Thyroid hormone (T3) regulation of mucin gene expression in normal human tracheobronchial epithelial (NHTBE) cells. Gray, T. E., Koo, J. S., Nettesheim, P. Laboratory of Pulmonary Pathobiology, NIEHS, RTP, NC 27709

We recently reported that mucin gene expression in NHTBE cells is controlled by retinoic acid (RA) via retinoic acid receptors (RARs) and that T3 downregulate the expression of the mucin gene MUC5AC and mucin secretion. The purpose of the studies reported here was to elucidate mechanisms by which T3 downregulates mucous production and mucin gene expression in NHTBE cells. T3 and RA both operate through the same superfamily of nuclear receptors, and both thyroid receptors (TRs) and RARs form heterodimers with RXRs for maximum activity. Using NHTBE cells grown in air-liquid interface cultures we confirmed our previous finding that continuous exposure of the cultures to T3 suppressed mucus secretion ~3 fold and MUC5AC expression ~2-fold. However we found that the mucin gene MUC5B was only marginally affected. In another series of experiments we found that T3 did not inhibit the development of the mucous phenotype and furthermore that T3 did not affect MUC5AC message stability. Taken together these data suggest that T3 suppresses (directly or indirectly) MUC5AC transcription in these culture. We also found that MUC5AC mRNA levels were significantly reduced within 6 hrs after addition of T3 and that the suppression of mucin genes was T3 concentration dependent and also dependent on the concentration of RA in the media (i. e. higher levels of RA inhibited the T3 suppression of mucin genes). These findings suggest that T3 might operate by modulating retinoid receptor signalling. Examination of the levels of retinoic acid receptor proteins in nuclear extracts showed that T3 caused a marked decrease in these proteins (but not TR protein), and gel shift assays revealed reduced RAR:RARE binding in the presence of T3. These studies indicate that in NHTBE cells, T3 reduces retinoid receptor levels thereby reducing transcription of mucin genes, which depends on activation of retinoid receptors by RA.

CHARACTERIZATION OF A SERUM COMPONENT THAT INDUCES IL-8 SECRETION BY AIRWAY EPITHELIAL CELLS. RA Brockman-Schneider, MK Schroth, WW Busse, JE Gern, University of Wisconsin-Madison.

Many infectious and inflammatory respiratory conditions cause exudation of serum into airway secretions. We have previously demonstrated that one or more serum components (MW > 50,000) is a potent inducer of IL-8 secretion by airway epithelial cells. To identify this component, experiments were conducted to assess whether the IL-8-inducing activity belongs to the lipid or protein fraction of serum. To digest serum proteins, serum was incubated (37°C, overnight) with protease K (PK), and following this procedure protein electrophoresis revealed only a few remaining low molecular weight bands. To test effects on IL-8 secretion, monolayers of bronchial epithelial (BE) cells in 24-well culture plates were incubated (48 hrs, 37°C) in serum-free medium, or in medium with either 5% serum or 5% PK-digested serum. IL-8 secretion was 1.7, 70, and 52 ng/ml, respectively. Thus, PK-digestion ı was a potent inducer of IL-ı xt, a separate aliquot of serı d silica (Aerosil, Sigma Co.), wı m 170 mg/dL to <10 mg/dL. Bı ıducers of IL-8 secretion (47 ı gest that the IL-8-inducing ı stant protein or peptide, or a p ımoval by Aerosil). Throu erum into the airway during ı of early neutrophilic inf

This abstract is funded

MECHANISMS OF HUMAN NEUTROPHIL ELASTASE-INDUCED MUCIN HYPERSECRETION BY HUMAN AIRWAY EPITHELIAL CELLS *IN VITRO* Fang He, Linda D. Martin and Kenneth B. Adler. North Carolina State University, College of Veterinary Medicine, Raleigh, NC.

Mucus hypersecretion is a common lesion in many respiratory diseases. Human neutrophil elastase (HNE) has been recognized as a major mediator in patients with inflammatory airway diseases, such as chronic bronchitis and cystic fibrosis. HNE can increase mucin (the glycoprotein component of mucus) secretion *in vivo* and *in vitro*. We examined possible mechanisms of HNE-induced mucin hypersecretion by airway epithelial cells, utilizing normal human bronchial epithelial (NHBE) cells maintained in air/liquid interface culture, a technique that allows for maintenance of differentiated structure and function. HNE increased, in a concentration-dependent manner, secretion of mucin by NHBE cells, with maximal stimulation occurring within a short (<15 minutes) time period after exposure. HNE was a potent mucin secretagogue, appearing to enhance secretion more than two-fold at this time point. Induction of mucin secretion required partial elastase activity, and a membrane-bound component of the cell may play a role. The mechanism of enhanced secretion appeared to involve activation of protein kinase C (PKC) as the PKC inhibitors, Calphostin C and Bisindolylmaleimide I, inhibited HNE-induced hypersecretion in a concentration-dependent manner. Results of translocation assays indicated that HNE activated PKCα in NHBE cells. The results suggest that HNE stimulates mucin secretion via a mechanism involving PKC. *Supported by grant HL36982 from NIH.*

