Assessment of respiratory mechanics in small animals: the simpler the better?

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THE RECENT INTEREST IN SMALL animal models, which are particularly suitable for genetic and translational studies on the pathophysiology of bronchial asthma, has prompted the search for measurements of lung function that are easy to implement and, if possible, noninvasive. In 1997, Hamelmann et al. (6) proposed a plethysmographic method to assess airway responsiveness in mice, which eventually gained wide popularity. The experimental setup of this method is indeed extremely attractive: the animal is awake, free to move, and restrained only in the sense that it is placed in the plethysmograph. Only box pressure (Pb) is measured, and a dimensionless parameter, called “enhanced pause” (Penh), is derived from the shape of the Pb decay during expiration and the ratio of the inspiratory and expiratory maxima of Pb. It was found that Penh increased during bronchoconstriction and, in anesthetized animals, correlated with pulmonary resistance, while it was apparently independent of breathing frequency and pattern (6). These observations led to the consideration of Penh as a valid surrogate for pulmonary resistance.

The enthusiasm about this “unrestrained plethysmography” (UP), however, was paralleled by some skepticism. This was because it was unclear how a mechanical property could be estimated on the basis of a single quantity not coupled to the respiratory system in any unique way or, in terms of systems analysis, assessed without any measured or standardized driving to which the response belongs. Mitzner and Tankersley (10) questioned the key assumptions of UP and also raised serious concerns about the appropriateness of its experimental validation. In a review article on mouse models of airway hyperresponsiveness, Drazen et al. (2) stressed the necessity to validate Penh with measurements of airway caliber based on known physical principles. Petá k et al. (12) compared UP with the low-frequency oscillation technique in mice exposed to 100% O2 and found a sharp increase in Penh, whereas the airway resistance (Raw) decreased and the tissue parameters remained unchanged. This implies that Penh may be completely unrelated to the mechanical properties of the lung but exclusively determined by the breathing pattern, which may differ widely between different species and under different experimental conditions.

The lack of a theoretical basis and the questionable specificity of UP have apparently been recognized by some of the authors of the original publication (6), who included standard (and invasive) measurements of respiratory mechanics in subsequent studies (e.g., Ref. 1). It should also be noted and appreciated that one of the authors of the original article is involved in a critical reevaluation of UP also published in this issue of the Journal of Applied Physiology (9), a paper representing a laudable mission intended to avoid further confusion about UP and to prevent its indiscriminate use in respiratory research.

The key issue about UP is the identification of the sources of Pb, i.e., the physical processes occurring when an animal is breathing unconditioned air and no signal but Pb is available. There is substantial agreement (6, 10) that Pb depends on 1) alveolar gas compression and expansion to generate flow through the airways, and 2) tidal volume (VT) plus any difference in temperature and humidity between the inspired and alveolar gases. The chief object of disagreement is the relative importance of these pressure sources, a question that badly needed to be addressed quantitatively. Lundblad and colleagues (9) point out that, when mice breathe spontaneously in a box where air is at room temperature and humidity, approximately two-thirds of Pb originate from gas conditioning and approximately one-third from gas compression and expansion.

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They also show that, under BTPS conditions, the time integral of P\textsubscript{b} over inspiration is accurately predicted by a term containing Raw, lung volume, and V\textsubscript{T}, which recalls the theory of plethysmographic measurement of Raw based on energy dissipation (8). The conclusion is that, unless lung volume and V\textsubscript{T} are measured or controlled, Penh will not be suitable to characterize airway mechanics (6). It is left to the reader to realize that this condition can hardly be fulfilled with an unrestrained animal in a box and to return to the reality of classic plethysmography as described by DuBois et al. in 1956 (3).

The need for noninvasive and repeatable measurements of airway mechanics in experimental animals cannot be met by technically demanding and sophisticated, yet specific and sensitive methods such as low-frequency oscillation technique (12). Meaningful mechanical variables can be obtained in restrained awake animals by using a double-chamber plethysmograph and forced oscillations (7, 11) or a head-out plethysmograph to measure respiratory flow (4). The article by Glaab et al. (5) in this issue of the *Journal of Applied Physiology* describes the use of head-out plethysmography in the assessment of bronchoconstrictor responses in conscious rats. The method is based on the measurement of expiratory flow (EF\textsubscript{50}) during tidal breathing and has been validated by establishing the relationships between EF\textsubscript{50} in conscious and anesthetized animals and between EF\textsubscript{50} and pulmonary conductance (GL) in anesthetized animals. Under a variety of experimental conditions, EF\textsubscript{50} and GL exhibited a fairly close relationship, which justifies the authors’ conclusion that EF\textsubscript{50} is an appropriate index of bronchoconstriction in a rat model of asthma, although, as noted by Mitzner and Tankersley (10), “almost all respiratory mechanics variables show qualitative correlations.” Despite some differences between EF\textsubscript{50} and GL, probably reflecting different sensitivities to airway and tissue components, this method has the advantages of being relatively simple and based on meaningful physical quantities.

The present commentary is not intended to create a vacuum in methodology by underlying the inadequacy of a technique and then to propose another method with which to fill the vacuum. There is nowadays a broad choice of experimental possibilities, including the few we mentioned above, which yield rather similar respiratory quantities; these techniques differ considerably as regarding the dimensions of sophistication, confirmed validity, and the ease of instrumentation.

**REFERENCES**