

Physiological responses of the airway wall and lung in hyperresponsive pigs

D.J. Turner*, P.R. Gray*, S.A. Taylor*, J. Thomas[#], H.W. Mitchell*

Physiological responses of the airway wall and lung in hyperresponsive pigs. D.J. Turner, P.R. Gray, S.A. Taylor, J. Thomas, H.W. Mitchell. ©ERS Journals Ltd 2001.

ABSTRACT: Airway hyperresponsiveness (AHR) might be driven by mechanisms inherent to the airway wall, and/or by factors arising from outside the airways. A porcine model of allergen-induced AHR was utilized to investigate physiological responses in intact airways *in vitro* and their contribution to responsiveness *in vivo*.

Responsiveness to acetylcholine (ACh) was measured in eight ovalbumin (OA)-sensitized/challenged pigs (tests) and eight saline-challenged controls. *In vivo* responsiveness to ACh was determined from pulmonary resistance (RL). *In vitro* responsiveness to ACh was determined from airway pressure in isovolumic bronchial segments, after exposure *via* the adventitial or the luminal surface.

Test pigs had lung ($255 \pm 26\%$ increase in RL, $p < 0.0001$) and skin responses to OA, and AHR to ACh ($p < 0.0001$). *In vitro*, test bronchi were less sensitive than controls to ACh applied to the airway adventitia (negative log of the ACh concentration producing half the maximum response (pD_2) = 4.18 and 4.58 respectively, $p < 0.01$), but not the lumen. Test bronchi had an increased amount of smooth muscle normalized for airway size *versus* controls ($p < 0.05$). Maximum responses to luminal ACh *in vitro* showed a weak positive correlation with maximum changes to ACh *in vivo* ($r = 0.599$, $p = 0.05$).

This study concludes that the effect of antigen challenge on bronchial responsiveness varies with the route of exposure to acetylcholine. *In vitro* responses to luminal acetylcholine are increased despite a possible reduction in responsiveness of airway smooth muscle. Responsiveness of the bronchial wall only partially explains responsiveness of the lungs *in vivo*.

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*Dept of Physiology, University of Western Australia and [#]Dept of Pathology, Murdoch University, Perth, Western Australia, Australia.

Correspondence: D.J. Turner, Dept of Physiology, University of Western Australia, Nedlands, WA 6907, Australia.
Fax: 61 893801025

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Despite the central role of airway narrowing in the response of the lung to provocative stimuli, its importance in airway hyperresponsiveness (AHR) has not been established. Since smooth muscle contraction is a major determinant of airway diameter, one proposal has been that AHR is due to altered contractile behaviour of airway smooth muscle (ASM). Studies in allergic animal models indicate that ASM contraction might be increased [1–3], while the responses of smooth muscle isolated from asthmatics are variable [4, 5]. *In vivo*, ASM is subject to additional factors arising from outside the airway, such as load from parenchymal tethering [6]. Some studies suggest that asthmatic bronchi have a reduced susceptibility to forces from parenchymal tethering, which could favour greater airway narrowing when the airway is stimulated [6, 7].

Many studies have focused on the responses of ASM after its isolation from the airway, usually the trachea [8]. In contrast, physiological responses described in intact bronchial airways isolated from asthmatics or animals models are scarce and the contributions that the bronchial wall may make to AHR *in vivo* remains controversial. A porcine model of AHR is presented in this study, which because of its size and ready availability allows the functional

properties of different size bronchi to be investigated [9, 10]. Early allergen-induced responses have previously been reported in the pig [7, 11]. The preliminary aim of this current study was, therefore, to determine whether sensitization and repeated allergen exposure in pigs subsequently led to AHR. A further aim was to establish for the first time whether or not physiological responses in intact bronchi isolated from hyperresponsive pigs were increased. It was hypothesized that if AHR *in vivo* is due to physiological or morphological changes to the bronchial wall, then there should be an increase in responsiveness of bronchi isolated from the lung. *In vitro* responses were assessed in a bronchial segment, which preserves the structural components of the wall in their normal geometric relationship. This allows the smooth muscle to be exposed to contractile stimuli directly, *via* the adventitial surface, or *via* the airway lumen to simulate *in vivo* aerosol challenge.