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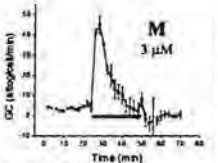
MASTOPARAN STIMULATES GLYCOCONJUGATE (GC) RELEASE FROM TRACHEAL SUBMUCOSAL GLAND CELLS (TSGC's). T. M Dwyer. Department of Physiology and Biophysics, UMMC, Jackson MS

A wide variety of inflammatory and nerve-derived signals trigger receptor-mediated exocytosis from tracheal submucosal glands, but the intracellular signaling pathways are not well understood. We therefore sought to investigate candidate second messengers. In this study, we examined the effect of directly raising intracellular calcium and also the effect of mastoparan (**M**), which directly activates pertussis (ptx) sensitive G-proteins by acting at the receptor-binding site. We used 7 cell isolates of swine TSGCs and measured mucin-like GC with a lectin-based assay. The rate of GC secretion was not significantly altered during 26 min exposures to 1 or 10 μM ionomycin (100% ± 1% and 131% ± 27% of baseline respectively; n = 3, P > 0.05). **M** (3 μM) caused a transient release of GC; during the first 10 min of exposure, the rate of release (25.3 ± 1.1 attog/cell/min; n = 4) increased 9 ± 4-fold over baseline (6.3 ± 1.5 attog/cell/min; n = 4, P < 0.05). A 90 min pretreatment of ptx reduced the **M** stimulus to 12 ± 7 attog/cell/ min over baseline (n = 4, P < 0.05 by paired t-test). Neither human neutrophil elastase (HNE) nor combined HNE/DMP-777 preincubation altered the action of **M**. Thus, **M** can stimulate secretion in TSGC's by a ptx-sensitive G-protein mechanism.



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EFFECTS OF LIPOPOLYSACCHARIDE (LPS) ON TRACHEAL EPITHELIAL BIOELECTRIC RESPONSES TO SEROSALLY- AND MUCOSALLY-APPLIED INDOMETHACIN AND HYPERTONIC SODIUM CHLORIDE. R.A. Johnston and J.S. Fedan. Dept. of Pharmacol. and Toxicol., West Virginia University, Morgantown, WV 26506 and PPRB, HELD, NIOSH, Morgantown, WV 26505 USA.

The purpose of this study was to determine whether LPS had any effect upon the bioelectric responses of guinea-pig tracheal epithelium to serosally- and mucosally-applied indomethacin and hypertonic NaCl. Guinea-pigs were injected with LPS (4 mg/kg, i.p.) or saline, and 18 hours later epithelial bioelectric responses were measured *in vitro* using the guinea-pig isolated perfused trachea apparatus, which allows addition of pharmacological agents separately to either the serosal or mucosal surfaces. Previously, our laboratory has shown that the basal transepithelial potential difference (V_{ms}) in LPS-treated animals is significantly hyperpolarized compared to control. In both saline- and LPS-treated animals, the simultaneous addition of indomethacin to the serosal and mucosal baths depolarized the V_{ms}, but significantly more so in the LPS-treated group: +1.88 mV (±0.31) and +5.57 mV (±1.37) for saline- and LPS-treated animals, respectively. In both saline- and LPS-treated groups, serosally- and mucosally-applied NaCl depolarized the V_{ms}. Within both treatment groups, NaCl was more potent serosally than mucosally. In addition, NaCl depolarized the V_{ms} more in the LPS-treated group than in the control group. These results indicate that cyclooxygenase products may contribute to the hyperpolarization of the V_{ms} in the airway epithelium after LPS-treatment. In addition, the epithelium acts as an osmotic sensor by depolarizing in response to serosally- and mucosally-applied hypertonic NaCl, with the serosal side being a more sensitive osmotic sensor.

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THE EFFECT OF HEAT SHOCK ON HUMAN INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) EXPRESSION BY A549 CELLS. G.Kohn, T.Shanley, M.Fiedler, J.M.Stark. Div. Of Pulmonary Medicine, Children's Hospital Medical Center, Cincinnati, OH, USA.

