Decreased airway narrowing and smooth muscle contraction in hyperresponsive pigs

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Smooth muscle contraction is a major determinant of airway diameter; therefore, AHR of asthma may be due to altered contractile behavior of ASM. However, the literature contains conflicting results, with only some studies indicating that ASM contraction is increased in animal models (11, 16, 30). Other studies provide clear evidence for a decrease in ASM contraction in sensitized animals (29). Responses of smooth muscle isolated from asthmatic patients show similar inconsistency (13, 28). Furthermore, attempts to show a relationship between the contractile properties of ASM and bronchial responsiveness in vivo have been unsuccessful (1, 27, 33). Despite extensive literature, the importance of ASM to hyperresponsiveness is still unclear, and it is not known whether ASM contraction in asthma is increased or not.

The effect of load on ASM shortening is well established (15, 31). Alterations to the ASM load may affect airway narrowing and could be responsible for the exaggerated airway narrowing and AHR seen in asthma. Such alterations include structural changes to the airway wall as a consequence of the inflammatory response, which could reduce ASM load and allow greater narrowing (7). Alternatively, factors arising from outside the airway, such as load from parenchymal tethering (14), may be altered in asthmatic airways. Some studies suggest that asthmatic bronchi have a reduced susceptibility to forces from parenchymal tethering, which could favor greater airway narrowing when the airway is stimulated (5, 21).

If AHR were caused by alterations to the airway wall, we would expect that airways isolated from animals with AHR would have increased airway narrowing. Alternatively, if AHR were associated more with external factors, then airway narrowing might not be increased. In this study, we investigate airway narrowing by using a porcine model of AHR. Previous data from this model suggest that there may be a reduction in ASM sensitivity in vitro, coupled with a stiffer airway wall (25, 32). The present study aims to determine by direct measurements whether airway narrowing is altered in individual airways from hyperresponsive allergen-exposed animals. We will also determine contractility of the ASM, both in isolated bronchial seg-

ASTHOMATIC AIRWAYS ARE CHARACTERIZED by inflammation and airway hyperresponsiveness (AHR). AHR in vivo in asthmatic patients suggests that narrowing to provocative stimuli is increased. The mechanisms responsible for airway narrowing are thought to involve either altered responses within the airway wall [e.g., airway smooth muscle (ASM) and wall loads] or alterations in factors external to the airway wall (e.g., parenchymal forces). Over recent years, a number of animal models have been developed to examine the role of the airway and the lung in AHR. However, many aspects of airway physiology in asthma remain unclear, for example, the extent of narrowing in airways from asthmatic patients compared with controls and mechanisms by which airway narrowing might be altered in asthma.

Turner, Debra J., Peter B. Noble, Matthew P. Lucas, and Howard W. Mitchell. Decreased airway narrowing and smooth muscle contraction in hyperresponsive pigs. J Appl Physiol 93: 1296–1300, 2002. First published June 21, 2002; 10.1152/japplphysiol.00150.2002.—Increased smooth muscle contractility or reduced smooth muscle mechanical loads could account for the excessive airway narrowing and hyperresponsiveness seen in asthma. These mechanisms were investigated by using an allergen-induced porcine model of airway hyperresponsiveness. Airway narrowing to electric field stimulation was measured in isolated bronchial segments, over a range of transmural pressures (0–20 cmH₂O). Contractile responses to ACh were measured in bronchial segments and in isolated tracheal smooth muscle strips isolated from control and test (ovalbumin sensitized and challenged) pigs. Test airways narrowed less than controls (P < 0.0001). Test pigs showed reduced contractility to ACh, both in isolated bronchi (P < 0.01) and smooth muscle strips (P < 0.01). Thus isolated airways from pigs exhibiting airway hyperresponsiveness in vivo are hyporesponsive in vitro. The decreased narrowing in bronchi from hyperresponsive pigs may be related to decreased smooth muscle contractility. These data suggest that mechanisms external to the airway wall may be important to the hyperresponsive nature of sensitized lungs.

bronchial hyperreactivity; respiratory mechanics; bronchoconstriction
RE gular measurements in situ and in tracheal smooth muscle strips. From these measurements, the contribution of the airway wall to AHR can be better assessed.

