447.5

QUANTITATION OF PHOSPHOLIPIDS AND LYSOPHOSPHOLIPIDS IN PULMONARY SURFACTANT USING HPLC/ELSD

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Hydrolysis of surfactant by secretory A_2 phospholipases ($sPLA_2$'s) is an important mechanism in inflammatory lung diseases. Accurate and sensitive methods for quantitation of phospholipids (PL's)and lysophospholipids (LPL's), the product of $sPLA_2$-mediated surfactant hydrolysis has been limited. One and two dimensional TLC requires large sample sizes ($\approx$ 350 nmoles), time and overlap of key compounds. Mass spectrometry is expensive. High performance liquid chromatography (HPLC) methods with ultraviolet detection are insensitive toward highly saturated compounds, such as pulmonary surfactant. Detection using evaporative light scattering device (ELSD) utilizes visible light scattering of particles vaporized from the effluent of the HPLC column. It is very sensitive ($\leq$ 1nmole/each PL), and does not depend on the presence of unsaturated compounds. We developed a method combining HPLC and ELSD to measure all major PL's and LPL's in pulmonary surfactant samples. Standard PL and LPL were used to develop binomial curves for accurate quantitation of unknown surfactant mixtures (all $R^2 \geq 0.995$). Phosphatidylbutanol was used as an internal standard. Analysis of animal and human surfactant samples with or without treatment by $sPLA_2$ has confirmed the method's speed, reproducibility and sensitivity (total sample size $\geq$ 12 nmoles). We conclude that this method should markedly improve the ability to measure PL's and LPL's in samples from small animal models of $sPLA_2$-mediated lung injury as well as alveolar and airway samples from humans with various inflammatory lung diseases.

447.6

LUNG SURFACTANT DECREASES IL-1β AND TNF-α PRODUCTION AT THE POST-TRANSCRIPTIONAL LEVEL IN LPS-STIMULATED ALVEOLAR MACROPHAGES

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We have shown previously that lung surfactant inhibits nitric oxide production at the post-transcriptional level in lipopolysaccharide(LPS)-stimulated rat alveolar macrophages (Miles et al., Am.J.Physiol. 276:L186, 1999). In this study, we examined the effect of lung surfactant (200 μg phospholipid/ml), isolated from rat lung lavage, on the production of two pro-inflammatory cytokines, IL-1β and TNF-α, by LPS-stimulated rat alveolar macrophages. After 22 h incubation with LPS plus lung surfactant, IL-1β and TNF-α production was reduced by 77% and 95%, respectively. mRNA levels were measured by Northern blot analysis, performed 4 hours after LPS-stimulation using digoxigenin-labeled probes. There was no difference in IL-1β or TNF-α mRNA levels between cells stimulated with LPS with or without lung surfactant. Nitric oxide, IL-1β and TNF-α are known to be regulated at both the transcriptional and post-transcriptional levels. It is interesting that lung surfactant inhibits the production of all three pro-inflammatory molecules at the post-transcriptional level. This suggests that the post-transcriptional regulation of these three molecules may share a similar mechanism.

447.7

NITRIC OXIDE STIMULATES PULMONARY SURFACTANT SECRETION FROM ISOLATED PERFUSED RAT LUNG VIA THE CGMP/PROTEIN KINASE G PATHWAY.

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Lung surfactant is secreted from epithelial type II cells into alveolar air space in response to airborn and circulating stimuli. Nitric oxide (NO) can be generated by constitutive and induced nitric oxide synthases (cNOS and iNOS) in pulmonary endothelial and epithelial cells. We therefore examined the effects of nitric oxide on lung surfactant secretion in an isolated perfused rat lung model. Infusion of L-NAME (100 μM), an inhibitor of cNOS and iNOS, via pulmonary circulation for 90 minutes resulted in a decrease of lung surfactant secretion (1.55 ± 0.15 % in control vs 0.79 ± 0.16% in L-NAME-treated, $P<0.05$). However, aminoguanide, an inhibitor of iNOS, had no effect, indicating that the decline of lung surfactant secretion is due to the specific blockage of cNOS rather than iNOS activity in the perfused lungs. A reduction of cGMP level by ODQ (25 μM), a specific inhibitor of guanylyl cyclase inhibited surfactant secretion by 54%. Furthermore, KT5823 (1 μM), an inhibitor of protein kinase G depressed surfactant secretion by 40%. Our results suggest that physiological concentrations of NO are required for lung surfactant secretion and NO-mediated secretion is at least partly via a rise of cGMP level and activation of protein kinase G (supported by grants from NIH HL 52146 and the UNC Institute of Nutrition).

447.8

Role of Acidic Ca^{++}-Independent Phospholipase A_2(aiPLA$_2$) Enzyme in Uptake and Degradation of Lung Dipalmitoyl Phosphatidylcholine.

