Peripheral Lung Inflammation and Enhanced Narrowing of Central Airways Combine Synergistically To Produce Extreme Hyperresponsiveness in Mice

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Rationale: Airway hyperresponsiveness in the acutely allergically inflamed mouse can be attributed to a thickened airway mucosa and an enhanced propensity for airway closure (J Appl Physiol 96:2019). While this may conceivably be a reasonable model of chronic allergic asthma, it likely does not reflect all the events relevant to the acute asthma exacerbation, which is known to involve central airway narrowing. We therefore hypothesized that central airway narrowing superimposed on a background of peripheral lung inflammation might lead to the severe compromise of lung function seen during an acute asthma attack. *Methods and Results*: We developed an anatomically accurate model of the mouse lung in which the diameter of each airway could be separately specified. The model also incorporated a mechanism for closing any airway that narrowed to a given critical radius. We found that thickening the airway mucosa by 20 microns (to simulate chronic inflammation) or doubling the degree of airway narrowing (to simulate excess smooth muscle shortening) each caused lung responsiveness to increase by *only* 2-3 fold. However, when both mucosal thickening and increased airway narrowing were incorporated into the model simultaneously, the responsiveness increased to about 10 times baseline. When these two mechanisms were combined *in vivo* by treating allergically inflamed mice with a tracheal instillation of Poly-L-lysine, all animals started to react to saline and died following exposure to the lowest dose of methacholine aerosol. *Conclusions*: When moderate degrees of allergic inflammation and increased airway narrowing are *both* present, they act synergistically to produce a catastrophic degradation in lung function. We speculate that this animal system may be a useful model of the acute asthma exacerbation.

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Effects of Electroporation on Lung Mechanics of Naive and Ovalbumin-Sensitized Mice

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Electroporation (EP) has been increasingly used *in vivo* as a method for non-viral DNA transfer to tissues and whole organs, including the lung. Electroporation causes no tissue injury or inflammatory response, but the effects of EP on airway and lung parenchymal mechanics are not known. We analyzed resting and methacholine-stimulated airway and parenchymal mechanics in naïve and ovalbumin (OVA)-sensitized mice. Female Balb/c mice were used for analysis. Plasmid DNA (100 μg, 50 μl) was delivered intratracheally, after which mice were electroporated transthoracically with 8 square-wave, 10 msec pulses at 200 V/cm field strength. Naïve, Tris-EDTA (TE)- and DNA-treated (no EP) or EP-only (no DNA) mice served as controls. Similar groups of OVA-sensitized animals were analyzed. Forty-eight hours after this procedure, animals were anesthetized, and their lung mechanics were studied using the Flexivent system. Using the forced oscillation technique, the Newtonian component of resistance (Rn), coefficients of tissue damping (G) and elastance (H), and hysteresivity (η) were measured at baseline and in response to nebulized methacholine (0.5 – 64 mg/ml). Concentrations of methacholine that resulted in 100% increase in Rn (PC_{100}Rn) were also calculated. There were no differences in Rn, G, H, or η between the untreated, DNA- or TE-treated, EP-only (no DNA), and DNA-treated and electroporated animals. As expected, ovalbumin-sensitized animals had higher baseline and methacholine-stimulated Rn, G, H at low methacholine concentrations (1-8 mg/ml), and significantly lower PC_{100}Rn ($p<0.05$). We conclude that electroporation does not affect resting and methacholine-stimulated lung mechanics of naïve and OVA-sensitized mice, which will allow for using the OVA model in our studies of gene delivery to the airways.

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Adenosine-Induced Airways Obstruction in Allergic Guinea Pigs

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We have previously reported that adenosine induces bronchoconstriction in an allergic guinea pig model (Rudman et al., 2004 AJRCCM, 169 (7), A188). This is consistent with clinical findings that inhaled adenosine induces bronchoconstriction in asthmatic but not healthy subjects (Cushley et al., 1983 Br J Clin Pharmacol 15:161-5.). The aim of the present study was to further investigate the mechanisms involved in adenosine-induced airways obstruction in allergic (passively sensitised) guinea pigs. *Method:* Male Dunkin Hartley guinea pigs were passively sensitised against ovalbumin. On day 7-10, animals were anaesthetised (1.5g/kg urethane) and mechanically ventilated (4ml/kg; 60 breaths/min). The increase in airways resistance above baseline (RL) was measured in response to various adenosine agonists. Results are expressed as mean ± s.e.m., n=4-6. *Results:* There was a significant increase in RL following aerosol administration of adenosine 5' monophosphate (AMP; 10mg/ml; 20 sec) and adenosine A1 agonist N6-cyclopentyl adenosine (CPA; 10mg/ml; 20 sec) compared with naive animals. The adenosine A2a receptor agonist CGS21680 (10mg/ml; 20 sec) and A3 receptor agonist IB-MECA (10mg/ml; 20 sec) failed to induce bronchoconstriction. The A1 receptor antagonist DPCPX (1mg/kg) as well as the 5-HT receptor antagonist ketanserin (1mg/kg) inhibited bronchoconstrictor response to AMP and CPA. However, the histamine H1 receptor antagonist mepyramine (1mg/kg) had no effect on AMP- or CPA-induced bronchoconstriction. *Conclusion:* These results suggest that adenosine acts via a A1 receptor dependent mechanism to induce airways obstruction that is independent of histamine release from mast cells although 5-HT receptors appear to be involved.