METHODS

Sensitization. White/Landrace female pigs (10 kg) were studied (14 test, 11 naive control). Test pigs were sensitized to ovalbumin (OA) on days 0 and 7, as previously described (32). Success of sensitization was determined from cutaneous responses to OA (0.001–1 mg/ml). Animals with positive responses (wheal > 5 mm diameter) at all concentrations received OA aerosol (50 mg/ml in saline) on days 14, 17, and 20 (32).

Bronchial segments. Preparation of isolated bronchi and endoscopic recording of luminal narrowing have been previously described (23, 26). Briefly, the right lower lobe stem bronchus was dissected free of parenchyma, the side branches ligated, and a 4-cm-long segment (0.6 cm outer diameter) cannulated at both ends and mounted in an organ bath filled with Krebs solution. Propanolol (10−6 M) was added to the organ bath to prevent the action of inhibitory neurotransmitter released by electrical field stimulation (EFS) (8). The lumen was filled with Krebs solution from a separate reservoir, the height of which could be varied to alter transmural pressure (Ptm). Airway narrowing was produced by EFS (299 mA, 30 Hz, 3 ms) and was visualized by placing a rigid fiber-optic endoscope into the center of the segment. Images of the airway lumen were recorded on videotape for subsequent analysis.

Bronchi were inflated to 20 cmH2O Ptm three times to establish a volume history and then inflated and deflated from 0 to 20 cmH2O Ptm in 5-cmH2O increments. Narrowing was determined at each pressure from the EFS-induced change in luminal cross-sectional area (CSA) (26).

Compliance was determined from the inflation and deflation limb of the Ptm-CSA curve. Ptm was varied in 5-cmH2O increments between −15 and 20 cmH2O, and the resulting change in luminal CSA was measured. At each Ptm, the change in CSA was divided by the CSA at 0 cmH2O (i.e., strain). Compliance was calculated from the slope of the strain-delta Ptm curve, between −5 and 5 cmH2O Ptm, with regression analysis. Commercial software was used, giving an $R^2 > 0.95$ (26).

Bronchi were maximally stimulated with ACh (10−1 M) at 5 cmH2O Ptm and then fixed in 4% formaldehyde for morphometric evaluation of ASM shortening. The airway was paraffin embedded, and 5-μm sections were cut and stained with hematoxylin and eosin. ASM shortening was calculated from the inner wall area, the outer perimeter of the ASM, and the perimeter of the epithelium, in four sections per airway, as detailed previously (17).

Tracheal smooth muscle. Smooth muscle strips were removed from the trachea at a point distal to the end of the endotracheal tube used to deliver OA. Muscle strips were mounted in an organ bath containing Krebs solution. The muscle strip was attached to an attachment post and an adjustable isometric force transducer. A passive and active (EFS 60 V, 0.5-ms pulse duration, 50 Hz) length-tension curve was constructed to determine the optimal length ($L_o$) of the muscle strips at which to perform dose-response curves to ACh (10−9 M to $3 \times 10^{-3}$ M). $L_o$ was identified as the muscle length at which the greatest active tension was generated. The CSA of the muscle was estimated from the length and weight of each muscle strip. Stress was determined from tension/CSA (g/mm²).

RESULTS

Airway narrowing. EFS-induced airway narrowing was quantified by the percent change in CSA and measured over a range of Ptm values. Narrowing Ptm curves for control ($n = 14$) and test ($n = 11$) airways are shown in Fig. 1. Airway narrowing was recorded both on inflation (in steps from 0 to 20 cmH2O) and on deflation back to 0 cmH2O. Test airways narrowed significantly less than did control ($P < 0.0001$, ANOVA). There was no significant difference in airway narrowing between inflation and deflation. Maximal narrowing occurred at 10 cmH2O Ptm ($P < 0.05$, Newman-Keuls post hoc test) for both control and test airways.

