differences in phospholipase C in the smooth muscle of airways in the various treatment groups.

172.5

Prostanoids Released by Activated Alveolar Macrophages Sensitize Acetylcholine Increased Short Circuit Current in Swine Tracheal Submucosal Gland Cells

Huiling Liu, Jerry M Farley. Pharmacology & Toxicology, University of Mississippi Medical Center, 2500 N State St, Jackson, MS 39216

To study the effect of mediators released by alveolar macrophages (**AM**) on the submucosal gland cell (**SGC**) secretion, supernatant from Zymosan A (0.1mg/ml) activated **AM**(**SZAM**) or PGE_2 was applied to the serosal side of confluent monolayers of **SGC** in Ussing chambers. **SZAM** at 0.1% ~ 10% dilutions (n=5), and PGE_2 dose-dependently increased **SGC** short circuit current (ΔI_{SC}), with estimated action for 100% **SZAM** equivalent to that of 100nM PGE_2, the EC50 for PGE_2 induced ΔI_{SC} dose response was 15.4 ± 0.3nM (n=4). The increased ΔI_{SC} was abolished by 1mM diphenylamine-2-carboxylate but not by 100μM 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid, indicating the involvement of cAMP activated CFTR Cl^- channel. Immunoassay detected a 20 fold increase in PGE_2 in the **SZAM** (195 ± 28ng/ml, n=3) compare with that in the supernatant from untreated **AM**(**Control**). Pretreatment of **SGC** with **SZAM** or PGE_2 sensitized acetylcholine (**ACh**) induced increases in ΔI_{SC}, 10% dilution of **SZAM** or 100nM PGE_2 shifted the EC50 for ACh induced ΔI_{SC} response from 715nM to 133nM (n=12, 10 respectively). Similarly 5uM forskolin (n=4) shifted the ACh EC50 to 93nM. However, **Control** supernatant had no effect on ΔI_{SC} and did not shift the ACh induced ΔI_{SC} dose response in **SGC**. These results suggest that during early airway inflammation triggered by inhaled particulates or pathogens, prostanoids secreted by activated **AM** may mediate increases in airway secretion by PGE_2 production and increasing cAMP in **SGC**.

172.6

Mechanism of hyperoxic cell injury: evidence for mitochondrial respiration as the main target

Jian Li[1], Xueshan Gao[2], Mingwei Qian[2], John W Eaton[2]. 1Pharmacology and Toxicology, University of Louisville, 580 S. Preston St, Baxter II Res. Bldg., #220, Louisville, KY 40202, 2Medicine, University of Louisville, Louisville, KY

Exposure of mammals to hyperoxia causes pulmonary and ocular pathology. Hyperoxic damage and cell death may derive from enhanced intracellular formation of reactive oxygen species (ROS), probably of mitochondrial origin. There is, however, controversy on this point. When wild-type and respiration-deficient (ρ^o) HeLa cells were cultured in 80% O_2, wild-type cells stopped growing after 5 days and died thereafter whereas ρ^ocells survived and grew to confluence. This tolerance of ρ^o cells to high oxygen was not associated with greater resistance to oxidants such as hydrogen peroxide and t-butyl hydroperoxide. Under both 20% and 80% O_2, ρ^o cells exhibited substantially decreased ROS production and, under 80% O_2, ρ^o cells showed no suppression of aconitase activity or mitochondrial protein carbonyl formation. Replacement of normal mitochondria in ρ^o cells restored ROS production and susceptibility to hyperoxia. Two other strategies which diminish mitochondrial ROS generation also increased tolerance to hyperoxia. HeLa cells constantly exposed to the protonophoric uncoupler, carbonyl cyanide m-chlorophenylhydrazone (which enhances respiration but decreases ROS production), showed preferential survival under 80% O_2 as did HeLa cells in the presence of chloramphenicol (which suppresses both respiration and mitochondrial ROS production). We conclude that interactions between respiring mitochondria and O_2 are primarily responsible for hyperoxic cell damage.

