Detection of Mediator-induced Airway Constriction by Barometric Plethysmography in Mice

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Abstract

The barometric method has recently been employed to detect airway constriction in small animals. This study was designed to evaluate the barometric method to detect mediator-induced central and peripheral airway constriction in BALB/c mice. First, the central airway constrictor carbachol and the peripheral airway constrictor histamine were employed to induce airway constriction, which was detected by both the conventional body plethysmography and the barometric method in anesthetized mice. Second, bronchoconstriction induced by aerosolized carbachol or other mediators was detected with the barometric plethysmography in conscious, unrestrained mice. Carbachol inhalation caused about four-fold increase in pulmonary resistance (R_L) and about two-fold increase in enhanced pause (Penh) in anesthetized mice. In contrast, in the same preparation, histamine aerosol induced a decrease in dynamic compliance (Cdyn), with no alteration in R_L or Penh. In awake mice, carbachol and methacholine caused increases in Penh, frequency, and tidal volume (V_T). On the other hand, histamine, histamine + bradykinin, and prostaglandin-D_2 did not alter Penh but decreased V_T in conscious mice. These data suggest that there was no sufficient evidence to indicate that Penh could be a good indicator of bronchoconstriction for the whole airways.

Key Words: barometric method, body plethysmography, bronchoconstriction

Introduction

The barometric method (9) is a useful technique to detect respiratory changes. Basically, it uses a detected pressure signal caused by alterations in humidity, temperature, airflow and chest movement during animal's breathing in a closed chamber. Using the barometric plethysmography to measure respiratory changes in awake animals can avoid the anesthetic effect and surgical artifact, and thus the obtained data have high physiological significance. However, the barometric method could not provide conventional parameters for bronchial constriction such as resistance or dynamic compliance. Enhanced pause (Penh), one of the parameters obtained from the barometric method, has been employed as an index of airway constriction (11). Since the publication of Hamelmann *et al*. (11), many studies on altered airway function in awake small animals have been carried out using the barometric method (2, 5, 21, 24).

Conventional body plethysmography can detect airway function mainly in anesthetized animals, and can provide pulmonary resistance (R_L) and dynamic compliance (Cdyn). Drazen (7) has postulated that R_L reflects an alteration in the central airway, and Cdyn reflects the peripheral airway. Accordingly, we investigated if the barometric method detects alterations in both central and peripheral airways. We compared central and peripheral airway constriction detected by the conventional body plethysmography and barometric method in anesthetized mice. Subsequently, we attempted to detect central and peripheral airway constriction using the barometric method in conscious, unrestrained

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mice. Our separation of large and small airway responses was mainly based on the theoretical reasons, using cholinergic agents to constrict the central airways (25) and histamine to constrict the peripheral airways (3). Our results suggested that the parameter Penh *via* the barometric method did not detect peripheral airway constriction. For the detection of central airway constriction, Penh showed the trend but did not match exactly R_L in mice.

Materials and Methods

This study was conducted according to NIH guidelines (Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86-23, Revised 1985, U.S. Government Printing Office, Washington, D.C.).

Determination of R_L and Cdyn Using the Conventional Body Plethysmography in Anesthetized Mice

In this portion, twenty six young BALB/c mice were used. According to our previous method (15), with some modifications, these animals were anesthetized with pentobarbital sodium (70 mg/kg, ip) and prepared to measure esophageal pressure (Pes), airway opening pressure (Pao), flow rate, and tidal volume. Transpulmonary pressure is the difference between Pao and Pes. During spontaneous breathing, R_L and Cdyn were determined using the method of Amdur and Mead (1).