Smooth muscle contraction. Smooth muscle contraction after a maximal dose of ACh (10−1 M) was determined morphometrically in several bronchial segments after the recording of airway narrowing. ACh-induced shortening was significantly reduced in test (30 ± 5%, $n = 7$) vs. control (47 ± 2%, $n = 8$) airways (Fig. 2, $P < 0.01$). Airway wall dimensions did not differ for control and test airways (Table 1).

Smooth muscle shortening was plotted against airway narrowing, with each recorded in the same airway segment. Muscle shortening was positively correlated with airway narrowing for both test and control groups.

Statistical analyses. Comparisons of airway narrowing between groups were made with ANOVA, with Bonferroni post hoc test. Unpaired Student’s $t$-tests were used to compare ASM contractility derived from morphological parameters. Correlations between narrowing vs. muscle shortening were performed by the method of least squares. Correlations used the maximum airway narrowing, obtained at any of the pressures on deflation, in each airway segment. Lines of best fit were generated by using the built-in equations in Graph Pad Prism software and were chosen on the basis of the highest $R^2$ value. Data are given as means ± SE; significant differences are where $P < 0.05$.

Fig. 1. Electrical field stimulation-induced narrowing in control ($n = 14$; ●) and test ($n = 11$; ○) bronchial segments recorded over a range of transmural pressures (Ptm) during airway inflation and deflation.
combined \( r = 0.55, P < 0.05 \), Fig. 3) but failed to reach significance when analyzed independently.

Isometric contraction of isolated tracheal smooth muscle was measured after stimulation with ACh \( (10^{-9} \text{ to } 3 \times 10^{-3} \text{ M}) \). Dose-response curves for control and test tracheal smooth muscle are shown in Fig. 4. There was no difference in CSA, \( L_w \), or mass of isolated tracheal smooth muscle between test and control animals (Table 2). Maximal smooth muscle stress was \( 8.8 \pm 0.9 \text{ g/mm}^2 \) in control strips and \( 4.7 \pm 0.2 \text{ g/mm}^2 \) in tests \( (P < 0.01) \). EC\(50\) was not significantly different between control and test smooth muscle.

Airway compliance. Airway-specific compliance, determined from the deflationary limb of the strain-Ptm curve, was \( 0.07 \pm 0.01 \text{ cmH}_2\text{O}^{-1} \text{ in control airways and } 0.06 \pm 0.01 \text{ cmH}_2\text{O}^{-1} \text{ in tests. There was no significant difference between control and test airways.}

**DISCUSSION**

This study investigates narrowing of bronchi harvested from pigs exhibiting some features common with asthma. Sensitized and challenged pigs are hyperresponsive to inhaled ACh and exhibit early skin and lung reactions to antigen. They also have mild airway inflammation and ASM hypertrophy or hyperplasia (32). Studies in other laboratories have shown the presence of late pulmonary reactions in sensitized pigs, with suppression of endogenous cortisol (12). In our pig model, the effects of sensitization on lung physiology in vivo are similar to those of several other animal models, including guinea pig (11, 16), rat (3), and rabbit (22). Despite in vivo hyperresponsiveness, described previously (32) and confirmed in a subgroup of animals from this study (data not shown), individual bronchi from sensitized pigs demonstrated reduced airway narrowing to a cholinergic stimulus. This agrees with and extends our laboratory’s previous findings, which showed, by indirect means, reduced in vitro airway responsiveness in bronchi isolated from pigs exhibiting AHR (32). This novel finding suggests that in vivo hyperresponsiveness could occur by a mechanism(s) external to the airway wall itself. It is not known whether similar findings are present in other models or in asthma, because direct measurements at the level of individual airways have not been made.