% Increase in RL

Treatment	AMP	CPA
Control	22.4 ± 1.1	29.1 ± 2.2
DPCPX	7.0 ± 2.1 *	8.1 ± 1.1 *
Mepyramine	26.8 ± 2.6	23.8 ± 5.4
Ketanserin	12.4 ± 1.3 *	11.4 ± 2.3 *

* $P<0.05$ versus control.

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Effects of Cytokines In Vitro on Mechanical and Bioelectric Responses to Methacholine (MCh) and Hyperosmolarity in Guinea-Pig Isolated, Perfused Trachea (IPT)

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Proinflammatory cytokines are involved in the development of lipopolysaccharide (LPS)-induced airway inflammation. Earlier we observed using IPT preparations that LPS (4 mg/kg, 18 h, i.p) increased the basal transepithelial potential difference (V_t), the hyperpolarizing response to methacholine (MCh), and the epithelium-derived relaxing factor (EpDRF)-mediated relaxation response to hyperosmolar solution, i.e., to D-mannitol (D-M). To identify the roles of cytokines in the LPS-induced changes, unmounted tracheas were incubated for 6 h with interleukin-1β (IL-1β, 10 ng/ml), interferon-γ (IFN-γ, 100 ng/ml) and tumor necrosis factor-α (TNF-α, 100 ng/ml) alone or in combination. IL-1β and IFN-γ reduced MCh-induced contractile responses and potentiated D-M-induced relaxation responses. However, TNF-α incubation enhanced the MCh contractions but had no effect on relaxations to D-M. When the cytokines were incubated together (cytomix) they had no effect on the contractile responses, but potentiated the D-M induced relaxations. MCh V_t concentration-response curves were biphasic. All the cytokines decreased the hyperpolarization responses at low MCh concentrations and increased the depolarization responses in higher concentrations. The individual cytokines had no effect on V_t responses to D-M. On the other hand, cytomix did not affect the V_t changes during MCh addition, but potentiated both the hyperpolarization and depolarization responses to D-M. The results indicate that incubation with cytokines affects airway smooth muscle reactivity to MCh and potentiates EpDRF-mediated relaxant responses to D-M. Thus, in the absence of inflammatory cells, the cytokines mimic the effect of LPS injection on D-M-induced relaxation, but do not recapitulate LPS' effects on V_t responses.

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Effect of Sildenafil and Ordonofil on Tracheal Muscle Relaxation Compared to That of Theophylline

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Introduction: Sildenafil (SILD) and Ordonfil (ORD) previously JPM8, are specific PDE 5 inhibitors, have been developed for erectile dysfunction. They accelerate NO-induced accumulation of cGMP leading to relaxation of corpus cavernosum during penile erection [1]. Since cGMP was demonstrated to produce 50 % relaxation in carbachol contracted tracheal muscle in guinea pig [2], we therefore tested the hypothesis that ORD and SILD could enhance tracheal relaxation, which could be of clinical significance for asthma. We compared their effect to that of Theophylline (non-specific PDE inhibitor) (THEO). **Methods:** Tracheae were excised from New Zealand rabbits (2-2.5kg) and cut spirally then into sections (4cm) at the middle of the C shaped cartilages. Each section contained two membranous parts of the trachea that contain smooth muscle. After 60 min of equilibration, the trachea was stimulated with carbachol (0.3 μM) twice and concentrations of the drugs (2.5 – 15 μM) were added individually at the peak of contraction. The induced relaxation was recorded for 30 min and calculated as % of control, see ref [3] for details. **Results:** While ORD had significantly greater effect than SILD, both induced significantly greater relaxation than THEO (61±22, 43±21, 33±16 % respectively at 15μM, Mean ± SD, n=14-18, $P<0.03$). The time course for the drugs over 30 min were different, ORD and SILD showed straight lines but steeper for ORD and parabolic for THEO. The presence Nitroprusside at 400 nM (NO donor) didn't alter the relaxation effect of any of the drugs. **Conclusion:** ORD and SILD produce significant relaxation in rabbit tracheal muscle, which seems to be independent of NO

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Tracheal Smooth Muscle Responses in a Murine Model of Respiratory Syncytial Virus

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Rationale: Studies in cotton rats and ferrets infected with respiratory syncytial virus (RSV) have shown increased responses in isolated tracheal smooth muscle (TSM) segments to electrical field stimulation (EFS) during the acute phase of infection (Larsen and Colasurdo (1999), J Pediatrics 135:21-27). Using *in vivo* techniques we have recently shown that adult (8 weeks old) and juvenile (3 weeks old) BALB/c mice infected with RSV have significantly increased responsiveness to methacholine (MCh) 7 days post infection. This study investigated whether the altered responsiveness seen *in vivo* could be attributed to altered responsiveness of the TSM. Methods: We examined the responsiveness of isolated TSM segments to EFS and MCh in BALB/c mice 7 days post infection with RSV. Adult and juvenile mice were infected with intranasal RSV or media control (MC). 7 days later TSM segments were assessed *in vitro* for responses to EFS (0.5 to 40 Hz) and MCh (10^{-8} M to 3×10^{-5} M). Results: RSV caused an increase in responsiveness to MCh (EC_{50}: $2.48 \pm 0.15 \times 10^{-7}$ M (RSV) vs $4.57 \pm 1.4 \times 10^{-7}$ M (MC)), but not EFS, in adult mice. Juvenile mice showed no heightened responses to either stimuli. Conclusions: The increased responsiveness to MCh in adult mice correlates to changes seen *in vivo*. In contrast, the lack of correlation in juvenile mice suggests an age effect and highlights the need for further investigation into the alterations in lung physiology associated with viral infection in mice.

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