

Repeated Allergen Inhalation Induces Cytoskeletal Remodeling in Smooth Muscle from Rat Bronchioles

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Airway hyperresponsiveness (AHR) is associated with airway wall structural remodeling and alterations in airway smooth muscle (ASM) function. Previously, in bronchioles from Brown Norway rats challenged by repeated ovalbumin (OVA) inhalation, we have reported increased force generation and depletion of smooth muscle contractile proteins. Here, we investigated if cytoskeletal changes in smooth muscle could account for this paradox. Sensitized rats ($n = 5/\text{group}$) were repeatedly challenged with OVA or saline, and the lungs were removed 24 h after the last challenge. Levels of globular (G) and filamentous (F) actin in bronchioles were determined by DNase I inhibition and contraction assessed in intact small bronchioles using a myograph. DNase I inhibition assays showed that G-actin monomers were more abundant ($\sim 1\text{F}:2\text{G}$) in extracts from resting small bronchioles from OVA- or saline-challenged animals. However, while contractile protein levels in bronchioles were reduced by OVA ($P < 0.05$), the proportion of F:G actin was 1.8-fold greater compared with saline challenge ($P < 0.05$). Consistent with induction of F-actin after OVA challenge, increases in maximum tension development to carbachol or KCl in small bronchioles from OVA-challenged animals were abrogated ($P < 0.01$) by actin cytoskeleton disruption with $0.5 \mu\text{M}$ latrunculin A. Cytoskeletal stabilization of F-actin with $0.1 \mu\text{M}$ jasplakinolide potentiated maximum contractions to carbachol or KCl ($P < 0.05$) in bronchioles from OVA- but not saline-treated rats. We conclude that alterations in the composition and/or arrangement of the contractile apparatus after OVA exposure confer enhanced contractile responses, possibly as a result of increased F-actin content. Such a mechanism may have relevance for AHR found in allergic asthma.

Keywords: airway hyperresponsiveness; airway smooth muscle; asthma; cytoskeleton

Airway hyperresponsiveness (AHR) is a key characteristic of asthma and describes the ability of the airways to narrow excessively in response to agonists and nonspecific stimuli (1, 2). AHR can be demonstrated by increased responses to inhaled bronchoconstrictors such as methacholine, and can be observed after allergen exposure. Mechanisms underlying AHR are multivariant. Induction and perpetuation of AHR may result from repeated inflammatory events involving a complex and coordinated response of multiple inflammatory and structural cells, mediators, connective tissue elements, and cytokines whose actions lead ultimately to persistent changes in airway wall structure (3). Links between inflammatory events leading to alter-

CLINICAL RELEVANCE

Allergen exposure in rats reduces airway smooth muscle contractile proteins, but enhances force generation. We examined this paradox and found that F-actin is doubled. Our data suggest that increased F-actin levels after chronic allergen exposure confer enhanced contractions.

ations in the mechanical phenotype of the airway remain poorly understood, and it is reported that AHR can be uncoupled from inflammatory cell infiltration (4–6). Equally, it is unclear whether AHR in asthma is dependent on structural and/or mechanical changes in the noncontractile elements of the airway wall, decreased coupling of the airway wall to the surrounding parenchyma, or persistent changes in the amount, phenotype, or plasticity of the airway smooth muscle (ASM) cell (7–9).

Current concepts of ASM contraction involve the myosin ATPase motor, where myosin exerts its contractile effects by interaction with actin within an integrated cytoskeletal scaffold that is highly dynamic and in a continuous state of remodeling (10). Remodeling of these internal microstructures including the cytoskeleton (CSK) produces efficient levels of muscle force over a wide range of muscle lengths (11–13), and is a major factor contributing to the excessive airway narrowing in asthma that characterizes AHR.

Repeated antigen challenge of the actively sensitized Brown Norway (BN) rat is a well-established model that replicates many of the pathologic features of individuals with chronic allergic asthma, including AHR, T cell and eosinophilic inflammation, and increased ASM content (14–16). Previously, in isolated bronchioles from sensitized BN rats challenged by repeated ovalbumin (OVA) inhalation, we have reported increased tension generation in response to contractile agonists despite depletion of contractile muscle protein content including smooth muscle (sm) myosin heavy chain (sm-MHC) and sm- α -actin (17).