Materials and methods

Sensitization procedure

Sixteen White/Landrace female pigs (10 kg) were studied. Eight test animals were sensitized to ovalbumin

(OA) on day 0 and 7 as previously described [7]. Four control animals were sham sensitized (saline and adjuvant) on corresponding days, while four naïve controls were housed for the same duration without receiving any injections.

Cutaneous responses

Success of sensitization was determined on day 14 and 20 using 50 μL intradermal injections of OA in 10-fold dilutions ($0.001\text{--}1\text{ mg}\cdot\text{mL}^{-1}$). The lowest concentration of OA at which a positive response (red wheal $>5\text{ mm}$ diameter) occurred was recorded and used as an index of reactivity to the allergen [12].

Aerosol challenge

Test animals received OA aerosol ($50\text{ mg}\cdot\text{mL}^{-1}$ in saline) on days 14, 17 and 20 while anaesthetized and intubated, as described previously [7]. Control animals received aerosols of saline alone. Pulmonary resistance (RL) and dynamic compliance (CL) were measured using an established protocol [7]. Measurements were made at baseline and for 30 min following challenge to detect early allergen responses.

Assessment of airways hyperresponsiveness in vivo

Airway responsiveness was determined from concentration-response curves (CRCs) to doubling concentrations of acetylcholine (ACh; Sigma Castlehill, NSW, Australia) dissolved in saline. Solutions were delivered as aerosols into the airways for 60 s, as described for allergen delivery [7]. RL and CL were measured before and after each concentration. Increasing concentrations of ACh were administered until RL reached a plateau. Baseline responsiveness was measured on day 14, prior to the first challenge, and again on day 20, 1 hr after the final challenge. Reactivity to ACh was determined from the maximal RL recorded during the CRC. Sensitivity to ACh was determined from the concentration of ACh required to increase RL 50% between baseline and the maximum RL value obtained (PC₅₀).

Assessment of airways hyperresponsiveness in vitro

The stem bronchus was dissected from the left and right lower lung lobes of euthanized pigs on day 20 following completion of *in vivo* measurements. The parenchyma was removed, side branches were ligated and a 25 mm-long segment was mounted in an organ bath, as previously described [7, 13]. These segments were classified as small/medium sized based on internal diameter (2–3 mm), location (spanning generations 9–14), and the presence of cartilage [14]. The segment adventitia was bathed in Krebs's solution and the lumen filled with Krebs's from a separate reservoir. Stopcocks at either end of the segment allowed the airway lumen to be sealed so that intraluminal

pressure could be monitored using a calibrated pressure transducer [10]. Segments were checked for pressure leaks throughout each experiment.

Bronchi were electrically field stimulated (EFS, 60V, 20 Hz and 3 ms pulses) using platinum ring electrodes and a Grass S44 stimulator (Grass Instruments, Quincy, MA, USA). EFS responses were used to assess the optimum passive pressure and stretch of segments (5 cmH_2O and 110% relaxed length, respectively, fig. 1) and to confirm that bronchial segments had recovered from the previous ACh concentration.

When a repeatable EFS response was obtained, a submaximal concentration of ACh, $1\times 10^{-4}\text{ M}$, was added to the bath, followed by washout, to provide a stable contraction history. Cumulative CRCs to ACh ($1\times 10^{-7}\text{--}1\times 10^{-2}\text{ M}$) were then obtained by adding ACh to the bath bathing the adventitial surface of the segment. After recovery, a noncumulative ACh