The number and body weight of animals used for each aerosol challenge were: carbachol, n = 7 (20.4 ± 0.6 g); histamine, n = 7 (19.6 ± 0.4 g); histamine + bradykinin, n = 5 (20.0 ± 0.5 g); PGD_2, n = 7 (21.2 ± 0.3 g). Aerosol was generated by placing a 10 ml saline, carbachol (7 mg/ml), histamine (200 mg/ml), histamine (100 mg/ml) + bradykinin (1 mg/ml), or PGD_2 (1 mg/ml) solution in the cup of an ultrasonic nebulizer (DeVilbiss, Somerset, PA, USA) and it was delivered *via* connecting tube and three-way connector to the inlet of the tracheal tube. The median size of the aerosol is approximately 3 μm; the range of the size is from 1 to 5 μm, according to the manufacturers information. About 12 min after anesthesia, each mouse inhaled saline for 3 min and then the R_L and Cdyn were measured for 3 min. Subsequently, the mouse inhaled aerosolized carbachol for 1 min or other agents for 3 min. R_L and Cdyn were then measured for 1 min right after carbachol exposure, or for 3 min immediately following exposure to other agents.

Determination of Penh Using the Barometric Plethysmography in Anesthetized Mice

First, we examined the anesthetic effect on Penh. Eight mice weighing 18.8 ± 0.2 g were anesthetized with sodium pentobarbital (70 mg/kg, ip) and then their respiratory parameters were continuously measured with the barometric plethysmography (Buxco Electronics, Troy, NY, USA). The plethysmograph has two chambers: one is the main or animal chamber (ID 7.5 cm and 5.5 cm height), and the another is the reference chamber (ID 7.5 cm and 3.5 cm height). A differential pressure transducer was employed to detect pressure difference between the above two chambers. The pressure signal was amplified, digitized *via* an A/D convert card, and sent to a computer with a BioSystem XA program (Buxco, Electronics, Troy, NY, USA), which sampled and calculated desired respiratory parameters. Similar to those reported by Hamelmann *et al*. (11), parameters of inspiratory time (Ti), expiratory time (Te), relaxation time (Tr), tidal volume (V_T), breathing frequency (f), minute ventilation, peak inspiratory flow (PIF), peak expiratory flow (PEF), Pause, and Penh were obtained. Tr is defined as the time where the area of pressure decays to 36% of the total expiratory period (area of pressure x time). Pause is (Te – Tr)/Tr and Penh is Pause x PEF/PIF. Soon after the anesthesia, breathing frequency decreased rapidly for a few min and then decreased slowly (Fig. 1A). The time course of anesthesia-induced changes in V_T (Fig. 1B) was similar to those of frequency. On the other hand, anesthesia caused a gradual increase in Penh (Fig. 1C). About 30 min after anesthesia, Penh increased to about three-fold of the awake value. According to the above data, agent-induced alterations were obtained 15-20 min after anesthesia.

A total of 21 mice were used to examine effects of airway constrictors on Penh. After anesthesia, the mouse was cannulated with a tracheal tube and placed inside the barometric plethysmograph. The number and body weight of animals used for each aerosol challenge were: carbachol, n = 7 (19.1 ± 0.8 g); histamine, n = 7 (19.6 ± 0.3 g); and histamine + bradykinin, n = 7 (19.4 ± 0.4 g). Aerosol was generated by the method mentioned above and the aerosol usually filled the chamber within 15-20 sec. About 12 min after anesthesia, each mouse inhaled saline aerosol for 3 min and then its respiratory parameters were measured for 3 min. Subsequently, the mouse inhaled aerosolized carbachol for 1 min. Immediately after the exposure, the aerosol in the chamber was drawn out for 15 sec using the bias flow (0.6 l/min). Respiratory parameters were then measured for 1 min right after carbachol aerosol inhalation. The sequence for histamine and histamine + bradykinin exposures was the same as that of carbachol challenge, except that both aerosol exposure time and respiratory determination time were 3 min.

