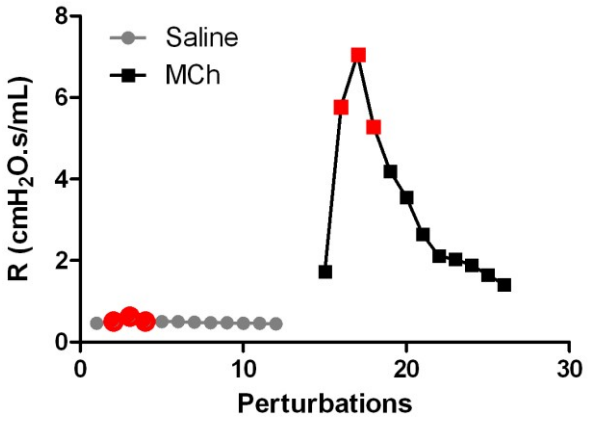

Figure 1.

Recipes

1. Prepare a solution of methacholine containing 50 mg/ml methacholine (0.26 M) diluted in sterile saline. Methacholine must be prepared extemporaneously.

2. Freshly prepare a solution of xylasine containing 0.25% xylasine diluted in sterile saline and a solution of pentobarbital containing 0.9% pentobarbital.

Acknowledgments

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References

1. Reber, L. L., F. Daubeuf, M. Plantinga, L. De Cauwer, S. Gerlo, W. Waelput, S. Van Calenbergh, J. Tavernier, G. Haegeman, B. N. Lambrecht, N. Frossard and K. De Bosscher (2012). A dissociated glucocorticoid receptor modulator reduces airway hyperresponsiveness and inflammation in a mouse model of asthma. *J Immunol* 188(7): 3478-3487.