In this study we examined if allergen exposure induces changes in the actin CSK that could account for this paradox. Thus, we examined if repeated exposure of sensitized BN rats to OVA induced persistent alterations in the actin CSK of smooth muscle present in small bronchioles, as assessed by the globular (G):filamentous (F) actin ratio as a surrogate marker of actin polymerization status. To investigate the functional significance of an altered actin CSK after allergen exposure, we have used pharmacologic agents that either promote actin depolymerization (latrunculin A) or its stabilization (jasplakinolide) during agonist-induced contractions of isolated small bronchioles. Some of the results of this study have been previously reported in the form of an abstract (18).

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MATERIALS AND METHODS

Animal Sensitization, Allergen Exposure, and Tissue Collection

After approval by local Institutional Review Boards, pathogen-free, male Brown Norway rats (Harlan, Bicester, UK) weighing 220–250 g were actively sensitized by intraperitoneal injections on three consecutive days of a suspension comprising 1 mg/kg OVA in 0.9% saline containing 100 mg of Al(OH)₃ as adjuvant (15). Rats were challenged on Day 6 and then every 3 d for a total of six 15-min exposures. Challenges took place in a 0.8-m³ chamber with free-breathing animals ($n = 5$ in each group) being exposed to either saline or 1% OVA in saline aerosol mist, generated by a DeVilbiss PulmoSonic nebulizer (DeVilbiss Healthcare Ltd, Feltham, UK). The aerosol mist was pumped into the box at 600 ml/min using a small animal ventilator. At all times animals were housed in a caging system that received clean filtered air only, with food and water supplied *ad libitum*. At 24 h after the last OVA exposure, animals were killed after intraperitoneal injection of pentobarbitone sodium (50 mg/kg), and the trachea and lungs were rapidly removed. Intact small bronchioles comprising branches of the distal subsegmental bronchus of the right lung were dissected free of parenchyma and connective tissue for assessment of contractile function or, with cartilage-free and epithelium-denuded trachealis, were either used fresh for contraction studies or snap-frozen and stored at -80°C for later determination of G:F actin composition. In some instances cultures of tracheal and bronchial smooth muscle cells were established from each cohort.

Contractile Tension Development

Intact small bronchioles (internal diameter 300–500 μm ; length 1–2 mm), free of all visible traces of parenchyma, were mounted onto the jaws of a Mulvany-Halpern small vessel myograph (Danish Myo Technology, Aarhus, Denmark) as described previously (17) and bathed with a physiologic salt solution (PSS) at 37°C , containing 118 mM NaCl, 24 mM NaHCO₃, 1 mM MgSO₄, 4 mM KCl, 5.56 mM glucose, 5 mM sodium pyruvate, 0.435 NaH₂PO₄, and 1.8 mM CaCl₂, pH 7.4, equilibrated with 5% CO₂ in air. After equilibration for 30 min with washing every 5 min, the degree of radial stretch was normalized for bronchiole size by setting each preparation to 80% of the stretch necessary to obtain the peak of the active length–tension relationship (80% L_{max}), which was determined by the response to 75 mM KCl (equimolar substitution for Na⁺) following each incremental stretch. At 80% L_{max} an equilibration period of 30 min was then allowed before the start of each experiment, after which radial contraction was induced by 75 mM KCl. This was repeated twice more at 10-min intervals and resulted in contractions of similar magnitude. After washing for 15 min, cumulative concentration–response relationships were generated for carbachol in the absence or presence (15 min) of actin cytoskeleton disruption with 0.5 μM latrunculin A or cytoskeletal stabilization with 0.1 μM jasplakinolide for 15 min. In parallel experiments, tension development was induced by treatment with 75 mM KCl. Developed tension was expressed in mN per mm length of bronchiole.

DNase I Inhibition Assay for Globular and Total Actin

Tracheal or bronchiolar smooth muscle tissues were snap-frozen in liquid nitrogen and pulverized using a liquid nitrogen pre-cooled Bessman Tissue Pulverizer (Fisher Scientific, Chicago, IL). Tissue protein extracts were prepared in buffer comprising 50 mM Tris-HCl, 2 mM MgCl₂, 1 mM EGTA, 0.2 mM dithiothreitol, and 1.0% Triton X-100, pH 7.4. Total actin and G:F ratios were determined spectrofluorimetrically by DNase I inhibition using a G-actin standard curve as described previously (19). For determination of G-actin content, 10 μl of lysate was added to the assay mixture (30°C) containing 10 μl of DNase I solution (0.1 mg/ml DNase I in 50 mM Tris-HCl, 10 mM PMSF, and 0.5 mM CaCl₂, pH 7.4) and 1 ml of DNA solution (80 $\mu\text{g}/\text{ml}$ DNA in 100 mM Tris-HCl, 4 mM MgSO₄, 1.8 mM CaCl₂, pH 7.4). DNase I activity at 30-s intervals over 7 min was monitored at 260 nm with a Philips PU8720 scanning spectrophotometer (Pye Unicam Ltd., Cambridge, UK).

