Tidal breathing pattern differentially antagonizes bronchoconstriction in C57BL/6J vs. A/J mice

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Chen, Bohao, Gustine Liu, Felix Shardonofsky, Maria Dowell, Oren Lakser, Richard W. Mitchell, Jeffrey J. Fredberg, Lawrence H. Pinto, and Julian Solway. Tidal breathing pattern differentially antagonizes bronchoconstriction in C57BL/6J vs. A/J mice. J Appl Physiol 101: 249–255, 2006. First published February 16, 2006; doi:10.1152/japplphysiol.01010.2004.—There is abundant evidence that tidal breathing, and especially tidal breathing at elevated minute ventilation, antagonizes the development and persistence of airflow obstruction during bronchoconstrictor stimulation in normal animals and people. Here, we studied the antiobstructive effect of different tidal breathing patterns in C57Bl/6J and A/J mice during bronchoconstriction induced by continuous or bolus infusion of methacholine. Anesthetized, paralyzed mice were mechanically ventilated at 1,500 ml·kg⁻¹·min⁻¹, using each of three breathing patterns: 5 ml/kg, 300 breath/min; 10 ml/kg, 150 breath/min; or 20 ml/kg, 75 breath/min. Changing from 10 ml/kg, 150 breath/min to 20 ml/kg, 75 breath/min, breathing functionally antagonized bronchoconstriction, reducing the level of airflow obstruction induced by methacholine infusion or boluses equivalently in both strains. In marked contrast, changing from 10 ml/kg, 150 breath/min to 5 ml/kg, 300 breath/min, breathing substantially exacerbated methacholine-induced airflow obstruction in A/J mice, whereas it had no significant effect in C57Bl/6J mice. Our results therefore demonstrate that 1) even at moderate, fixed minute ventilation, the precise breathing pattern can influence the degree of airflow obstruction substantially, and 2) the influence of breathing pattern on bronchoconstriction differs considerably between genetically diverse inbred mouse strains. These findings imply that differences in antiobstructive effects of breathing can contribute to differences in apparent airway constrictor responsiveness. Much attention has been placed on dysregulation of contractile function of airway smooth muscle in human disease. We suggest that important pathophysiology might also be found in impairment of the functional antagonist effect of tidal breathing on airflow obstruction.

asthma; deep inhalation; airway; mouse

“BREATHING IS GOOD FOR BREATHING.” With these words, Fredberg (9) summarized abundant evidence that tidal breathing suppresses experimentally induced bronchoconstriction in normal animals and people. Shen et al. (26) demonstrated that the bronchoconstriction induced by a single intravenous dose of methacholine (MCh) to apneic rabbits was progressively ameliorated, and ultimately could be prevented, by mechanically ventilating animals at progressively greater minute ventilation by increasing tidal volume at fixed breathing frequency or by increasing respiratory rate at fixed tidal volume. In dogs, Salerno et al. (25) showed the magnitude of bronchoconstriction induced by MCh infusion decreased when tidal volume was increased at fixed frequency, even if mean bronchial pressure was maintained constant; Brown and Mitzner (1) found that tidal breathing speeds the resolution of experimentally induced canine bronchoconstriction. Studying guinea pigs, Murphy et al. (23) showed that eucapnic hyperpnea of warm humidified gas (induced by raising breathing frequency with no elevation of tidal volume) markedly reduced bronchoconstrictor responsiveness to intravenous MCh in both immature and adolescent animals. In normal humans, too, breathing antagonizes bronchoconstriction. For example, Freedman et al. (11) found that elevation of minute ventilation accelerated the resolution of MCh-induced bronchoconstriction in normal people, even while their tidal volumes remained <60% of inspiratory capacity. Together, these data indicate that tidal breathing, and especially tidal breathing at elevated minute ventilation, suppresses bronchoconstriction in normal animals and people.

The airflow obstruction induced by a bronchoconstrictor agonist must therefore reflect a balance between the pro-obstruction effect of that agonist and the antiobstruction effect of breathing. Among individuals, differences in apparent airway constrictor responsiveness could thus in theory reflect differences in either of these two components. For example, even if the pro-obstruction effects of a constrictor agonist were identical in two individuals, the apparent constrictor responsiveness to that agent should appear smaller in one if the antiobstructive effect of breathing were greater in that individual.