172.7

Tracheoplegia induced by hypothermia and a cardioplegic solution

Hugo Galera-Ruiz, J. Garcia-Palomeque, M. Serrano-Molina, F. Munoz-Borge, E. Berrocal-Postigo, J. Serrano-Molina. Clinical Pharmacology Unit, Pharmacology, Pediatrics & Radiology Dept., University of Seville School of Medicine, Viren Macarena University Hospital, Seville, 41000 Spain

The present study intended to evaluate a method of tracheal preservation to be used in orthotopic transplantation . Hypothermia and legisol solution were combined as means of preserving an adequate histological condition of the excised trachea. The effect of preservation was primarily to be assessed in the capacity for maintaining cellular integrity of the mucosal epithelium and for avoiding tracheal stenosis. In the experimental groups we used plegisol solution with the following composition (mg/dl): calcium cloride 17.6, magnesium chloride hexahydrate 325.3; potassium chloride 119.3; sodium chloride 643. The osmorality of the cryopreservant solution was 304 mosm/ml and its mean pH was 3.8 (3.5-3.9). Tracheas were obtained from Wistar rats and assigned to the following groups. I.Control (n=25), normal tracheas were mantained in formol for the histological study .II Experimental unpreserved tracheas(n=25) were maintained during 3 h at 37 ° C; III. Experimental preserved (n=25), the same setting in plegisol solution. The histological study of the organs showed that no damage was produced in the mucosal epithelium nor tracheal stenosis was induced under the experimental conditions described. This may be taken as a preliminary indication that the method might be adequate for cryopreservation in tracheal transplants after its validation in human tracheas.

172.8

Role of CD38/cADPR signaling in muscarinic activation of airway smooth muscle cells

Deepak A Deshpande1, Soner Dogan[1], Mathur S Kannan[1], Timothy F Walseth[2]. [1]Veterinary Pathobiology, University of Minnesota, 1971 Commonwealth Avenue, Saint Paul, MN 55108, [2]Pharmacology, University of Minnesota, Minneapolis, MN

Cyclic-ADP-ribose (cADPR), a β-NAD metabolite, mobilizes calcium from intracellular stores in airway smooth muscle (ASM). We determined the second messenger role of CD38/cADPR pathway in the muscarinic activation of ASM cells. Pig ASM strips were stimulated with 1 μM acetylcholine (Ach) for 30, 60 and 120 seconds and cADPR levels and ADP-ribosyl cyclase activities were determined. ADP-ribosyl cyclase activity and cADPR levels were significantly higher after 60 seconds of Ach stimulation compared to controls, demonstrating the activation of CD38/cADPR pathway. Pretreatment of ASM strips with methoctramine, a M2 muscarinic receptor antagonist, attenuated Ach-induced ADP-ribosyl cyclase activation at 60 and 120 seconds compared to control ASM strips, demonstrating that the CD38/cADPR signaling is coupled to M_2 muscarinic receptors. The mechanism of CD38 activation upon Ach stimulation is not understood. Treatment of ASM strips with 50 mM sodium fluoride, a non-specific phosphatase inhibitor, for 30 minutes before and during Ach stimulation resulted in higher ADP-ribosyl cyclase activity compared to ASM strips treated with vehicle, suggesting phosphorylation of CD38 as a potential mechanism of activation. In addition, Ach-induced contractions of ASM strips were attenuated by a cADPR antagonist. Together these findings suggest a second messenger role for CD38/cADPR pathway in muscarinic activation of ASM cells. (Supported by NIH and Univ.of MN to TFW and MSK).

172.9

Effects of protein kinase (PK) inhibitors on bioelectric and mechanical responses of guinea-pig isolated, perfused trachea (PT) to hyperosmolar (HO) D-mannitol (D-M)

Y. Jing[1], M. R. Van Scott[2], J. S. Fedan[3]. [1]Department of Biochemistry and Molecular Pharmacology, West Virginia University, Robert C. Byrd Medical Center, Morgantown, WV 26506, [2]Department of Physiology, East Carolina University, Greenville, NC, [3]PPRB, HELD, National Institute for Occupational Safety and Health, Morgantown, WV