Actin was measured by reference to a standard curve for the inhibition of DNase I activity, prepared with bovine muscle actin (Sigma-Aldrich, Poole, UK). A linear relationship was observed over the range of 20–70% inhibition of DNase I activity. For estimation of total actin,

standards or samples were treated on ice for 15 min with an equal volume of guanidine hydrochloride solution to depolymerize F-actin. The depolymerizing solution contained 1.5 M guanidine hydrochloride, 1 M sodium acetate, 1 mM CaCl₂, 1 mM ATP, and 20 mM Tris-HCl (pH 7.4). After centrifugation, aliquots were combined in a cuvette with DNase I and DNA solutions for actin assay. F-actin was calculated as the difference between total actin and initially measured G-actin. Both G-actin and F-actin levels were related to total protein content, measured with the BCA protein assay reagent kit (Sigma-Aldrich).

Detection of Smooth Muscle Proteins by Western Immunoblot

For each treatment group (i.e., sensitized control or sensitized OVA-challenged), snap-frozen bronchioles (five bronchioles per animal) and trachealis muscle (epithelium-denuded) from each of five animals were pulverized using a liquid nitrogen pre-cooled Bessman Tissue Pulverizer and tissue protein extracts prepared as described previously (17). Proteins (5–10 $\mu\text{g}/\text{lane}$) were separated by SDS/PAGE on 10% or 4 to 12% acrylamide pre-cast gels (Invitrogen Ltd., Paisley, UK), transferred to nitrocellulose membranes, and detected with monoclonal antibodies (Sigma-Aldrich) against sm- α -actin (clone 1A4, 1:10,000), β -actin (clone AC15, 1:10,000), and sm-MHC (clone G4, 1:200; Santa Cruz, Santa Cruz, CA). Signals were visualized by enhanced chemiluminescence (Amersham-Pharmacia, Amersham, UK) and were quantified using ImageQuant software (Molecular Dynamics, Sunnyvale, CA) on autoradiographs that depicted bands within a linear range of exposure.

Flow Cytometric and Cytochemical Protein Detection in Cultured Smooth Muscle Cells

Tracheal and bronchiolar smooth muscle cells from saline- or OVA-challenged BN rats were grown from tissue fragment explants using methods described previously (20). Cell passages 3–6 were used in all experiments. After harvesting using trypsin/EDTA (0.02%), 4% formaldehyde-fixed, 0.1% Tween 20–permeabilized cultured cells were treated with 1% goat serum; incubated with monoclonal antibodies (Sigma-Aldrich) to sm- α -actin (clone 1A4, 1:250), calponin (clone hCP 1:500), or desmin (clone DE-U-10 1:50); and then incubated with an anti-mouse fluorescein isothiocyanate–conjugated secondary antibody. Labeling was analyzed on a FACS Calibur flow cytometer (Becton Dickinson, Oxford, UK) as described previously (21). For cytochemistry, adherent permeabilized cells were labeled with rhodamine-phalloidin (0.5 μM) and counterstained with Hoechst 33342.

Data and Statistical Analysis

Effective concentrations inducing 50% maximal responses (EC_{50}) and extrapolated maximum responses were estimated for individual concentration–response curves using nonlinear least-squares regression (r^2 values were > 0.9 and the curve fit described by the equation $y = y_0 + [ax/b + x]$, SigmaPlot; SPSS Inc., Chicago, IL). EC_{50} values were converted to negative logarithmic values (PD_2) for all statistical analysis, although for ease of comprehension EC_{50} values ($\pm 95\%$ confidence intervals) are given in the text. All other values are given as mean $\pm$ SE of observations obtained in tissues from n animals in each treatment group. Data were compared using Student's t test or one- or two-way ANOVA with a Bonferroni *post hoc* test as appropriate (SigmaStat; SPSS Inc.). A P value of < 0.05 was considered significant.