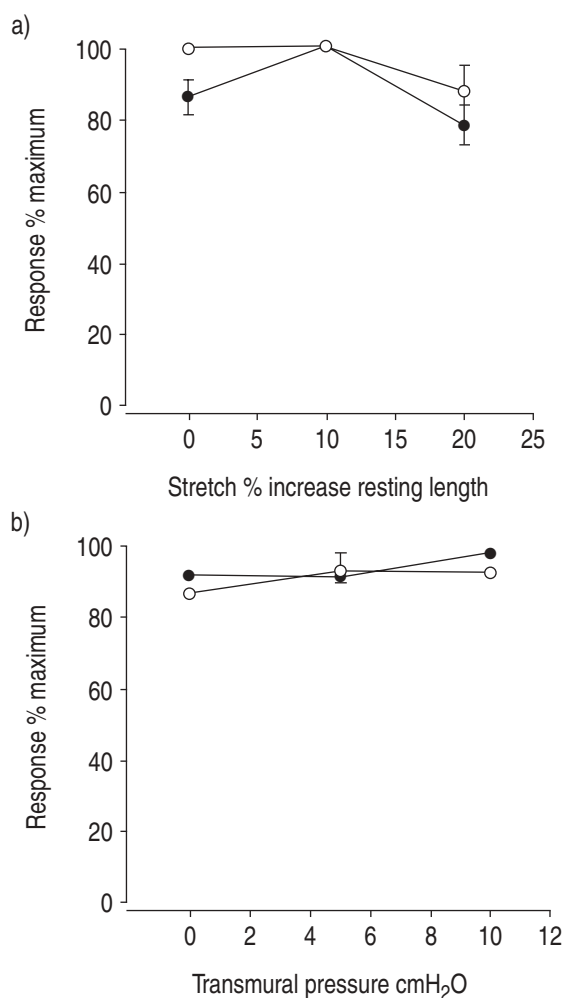


Fig. 1.—Effect of stretch (a) and passive transmurial pressure (b) on responses to electrical field stimulation (EFS) in bronchial segments from test (●) and control (○) pigs. Responses were calculated as percentages of maximum EFS response in each bronchus and are expressed as a group mean \pm SEM. a) Responses varied with length in test bronchi, but not control (analysis of variance (ANOVA), $p < 0.02$). b) There were no significant differences at different transmurial pressures or between control or test bronchi at individual pressures (ANOVA and two-tailed unpaired t-test).

CRC (1×10^{-7} – 1×10^{-1} M) was obtained by adding ACh to the fluid bathing the segment lumen [10, 13]. Bronchial responsiveness *in vitro* was quantified as the maximum pressure developed in response to ACh (E_{\max}) and the negative log of the ACh concentration that produced half the maximum pressure (pD₂).

Morphology

Bronchi were fixed at an intraluminal pressure of 5 cmH₂O with 10% buffered formalin solution. Fixed tissues were paraffin embedded, 5 µm sections were cut and stained with haematoxylin and eosin. ASM area, inner and outer wall area [15] and the lumen perimeter (Pi) were determined using image analysis software (Leading Edge Pty Ltd, Adelaide, South Australia). Measurements were averaged from five randomly-selected sections of each bronchus. Area was normalized [16] for differences in airway size by $\sqrt{\text{area}/\text{Pi}}$. The intra-observer coefficient of variation was <1.3%.

Histology

Representative sections of lower lung lobes were fixed in 10% buffered formalin. Fixed tissues were paraffin embedded, sectioned (5 µm) and stained with haematoxylin and eosin. Tissue samples were assigned a code number and examined by the pathologist. Visual assessment of the overall architecture of the alveolar and bronchiolar tissue, degree of vascular disturbance (oedema, hyperaemia and haemorrhage) and the presence of inflammatory cells (neutrophils, eosinophils, macrophages and lymphocytes) were noted.

Statistical analyses

Cutaneous and lung responses to OA or saline, and the reactivity and sensitivity to ACh were compared within groups using paired t-tests and between groups using unpaired t-tests. *In vivo* and *in vitro* CRCs to ACh were compared between groups using nonlinear regression (F-test) Unpaired t-tests were used to compare morphological parameters.

Correlations were determined from data obtained in controls, test and nonresponder animals, using the method of least squares. A p-value <0.05 was regarded as significant. Data are shown throughout as mean ± SEM, unless otherwise stated

Results

Cutaneous responses

Seven of eight test animals showed strong skin reactivity to intradermal OA on days 14 and 20. Positive responses occurred at a lower concentration on day 20 (geometric mean $0.019 \text{ mg} \cdot \text{mL}^{-1}$, range 0.001 – $0.01 \text{ mg} \cdot \text{mL}^{-1}$) than day 14 ($0.037 \text{ mg} \cdot \text{mL}^{-1}$,

range 0.01 – $0.1 \text{ mg} \cdot \text{mL}^{-1}$, $p < 0.02$). The remaining test animal had a weak response on both days ($1 \text{ mg} \cdot \text{mL}^{-1}$). Control animals showed no response to intradermal OA.

In vivo responses

Naïve and sham-sensitized control animals showed no differences in lung function or responsiveness to ACh (table 1). The data was combined into one group (n=8) for comparison with test animals.