Exercise causes evaporative water loss and hyperosmolarity in airway surface liquid. Mucosal application of HO D-M to the PT elicits transepithelial potential difference (V_t) changes and epithelium-dependent airway smooth muscle (SM) relaxation mediated by epithelium-derived relaxing factor. We examined the possible roles of PKs, which are reported to have regulatory effects on epithelial ion transport, on 30 mosM D-M-induced V_t and relaxation responses. The PK inhibitors applied mucosally were: chelerythrine (PKC inhibitor), LY 294002 (PI-3-K inhibitor), KN-62 (CaM-K-II inhibitor) and ML-7 (MLCK inhibitor). All PK inhibitors caused depolarization upon application. Chelerythrine inhibited methacholine (MCh)- and D-M-induced hyperpolarization responses; it also caused SM contraction. LY 294002 did not affect V_t responses to MCh or D-M, but inhibited MCh-induced SM contraction. KN-62 and ML-7 had no effect on the

bioelectric and mechanical responses to MCh or D-M. The phosphatase inhibitor, Na_3VO_4, caused hyperpolarization and inhibited D-M-induced hyperpolarization and MCh-induced contraction responses. None of the tested inhibitors had any effect on D-M-induced SM relaxation response. The results indicate that a common PK signaling pathway is not involved in both airway bioelectric and mechanical responses to HO challenge. Funded by NIOSH.

172.10

Opposite regulation of epidermal growth factor receptors by lysophosphatidic acid and isoproterenol

Karen M. Kassel, Aaron D. Lanik, Bryan L. Danforth, Myron L. Toews. Department of Pharmacology, University of Nebraska Medical Center, 985800 Nebraska Medical Center, Omaha, NE 68198-5800

We have reported previously that the G protein-coupled receptor mitogen lysophosphatidic acid (LPA) stimulates proliferation of human airway smooth muscle (HASM) cells synergistically with the receptor tyrosine kinase mitogen epidermal growth factor (EGF) and that LPA up-regulates expression of the EGF receptor (EGFR) [Toews et al., Biochim. Biophys. Acta 1582, 240-250, 2002]. Because the beta-adrenergic receptor agonist isoproterenol (ISO) inhibits HASM cell proliferation, the opposite effect of LPA, we tested the hypothesis that ISO would also act opposite of LPA to inhibit EGFR binding. Pretreatment of HASM cells for 18 hr with 10 μM ISO decreased binding of ^{125}I-EGF to intact cells on ice by approximately 50%. The decrease in binding induced by ISO was apparent by 1-2 hr and maximal by 4-6 hr, markedly faster than the EGFR up-regulation by LPA, which began only after an 8-hr lag. The adenylyl cyclase activator forskolin also inhibited HASM cell proliferation and decreased EGFR binding, suggesting cyclic AMP as a likely mediator of the ISO effects. The effects of LPA and ISO on EGFRs were also opposite from each other in HFL-1 human fetal lung fibroblasts, another airway mesenchymal cell type, with LPA causing a 3-fold increase and ISO causing a 50% decrease in binding. These results demonstrate the ability of G protein-coupled receptors to modulate EGFRs bi-directionally in airway mesenchymal cells, a mechanism that may contribute to their opposite effects on proliferation of these cells. *Supported by University of Nebraska Medical Center funds.*

DRUG METABOLISM: ENZYME REGULATION (173.1-173.7)

173.1

Induction of detoxifying enzymes by garlic organosulfur compounds is mediated by Nrf2, chemical structure and stress signals

Chi Chen, Ah-Ng Tony Kong. Pharmaceutics, Rutgers University, 160 Frelinghuysen Road, Piscataway, NJ 08854

Garlic organosulfur compounds (OSCs) have been recognized as potential chemopreventive agents. It is known that garlic OSCs can differentially affect drug metabolizing system, but the mechanism behind their inducing effect on detoxifying enzymes still remained largely unknown. In this study, influences of three major garlic OSCs, DAS, DADS and DATS, on the transcriptional level of NQO1 and HO1, the activity of antioxidant response element (ARE)-mediated reporter, and the level of Nrf2 protein, were measured. The activation of ARE and the accumulation of Nrf2 protein were well correlated with the induction of phase II genes. The structure-activity relationship (SAR) study indicated that the third sulfur in the structure of OSCs had major contribution to the bioactivity, and allyl-containing OSCs were more potent than propyl-containing OSCs. DATS-induced ARE activity was blocked by the transfections of dominant-negative Nrf2 (Nrf2-DN) and Keap1, and the co-treatments of thiol antioxidants or epoxide hydrolase decreased the ARE activity and Nrf2 protein level induced by DATS. Three major MAPKs, were activated by DATS treatment, but the inhibition of MAPKs did not decrease DATS-induced ARE activity. Inhibition of upstream protein kinases showed that DATS-induced ARE activity was decreased by the pretreatment of staurosporine, but not by specific PKC inhibitor, and calcium-dependent signaling pathway might contribute to DATS-induced cytoprotective effect.