RESULTS

In separate experiments, the maximum developed contractile force induced by carbachol in isolated bronchioles obtained from the lungs of sensitized BN rats 24 h after challenge with either OVA or saline was significantly greater in OVA- compared with saline-treated animals (saline 2.52 ± 0.38 mN/mm versus OVA 3.92 ± 0.24 mN/mm, $P < 0.01$, $n = 5$) (Figure 1A). Paradoxically, the content of sm- α -actin and sm-MHC was significantly reduced in bronchioles from OVA-challenged animals ($P < 0.05$ - $P < 0.01$, $n = 5$) (Figure 1B). Changes in β -actin, a nonmuscle isoform, were not detected in lysates from bronchioles, demonstrating that equal amounts of total lysate protein were blotted. These findings confirmed our previous observations with this

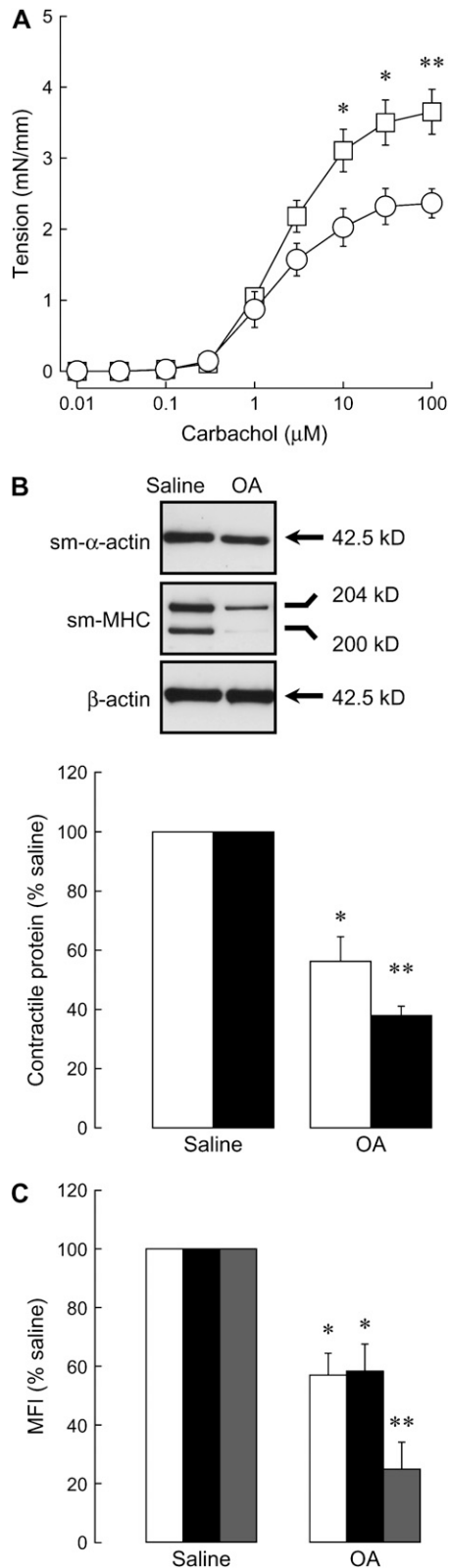


Figure 1. (A) Isometric tension development induced by carbachol *ex vivo* 24 h after repeated challenge of sensitized BN rats with saline (open circles) or aerosolized 1% OVA (open squares). (B) Western immunoblot detection and densitometry of smooth muscle contractile proteins (open bars, sm-α-actin; solid bars, sm-MHC, 204 kD) in isolated small bronchioles 24 h after saline or OVA challenge. (C) Flow cytometric evaluation of myocytes (open bars, sm α-actin; solid bars, sm-MHC; shaded bars, desmin) cultured 24 h after saline or OVA challenge. Data points and vertical bars indicate mean $\pm$ SE from duplicate preparations from five separate animals in each treatment group. * $P < 0.05$, ** $P < 0.01$ compared with saline by two-way ANOVA (A) and unpaired Student's *t* test (B and C).

OVA exposure was seen in cultured tracheal myocytes but the reduction was less marked ($\sim 15\%$ of saline-treated) and did not reach significance (data not shown). In addition, while there was a reduction in the intensity of fluorescence labeling with these contractile markers in bronchiolar myocytes cultured from OVA-treated animals, the number of cells that were positive did not vary between the two treatment groups, and labeling was not less than 95% for any of the markers examined (data not shown).

Airway Myocyte F-actin Content after Repeated Allergen Inhalation

Because of the paradoxical finding of increased maximal developed force in bronchioles from OVA-treated rats accompanied by decreased contractile protein content, we examined the hypothesis that actin polymerization status and hence the composition and/or organization of the actin CSK in bronchiolar smooth muscle from OVA-exposed cells differed from saline-treated animals. DNase I inhibition assays examining the G:F ratio, as a surrogate marker of actin polymerization status, showed that G-actin monomers were more abundant in extracts from resting bronchioles from either OA- or saline-challenged animals ($P < 0.05$, $n = 5$). However, the proportion of F-actin was ~ 1.8 -fold greater in bronchioles from OVA-challenged animals compared with saline ($P < 0.05$, $n = 5$). In contrast, in trachealis the G- and F-actin content was similar, and there was no change in actin G:F ratio after OVA exposure ($P > 0.05$, $n = 5$) (Figure 2). Levels of total actin did not differ significantly ($P > 0.05$, $n = 5$) with OVA or saline exposure between bronchiole tissue (mg/ml: saline 0.069 ± 0.012 versus OVA 0.087 ± 0.015) or trachealis (mg/ml: saline 0.086 ± 0.010 versus OVA 0.082 ± 0.015).