It is well established that various inbred mouse strains exhibit different degrees of native cholinergic airway constrictor responsiveness that is manifest even in the absence of induced or spontaneous airway inflammation (7). Among strains, A/J mice are famously hyperresponsive, whereas C57Bl/6J mice exhibit much more blunted bronchoconstriction in response to MCh (3, 18). These differences in native constrictor responsiveness are genetically determined (3) and may stem in part from differences in airway smooth muscle shortening velocity (5), but other potentially contributing mechanisms have not been reported. In particular, it is unknown whether differences in the antiobstructive effect of breathing noted above contribute to the apparent differences in constrictor responsiveness between these strains. In the present study, we tested the hypothesis that differential antiobstructive effects of breathing could contribute in part to the apparent
differences in cholinergic airway constrictor responsiveness of A/J vs. C57Bl/6J mice. Our results support this possibility.

METHODS

We performed two series of experiments. In the first, 22 C57Bl/6J and 20 A/J mice of either sex were purchased from the Jackson Laboratory and housed in a specific pathogen-free facility maintained by The University of Chicago Animal Resources Center. Animal care and use were in accordance with institutional and National Institutes of Health guidelines and were approved by the University of Chicago Institutional Animal Care and Use Committee. All animals were anesthetized with ketamine hydrochloride (200 mg/kg ip) and xylazine (40 mg/kg ip) and paralyzed with pancuronium bromide (0.2 mg/kg ip). A stable depth of anesthesia was maintained by supplemental administration of 30% of the initial anesthetic dose at 25-min intervals. After tracheostomy, the trachea was cannulated with a blunt 18-gauge metal needle. The mouse was ventilated with a computer-controlled small-animal ventilator (flexiVent; SCIREQ, Montreal, Quebec, Canada) using tidal volume (VT) of 10 ml/kg and respiratory frequency (f) of 150 breaths/min (breath/min). Positive end-expiratory pressure (PEEP) of 2 cm H2O was applied throughout. An external jugular vein was isolated for intravenous infusion of MCh as described below. Heart rate was monitored by electrocardiogram with needle electrodes to provide evidence that each animal remained alive throughout the experiment.

To gauge the influence of VT-f combination on airway caliber at baseline or during MCh-induced bronchoconstriction, we varied VT (10, 5, or 20 ml/kg) while adjusting frequency (150, 300, or 75 breath/min, respectively) to keep minute ventilation constant. First, mice were studied without MCh infusion. Each mouse received two breaths/min, respectively) using the 10 ml/kg, 150 breath/min breathing, and in 16 A/J and 17 C57BL/6J mice, MCh (0.1 mg/ml iv) was infused by syringe pump at a rate of 4.2 ml/min) to measure CO2 concentration in exhaled gas, and positioned the sampling catheter in the airway colinear with the trachea (i.e., beyond the inspiratory and expiratory limbs of the ventilator circuit, into the common inspiratory and expiratory path of the mouse). Each mouse was ventilated for 5 min using one of the three ventilation patterns, then end-tidal CO2 (ETCO2) was measured in a standard way, during two breaths of the 20 ml/kg, 75 breath/min pattern. In so doing, we excluded any possibility of introducing error in the way of ETCO2 measurement. For each Rrs measurement, tidal ventilation was stopped, the mouse exhaled for 1 s, Rrs was again measured as noted above, and ventilation was resumed. In this way, Rrs was always measured with the same perturbation and at the same lung volume (FRC with 2 cm H2O PEEP), with only brief (2 s) interruptions of tidal breathing pattern. Next, each mouse was returned to 10 ml/kg, 150 breath/min breathing, and in 16 A/J and 17 C57Bl/6J mice, MCh (0.1 mg/ml iv) was infused by syringe pump at a rate chosen to cause modest or more severe bronchoconstriction. Once the airflow obstruction reached a steady level of severity, MCh infusion was continued and the entire ventilation pattern sequence (2 deep inflations; 3’20’’ 10 ml/kg, 150 breath/min; 3’20’’ 5 ml/kg, 300 breath/ min; and 3’20’’ 20 ml/kg, 75 breath/min) was repeated, with Rrs recorded every 20 s as above. In some mice, the MCh infusion rate was increased yet more to establish even greater steady bronchoconstriction, and the ventilation pattern sequence and Rrs recordings were again repeated. For each 3’20’’ epoch of breathing pattern, the last two Rrs measurements were averaged and used as the Rrs datum for that epoch. In some mice, during each rate of MCh infusion, the ventilation pattern sequence was extended (2 deep inflations, 3’20’’ each of 10, 5, 20, 5, 20, and 10 ml/kg, with frequency adjusted accordingly) to explore the possibility that the order of breathing pattern imposition influenced our results. In these cases, the pattern of Rrs variation with breathing pattern was similar for the first three epochs and the second three epochs, and so values for corresponding breathing patterns from the first three and second three epochs were averaged. Including these measurements (36 ventilation pattern sequence observations in 11 A/J mice and 26 observations in 10 C57Bl/6J mice), a total of 56 observations in 20 A/J mice and 52 observations in 22 C57Bl/6J mice comprised the data set analyzed.

