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SHEAR STRESS STIMULATES PROSTACYCLIN PRODUCTION BY CULTURED PLEURAL MESOTHELIAL CELLS. N. DePaola, C.M. Waters, M.R. Glucksberg, and J.B. Grotberg. Depts. of Biomedical Engineering and Anesthesia, Northwestern University, Evanston, IL 60208.

Prostacyclin production has been recognized as a potentially important lung homeostatic mechanism. Mesothelial cells forming the pleural membranes of the lung are continuously subjected to shear forces during the respiratory cycle. We investigated the effects of fluid shear stress on prostacyclin production by cultured mesothelial cells. Although the precise role of prostaglandins in pleural mesothelial cells is yet to be determined, prostaglandin secretion may be involved in the normal and pathological control of pleural fluid fluxes. Rat visceral pleural mesothelial cells were cultured on glass coverslips and exposed to well-defined shear stress forces in a parallel plate flow chamber. Samples from the circulating medium were collected after 2, 5, 10, 20, and 30 minutes, and after 1, 2, 3, 6, 20, 22, and 26 hours of exposure to fluid shear stresses of 15 dynes/cm^2. Enzyme immunoassays were used to quantify the amount of 6-keto Prostaglandin $F_{1\alpha}$ (the stable metabolite of prostacyclin) present in the samples. The cumulative amount of 6-keto- $PGF_{1\alpha}$ in the circulating medium (pg/ml) showed a sharp increase in the production of prostacyclin within the first 30 minutes of flow; the production rate was approximately 4 pg of 6-keto- $PGF_{1\alpha}$ per 10^7 cells per minute. After 6 hours, it decreased by a factor of 10 to a constant rate of 0.47 pg per 10^7 cells per minute. The same number of cells under static conditions (controls) showed an average production rate of 0.13 pg per 10^7 cells per minute. No morphological alterations were observed on mesothelial monolayers exposed to 15 dynes/cm^2 for up to 26 hours. Continuous phase-contrast microscopy monitoring of the mesothelial monolayers indicated that mechanical injury was not the stimulus for the decline in prostacyclin production seen after few hours of flow. Our data indicate that fluid shear stresses may modulate arachidonic acid metabolism by pleural mesothelial cells. Supported by the Whitaker Foundation.

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EFFECT OF ENDOTOXIN ON CARDIOPULMONARY FUNCTION IN CALVES DEPLETED OF COPPER (Cu). T.T. Brown, Jr., G.P. Gengelbach, J.W. Spears, L.W. Johnson, P.W. Hellyer, J.R. Dodam and N.C. Olson. North Carolina State University, Raleigh, NC 27606.

We evaluated the acute cardiopulmonary response to an infusion of *Escherichia coli* endotoxin (8 µg/kg from 0-0.5 h IV) into cattle with normal and low plasma concentrations of Cu. Holstein bull calves (194±5.1 kg) were fed diets containing normal Cu (n=4) or low Cu (n=14) for ~6 months. The Cu deficient diet resulted in a significant (P<0.05) decrease in plasma Cu concentration (0.26±0.04 µg/ml versus 0.81±0.08 µg/ml in normal control). Calves were anesthetized with thiopental and maintained with halothane (1% end tidal concentration) in 100% O_2 while in sternal recumbency. Endotoxin caused a transient, early (at 20 min) increase in mean pulmonary arterial pressure to 114% and 147% of baseline in normal and Cu-depleted calves, respectively (P<0.05). In both groups of calves, mean pulmonary arterial pressure, mean aortic pressure, systemic vascular resistance, and peripheral neutrophil and lymphocyte counts decreased from 0.5-4 h. In normal and Cu-depleted calves, plasma levels of thromboxane (TX) B_2, prostaglandin (PG) $F_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and tumor necrosis factor-α increased at ~0.5-2 h, whereas blood gas tensions and hematocrit were unaffected. However, at 0.5 h in Cu-depleted calves, endotoxin significantly decreased heart rate and cardiac index (as compared to normal calves) while endotoxin-induced systemic hypotension and increased TXB_2 production were potentiated. We conclude that chronic dietary depletion of Cu potentiates TXB_2 biosynthesis and worsens the acute cardiovascular response to endotoxin challenge in cattle. Supported by NIH HL32726, UNC Institute of Nutrition, and the State of North Carolina.

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LACTIC DEHYDROGENASE (LDH) FROM LUNG LAVAGE: A POSSIBLE MARKER FOR VENTILATOR-INDUCED LUNG INJURY. R. Behnia, A Molteni, C.M. Waters, W.F. Ward, B.A. Shapiro, J. Taylor. Departments of Anesthesia, Radiology and Pathology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Studies in rats and mice have shown that positive pressure ventilation with hyperdistention of the lungs leads to microscopic lung injury. We used LDH as a marker for detection of lung injury following positive pressure ventilation with hyperdistention in rats. Eight Sprague Dawley rats were anesthetized with pentobarbital (40 mg/kg, PI.). Control rats (N=4) were spontaneously ventilated in room air. Study rats (N=4) were ventilated with a tidal volume of 25 ml/kg, respiratory rate of 66, and FiO_2 of 21%. A dead space tube was added to respiratory circuit to keep rats isocapnic. These ventilatory methods generated an airway plateau pressure of 40 cm H_2O. We observed a progressive increase in airway pressure indicating a decrease in lung compliance following hyperdistention. After seven hours, rats were sacrificed with pentobarbital overdose and lungs were lavaged with normal saline. LDH levels in lavage fluid in hyperdistended lungs were significantly higher than control lungs (79.3±23.9 IU/ml vs 47.0±4.6, means ±SD) and some red blood cells were found in the sediment of the lavage fluid. In addition, analysis of LDH for isoenzymes showed a marked peak five upon electrophoresis, the same peak evident in endothelial cells exposed to acute radiation injury. Analysis of lung and pleural lavages for endothelin - I were negative. LDH levels in bronchial lavage could be an early marker for ventilator-induced lung injury. Supported in part by the Whitaker Foundation and grant HL 25106 from NHLBI.