Baseline RL and CL did not differ between test and controls on any study day Seven of the eight test pigs showed a strong increase in RL ($255 \pm 26\%$, $p < 0.0001$) and a corresponding decrease in CL ($90 \pm 3\%$, $p < 0.0001$) in response to OA aerosol on day 20 (fig. 2). Control animals showed no changes to saline aerosol (RL increased $11 \pm 4\%$, CL decreased $1 \pm 6\%$). Peak responses occurred in all animals within 5 min of completing the aerosol.

Baseline RL did not differ between preallergen/saline exposure and the start of the CRC to ACh 1 hr later (tests: $18.1 \pm 1.8 \text{ cmH}_2\text{O} \cdot \text{L}^{-1} \cdot \text{s}$ and 17.8 ± 1.5 ; controls: 18.7 ± 1.4 and 18.3 ± 1.7 , respectively). Test pigs showed a significant left shift in the CRC to ACh on day 20 ($p < 0.0001$) compared to baseline values on day 14 (fig. 3). In contrast, control animals showed no change between day 14 and 20 (fig. 3).

Sensitivity to ACh doubled in test animals from day 14 to 20, but showed no change in controls. As illustrated in figure 3, the concentration of ACh causing a 50% increase between baseline and maximal RL (PC₅₀) decreased in test pigs from a baseline of $9.9 \pm 1.5 \text{ mg} \cdot \text{mL}^{-1}$ on day 14 to 5.1 ± 0.9 on day 20, $p = 0.01$, while controls showed no change ($8.3 \pm 0.6 \text{ mg} \cdot \text{mL}^{-1}$ and 8.4 ± 1.1). PC₅₀ was significantly different between the groups on day 20 ($p < 0.05$), but not on day 14.

Table 1.—Comparative data from naïve (n=4) and sham-sensitized (n=4) control animals

	Naïve	Sham-sensitized
Baseline RL cmH ₂ O·L ⁻¹ ·s		
Day 14	22.2±2.7	17.9±2.0
Day 20	20.2±1.8	16.8±2.3
Baseline CL mL·cmH ₂ O ⁻¹		
Day 14	12.9±2.2	16.2±3.9
Day 20	14.4±1.4	19.1±5.0
Max RL cmH ₂ O·L ⁻¹ ·s		
Day 14	52±7	65±13
Day 20	57±7	57±9
Log PC ₅₀		
Day 14	3.26±0.04	2.80±0.78
Day 20	3.22±0.33	2.74±0.15

Data are presented as mean ± SEM. RL: pulmonary resistance; CL: dynamic compliance; baseline RL and CL represents the baseline measurement prior to acetylcholine (ACh) exposure. Max RL: the maximum RL obtained from *in vivo* ACh concentration-response curve; PC₅₀: ACh concentration required to increase RL 50% between baseline and maximum, obtained from *in vivo* ACh concentration-response curve. No significant differences were found between the two group (unpaired t-test).