173.2

Lithocholic acid (LCA) inhibits human cholesterol 7a hydroxylase gene transcription via Pregnane X receptor (PXR) mediated pathway

tiangang li, John Chiang. Biochemistry and Molecular Pathology, Northeastern Ohio Universities College of Medicine, 4209 state route 44, rootstown, ohio 44272

Cholesterol 7a hydroxylase (CYP7A1) is the rate-limiting enzyme in bile acids biosynthesis and is feedback suppressed by bile acids. Here, we demonstrate that human PXR can act as a LCA receptor to mediate bile acids feedback inhibition of CYP7A1 gene transcription. Transient transfection assays show PXR and Hepatocyte nuclear factor 4a (HNF4a) stimulate CYP7A1 promoter activity, and addition of a PXR agonist, LCA or rifampicin strongly inhibits CYP7A1 transcription. Electrophoretic mobility shift assay and mutagenesis analysis identified a functional PXR binding site in the bile acids response element-I of the CYP7A1 promoter. Mammalian two hybrid assays show that Peroxisome proliferators-activated receptor g (PPARg) coactivator-1a (PGC-1a) interacts with both HNF4a and PXR in the absence of a PXR ligand. PXR interacts with HNF4a and Small heterodimer partner (SHP) in a ligand -dependent manner. These results suggest that in the absence of a PXR ligand, PXR, HNF4a, PGC-1a and other coactivators may form a transcriptionaly activating complex and coordinately regulate CYP7A1 basal transcriptional activity. Ligand binding of PXR may lead to the disruption of this complex and result in inhibition of CYP7A1 gene transcription. (Supported by NIH grants DK58379 and DK44442).

173.3

Association of the 7520C>G single nucleotide mutation in the 3'-untranslated region of the *CYP2A13* gene with decreased transcript abundance in human lung

Xiuling Zhang1, Michele Caggana[1], Tracey L Cutler[2], Xinxin Ding[1]. [1]Wadsworth Center, New York State Department of Health, Empire State Plaza, Albany, New York 12201, [2]School of Public Health, State University of New York at Albany, Albany, New York

Genetic polymorphisms in the *CYP2A13* gene may be associated with interindividual differences in tobacco-related tumorigenesis risk. The aim of this study is to explore potential roles for selected SNPs in CYP2A13 expression in human lung. Expression levels of CYP2A13 mRNA in normal human lung displayed significant interindividual variation (approximately 50-fold). Preliminary sequence analysis of CYP2A13 RNA-PCR products suggested that the 7520C>G mutation, which is located in the 3'-untranslated region, may be associated with low transcript abundance. The CYP2A13 transcript derived from the 7520G mutant allele was detected in only five of 16 lung samples heterozygous for the 7520C>G mutation. Subsequent quantitative analysis, using a newly developed method for quantification of allele-specific gene expression, indicated that transcripts from the mutant allele were approximately 5-10-fold less abundant than transcripts from the wild-type allele. The frequencies of the 7520C>G variant allele in anonymous white, black, Hispanic, and Asian newborns from New York State are 5.2%, 26.8%, 17.7%, and 4.3%, respectively. It appears that the 7520C>G SNP may contribute to interindividual differences in CYP2A13 mRNA levels in the lung. Additional studies to address the mechanisms underlying the observed low transcript abundance of the 7520G mutant allele are underway. (Supported in part by NIH grant CA92596)

173.4

Microarray Analysis Of Hepatic Gene Expression In Liver-Specific NADPH-Cytochrome P450 Reductase (*Cpr*)-Knockout And Global *Cpr*-Knockdown Mouse Models

Yan Weng, Andrew A. Reilly, Xinxin Ding. Wadsworth Center, New York State Department of Health, Empire State Plaza, Box 509, Albany, NY 12201-0509

Our aim was to identify genetic bases for observed phenotypes in two mutant mouse models, *Cpr-low*, with globally decreased CPR expression, and *liver-Cpr-null*, with liver-specific deletion of the *Cpr* gene; the phenotypes include a reduced serum cholesterol and an induction of hepatic cytochrome P450s (CYP) in both strains, and hepatomegaly and fatty liver in the *liver-Cpr-null* mice. Microarray analysis of hepatic gene expression revealed that multiple enzymes in the cholesterol biosynthetic pathways were induced; however, their

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ABSTRACTS

PART I

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