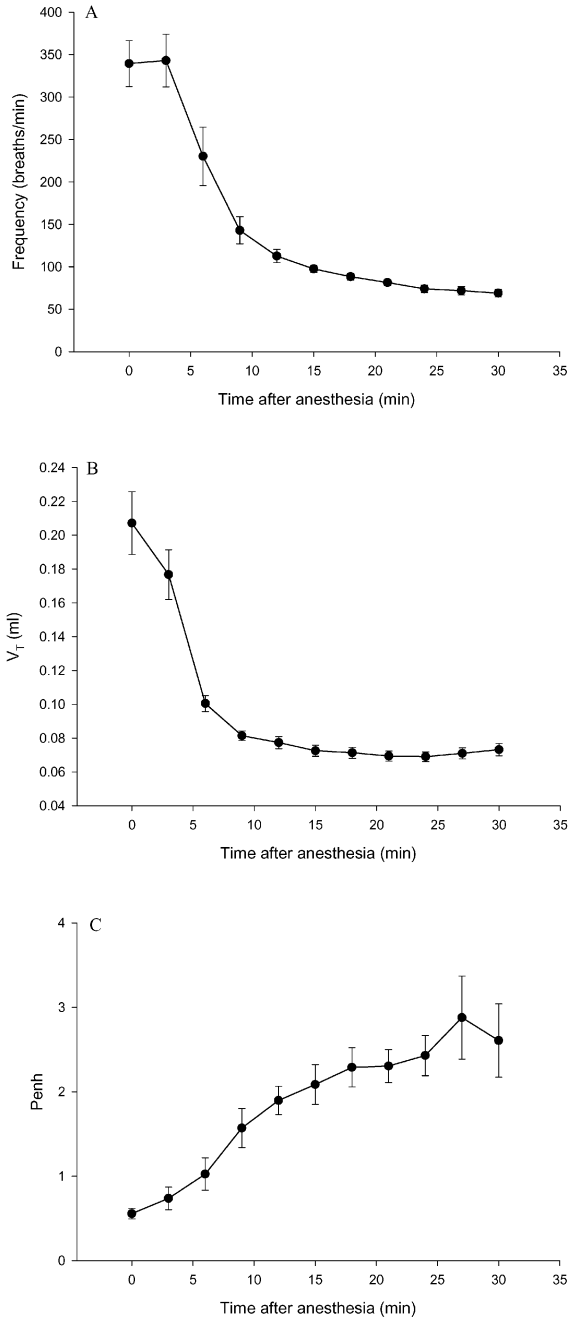

Fig. 1. Sodium pentobarbital-induced change in breathing frequency (A), tidal volume (V_T) (B), and enhanced pause (Penh) (C) in mice (n = 8).

Determination of Penh Using the Barometric Plethysmography in Conscious, Unrestrained Mice

Several agents were aerosolized into the barometric plethysmograph and their effects were compared to that of saline in conscious, unrestrained mice. Each mouse inhaled saline aerosol for 3 min and then the respiratory parameters were measured for 3 min. Then, an aerosol of a pharmacological agent was delivered into the plethysmograph for 3 min. The aerosol in the chamber was cleared immediately after the exposure. Respiratory parameters were then measured for 3 min following the inhalation of the pharmacological agent.

Concentrations of pharmacological agents and the number of animals used were: carbachol (7 mg/ml, n = 10); methacholine (50 mg/ml, n = 9); propranolol (3.5 mg/ml, n = 12); histamine (200 mg/ml, n = 10); bradykinin (1 mg/ml, n = 8); histamine (100 mg/ml) + bradykinin (1 mg/ml) (n = 8); and prostaglandin D_2 (1 mg/ml, n = 13). Histamine and bradykinin were mixed together in one solution for generation of the sixth aerosol. We usually performed challenge with an agent for all animals within one day. Following the challenge with an agent, we cleaned the plethysmograph and connecting tubes. We normalized respiratory values from pharmacological treatment with those of saline exposure and expressed as percent change. In order to test whether the parasympathetic action of methacholine can be blocked by ipratropium bromide (0.25 mg/ml), ipratropium bromide aerosol was first inhaled for 3 min in eight mice. After 15 min, methacholine aerosol was then inhaled for 3 min. Respiratory parameters were monitored for 6 min and two averaged values were obtained for each parameter during the first (0-3 min) and second (3-6 min) 3-min periods.

Statistical Analysis

Values are means ± SEM. Differences in parameters among groups were analyzed with one-way analysis of variance. If significant differences existed among groups, statistical differences between any two groups were analyzed by the Newman-Keuls test (27). Differences were considered significant if $P < 0.05$. Differences between values before and after a pharmacological treatment were analyzed by paired *t*-test.