In a second series of experiments, nine C57Bl/6J and six A/J mice were anesthetized, tracheostomized, mechanically ventilated, and paralyzed as above. In these mice, three individual but identical intravenous boluses (50 µl each) of MCh in normal saline (20 µg for C57Bl/6J mice, 4 µg for A/J mice) were given, each during one of the three breathing patterns described above; the sequence of three respiratory patterns was randomly picked for each mouse. Before each dose was administered, the mouse was ventilated with the first ventilation pattern for 1 min. Rrs was recorded every 12 s for 2 min thereafter using the flexiVent “Snapshot-6Hz” protocol as described above. In this way, the bronchoconstrictor response to identical but individual doses of MCh during each breathing pattern was assessed. The different doses for C57Bl/6J and A/J mice were chosen to induce similar levels of bronchoconstriction during 10 ml/kg, 150 breath/min breathing.

To evaluate whether alveolar ventilation was adequate during mechanical ventilation using the 5 ml/kg, 300 breath/min pattern, we performed additional experiments in which we directly assessed alveolar ventilation. We used an AirSpec gas mass spectrometer (sampling rate 4.2 ml/min) to measure CO2 concentration in exhaled gas, and positioned the sampling catheter in the airway colinear with the trachea. As such, the ETCO2 values were obtained for each pattern. As such, the ETCO2 values were obtained for each pattern. In so doing, we excluded any possibility of introducing error if our mass spectrometer could not follow the rapid tidal fluctuations of 100 breath/min breathing; and because ETCO2 was measured on the first of these two breaths, alveolar gas concentrations should not have changed importantly from their values during the breathing pattern under study. The whole sequence was repeated five times, and all five ETCO2 measurements were averaged to yield the value for that mouse and that breathing pattern. Then, the other two breathing patterns were imposed (random order), and ETCO2 was measured for those patterns. As such, the ETCO2 values were obtained for each mouse and each breathing pattern (done in random order); four A/J and four B6 mice were studied.

All data are presented as means ± SD. Comparisons between strains were made using unpaired t-test or the F-test as appropriate. For all statistical tests, P < 0.05 was considered statistically significant.

RESULTS

A/J and C57Bl/6J mice in the first experimental series were of similar weight (A/J, 21.3 ± 3.0 g; C57Bl/6J, 24.1 ± 4.6 g; P = 0.097, unpaired t-test) and had similar baseline respiratory system resistances (A/J, 0.5984 ± 0.1852 cmH2O·ml−1·s; C57Bl/6J, 0.6631 ± 0.1523 cmH2O·ml−1·s; P = 0.2173, unpaired t-test).

Typical primary data from one mouse are shown in Fig. 1. Two patterns were evident. First, Rrs was lower in either strain

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1 Tidal volumes reported in this manuscript are those actually delivered to the lungs, after accounting for gas compression in the ventilator circuit. To achieve these tidal volumes, the flexiVent ventilator was set to a nominal tidal volume one-third greater than the delivered tidal volume.