Several aspects of the experimental methodology need discussing to assist in interpretation of the results. The technique for recording lumen narrowing in isolated airway segments has been described in several previous publications (23, 26). The experimental de-

**Table 1. Mean airway wall dimensions**

<table>
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<tr>
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<th>Control</th>
<th>Test</th>
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<tr>
<td>Inner wall area, mm(^2)</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>CSA at 20 cmH(_2)O, mm(^2)</td>
<td>17 ± 1</td>
<td>19 ± 2</td>
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Values are means ± SE. Internal perimeter \( (P_i) \) and inner wall area (defined as the area of airway wall between the outer layer of the smooth muscle and the perimeter of the lumen) were measured by using morphometric techniques. Cross-sectional area (CSA) at 20 cmH\(_2\)O was measured by using an endoscope. No significant differences were found between control and test groups.

**Fig. 2.** Smooth muscle (SM) shortening in control \(( n = 8 \) ) and test \(( n = 7 \) ) bronchial segments. Segments were fixed after a maximal dose of ACh \(( 10^{-9} \text{ to } 3 \times 10^{-3} \text{ M}) \), and SM shortening was determined morphometrically. Values are means ± SE. SM shortening was significantly reduced in test airways \( (P < 0.01) \) compared with controls.

**Fig. 3.** Relationship between SM shortening to a maximum dose of ACh and airway narrowing to electrical field stimulation in isolated bronchial segments \( ( n = 8 \) control, \( \bullet \), and \( n = 7 \) test, \( \square \) ). SM shortening and airway narrowing, quantified by the \( \Delta \text{CSA and expressed as } \text{CSA}_{20} \), were positively correlated in test and control animals combined \( r = 0.55, P < 0.05 \).

**Fig. 4.** ACh dose-response curves in isolated tracheal SM. Isometric contraction of isolated tracheal smooth muscle was measured after stimulation with ACh \( (10^{-9} \text{ to } 3 \times 10^{-3} \text{ M}) \). Dose-response curves for control and test tracheal smooth muscle are shown in Fig. 4. There was no difference in CSA, \( L_w \), or mass of isolated tracheal smooth muscle between test and control animals (Table 2). Maximal smooth muscle stress was \( 8.8 \pm 0.9 \text{ g/mm}^2 \) in control strips and \( 4.7 \pm 0.2 \text{ g/mm}^2 \) in tests \( (P < 0.01) \). EC\(50\) was not significantly different between control and test smooth muscle.

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sign allows several crucial aspects of the airway to be tightly controlled, including airway location and size, PtM, and the stimulation parameters used to produce bronchoconstriction. EFS-induced contractions are atropine sensitive (24); however, there is also an abundant adrenergic response to EFS in pig bronchi (8), which, in this study, was blocked with propranolol so that the size of bronchoconstriction was predominantly due to the release and effect of ACh. Our study provides no information on possible changes in sensitivity (EC50) of individual bronchi because narrowing was recorded only to maximum EFS. However, previous work in pig-isolated bronchi from our laboratory suggests that sensitivity to ACh may be reduced by sensitization (32); as shown in the present study, tracheal smooth muscle sensitivity was not affected by sensitization.

An important aspect of the protocol was recording airway narrowing over a range of PtM values. This ensures that responses are obtained under optimum conditions of preload. The applied preload sets the operating length of the ASM and, therefore, ASM force during contraction. As seen previously in this species, airway narrowing is not greatly affected by different pressures, possibly because abundant cartilage limits the amount of ASM stretch in response to applied luminal pressure (26). Once activated, ASM contracts against afterload from elastic properties of the airway wall and from the pressure applied to the lumen. Therefore, airway narrowing in isolated bronchi may be influenced by structural remodeling of the airway, where this leads to altered airway stiffness and/or altered ASM contraction.