Results

Comparison between the Conventional and Barometric Plethysmography in Anesthetized Mice

Using conventional plethysmography, carbachol aerosol inhalation increased R_L and decreased Cdyn (Table 1). In the barometric method, the same aerosol increased Penh. Using conventional plethysmography, histamine aerosol inhalation decreased Cdyn but did not increase R_L (Table 1). In the barometric method, the same histamine aerosol caused no change in Penh. Aerosol of the mixture of histamine and bradykinin induced an increase in R_L, no change in Penh, and a decrease in Cdyn in anesthetized mice (Table 1). For anesthetized mice using the conventional body

Table 1. Changes in respiratory mechanics and Penh induced by carbachol, histamine, and histamine + bradykinin in anesthetized mice

Aerosol	Conventional body plethysmography		Barometric method
	R_L ($cmH_2O \cdot s/ml$)	Cdyn (ml/cmH_2O)	Penh
Saline	1.11 ± 0.26	0.028 ± 0.004	1.98 ± 0.14
Carbachol	4.68 ± 1.13*	0.014 ± 0.003*	4.40 ± 0.47*
Saline	1.75 ± 0.35	0.024 ± 0.003	1.23 ± 0.10
Histamine	1.70 ± 0.28	0.016 ± 0.002*	1.20 ± 0.09
Saline	0.83 ± 0.21	0.027 ± 0.003	1.17 ± 0.11
Histamine+ bradykinin	1.31 ± 0.28*	0.020 ± 0.003*	1.07 ± 0.07

Values are mean ± SEM. R_L, pulmonary resistance; Cdyn: dynamic compliance of the lung; Penh: enhanced pause. *Significant difference ($P < 0.05$), as compared to the respective saline (baseline) value.

plethysmography, however, PGD_2 only decreased Cdyn (saline 0.038 ± 0.005 ml/cmH_2O *vs.* PGD_2 0.026 ± 0.004 ml/cmH_2O, $P < 0.05$).

Changes in Respiratory Parameters Induced by Pharmacological Agents in Conscious, Unrestrained Mice

We explored respiratory changes caused by various pharmacological agents using the barometric plethysmography. The base level values for respiratory parameters were: Pause, 0.748 ± 0.019; Penh, 0.528 ± 0.025; f, 280 ± 13 breaths/min; V_T, 0.142 ± 0.004 ml; PIF, 3.137 ± 0.162 ml/s; PEF, 1.914 ± 0.09 ml/s; Te, 0.174 ± 0.014 s; and Tr, 0.086 ± 0.006 s. Both carbachol and methacholine increased Pause (Fig. 2A), Penh (Fig. 2B) and f (Table 2), whereas propranolol, histamine, histamine + bradykinin, and PGD_2 did not alter Penh or f in conscious mice. In most cases, changes in PIF (Table 2) and PEF (Table 2) induced by the above pharmacological agents were similar to those of Penh. The above agents did not affect Ti, but Te (Table 2) and Tr (Table 2) were decreased by carbachol and methacholine. Both carbachol and methacholine caused an increase in V_T, whereas histamine, histamine + bradykinin, and PGD_2 decreased V_T in conscious mice (Table 2). However, bradykinin alone did not induce a change in any parameter (ranged from 93.0 ± 5.5% to 104.9 ± 9.0% of saline baseline values). In addition, the methacholine aerosol-induced increase in Penh was blocked by ipratropium bromide (Fig. 3).