Effect of Modulation of the CSK on Allergen-Augmented Contractile Responses

Observed changes in the actin G:F ratio in sensitized BN rats repeatedly exposed to allergen suggested that persistent alterations were present in the actin CSK of smooth muscle present in bronchioles. To investigate the functional significance of an altered actin CSK after allergen exposure, isolated bronchioles were treated for 15 min with pharmacologic agents that either promoted actin depolymerization (latrunculin A) or stabilized the CSK (jasplakinolide) before evoking contractile responses with the muscarinic agonist carbachol or induction of depolarization by KCl. Consistent with previous findings, OVA treatment increased the maximal force generation induced by carbachol on isolated bronchioles. Treatment with latrunculin A ($0.5 \mu\text{M}$) disrupted the actin CSK in cultured cells as indicated by the absence of stress fibers labeled by rhodamine-phalloidin (Figure 3B). Based on findings in Figure 2 that F-actin levels were enhanced

antigen-driven animal model of chronic asthma (17) and were in keeping with the observed reduction in mean fluorescence intensity for multiple contractile markers (sm-α-actin, calponin, and desmin) expressed by bronchiolar myocytes cultured from OVA-challenged BN rats compared with saline-treated animals ($P < 0.05$ - $P < 0.01$, $n = 5$) (Figure 1C). A similar trend with

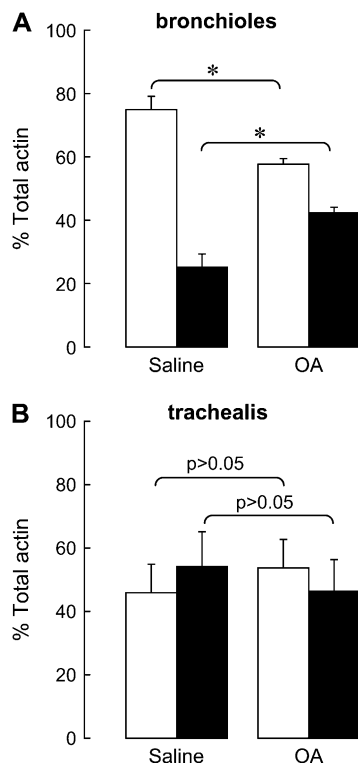


Figure 2. Total actin and globular (G):filamentous (F) ratios in (A) isolated small bronchiole preparations and (B) trachealis obtained 24 h after repeated challenge of sensitized BN rats with saline (open bars) or aerosolized 1% OVA (solid bars). Vertical bars indicate mean $\pm$ SE from duplicate preparations from five separate animals in each treatment group. * $P < 0.05$ compared with saline by unpaired Student's *t* test.

in bronchioles from OVA-treated animals, it was hypothesized that latrunculin A would be less effective in suppressing responses in tissues from the OA group. Latrunculin A markedly suppressed ($P < 0.05$, $n = 5$) contractile responses at concentrations of carbachol exceeding $1 \mu\text{M}$ in bronchioles from either OVA- or saline-treated animals, but residual contractions of bronchioles from OVA- or saline-treated animals did not differ ($P > 0.05$, $n = 5$) (Figure 4A). No change in the contractile sensitivity to carbachol was detected after latrunculin A (data not shown). To ensure that latrunculin A was not nonselectively affecting muscarinic receptor-mediated contractile responses, the effect of this agent was also examined on KCl-induced contractions, and a similar marked suppression of force generation was found (Figure 4B).

In contrast to CSK disruption, stabilization of the actin CSK by treatment with jasplakinolide ($0.1 \mu\text{M}$) enhanced carbachol-evoked contractions, but only in bronchioles isolated from OVA-treated animals ($P < 0.05$, $n = 5$) (Figure 5A). The major effect involved increasing the contractile sensitivity of OVA-treated tissues to carbachol (EC_{50} : control $2.49 \pm 1.57 \mu\text{M}$ versus jasplakinolide $0.77 \pm 0.82 \mu\text{M}$, $P < 0.05$, $n = 5$). A similar increase occurred with KCl-induced contractions after jasplakinolide treatment, which again was restricted to bronchioles from OVA-treated rats ($P < 0.001$, $n = 5$) (Figure 5A).