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MECHANISM OF PMA LUNG INJURY INVOLVES ADP-RIBOSYLATION. W.K. Adkins, T.M. Moore, and A.E. Taylor. Univ. South Alabama, Mobile, AL.

Recent studies have shown that TNF-mediated endothelial cytotoxicity involves ADP-ribosylation of the intracellular actin pool, resulting in actin filament disassembly and endothelial barrier dysfunction. The present study investigated the role of the actin cytoskeleton and ADP-ribosylation in a PMA-induced lung injury model. Isolated, blood perfused canine lung lobes were challenged with PMA after pretreatment with the ADP-ribosyltransferase inhibitors nicotinamide (NIC) and 3-aminobenzamide (ABA), the actin stabilizing agent phalloidin (PHAL), or vehicle alone (saline, ethanol). Endothelial injury was evaluated by measuring the capillary filtration coefficient (Kfc) in each lung under control conditions, after pretreatment with inhibitor, and after challenge with PMA. Kfc remained unchanged from baseline after pretreatment with vehicle alone, but then increased 4-fold after PMA challenge (0.124±0.011 to 0.495±0.124 ml/min/cmH_2O/100g). Pretreatment with NIC (100mM) and ABA (10mM) prevented the PMA-induced increase in Kfc (NIC 0.099±0.006 to 0.161±0.045 ml/min/cmH_2O/100g, ABA 0.103±0.027 to 0.299±0.126 ml/min/cmH_2O/100g). Kfc was unchanged after PMA challenge in lungs pretreated with Phalloidin (0.068±0.007 to 0.079±0.010 ml/min/cmH_2O/100g). These data demonstrate that PMA lung endothelial injury may be mediated by effecting the cell cytoskeleton through ADP-ribosylation of actin. (NIH HL22549)

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LUNG INJURY DUE TO *N*-METHYL-D-ASPARTATE (NMDA) GLUTAMATE RECEPTOR ACTIVATION: PREVENTION BY BENZAMIDE, AN INHIBITOR OF POLY(ADP-RIBOSE) POLYMERASE. H.I. Berisha, H. Pakbaz, and S.I. Said. VAMC, Northport, NY and University Medical Center, Stony Brook, NY, 11794-8172.

Poly(ADP-ribose) polymerase (PARP), a nuclear enzyme that catalyzes the synthesis of poly(ADP-ribose), is involved in cell differentiation, proliferation and repair. Its activation, by DNA damage, leads to depletion of cellular NAD and ATP, and eventual cell lysis. We have investigated the possible role of PARP stimulation in the production of lung injury induced by activation of NMDA glutamate receptors. The injury was produced by the addition of NMDA (1 mM), together with L-arginine (10 mM), in isolated, perfused and ventilated rat lungs. After 1 h perfusion the injury was evidenced by increases in peak airway pressure, pulmonary perfusion pressure, wet/dry lung weight ratio, and broncho-alveolar lavage protein concentration. Benzamide (1 mM), a specific inhibitor of PARP, added to the perfusate, prevented all signs of injury (n=4,<0.01). Benzamide also attenuated paraquat-induced injury of guinea pig lungs, and others found it to reduce cell lysis by H_2O_2 (Schraufstatter *et al.*, 1986) and glutamate-induced neuronal death (Cosi *et al.*, 1994; Zhang *et al.*, 1994). PARP activation thus appears to be a common and essential pathway of cell death in different types of tissue injury, and its inhibition can protect against some forms of lung injury. (Supported by NIH Grant HL-30450 and by VA research funds.)

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EXPOSURE TO CRYSTALLINE SILICA OR TREATMENT WITH CHLORPHENTERMINE INCREASES ALVEOLAR LAVAGE VITAMIN E LEVELS. P.R. Miles, L. Bowman, and M.J. Reasor. Div. Resp. Dis. Stud., NIOSH and West Virginia Univ., Morgantown, WV 26505.

Vitamin E may be an integral part of lung surfactant (Am. J. Physiol. 265:L133-L139, 1993). Therefore, we measured vitamin E levels in alveolar lavage materials from rats exposed to silica or treated with chlorphentermine (CP), two treatments which are known to increase surfactant phospholipids (PL) by different mechanisms. Silica exposure leads to increased PL synthesis, and CP treatment causes reduced PL degradation. HCl-washed and unwashed silica were used because exposure to each of them leads to different degrees of phospholipidosis. Exposure to HCl-washed silica results in a 17-fold increase in lavage PL and protein levels and a 12.2-fold increase in the amount of vitamin E. Exposure to unwashed silica leads to a 7-fold increase in PL and proteins and a 5.8-fold increase in lavage vitamin E. Following treatment of rats with CP, there are 15- to 19-fold increases in lavage PL and proteins and a 13.6-fold increase in vitamin E. Because surfactant synthesis occurs in the endoplasmic reticulum, we also measured vitamin E in lung microsomes. Both silica exposure and CP treatment lead to 1.8- to 2.5-fold increases in the lung microsomal levels of vitamin E. These results demonstrate that alveolar lavage vitamin E levels are elevated along with lavage PL and proteins and lung microsomal vitamin E levels are increased following exposure of rats to silica or treatment of the animals with CP.

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ABSTRACTS

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