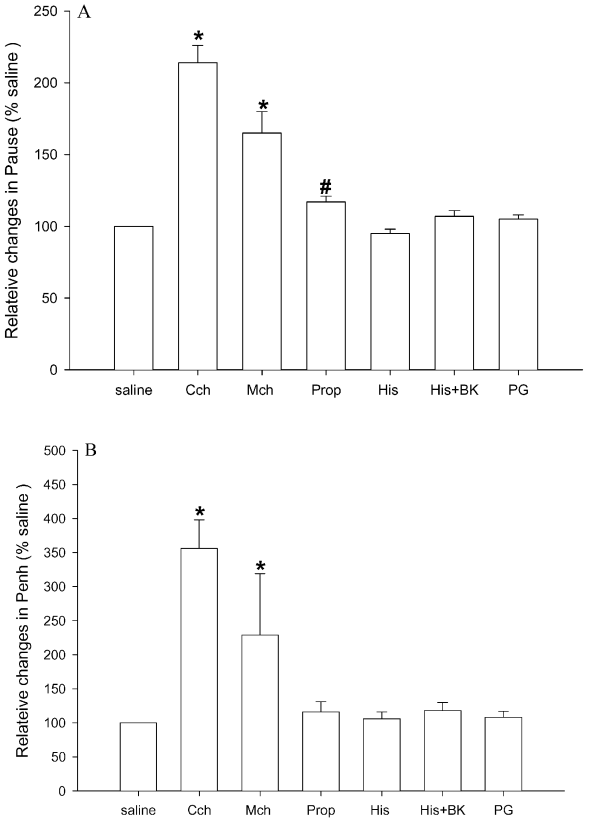

Fig. 2. Relative changes in pause (A) and enhanced pause (Penh) (B), expressed as percent of saline (baseline) value, in six groups of conscious, unrestrained mice. Cch, carbachol; Mch, methacholine; Prop, propranolol; His, histamine; His + BK, histamine + bradykinin; PG; prostaglandin D_2. *Significant difference ($P < 0.05$), as compared to all other groups. #Significant difference ($P < 0.05$) compared to the saline value.

Discussion

We demonstrated that carbachol increased R_L and Penh but decreased Cdyn in anesthetized mice. Histamine, in anesthetized mice, decreased Cdyn but did not affect R_L or Penh. In conscious and unrestrained mice, carbachol and methacholine caused increases in Penh, f, and V_T. In contrast, histamine, histamine + bradykinin, and PGD_2 decreased V_T but did not alter Penh in conscious mice. Several features of the relationship between alterations in respiratory parameters and estimated central/peripheral airway constriction will be discussed as follows.

In an attempt to induce central and peripheral

Table 2. Pharmacological agent-induced relative changes, expressed as percentage of the baseline value, in respiratory parameters of conscious, unrestrained mice

	Saline	Cch	Mch	Prop	His	His+BK	PGD_2
f	100.0	117.0[§]	122.9[*]	106.8	104.9	99.6	95.9
	±0.0	±1.5	±4.9	±4.9	±1.1	±2.0	±3.0
V_T	100.0	151.6[¶]	157.0[¶]	104.6	85.0[|]	85.6[|]	92.1[+]
	±0.0	±7.1	±0.9	±3.6	±0.8	±0.8	±1.3
PIF	100.0	167.2[¶]	165.1[¶]	114.2	94.1	81.3[|]	94.6
	±0.0	±9.1	±2.6	±5.3	±0.7	±3.3	±2.9
PEF	100.0	271.5[¶]	245.8[¶]	118.8	94.5	86.6	93.9
	±0.0	±77.7	±16.9	±19.3	±2.9	±3.9	±3.5
Te	100.0	72.4[‡]	67.6[¶]	97.9	90.1	93.1	108.6
	±0.0	±0.8	±0.4	±8.2	±3.4	±2.8	±5.8
Tr	100.0	47.2[¶]	54.2[¶]	105.0	93.4	91.5	110.1
	±0.0	±2.5	±1.3	±3.1	±5.2	±3.7	±6.5

Values are means ± SEM. Cch,carbachol; Mch, methacholine; Prop, propranolol; His, histamine; BK, bradykinin; PGD_2, prostaglandin D_2; f, breathing frequency; V_T, tidal volume; PIF, peak inspiratory flow; PEF, peak expiratory flow; Te, expiratory time; and Tr, relaxation time.

*Significant difference ($P < 0.05$) compared to the PGD_2 and saline groups.

[+] Significant difference ($P < 0.05$) compared to the Cch, Mch, and saline groups.

[‡] Significant difference ($P < 0.05$) compared to the Prop, PGD_2, and saline groups.

[§] Significant difference ($P < 0.05$) compared to the His+BK, PGD_2, and saline groups.

[|] Significant difference ($P < 0.05$) compared to the Cch, Mch, Prop, and saline groups.