DISCUSSION

Previously, in isolated bronchioles from sensitized BN rats challenged by repeated OVA inhalation, we have reported increased tension generation in response to multiple contractile agonists *ex vivo*, which paradoxically was accompanied by a reduction in contractile muscle protein content (17). Here, we confirmed this finding in an independent series of experiments and examined if allergen exposure induced remodeling of the actin CSK that could account for this anomaly. DNase I inhibition assays revealed that the proportion of F:G actin, a surrogate marker

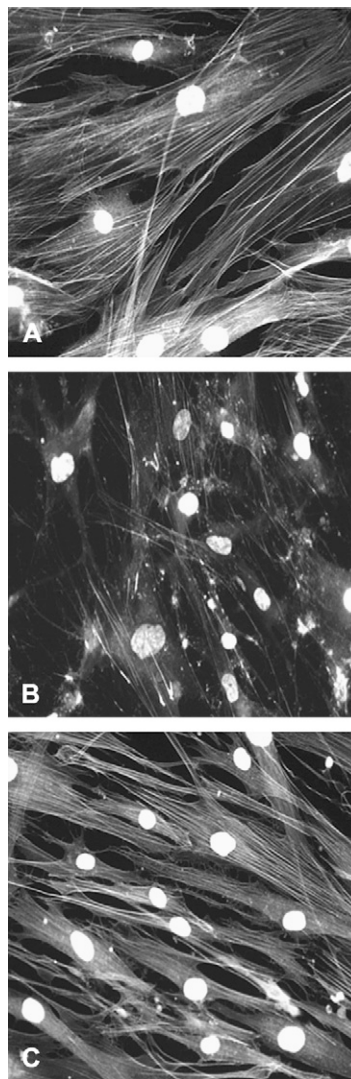


Figure 3. Representative photomicrographs depicting total actin stress fibers using rhodamine-phalloidin of (A) control cultured myocytes and (B) stress fiber disruption after treatment with latrunculin A ($0.5 \mu\text{M}$) for 15 min. Total actin stress fibers persisted after treatment with (C) the actin polymerization-stabilizing agent, jasplakinolide ($0.1 \mu\text{M}$), for 15 min. Cell nuclei were counterstained with Hoechst 33258 and photomicrographs obtained using a $\times 40$ objective.

of actin polymerization status, was almost double that found in bronchioles from OVA-treated animals compared with saline-exposed controls. In keeping with induction of F-actin after OVA challenge, increases in maximum tension development to either carbachol or KCl in small bronchioles from OVA-challenged animals were abrogated by disruption of the actin CSK with latrunculin A. Moreover, stabilization of F-actin with jasplakinolide potentiated maximum contractions to carbachol or KCl in bronchioles from OVA-treated rats, but had no effect on similar contractions in bronchioles from saline-treated rats. In addition, while numbers of cells subsequently cultured from bronchioles of OVA-exposed animals that were positive for smooth muscle proteins did not differ from saline controls, we demonstrate that levels of expression of smooth muscle marker proteins in cells from OVA-exposed animals are reduced by around 50%. We propose that persistent changes in the composition and/or arrangement of actin CSK dynamics result from chronic allergen exposure that confer enhanced contractile responses, possibly as a result of the increased F-actin:G-actin content, and that this may contribute in part to the AHR found in allergic asthma.

Proposed mechanisms underlying AHR include structural and/or mechanical changes in ASM, as well as airway nerve activation, airway wall inflammatory events, and changes in