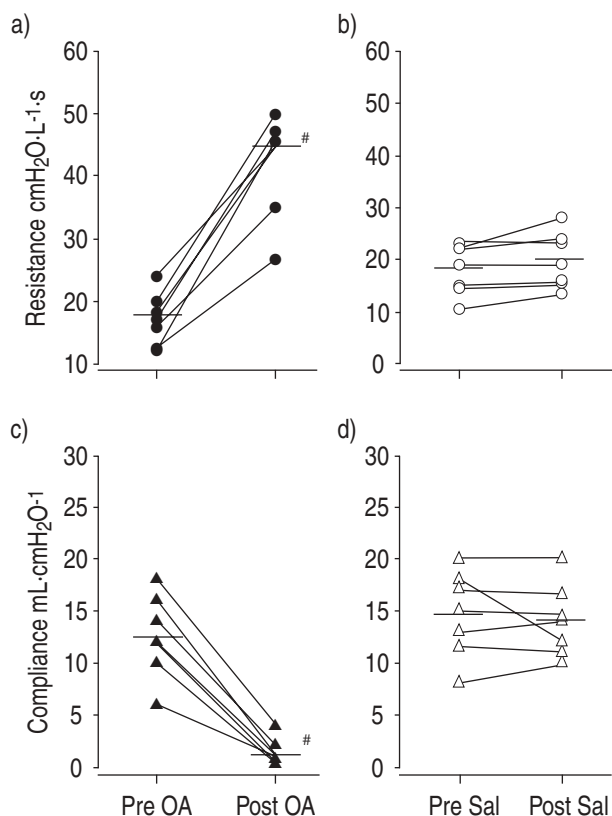


Fig. 2. – Paired measurements of resistance (a) test: ●; b) control: ○ and compliance (c) test: ▲; control: △) in individual pigs, pre and post *in vivo* aerosol challenge on day 20. Test animals received an ovalbumin (OA) aerosol (● and ▲, n=7), control animals received a saline (Sal) aerosol (○ and △, n=8). Mean responses are indicated with a horizontal bar. Test animals had a significant increase in both resistance and compliance following OA exposure (#; $p < 0.0001$).

Mean maximal RL increased from day 14 to 20 ($289 \pm 36\%$ and $387 \pm 68\%$ respectively, $p < 0.05$) in test animals but did not change in controls ($295 \pm 49\%$ on day 14 to $332 \pm 60\%$ on day 20).

Nonresponder

One of the eight sensitized pigs showed minimal responses to aerosol OA on day 20 (fig. 4), no change in responsiveness following OA exposure (fig. 4), and had a very weak response to skin-prick testing ($1 \text{ mg} \cdot \text{mL}^{-1}$). The animal was classed as a nonresponder and excluded from the test group for analyses.

Responsiveness of control and test bronchi *in vitro*

Each pig yielded *in vitro* CRCs to adventitially applied ACh; however, only five animals per group produced maximum CRCs to luminal ACh due to fatigue or development of a leak. The data from right and left bronchi were averaged, as no differences in responsiveness (pD_2 and E_{max}) to ACh were found (table 2).

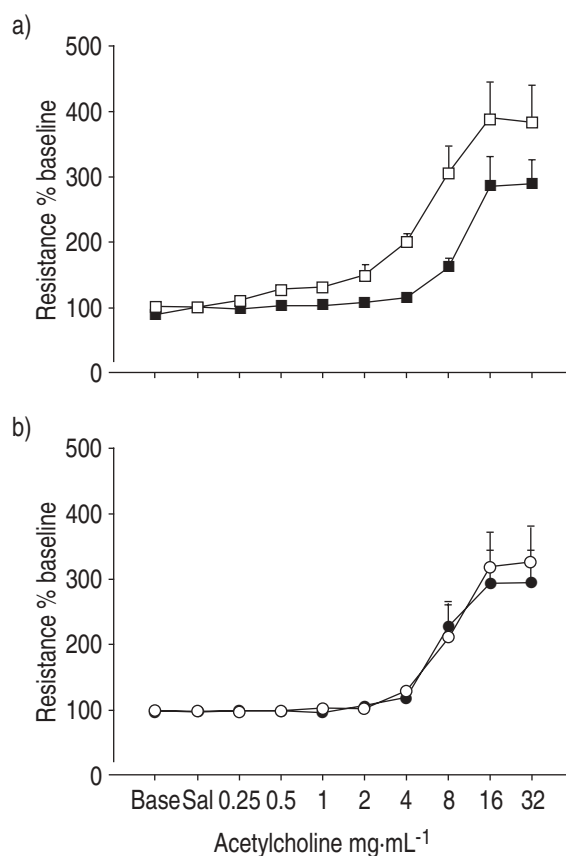


Fig. 3. – Mean \pm SEM resistance, expressed as a percentage of baseline (base), in response to inhaled acetylcholine (ACh) on days 14 and 20. a) Test animals (n=7) showed a significant shift of the *in vivo* concentration response curve (CRC) on day 20 (□) compared to baseline measurements on day 14 (■; F-test, $p < 0.0001$). The concentration of ACh causing a 50% increase between baseline and maximal pulmonary resistance (RL) was significantly decreased (paired t-test, $p=0.01$) and maximal RL significantly increased (paired t-test, $p < 0.05$) on day 20 *versus* day 14. b) Control animals (n=8) showed no change between day 14 (●) and day 20 (○). The CRC to ACh of test animals was different to that of controls on day 20 (F-test, $p=0.005$), but not on day 14. Sal: saline.