2 These mice also received lower doses of MCh using the first randomly chosen breathing pattern, and prior to the high doses noted here. However, the precise doses given were variable due to technical difficulty, and so results from those doses are not reported.
during ventilation with high tidal volume and low frequency (20 ml/kg, 75 breath/min, “Rrs20”) than during ventilation at equivalent minute ventilation, but using low tidal volume and high frequency (5 ml/kg, 300 breath/min, “Rrs5”). Second, in each strain, the difference between Rrs5 and Rrs20 increased with the level of bronchoconstriction as judged during 10 ml/kg, 150 breath/min breathing, “Rrs10” (Fig. 2); the slopes of the Rrs5-Rrs20 vs. Rrs10 relationships were each significantly ($P < 0.0001$, $F$-test) greater than zero. The dependence of Rrs5-Rrs20 on bronchoconstriction shown for all data points in each strain in Fig. 2 also holds for each individual animal. For each strain, we compared the value of Rrs5-Rrs20 at baseline (BL) before bronchoconstriction with Rrs5-Rrs20 during bronchoconstriction by paired t-test in all mice who received MCh ($n = 16$ A/J, $n = 17$ C57Bl/6J); because some mice had multiple levels of bronchoconstriction, in such mice we averaged the Rrs5-Rrs20 values during all levels of bronchoconstriction together to yield a single average Rrs5-Rrs20 during bronchoconstriction datum for each animal. In both strains, Rrs5-Rrs20 was substantially and significantly greater during bronchoconstriction than at baseline [A/J: 0.82±0.75 cmH$_2$O·ml$^{-1}$·s (MCh) vs. 0.09±0.05 (BL), $P < 0.002$; C57Bl/6J: 0.40±0.19 cmH$_2$O·ml$^{-1}$·s (MCh) vs. 0.10±0.04 (BL), $P < 0.001$]. Together, these results indicate that in each strain, MCh-induced bronchoconstriction exaggerates the ability of breathing pattern to influence the apparent level of bronchoconstriction.

Furthermore, the dependences of Rrs5-Rrs20 on Rrs10 were significantly different between C57Bl/6J and A/J strains ($P = 0.00016$, $F$-test), with A/J mice exhibiting a significantly ($P < 0.001$) steeper dependence of Rrs5-Rrs20 on level of bronchoconstriction (Rrs10), with over twice the slope. Thus breathing pattern exerts considerably more influence on the apparent level of bronchoconstriction in A/J than in C57Bl/6J mice.

We further analyzed this difference between A/J and C57Bl/6J strains by partitioning the entire Rrs5-Rrs20 difference into two components, Rrs5-Rrs10 and Rrs10-Rrs20, and examining their dependences on level of bronchoconstriction in each strain. As shown in Fig. 3, this analysis discloses important similarities and differences between A/J and C57Bl/6J mice. As seen in Fig. 3B, Rrs10-Rrs20 increased with the level of bronchoconstriction, but it did so almost identically in both strains. The slopes of these relationships were not different between the two strains ($P = 0.24$, $F$-test), and there was a tiny but statistically significant ($P = 0.041$) difference in their Y-intercepts (A/J: $-0.200 ± 0.048$ cmH$_2$O·ml$^{-1}$·s; C57Bl/6J: $-0.1824 ± 0.04479$ cmH$_2$O·ml$^{-1}$·s). Thus changing from the 10 ml/kg, 150 breath/min pattern sequence at a given rate of MCh infusion. Arrows identify the penultimate Rrs measurement in each epoch, which, together with the last Rrs measurement of each epoch, was averaged to yield the datum for that epoch.

![Fig. 1. Typical primary data from one A/J mouse in the first series of experiments. Methacholine infusion (MCh) typically increased respiratory system resistance (Rrs). At a given rate of MCh infusion, Rrs was generally higher during breathing at 5 ml/kg, 300 breath/min (“5”) and lower during breathing at 20 ml/kg, 75 breath/min (“20”) than during breathing at 10 ml/kg, 150 breath/min (“10”). Also, Rrs values were reproducible within a ventilation pattern sequence at a given rate of MCh infusion. Arrows identify the penultimate Rrs measurement in each epoch, which, together with the last Rrs measurement of each epoch, was averaged to yield the datum for that epoch.](http://jap.physiology.org/Downloaded from http://jap.physiology.org)

![Fig. 2. Breathing pattern dependence of bronchoconstriction (Rrs5-Rrs20) increases with the level of bronchoconstriction (Rrs10) in both C57Bl/6J and A/J strains. Rrs5, Rrs10, and Rrs20 designate respiratory system resistances recorded during ventilation with tidal volumes of 5, 10, and 20 ml/kg, and frequencies of 300, 150, and 75 breath/min, respectively. Slopes of each Rrs5-Rrs20 vs. Rrs10 relationship were each positive ($P < 0.0001$, $F$-test), indicating that bronchoconstriction exaggerates the influence of breathing pattern on apparent airflow obstruction. In addition, the dependences of Rrs5-Rrs20 on Rrs10 were significantly different between C57Bl/6J and A/J mice ($P = 0.00016$, $F$-test), and the slope of the relationship for A/J mice was over twice that for C57Bl/6J mice ($P < 0.001$, $F$-test). ○ and broken regression line, C57Bl/6J mice; ● and solid regression line, A/J mice. $r^2$ values for regression lines: 0.62 for A/J, 0.49 for C57Bl/6J.](http://jap.physiology.org/Downloaded from http://jap.physiology.org)