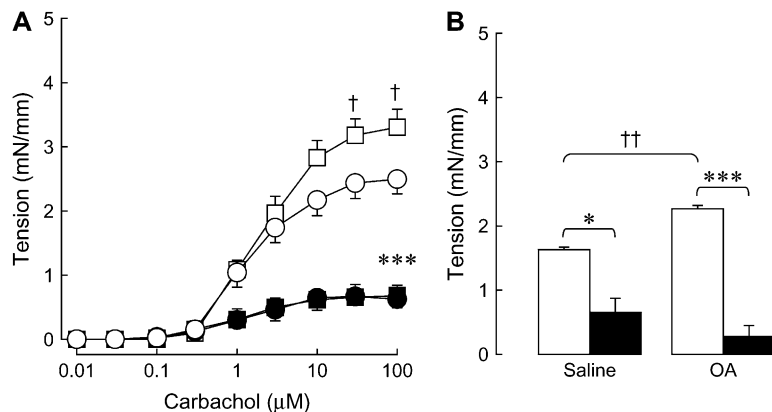


Figure 4. Effect of treatment with latrunculin A ($0.5 \mu\text{M}$, 15 min, solid symbols) on isometric tension development induced by (A) carbachol or (B) 75 mM KCl in isolated small bronchioles obtained 24 h after repeated challenge of sensitized BN rats with saline (open circles, open bars) or aerosolized 1% OVA (open squares, solid bars). Vertical bars and data points indicate mean $\pm$ SE from duplicate preparations from five separate animals in each treatment group. * $P < 0.05$, *** $P < 0.01$ compared with the absence of latrunculin A by two-way ANOVA. † $P < 0.05$, †† $P < 0.01$ compared with saline by two-way ANOVA.

airway epithelial function and/or integrity (2–4). Repeated OVA challenge in sensitized BN rats increases epithelial cell DNA synthesis (14), supporting activation of injury and repair mechanisms (15). Mechanical disruption of the airway epithelium increases the sensitivity of opened (i.e., nonperfused) tracheal or bronchial preparations to cholinesterase-sensitive cholinomimetics (acetylcholine and methacholine), but not to KCl or cholinesterase-resistant cholinomimetics (carbachol, bethanechol) (22, 23). This contrasts with the profile of increased agonist responsiveness we observed with both carbachol and KCl, suggesting that altered epithelial function is unlikely to be a major factor in determining increased force generation after OVA challenge in open bronchiole preparations. Indeed, AHR to inhaled muscarinic agonists after repeated OA challenge (15, 24) may be due to increased maximum force generation of the ASM itself in small airways, since we observed an increase in maximal isometric tension with both KCl and the cholinesterase-resistant cholinomimetic, carbachol. KCl initiates smooth muscle contraction largely independently of receptor activation by direct depolarization of the plasma membrane, allowing opening of voltage-dependent channels and influx of extracellular Ca^{2+} , whereas carbachol requires a predominately receptor-mediated phosphoinositide turnover to induce Ca^{2+} release (25). Both contractile agonists converge on a final pathway in smooth muscle with the interaction of sm- α -actin and myosin and the onset of cross-bridge cycling (10). Thus, the quantitative and qualitative similarities between KCl and carbachol, agonists that induce contraction by receptor and nonreceptor constrictor mechanisms, probably result from allergen-induced modulation of contraction downstream of Ca^{2+} release. Alterations at the level

of the contractile machinery, perhaps related to changes in its composition (Figure 1) (17) and/or in its arrangement (Figure 2), are the most probable explanation and reflect a generalized intrinsic change conferred by allergen exposure in the ability of the bronchiole preparations to generate force to these agonists.

It was anticipated that latrunculin A would be less effective in suppressing responses in tissues from the OVA group because F-actin levels were higher than in saline-treated tissues but latrunculin A was found to be equally effective. It is possible that latrunculin A would have been less effective against contractions in bronchioles from OVA-treated animals if it had been used at a lower submaximal concentration. Our observation that actin CSK disruption by latrunculin A treatment reduced both carbachol- and KCl-induced isometric force generation, whereas CSK stabilization by jasplakinolide was without effect in bronchioles isolated from saline-treated control rats accords with similar findings from Dowell and colleagues (26) in unsensitized canine trachealis strips. Similarly, concentrations of the CSK modifying reagents we used in the present study were similar to those used Dowell and colleagues (26) and their efficacy was subsequently confirmed in cultured ASM cells by rhodamine-phalloidin labeling (Figure 3). Furthermore, the observed selectivity of jasplakinolide on bronchioles from OVA-treated rats causing potentiation of agonist-induced contractions, as well as a near-doubling in the proportion of F-actin levels in resting bronchioles from these animals, is novel and unrelated to sensitization status because all animals were sensitized to OVA in this study. Instead, the findings support the notion that repeated allergen exposure induces persistent changes in actin CSK dynamics in addition to those occurring acutely with agonist stimulation (26). Ultimately, changes in CSK