Tumor necrosis factor-alpha (TNF) induces expression of ICAM-1 in respiratory epithelial cells through activation of Nuclear Factor Kappa B (NF-κB). Expression of Heat Shock Proteins (HSP) is protective against inflammation in several models. Our hypothesis is that induction of HSP in respiratory epithelial cells (A549) will decrease expression of ICAM-1 by altering NF-κB activation. A549 cells were subjected to heat shock, then treated with TNF (HS-TNF), and a control group was treated with TNF alone (C-TNF). Analysis by flow cytometry demonstrated no significant difference in surface ICAM-1 following HS-TNF and C-TNF treatments. These data were supported by transcriptional analysis of the ICAM-1 promoter (firefly luciferase reporter gene) using the same treatment regimen. Surprisingly, Electrophoretic Mobility Shift Assays (EMSA) demonstrated decreased NF-κB activation following heat shock. We conclude that ICAM-1 expression in A549 cells is not down-regulated by heat shock despite the effect of heat shock on NF-κB activation, suggesting an alternative mechanism of ICAM-1 upregulation in response to TNF in these cells. *Supported by a grant from the ALA (JMS)*

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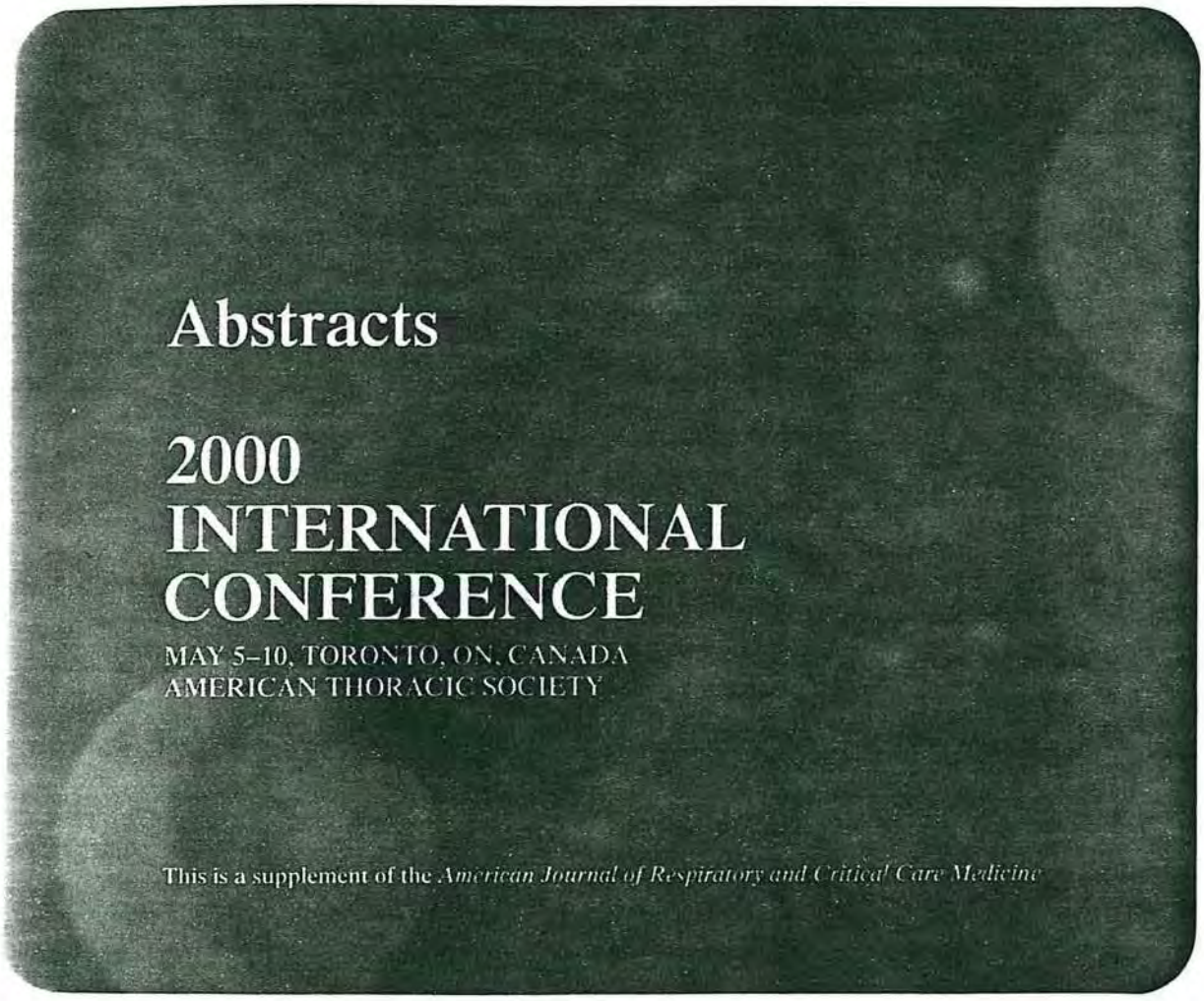

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