![Fig. 3. Partitioning of breathing pattern dependence of bronchoconstriction shown in Fig. 2 into upper tidal volume range (10–20 ml/kg; B) and lower tidal volume range (5–10 ml/kg; A). Rrs5, Rrs10, and Rrs20 designate respiratory system resistances recorded during ventilation with tidal volumes of 5, 10, and 20 ml/kg, and frequencies of 300, 150, and 75 breath/min, respectively. Slope of the Rrs5-Rrs10 vs. Rrs10 relationship in A/J mice was 4.2 times that found in the C57Bl/6J strain ($P < 0.001$, $F$-test), but there was no significant difference in the slope of the Rrs10-Rrs20 vs. Rrs10 relationship between strains ($P = 0.24$, $F$-test). ○ and broken regression lines, C57Bl/6J mice; ● and solid regression lines, A/J mice. $r^2$ values for regression lines: A, 0.52 for A/J, 0.11 for C57Bl/6J; B, 0.65 for A/J, 0.54 for C57Bl/6J.](http://jap.physiology.org/Downloaded from http://jap.physiology.org)
Fig. 4. Effect of breathing pattern on peak Rrs after 3 identical intravenous boluses of MCh in A/J and C57Bl/6J mice (A/J mice received 4 μg/bolus; C57Bl/6J mice received 20 μg/bolus). Changing breathing pattern from 10 ml/kg, 150 breath/min to 20 ml/kg, 75 breath/min reduces bronchoconstrictor responses equivalently in both strains, but compared with the 10 ml/kg, 150 breath/min pattern, breathing at 5 ml/kg, 300 breath/min significantly \( (P < 0.004) \) accentuates bronchoconstrictor response only in A/J mice.

In marked contrast, changing from the 10 ml/kg, 150 breath/min breathing pattern to the 5 ml/kg, 300 breath/min breathing pattern had a substantially different effect on bronchoconstriction in A/J vs. C57Bl/6J mice. As seen in Fig. 3A, C57Bl/6J mice exhibit little dependence of Rrs5-Rrs10 on Rrs10, whereas the slope of this relationship is 4.2-fold greater \( (P < 0.0001, \ F\text{-test}) \) in A/J mice. This result indicates that A/J mice exhibit much greater exaggeration of bronchoconstriction during low tidal volume breathing than do C57Bl/6J mice, and the degree of exaggeration increases with the level of bronchoconstriction. Together, the data in Fig. 3 indicate that greater dependence of airway caliber on breathing pattern evident in A/J mice (Fig. 2) actually stems from particular sensitivity to low tidal volume breathing. [A similar physiological message emerged when data were analyzed after normalization for the level of bronchoconstriction [i.e., dividing both (Rrs5-Rrs10) and (Rrs10-Rrs20) by Rrs10]; data not shown.]

To test this dependence more directly, we performed a second series of experiments in which mice received three identical boluses of intravenous MCh during ventilation with each of the three breathing patterns. The peak Rrs responses recorded after each MCh dose are shown in Fig. 4. As noted above, the MCh dose given to C57Bl/6J or A/J mice was chosen to induce approximately the same level of bronchoconstriction in each strain during 10 ml/kg, 150 breath/min breathing, as is evident in Fig. 4. The influence of changing from this breathing pattern to 20 ml/kg, 75 breath/min breathing was similar in both strains: during the latter breathing pattern, the severity of airway narrowing was equivalently reduced. In marked contrast, breathing with shallow breaths and high-frequency significantly accentuated bronchoconstriction only in A/J mice \( (P < 0.004) \), and A/J mice exhibited significantly greater accentuation than did C57Bl/6J mice \( (P = 0.012) \), unpaired t-test on individual differences in Rrs between 10 ml/kg, 150 breath/min and 5 ml/kg, 300 breath/min breathing patterns. These results thus confirm that the greater breathing pattern dependence of airway constrictor responsiveness seen in A/J mice reflects their particular accentuation of bronchoconstrictor responses during low tidal volume breathing.

Figure 5 presents ETCO2 values observed during ventilation in each strain during each of the three breathing patterns. Rather than hypoventilating the mice, the ventilation patterns used actually hyperventilated every mouse. The A/J mice were systematically more hyperventilated than were the C57Bl/6J mice, but the C57Bl/6J mice were hyperventilated too (Fig. 5A). In addition, the breathing pattern mattered: 20 ml/kg, 75 breath/min resulted in the greatest degree of hyperventilation and 5 ml/kg, 300 breath/min in the least hyperventilation. However, when the ETCO2 of each mouse was normalized to that mouse’s own 10 ml/kg, 150 breath/min ETCO2 value, there was no difference between A/J and C57Bl/6J mice in the relative ETCO2 during ventilation using any one of the three patterns (Fig. 5B).