Statistical analysis of the luminal ACh CRC show a small, but significant, difference between test and control groups ($p < 0.03$, fig. 5); however, neither pD_2 nor E_{max} differed between the groups (pD_2 : controls 2.84 ± 0.13 and tests 3.05 ± 0.17 ; E_{max} : controls $60.1 \pm 4.1 \text{ cmH}_2\text{O}$ and tests $61.1 \pm 4.9 \text{ cmH}_2\text{O}$). In contrast to luminal data, test bronchi showed a significant 2.5-fold loss in contractile potency to adventitial ACh compared to controls ($p < 0.0001$, fig. 5). pD_2 was significantly reduced (control 4.58 ± 0.10 , test 4.18 ± 0.07 , $p < 0.01$), although E_{max} was not different between the two groups (control $82.1 \pm 3.4 \text{ cmH}_2\text{O}$, test $75.6 \pm 7.5 \text{ cmH}_2\text{O}$, fig. 5).

Morphometry

ASM area was increased in test pigs ($0.064 \pm 0.004 \text{ mm}$, $p < 0.05$) compared to controls (0.054 ± 0.001) (table 3). There were no differences in

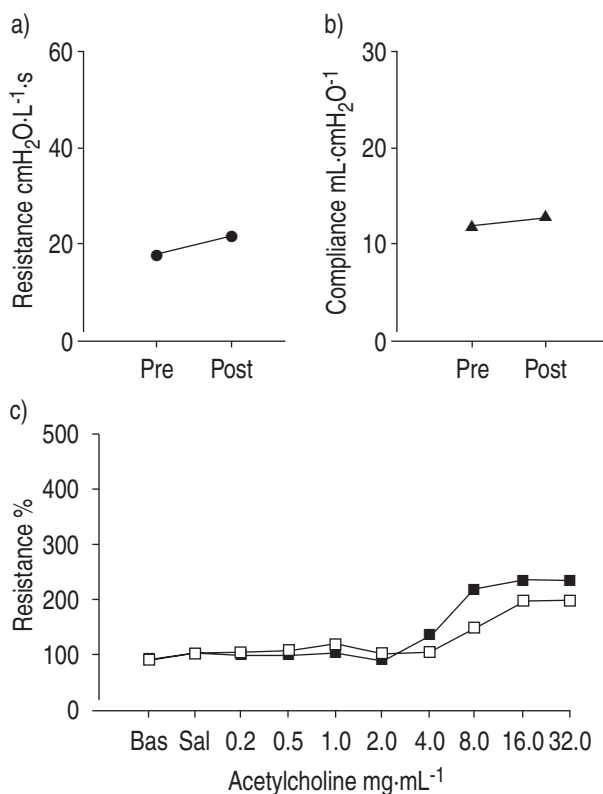


Fig. 4.—Resistance (a) and compliance (b) before (pre) and after (post) exposure to ovalbumin aerosol (OA) on day 20 in a "nonresponder" test animal. c) Resistance, expressed as a percentage of baseline (base), was in response to inhaled acetylcholine (ACh) on days 14 (■) and 20 (□). This animal showed no airway response to OA and did not develop hyperresponsiveness to ACh by day 20. Sal: saline.

inner, outer or total wall area between the two groups (table 3). The epithelium was >85% intact in both control and test bronchi, and of normal appearance.

Table 2.—Comparison of physiological data obtained from paired left and right lower lobe bronchial segments from control and test pigs (n=3–5)

Bronchi		Left	Right	
pD ₂	Lumen	Control	2.76±0.21	2.73±0.14
		Test	2.88±0.51	3.05±0.36
	Adventitia	Control	4.75±0.12	4.54±0.19
		Test	4.24±0.13	4.20±0.10
E _{max} cmH ₂ O	Lumen	Control	63.9±3.6	67.2±2.8
		Test	53.9±3.4	56.0±3.1
	Adventitia	Control	79.0±4.9	74.7±8.8
		Test	78.9±15.7	66.0±11.4

Data are presented as mean±SEM. E_{max}: maximum response; pD₂: the -log Concentration of Acetylcholine (ACh) producing half E_{max}. E_{max} and pD₂ were recorded in response to ACh administered either to the airway lumen or adventitia. There were no significant differences between left or right lobe bronchi.