[¶] Significant difference ($P < 0.05$) compared to the Prop, His, His+BK, PGD_2, and saline groups.

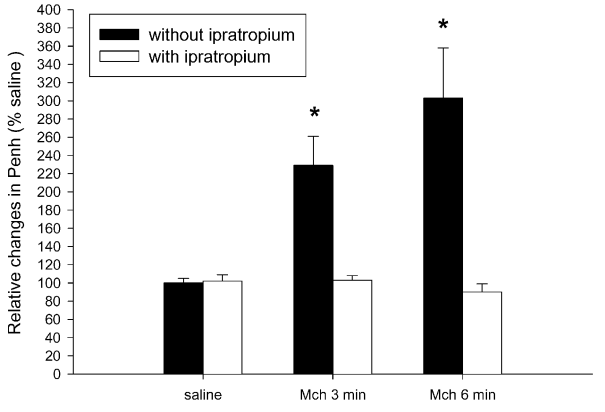

Fig. 3. Methacholine (Mch)-induced relative changes in enhanced pause (Penh), expressed as percent of saline (baseline) value, in the presence or absence of ipratropium bromide. Mch 3 min: averaged values were obtained during the first (0-3 min) 3-min period after Mch exposure; Mch 6 min: averaged values were obtained during the second (3-6 min) 3-min period after Mch exposure. *Significant difference ($P < 0.05$), as compared to the saline (baseline) value in the condition without ipratropium bromide.

airway constriction, two types of constrictors were employed in this study. Cholinergic agonists, carbachol and methacholine mainly cause central airway constriction (25). This central action was demonstrated in the *in vitro* (8) and the *in vivo* (22) experiments. In contrast, histamine acts mainly on peripheral airway and parenchyma (3). Both the *in vitro* (8) and *in vivo* (10) experiments showed this peripheral action of histamine. Similar to histamine, PGD_2 induced much more constriction of the parenchymal strip than that of the tracheal strip (23, 26).

We found that pentobarbital anesthesia decreased f and V_T but increased Penh in mice. These anesthesia-induced alterations in f and V_T were the same as our previous data in anesthetized-tracheostomized rats (14), but were somewhat different from those observed by Hamelmann *et al*. (11). Hamelmann *et al*. (11) found that avertin anesthesia induced a decrease in f and an increase in V_T, with no alteration in Penh. These differences between our study and the paper of Hamelmann *et al*. (11) could be mainly due to different anesthetic agents used. Because of the observed rapid changes in respiratory parameters during the early period following pentobarbital anesthesia, we thus measured pharmacological agent-induced respiratory responses at 15-30 min following the anesthesia.

The barometric method detected respiratory parameters in conscious, unrestrained mice. However, the conventional body plethysmography usually detected respiratory parameters only in anesthetized animals. Since anesthesia affected respiratory parameters (Fig. 1), the barometric method could

avoid the effects of anesthesia. In addition, the conventional method was limited to short periods of time in anesthetized animals. Therefore, in order to analyze sustained changes caused by a single factor, by using the barometric method, the same group of animals could be used in a temporal study. Furthermore, respiratory responses to a stimulus are often inhibited by anesthesia. Taken together, there were several advantages of using conscious, unrestrained animals for study if the method employed could detect all the desired parameters.

The barometric method could not obtain R_L and Cdyn for airway function. In the conventional body plethysmography, it is well established that alteration in R_L indicates a change in the central airways (7, 17), whereas Cdyn is influenced by the state of the peripheral airways (7). It is not clear whether the barometric method, measuring pressure signal caused by alterations in humidity, temperature, airflow and chest movement during animal's breathing in a closed chamber, detects also both central and peripheral airway constriction. By using both methods in anesthetized mice, carbachol challege induced increases in both R_L and Penh (Table 1). However, histamine challenge decreased Cdyn, with no significant change in either R_L or Penh (Table 1). Similar to histamine, PGD_2 induced a decrease in Cdyn, but no change in R_L in anesthetized animals. Therefore, the change in Penh detected by the barometric method seemed to be parallel to the change in R_L detected by the conventional body plethysmography. This fact may imply that Penh may be used as an indication of the central, but not the peripheral, airway constriction. Similarly, Petak *et al*. (21) showed that Penh only relates to R_L insofar as airway constriction leads to alterations in breathing pattern. However, the mediator-induced change in Penh did not exactly match that of R_L. In addition, histamine + bradykinin induced a significant increase in R_L with no significant change in Penh (Table 1).