**DISCUSSION**

There is abundant evidence that tidal breathing, and especially tidal breathing at elevated minute ventilation, antagonizes the development and persistence of airflow obstruction during bronchoconstrictor stimulation in normal animals and people (1, 11, 23, 25, 26). The present study extends current knowledge by demonstrating that 1) even at moderate, fixed minute ventilation, the precise breathing pattern can influence the degree of airflow obstruction substantially, and 2) the

![Fig. 5. Alveolar ventilation during 3 breathing patterns, as reflected in exhaled end-tidal CO2 (ETCO2) measurements. Open bars, C57Bl/6J mice; solid bars, A/J mice. A: ETCO2 values are low, reflecting alveolar hyperventilation, during ventilation with each tidal volume (Vt), frequency (F) combination, in both C57Bl/6J and A/J mice. Two-way ANOVA demonstrates that breathing pattern significantly influenced ETCO2 \( (P < 0.001) \) and that A/J mice were more hyperventilated than were C57Bl/6J mice \( (P = 0.002) \); however, there was no significant interaction between breathing pattern and mouse strain \( (P = 0.69) \). B: ETCO2 values normalized (in each mouse) to the value observed during ventilation using the 10 ml/kg, 150 breath/min pattern (“Relative ETCO2”). There were no differences in relative ETCO2 between C57Bl/6J and A/J mice during ventilation with either the 5 ml/kg, 300 breath/min \( (P = 0.288) \) or 20 ml/kg, 75 breath/min \( (P = 0.19) \) patterns (unpaired t-tests). Four C57Bl/6J and four A/J mice were studied.](http://jap.physiology.org/)

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influence of breathing pattern on bronchoconstriction differs considerably between genetically diverse inbred mouse strains.

Previously, a number of groups reported that increasing minute ventilation during tidal breathing progressively suppressed the development or persistence of airflow obstruction induced by cholinergic bronchoconstrictor stimulation (11, 23, 25, 26). This effect of increasing minute ventilation was manifest when either breathing frequency or tidal volume was increased to achieve elevated minute ventilation. Our results, however, suggest that elevated tidal volume is the more effective antagonist to bronchoconstriction, for when minute ventilation was held constant at 1,500 ml·kg⁻¹·min, Rrs was consistently lower when breathing with large Vt and low f than when breathing with small Vt and high f. Although Shen and colleagues (26) studied the effects of varying both tidal volume (at fixed frequency) and frequency (at fixed tidal volume) in their rabbits, the level of bronchoconstriction in the absence of any ventilation differed markedly between their two experimental series. Because the effect of breathing pattern on airflow obstruction depends greatly on the intensity of bronchoconstrictor stimulation (Fig. 1), it is difficult to infer from their studies whether increased tidal volume or increased frequency had the relatively greater antiostructive effect. We are not aware of any other in vivo studies that address this question, but recent theoretical and in vitro studies may shed light. Changes of amplitude and frequency of length oscillations do not have equivalent effects on myosin binding dynamics in contracted airway smooth muscle (21). Previously it had been widely assumed that during bronchospasm, the airway smooth muscle length is set by a balance of static forces, with contracting muscle becoming equilibrated at the length for which active isometric force that it generates equals the elastic load against which it is contracting (16, 19, 22). By contrast, there is now substantial evidence demonstrating that during tidal loading (as occurs during breathing) the length of airway smooth muscle becomes equilibrated dynamically, not statically, and that this dynamically equilibrated length does not approximate the length that would be expected based on a balance of static forces (8–10, 17, 21, 26). As such, during bronchospasm, airway caliber is equilibrated dynamically, not statically. Recent unpublished results (J. Fredberg, personal observation) indicate that the extent of force fluctuation-induced relengthening of contracted, isotonically shortened airway smooth muscle (4, 10, 15) depends relatively more on the magnitude of applied force fluctuations than on their frequency. Thus it seems likely that the greater antiostructive influence of tidal volume than of frequency observed in our mice stems from properties of contracted smooth muscle. Later, we consider other factors that conceivably might have contributed to this result.