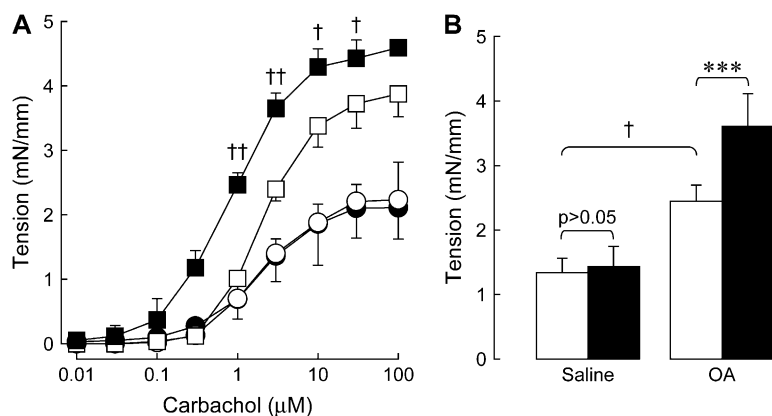


Figure 5. Effect of treatment with jasplakinolide ($0.1 \mu\text{M}$, 15 min, solid symbols) on isometric tension development induced by (A) carbachol or (B) 75 mM KCl in isolated small bronchioles obtained 24 h after repeated challenge of sensitized BN rats with saline (open circles, open bars) or aerosolized 1% OVA (open squares, solid bars). Vertical bars and data points indicate mean $\pm$ SE from duplicate preparations from five separate animals in each treatment group. *** $P < 0.01$ compared with the absence of jasplakinolide by two-way ANOVA. † $P < 0.05$, †† $P < 0.01$ compared with saline by two-way ANOVA.

stiffness and/or CSK remodeling rates may be reflected in the overall composition and/or arrangement of the CSK, thereby affecting smooth muscle optimal force generation during shortening and contraction maintenance, and in turn being of relevance for mechanisms underlying AHR in asthma (11, 27).

In keeping with the importance of actin CSK dynamics modulating AHR *in vivo*, a recent study comparing the biophysical properties of ASM cells isolated from the relatively hyporesponsive Lewis rat strain with the intrinsically hyperresponsive Fisher rat showed greater CSK stiffening, bigger contractile forces, and faster CSK remodeling, the latter being a function of increased cellular ATP content (10). Further, as pointed in the article by An and colleagues (10), while Fisher rats have more ASM in their airways (28) and show a greater capacity to proliferate in cell culture (29), consistent with properties of ASM cells cultured from individuals with asthma (30, 31), an increase in ASM content and contractility would be expected to predispose toward AHR (7, 28, 32). However, under such co-varying conditions of increased muscle content and contractility, an ASM cell in a proliferative or synthetic state might be less contractile than its neighbor existing in a differentiated and fully contractile state. Such a situation would be compensatory but no published mechanical data are available to support this possibility. However, we and others (14, 17) have shown that repeated allergen exposure induces a modest (~ 1.5 -fold) increase in bronchiolar smooth muscle content together with a reduction in contractile protein expression (17), features that are consistent with modulation of ASM phenotype toward a proliferative/synthetic state (8, 9). However, we now demonstrate that mechanically these bronchiolar preparations from allergen-challenged animals are in a state of enhanced isometric force generation in which baseline levels of actin polymerization were found to be approximately doubled. When viewed in isolation these changes appear subtle. However, when considered in the context of increased muscle content and airway wall thickening resulting from tissue inflammation and remodeling, modest changes in muscle cell contractility interacting with these and other variables may result in more summative increases in airway reactivity, but this requires further investigation.

It is unclear why the proportion of F-actin was elevated in bronchioles but not in trachealis from OVA-exposed rats. Possible explanations include preferential deposition of OVA in small airways as a result of OVA inhalation via the nose resulting in the scrubbing of larger particles in the aerosol to permit a greater proportion of small particles to reach more distal airways. Additional factors might include heterogeneity in the capacity of ASM between large and small airways to respond to OVA, or the release of different proinflammatory mediators at varying levels of the tracheo-bronchial tree (33). Whichever the explanation, the bronchiole-selective increase in F-actin agrees with our previous observation in which the content of multiple contractile proteins but not nonmuscle proteins was reduced after OVA exposure in bronchioles but not in trachealis (17).

In summary, repeated OVA exposure of adult BN rats resulted in increased maximal tension development of bronchiolar smooth muscle preparations *ex vivo*, decreased the abundance of smooth muscle contractile proteins but increased baseline levels of F-actin polymerization. Consistent with induction of F-actin after OVA challenge, increases in maximum tension development to carbachol or KCl in small bronchioles from OVA-challenged animals were abrogated by disruption of the actin cytoskeleton, whereas stabilization of F-actin potentiated agonist induced contractions in these preparations. We conclude that the alterations composition and/or arrangement of the contractile apparatus after OVA exposure confer enhanced contractile responses, possibly as a result of the increased F-actin:

G-actin content. Such a mechanism may contribute in part to the AHR found in allergic asthma.

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