The present study is the first to examine airway narrowing in conjunction with ASM contraction in allergen-sensitized hyperresponsive animals. As the airways were separated from lung parenchyma, the factor(s) reducing airway narrowing must arise from within the airway wall. In the present study, ASM contraction both in bronchial segments and in smooth muscle strips removed from the trachea was reduced. Bronchial smooth muscle shortening was assessed morphometrically (17) in the same bronchial segments used for recording airway narrowing, so that muscle contraction and airway narrowing were assessed under identical conditions of load. There was a significant, but weak, correlation between airway narrowing to EFS and ASM shortening to ACh in test and control animals combined, linking hyporesponsiveness in the intact airway to ASM responsiveness, although the correlation failed to reach significance when the groups were analyzed independently because of the small sample numbers. In the second approach, the force produced by tracheal ASM was normalized for the CSA of muscle and carried out at each tissue’s resting $L_o$. The importance of controlling for muscle $L_o$ and area is now well recognized and is especially pertinent as changes in muscle mechanics or area may occur in remodeling (30). Measurements carried out under these controlled conditions indicated that sensitization affected (reduced) the size of the contractions but not the sensitivity. Findings on ASM force or shortening in other sensitized animal models show marked variability between studies. In some, there is increased contraction (11, 16, 30), whereas, in others, contractions are either unchanged (20) or reduced (29), as in the present study. In asthma, the effects of possible changes to $L_o$ or ASM mass on airway contraction are not known, and it is still unclear whether ASM contraction is altered in this disease (2, 13, 28).

An additional mechanism considered for producing hyporesponsiveness in vitro was the stiffening of the airway wall shown in a previous study in sensitized pig bronchi (25). A stiff wall loads ASM, reducing its capacity to produce airway narrowing (21, 26, 30). However, in contrast to our previous results (25), the airways studied here showed no statistically significant stiffening. The difference between these two studies may well be due to methodological issues, as the airways studied in the present experiment differed in location and size to those in the previous study. Despite these differences, we can conclude from the present study that the decrease in narrowing seen in isolated bronchi cannot be attributed to wall loads and is, therefore, more likely attributable to the decreased ASM contractility seen in these animals.

There are two further possible explanations as to why animals from which bronchi were harvested show AHR in vivo yet are hyporesponsive in vitro. First, airway narrowing is measured by an endoscope inserted into the lumen; therefore, only mid-sized bronchi, or larger, can be studied. In past studies, these bronchi have been shown to make a contribution to airway resistance (6). However, recent studies also implicate peripheral airways and lung tissue in lung responsiveness (19), and these may have made a significant contribution to in vivo AHR in the pig. Second, mucosal swelling or edema could also contribute to in vivo hyperresponsiveness; however, there is little evidence to support this, as baseline resistance and compliance are the same in test and control animals, and no wall swelling was evident based on morphological assessment (Table 1).

What is the relevance of the present findings in animals to human lung function in disease? On the surface, this paradoxical reduction in airway narrowing is surprising, but a similar contradiction may also exist in human asthma, as there is emerging evidence that airways of asthmatic patients have reduced compliance (4, 18). We know from previous studies that in vitro airway stiffness limits airway narrowing (26). An untested possibility is that a stiffer airway uncouples

| Table 2. Mean data from porcine isolated tracheal smooth muscle |
|------------------|------------------|
|                  | Control          | Test             |
| CSA, mm$^2$      | 1.2 ± 0.1        | 1.3 ± 0.1        |
| $L_o$, mm        | 9.5 ± 1.2        | 6.8 ± 0.6        |
| Mass, mg         | 10.8 ± 1.6       | 8.8 ± 0.9        |

Values are means ± SE. $L_o$, optimal length.

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the ASM from the effects of parenchymal load, which is normally exerted via airway-parenchymal interdependence. Given that airway stiffness limits airway narrowing in vitro, any reduction in airway narrowing may be more than compensated for by a reduced transmission of parenchymal load to the ASM in vivo, resulting in increased airway narrowing. An effect of ASM-parenchymal uncoupling in bronchial responsiveness has been suggested previously with a focus on the effects of airway wall remodeling or edema (21). The present study suggests that mechanisms external to the airway wall may be important to the origin of hyperresponsiveness.

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