Mitzner and Tankersley (19, 20) questioned the use of Penh as an index of airway constriction. Lundblad *et al*. (16) reevaluated the barometric method and found that too much more work is needed to accomplish before using the barometric method to detect airway constriction. Similarly, we found that the barometric method could not detect the peripheral airway constriction, and could not always match that of R_L. Therefore, there is no sufficient evidence to indicate that Penh could be a good indicator of airway constriction for the whole airways.

Respiratory changes detected by the barometric method were further examined in conscious unrestrained mice exposed to several pharmacological agents including carbachol, methacholine, propranolol, histamine, bradykinin, histamine + bradykinin, and PGD_2. Their respiratory responses to the above agents can be roughly divided into three types. [1] Carbachol and methacholine increased Penh, pause, PIF, PEF, f, and V_T, and decreased Te and Tr, with no significant change in Ti. Therefore, these alterations were similar to those induced by carbachol in anesthetized mice. According to the analysis mentioned above, carbachol and methacholine aerosol inhalation should induce central airway constriction. Since this constriction was eliminated by a cholinergic blocking agent ipratropium (Fig. 3), this central airway constriction is thus mediated *via* cholinergic mechanism. [2] Histamine, histamine + bradykinin, and PGD_2 induced a decrease in V_T, with no change in either Penh, pause, PIF, PEF, f, Ti, Te or Tr. Basically, this group of agents induced the same action (peripheral airway constriction) as that of histamine, although it was shown that bradykinin's action is controversial (6, 12). [3] Propranolol induced only a significant increase in pause, with no significant change in either Penh, PIF, PEF, f, Ti, Te, Tr, or V_T. Propranolol has been demonstrated to induce constriction in the central (13) and peripheral (25) airways. However, a considerable dose is needed to induce these alterations in the airway (18). It is possible that higher doses of propranolol are required to induce an increase in Penh in our study (4).

In summary, we used several pharmacological agents to induce central and peripheral airway constriction in conscious and anesthetized mice. Our data suggest that there is no sufficient evidence to indicate that Penh could be a good indicator of airway constriction for the whole airways.

Acknowledgments

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References

1. Amdur, M.O. and Mead, J. Mechanics of respiration in unanesthetized guinea pigs. *Am. J. Physiol*. 192: 364-368, 1958.
2. Bergren, D.R. Chronic tobacco smoke exposure increases airway sensitivity to capsaicin in awake guinea pigs. *J. Appl. Physiol*. 90: 695-704, 2001.
3. Burka, J.F. The interaction of histamine with other bronchoconstrictor mediators. *Can. J. Physiol. Pharmacol*. 65:4 42-447, 1987.
4. Chapman, R.W., Danko, D. and Siegel, M.I. Effect of propranolol on pulmonary function and bronchoconstrictor responsiveness in guinea pigs and rats. *Pharmacol. Res. Commun*. 17: 149-163, 1985.
5. Cieslewicz, G., Tomkinson, A., Adler, A., Duez, C., Schwarze, J., Takeda, K., Larson, K.A., Lee, J.J., Irvin, C.G. and Gelfand, E.W. The late, but not early, asthmatic response is dependent on IL-5 and correlates with eosinophil infiltration. *J. Clin. Invest*. 104: 301-308, 1999.
6. Drazen, J.M. and Austin, K.F. Atropine modification of the pulmonary effects of chemical mediators in the guinea pig. *J. Appl*.