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Lung tissue has been shown to contain acidic (pH 4 max) calcium-independent phospholipase A_2(aiPLA$_2$) activity localized to lysosomes and lamellar bodies. A transition state phospholipid analogue inhibitor, MJ33, significantly inhibits aiPLA$_2$ activity of lung homogenate (2.39± 0.1 vs 15.33 ± 0.2 nmol/hr/mg prot.). Using MJ33 as a probe we studied the uptake and degradation of DPPC by isolated perfused rat lung. Unilamellar liposomes (0.5 ^{3}H DPPC, 0.25 PC, 0.15 cholesterol, 0.1 PG, mol/mol) ± 3mol% MJ33 were prepared by extrusion. Liposomes (0.1μmol DPPC) were instilled into the trachea of an anesthetized rat. Lungs were removed, continuously ventilated and perfused via the pulmonary artery ± 0.1mM 8-Br cAMP for 5min, 0.5,1,2,and 3 hrs. DPPC content of lung lavage fluid was 867 ± 5 nmol in control lungs and was unchanged by MJ33 or 8-Br cAMP. Uptake of DPPC was defined as post- lavage accumulation of radioactivity in the lung. Degradation of DPPC was calculated from the sum of dpm in aqueous, lyso PC, and unsaturated PC measured in homogenate, lavage fluid and perfusate. Uptake and degradation were linear for 3 hrs under all conditions. DPPC uptake, 110 nmol/hr in control and 180nmol/hr with 8-Br cAMP, was unchanged with MJ33. Degradation in control and 8-Br cAMP lungs was 58 and 102 nmol/hr,respectively, representing 54 and 57% of uptake. MJ33 inhibited the rate of degradation by 63 to 65%.These studies demonstrate that lung degradation exceeds 50% of internalized alveolar surfactant phospholipid and indicates a role for aiPLA$_2$ in the process. (Supported by HL19737)

LUNG AIRWAY REACTIVITY (448.1-448.2)

448.1

20-HETE INDUCED RELAXATION OF RABBIT BRONCHIAL RINGS IS MEDIATED BY CAMP/PKA DEPENDENT MECHANISMS.

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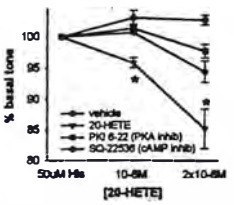
We have reported that the CYP4A product 20-HETE (hydroxyeicosatrienoic acid) relaxes rabbit bronchial rings in a cyclooxygenase- and epithelial-dependent manner, but little else is known about the factors which mediate bronchorelaxation. Airway relaxation to many physiologic and pharmacologic agents is mediated largely by cAMP/PKA (cyclic AMP/Protein Kinase A) dependent mechanisms; therefore, our studies were undertaken to examine the contribution of these pathways to 20-HETE- induced bronchial relaxation. Rabbit bronchial rings (2-3 mm diameter) pretreated with vehicle, the PKA inhibitor PKI-6-22 (1 μM), or the cAMP inhibitor SQ22536 (100 μM) were constricted with 50 μM histamine. Relaxation to 20-HETE was blunted by inhibition of cAMP or PKA. These data suggest that 20-HETE- induced broncho-relaxation is mediated at least in part by second messenger systems cAMP and PKA. Grant: HL 49294, ERJ.



448.2

Exacerbation of airway hyperresponsiveness and lung fibrosis by *in vivo* IL-18 neutralization in a chronic model of Aspergillus fumigatus-induced allergic airway disease

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Hyper-sensitivity responses to *A. Fumigatus*, commonly referred to as allergic bronchopulmonary aspergillosis, can be characterized by asthma-like responses such as elevated IgE levels, eosinophilia, mucus hyper-secretion and pulmonary fibrosis. IL-18, a cytokine that directs Th1 type responses by increasing IFN-γ production and preventing IgE isotype switching, may be an important early mediator in the host response to conidia. Culture of airway epithelial cells *in vitro* with *A. fumigatus* conidia (16h) caused a significant increase in IL-18 release. *In vivo* neutralization of IL-18, 1h prior and 3 days post conidia challenge, in mice previously sensitized to *A. fumigatus* caused a significant increase in eosinophil numbers in the BAL and exacerbated airway hyperresponsiveness to methacholine 3 days post conidia an effect that was still evident at 30 days post conidia compared to control serum treated animals. Histological analysis at day 30 displayed evidence of increased mucus secretion, collagen deposition and fibroblast proliferation in the anti-IL-18 treated group compared to controls. Furthermore, persistence of conidia was observed in granuloma formations present in the anti-IL-18 treated animals but not control groups. These results indicate that IL-18 plays a key role in the inflammatory response to inhaled conidia in *A. fumigatus* sensitized animals. This abstract is funded by the American Lung Association.

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