Both A/J and C57Bl/6J mice exhibited breathing-related functional antagonism of bronchoconstriction, but the influence of breathing pattern on airflow obstruction was considerably more marked in A/J mice (Fig. 2). Their greater sensitivity to breathing pattern was entirely attributable to a differential effect of breathing with the low Vt, high f pattern. Changing from 10 ml/kg, 150 breath/min to 20 ml/kg, 75 breath/min breathing reduced airflow obstruction equivalently in both strains (Figs. 3 and 4). In marked contrast, changing from 10 ml/kg, 150 breath/min to 5 ml/kg, 300 breath/min breathing had minimal effect on bronchoconstriction in C57Bl/6J mice, but substantially exacerbated MCh-stimulated airflow obstruction in A/J mice. This result was evident during both continuous MCh infusion (Fig. 3) and during bolus MCh delivery (Fig. 4). Because, as discussed above, the net airflow obstruction induced by a constrictor agonist reflects the balance of its pro-obstructive contractile activation vs. the antiostructive consequences of breathing, and because agonist administration was held constant among breathing patterns, our results indicate that breathing at low Vt and high f more effectively antagonizes airflow obstruction in C57Bl/6J than in A/J mice. It should be noted that the mechanism resulting in airflow obstruction in our mice might include closure of some airways as well as concentric bronchoconstriction, although both mechanisms would be enhanced by greater smooth muscle shortening. Given the strain difference observed during low Vt, high f breathing, it is interesting that high Vt, low f breathing was similarly effective in antagonizing bronchoconstriction in both A/J and C57Bl/6J mice. Perhaps there is a threshold for this antagonist effect of breathing that is greater in A/J than in C57Bl/6J mice. If so, then 5 ml/kg, 300 breath/min appears sufficient to exceed the threshold and so provide effective antagonism of airflow obstruction in C57Bl/6J mice, although this “low-intensity” breathing-antagonist effect is insufficient to exceed the threshold in A/J mice and so does not protect their airways against bronchoconstriction.

What might account for this interstrain difference? Inasmuch as parenchymal tethering transmits the distending forces of tidal breathing to the airways, differences in lung elasticity over the lung volume range traversed during low Vt breathing or differences in parenchymal-airway attachments (6) between strains could hypothetically lead to this finding. However, the pulmonary pressure-volume-relationships are essentially identical in A/J and C57Bl/6J mice (12, 29), and alveolar size is also closely similar in these strains (12). It therefore seems unlikely that differences in static parenchymal mechanics or numbers of alveolar septal-airway attachments account for our findings, although we acknowledge that the latter have not been specifically reported among strains. Second, A/J mice have thinner airway wall mucosal and smooth muscle layers than do C57Bl/6J mice (28), so although the same stress may be applied by the parenchyma to the airway walls of A/J or C57Bl/6J mice, the thinner airway walls of A/J mice might experience greater tension. A third possibility is that intrinsic differences in airway smooth muscle function between strains account for the greater sensitivity of A/J mice to low Vt breathing. Duguet et al. (5) has already shown that in explanted lung sections, the airways of A/J mice narrow faster than those of C57Bl/6J mice after contractile activation. Because the speed of narrowing was undoubtedly determined by the velocity of contraction of airway smooth muscle, their results strongly implicate intrinsic differences in airway muscle behavior between these strains. We recently showed that the extent of force fluctuation induced relengthening (FFIR) of contracted airway smooth muscle varies with physiologically regulated cellular properties, including actin filament dynamics (4) as well as p38 MAPK (15) and ERK1/2 (R. Mitchell and J. Solway, unpublished observation) signaling. It therefore seems plausible that differences in force fluctuation-induced relengthening in A/J vs. C57Bl/6J mice might account for their differential sensitivity to low Vt breathing. This possibility might be testable by direct measurement of FFIR in isolated airways.
from these strains, although this could be a technically daunting task. Identification of differences in FFIR across strains (if present), and of the molecular and ultimately genetic mechanisms that underlie those differences, would provide substantial insight into how the antiobstructive effect of breathing modulates airway responsiveness. It is interesting to note that spontaneously breathing A/J mice choose larger tidal volumes and lower frequencies than do spontaneously breathing C57Bl/6 mice (13).

Four methodologic issues are worth considering. First, it is important to note that our experiments were performed in naive mice of either strain, in the absence of allergen-induced inflammation. Prior studies from Ewart et al. (7), Wesselkamper et al. (30), and Guerassimov et al. (12) demonstrate that neither C57Bl/6J nor A/J mice have spontaneous airway inflammation. Thus our results reflect differences in native airway function between these two strains (3, 18).