Physiol. 38: 834-838, 1975.
7. Drazen, J.M. Physiologic basis and interpretation of common indices of respiratory mechanical function. *Environ. Health. Persp.* 16: 11-16, 1976.
8. Drazen, J.M. and Schneider, M.W. Comparative responses of tracheal spirals and parenchymal strips to histamine and carbachol *in vitro*. *J. Clin. Invest*. 61: 1441-1447, 1978.
9. Drorbaugh, J.E. and Fenn, W.O. A barometric method for measuring ventilation in newborn infants. *Pediatrics* 16: 81-87, 1955.
10. Habib, R.H., Suki, B., Bates, J.H.T. and Jackson, A.C. Serial distribution of airway mechanical properties in dogs: effects of histamine. *J. Appl. Physiol*. 77: 554-566, 1994.
11. Hamelmann, E., Schwaze, J., Takeda, K., Oshiba, A., Larsen, G.L., Irvin, C.G. and Gelfand, E.W. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med*. 156: 766-775, 1997.
12. Homma, T. and Irvin, C.G. Bradykinin-induced bronchospasm in the rat *in vivo*: a role for nitric oxide modulation. *Eur. Respir. J*. 13: 313-320, 1999.
13. Kress, M., Reeh, P.W. and Vyklicky, L. An interaction of inflammatory mediators and protons in small diameter dorsal root ganglion neurons of the rat. *Neurosci. Lett*. 224: 37-40, 1997.
14. Lai, Y.-L., Tsuya, Y. and Hildebrandt, J. Ventilatory responses to acute CO_2 in the rat. *J. Appl. Physiol.* 45: 611-618, 1978.
15. Lai, Y.-L. and Chou, H.-C. Respiratory mechanics and maximal expiratory flow in the anesthetized mouse. *J. Appl. Physiol*. 88: 939-943, 2000.
16. Lundblad, L.K.A., Irvin, C.G., Adler A. and Bates, J.H.T. A reevaluation of the validity of unrestrained plethysmography in mice. *J. Appl. Physiol.* 93: 1198-1207, 2002.
17. Macklem, P.T. and Mead, J. Resistance of central and peripheral airways measured by a retrograde catheter. *J. Appl. Physiol*. 22: 395-401, 1967.
18. MacLagan, J. and Ney, U.M. Investigation of the mechanism of propranolol- induced bronchoconstriction. *Br. J. Pharmacol*. 66: 409-418, 1979.
19. Mitzner, W. and Tankersley, C. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography (letter to the editor). *Am. J. Respir. Crit. Care Med*. 158: 340-341, 1998.
20. Mitzner, W. and Tankersley, C. A reevaluation of the validity of unrestrained plethysmography in mice (letter to the editor). *J. Appl. Physiol*. 94: 828-832, 2003.
21. Petak, F., Habre, W., Donati, Y.R., Hantos, Z. and Barazone-Argiroffo, C. Hyperoxia-induced changes in mouse lung mechanics: forced oscillations *vs*. barometric plethysmography. *J. Appl. Physiol.* 90: 2221-2230, 2001.
22. Romero, P.V. and Ludwig, M.S. Maximal methacholine-induced constriction in rabbit lung: interaction between airways and tissue? *J. Appl. Physiol.* 70: 1044-1050, 1991.
23. Schneider, M.W. and Drazen, J.M. Comparative *in vitro* effects of arachidonic acid metabolites on tracheal spirals and parenchymal strips. *Am. Rev. Respir. Dis*. 121: 835-842, 1980.
24. Sur, S., Wild, J.S., Choudhury, B.K., Sur, N., Alam, R. and Klinman, M. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligo-deoxynucleotides. *J. Immunol*. 162: 6284-6293, 1999.
25. Vijayaraghavan, R., Shaper, M., Thompson, R., Stock, M.F. and Alarie, Y. Characteristic modifications of the breathing pattern of mice to evaluate the effects of airborne chemicals on the respiratory tract. *Arch. Toxicol*. 67: 478-490, 1993.
26. Wasserman, M.A., Griffin, R.L. and Marsalisi, F.B. Potent bronchoconstrictor effects of aerosolized prostaglandin D_2 in dogs. *Prostaglandins* 20: 703-715, 1980.
27. Zar, J.H. Biostatistical Analysis. Englewood Cliffs, NJ: Prentice-Hall, USA, 1984. pp. 162-191.