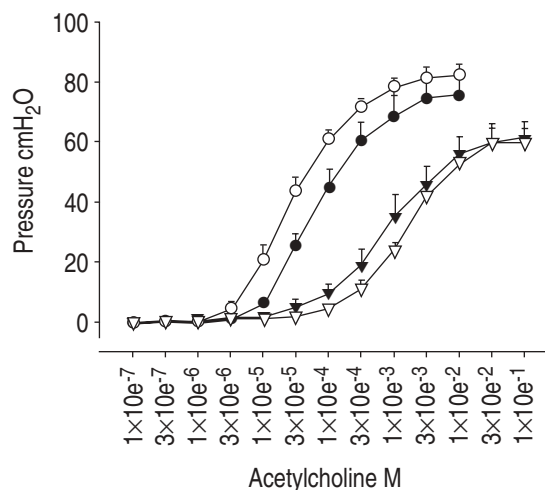


Fig. 5.—Increases in pressure (cmH₂O) in response to acetylcholine (ACh) in bronchial segments from test (adventitia: ●; lumen: ▼; n=7) and control (adventitia: ○; lumen: ▽; n=8) pigs. Mean±SEM control and test concentration-response curves were significantly different for both adventitial (p<0.0001, F-test) and luminal (p<0.03, F-test) administration of ACh. The -log concentration of ACh producing half the maximum pressure developed in response to ACh (E_{max}) was significantly reduced in test animals (p<0.01) compared to controls in response to adventitial ACh only.

Responsiveness in vivo versus in vitro

Responsiveness of isolated bronchial segments to luminal ACh was compared with *in vivo* responsiveness to aerosol ACh in control, test and nonresponder pigs. Reactivity to ACh *in vivo* (R_{max}) showed a weak positive correlation with reactivity *in vitro* (E_{max}) (r=0.599, p=0.05). However, there was no correlation between sensitivity (log PC₅₀) values *in vitro* and *in vivo* (r=0.441, p=0.175). Adventitial responses to ACh were also compared with *in vivo* responses. No correlation was found in reactivity (R_{max} versus E_{max}, r=0.079, p=0.77) or sensitivity (log PC₅₀, r=0.381, p=0.146).

Histology

Lymphocytes, neutrophils and eosinophils were evident in the parenchyma of test pigs. There was frequent, mild granulocytic cuffing around small bronchioles and an increase in cellularity within interstitial areas. Control animals showed occasional areas of increased cellularity, primarily due to atelectasis of

Table 3.—Morphometric analysis of the airway wall in bronchi from test (n=4) and control (n=4) pigs

	Inner airway wall	Outer airway wall	Smooth muscle
Test	1.046±0.211	4.144±0.880	0.064±0.004*
Control	1.206±0.344	3.817±0.682	0.054±0.001

Data were standardized for airway size and are presented as mean±SEM mm/mm. Measurements were made of inner airway wall, other airway wall, and airway smooth muscle areas. *: p<0.05, unpaired t-test for test versus control.

the parenchyma. Larger airways were normal in appearance in both test and control pigs. No differences were observed in overall architecture of the tissue, or the degree of vascular disturbance between the two groups.

Discussion

This study is the first to report functional properties of whole bronchi in a model of AHR. The model incorporates the primary characteristics of asthma, namely airway narrowing, AHR and inflammation. A unique feature of this model is that the size and ready availability of the animal allows the airway physiology of small to medium size bronchi to be studied *in vitro* after demonstration of AHR *in vivo*. This allows comparisons to be drawn between physiological properties of the airway wall *in vitro* and lung responsiveness. In addition, the use of bronchial segments to assess airway function *in vitro* allows responsiveness to be determined separately for ASM (*via* adventitial application of ACh) and for the airway wall (*via* the lumen). The results from the present study imply that repeated allergen challenge in sensitized pigs produces complex changes affecting several components of the bronchial wall. Evidence suggests that ASM is less responsive in allergen exposed bronchi than controls, indicating that AHR *in vivo* may be associated with other factors which regulate ASM contraction, such as airway structure or external loads from the parenchyma.