Second, Rrs was always measured at the same lung volume (FRC, artificially elevated by the external PEEP), because mice were allowed to exhale passively for 1 s before Rrs measurement. Rrs was also always measured using the same frequency (6 Hz) and tidal excursions (0.08 ml) during brief interruptions of the tidal breathing pattern being studied. As such, artificial effects related to Rrs measurement technique could not have influenced our results.

Third, mean lung volume was undoubtedly greater during high Vt, low f breathing than during low Vt, high f breathing. As a consequence, mean (time averaged) airway smooth muscle length may have been longer during high Vt, low f breathing and shorter during low Vt, high f breathing. Youn et al. (31) previously showed that the degree of contractile activation is influenced by muscle length (longer muscles exhibited greater activation than shorter muscles), but were this an important factor in our study, the direction of this effect should have resulted in greater airflow obstruction during high Vt, low f breathing. Because we found just the opposite (i.e., we observed reduced airflow obstruction with this breathing pattern), it is unlikely that any effect of muscle length on degree of contractile activation led to our findings. Although it seems conceivable that the increased mean load that may have been applied during high Vt, low f breathing could have reduced smooth muscle shortening and airflow obstruction, Salerno et al. (25) showed that increasing Vt enhanced the antiobstruction effect of breathing in mechanically ventilated dogs receiving MCh even when mean bronchial pressure was held constant. As such, any change in mean lung volume that accompanied the different breathing patterns was probably not the dominant factor in determining our results. Interestingly, Dandurand et al. (2) found that acutely raising lung volume (by raising PEEP) partially ameliorated MCh-induced bronchoconstriction in Lewis rats, whereas raising lung volume had little effect on bronchoconstriction in Fisher rats; that latter strain exhibits the greater airway constractor responsiveness. To the extent that increasing Vt mimics increasing lung volume, our results in mice might seem at odds with Dandurand’s results, for the more responsive A/J mice exhibited greater reduction in MCh-induced airflow obstruction than did the less responsive C57Bl/6J mice on changing from 5 ml/kg, 300 breath/min to 10 ml/kg, 150 breath/min breathing in our study. It is intriguing that, as noted above, the mucosal and smooth muscle layers of the airway walls of the hyperresponsive A/J mice are thinner than those of C57Bl/6 mice (28), whereas the less responsive Lewis rats seem to have thinner airway walls than do Fisher rats (20). Thus the mouse and rat strains with the thinner airway walls are the ones whose bronchoconstrictor responses increase more when breathing conditions associated with lower lung volumes are imposed.

Fourth, our mice were substantially hyperventilated during mechanical ventilation using all three breathing patterns. Hypocapnia causes bronchoconstriction in people (24) and dogs (14), and increases phosphatidylinositol turnover in rat airway smooth muscle (27); we are not aware of any reports directly addressing whether hypocapnia enhances airway tone in mice. However, we think that it is unlikely that hypocapnia accounts for our finding that A/J mice exhibit a greater increase in bronchoconstriction during 5 ml/kg, 300 breath/min breathing than do C57Bl/6J mice, because in both strains hypocapnia was least severe during this breathing pattern. So, it is hard to reconcile how less severe hypocapnia should cause more severe bronchoconstriction. Furthermore, baseline Rrs values were similar between A/J and B6 mice during 10 ml/kg, 150 breath/min breathing, despite the somewhat greater hyperventilation in A/J mice. In all, these results suggest that the level of alveolar ventilation did not account for our key finding.

Our study has important implications regarding the interpretation of relative airway constractor responsiveness among individuals or populations. Our results show that differences in apparent constractor responsiveness can be substantially influenced by the breathing pattern used during its quantification. For example, whereas A/J mice would appear more responsive than C57Bl/6J mice at any breathing pattern, the magnitude of this difference would seem much greater when measured during breathing at 5 ml/kg, 300 breath/min than when measured during breathing at 10 ml/kg, 150 breath/min. Because, as shown here, biological variation in the antiobstructive effect of breathing can be substantial, it follows that differences among individuals in apparent airway constractor responsiveness also could stem (sometimes exclusively?) from differences in their individual antiobstructive effects of breathing. Much attention has been placed on dysregulation of contractile function of airway muscle in disease. We suggest that important pathophysiology might also be found in the functional antagonist effect of tidal breathing on airflow obstruction.

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