Bronchi from sensitized pigs had increased ASM, which is consistent with airway remodelling as seen in asthmatics [16]. However, when assessed *in vitro* bronchi from sensitized pigs were less responsive to adventitial ACh than controls. Responsiveness could be influenced by inflammatory stimulation and also by the presence of smooth muscle cells with different contractile or secretory phenotypes [17]. In some animal models, tracheal smooth muscle contraction is increased [1–3]. There are fewer studies in bronchi, but one human study showed decreased smooth muscle force in bronchi obtained *post mortem* [5]. The reduced bronchial response to adventitial ACh in the present study suggests that ASM force and sensitivity is decreased despite the presence of more ASM. At present, the authors have no information on contractions in more peripheral airways, which might show different levels of responsiveness. In contrast to the adventitial route, there was a modest rightward shift in the luminal CRC of sensitized bronchi, the route considered to be more physiological. The small increase in luminal sensitivity suggests the presence of some mechanism(s) within the airway wall which act to increase activation of ASM. Of particular importance is the lining epithelium, which if damaged leads to major increases in luminal responsiveness due to increased drug penetration to the underlying smooth muscle [13]. Although no gross alterations to epithelial morphology were observed in the test bronchi of this study, more subtle changes in epithelial permeability may have been present.

The prevalence of early airway responses to inhaled

allergen using this porcine model was 87%, which compares favourably with other allergic animal models [18]. One sensitized pig failed to respond to OA aerosol and showed a poor cutaneous response compared to the other seven test animals. This biological variation is typical of species that are not highly inbred, hence some research groups use screening tests, such as cutaneous responses, to preselect the animals they study [11, 18–20]. The antigen-sensitized animals developed a typical wheal and flare response, suggesting a production of antigen-specific immunoglobulin-E (IgE) antibodies [12]; whereas, neither naïve nor sham-sensitized control pigs responded to cutaneous antigen. A more sensitive index of sensitization would be advantageous. To date, it is not possible to measure porcine IgE concentrations directly, as there are no commercially-available anti-porcine IgE monoclonal antibodies. However, significant advances have recently been made with the isolation of the pig IgE epsilon chain [21].

All control animals were exposed to an aerosol of saline, rather than OA in saline diluent, as repeated OA aerosol exposure may be sufficient to sensitize the animal in the absence of an initial antigen injection [22]. Sham-sensitized pigs showed the same responses as naïve pigs (table 1). A third control group of OA-sensitized and saline-challenged pigs had been previously assessed in the present authors' laboratory with similar results (unpublished data).

Multiple allergen exposure has been used in several animal models [23–25] to induce some of the functional and structural changes seen in asthma. In this study, three aerosol exposures to OA resulted in a mild inflammation in test animals particularly in the small airways. There was also an increase in ASM area, but no other changes in overall architecture of the tissue or degree of vascular disturbance distinguished test animals from controls.

An aim of the present study was to determine whether differences in the responsiveness of lungs *in vivo* could be accounted for by properties of the isolated airway wall. Data from control, test and nonresponder pigs were combined providing a broad range of responses. The present study demonstrates a relationship between maximal responses to luminal challenge *in vivo* and *in vitro*. Previous studies using rings or strips of smooth muscle [8] have failed to show such an association, possibly because the dissection procedure may compromise the epithelium and other structural features that contribute to responsiveness *in vivo*. More recently, MASAKI *et al.* [26] showed a positive correlation between the effect of lumenally-applied ACh on tracheal narrowing *in vitro* and insufflation pressure *in vivo* in an OA-sensitized cohort of guinea pigs. In contrast, a study by WOISIN *et al.* [27] found no correlation between *in vivo* and *in vitro* responsiveness in control and sensitized rabbits; however, the sensitized cohort failed to show hyperresponsiveness when compared to the controls. The present study extends these findings as it includes a cohort of normal pigs together with sensitized animals exhibiting clear AHR.

The present results suggest that reactivity *in vivo* may be related to reactivity in the individual

bronchus, as determined *in vitro*. There was no such relationship between sensitivities *in vivo* and *in vitro*. These findings are consistent with sensitivity arising as a result of more than one mechanism. Extrinsic airway mechanisms, as well as local airway alterations, could contribute to responsiveness as suggested by others [6]. The present authors have previously shown that bronchi from test pigs are less compliant than controls [7], which is consistent with structural remodelling. In the present study, test animals showed airway wall remodelling in the form of increased airway smooth muscle area. This could lead to stiffer airways, which could be less prone to forces of interdependence in the lungs. Any reduction in the afterload on airway smooth muscle contraction, arising from forces of interdependence, might favour airway narrowing *in vivo*, but would not be apparent in the individual airway *in vitro*. Subsequent studies using this model may uncover, at the cellular level, mechanisms producing changes to airway function in the bronchial wall.

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