CYSTIC FIBROSIS AND NORMAL HUMAN AIRWAY RESPONSES TO HYPERTONIC SOLUTIONS

Middleton PG, Herok GH & Donohoo E
Ludwig Engel Centre for Respiratory Research, Westmead Millenium Institute and University of Sydney, Westmead Hospital, 2145, Sydney, NSW, Australia.

We have recently demonstrated that the normal human airway responds to addition of hypertonic saline to the airway surface liquid (ASL) with a decrease in potential difference (PD), whilst a similar osmotic stimulus with mannitol did not decrease PD. To further investigate the mechanisms underlying these responses, we have now compared the effects of these hypertonic solutions on nasal PD in 6 subjects with cystic fibrosis (CF) and 7 normal subjects. ***Methods:*** Nasal PD was measured using standard techniques (*Eur Respir J* 1994:7:2050). On separate days, addition of 500 mM NaCl or 1M mannitol to the Krebs HEPES diluent was tested, either with or without amiloride (am) pre-treatment. ***Results:*** Mannitol significantly decreased PD in CF (became less negative), with a mean (SEM) decrease of 22.1 (4.6) mV; and 5.7 (1.2) mV after am. In the normals mannitol tended to increase PD by 1.4 (0.8) mV; after am mannitol significantly increased PD by 3.9 (1.0) mV. In the CF subjects saline significantly decreased nasal PD by 33.7 (5.2) mV; 15.8 (2.0) mV following am. In normals saline decreased PD by 8.6 (1.4) mV; 0.9 (0.4) mV following am. ***Conclusions:*** This suggests that a large hypertonic stimulus (mannitol) decreases Na^+ absorption across the CF airway, but in normals this is balanced by another ion transport process. Whilst in normals the saline response appears related to amiloride-sensitive Na^+ absorption, in CF there is a dual response - both to the osmolarity and the increased saline in the ASL. This study demonstrates that the human airway response to hypertonicity depends both on the composition of the solution and the underlying ion transport processes.

This abstract is funded by: Australian CF Trust, Westmead Institute Research Trust

THE EFFECT OF ION TRANSPORT INHIBITORS ON THE BIOELECTRIC RESPONSES OF GUINEA-PIG TRACHEAL EPITHELIUM TO HYPERTONIC D-MANNITOL SOLUTION.

*†R.A. Johnston, ‡M.R. Van Scott, †A. Rengasamy and *†J.S. Fedan. *Dept. of Pharmacol. and Toxicol., West Virginia Univ., Morgantown, WV 26506; †PPRB, HELD, NIOSH, Morgantown, WV 26505; and ‡Dept. of Physiol., East Carolina Univ., Greenville, NC 27858 USA.

Elevation of serosal or mucosal tonicity induces the release of the non-nitric oxide, non-prostanoid epithelium-derived relaxing factor (EpDRF) from guinea-pig tracheal epithelium, which subsequently diffuses to the underlying airway smooth muscle to initiate relaxation. Hypertonicity-induced smooth muscle relaxation *via* EpDRF is preceded by a depolarization of the transepithelial potential difference. This study sought to characterize further the ion channels and/or transporters involved in the response of guinea-pig tracheal epithelium to hypertonic solution by determining the effects of inhibitors on transepithelial short-circuit current (I_{sc}) responses. I_{sc} responses of tracheal segments were measured *in vitro* using a Ussing chamber. Methacholine (3×10^{-7} M) was added to the serosal surface to mimic conditions used in previous studies of EpDRF. Hypertonic D-mannitol solution (120 mOsm) added to the mucosal solution decreased the I_{sc}. Pretreatment with amiloride (3×10^{-5} M; mucosal), NPPB (10^{-4} M; mucosal), or bumetanide (10^{-5} M; serosal) inhibited 36±2%, 77±8% or 7±2% of the baseline I_{sc}, respectively. Amiloride inhibited 30±10% of the ΔI_{sc} induced by D-mannitol. NPPB almost completely abolished the ΔI_{sc} induced by D-mannitol while bumetanide inhibited 34±9% of the ΔI_{sc} response. Iberiotoxin (10^{-7} M; mucosal and serosal) had no apparent effect. Ouabain (10^{-5} M; serosal) itself caused a progressive reduction in I_{sc} which prevented assessment of the response to D-mannitol. These results indicate that apical membrane Na^+ and Cl^- channels and the basolateral Na^+-K^+-$2Cl^-$ cotransporter mediate the epithelial response to elevated mucosal tonicity.

NIOSH
This abstract is funded by:

NRAMP-2 IS INCREASED IN AIRWAYS EXPOSED TO EITHER IRON OR PARTICLES.

Xinchao Wang[1], AJ Ghio[2], LA Dailey[2], JD Carter[2], J Stonehuerner[2], KG Dolan[3], MD Garrick[3]. [1]CEMLB, University of North Carolina, Chapel Hill, NC 27514, [2]NHEERL, U. S. EPA, Research Triangle Park, NC and [3]SUNY, Buffalo, NY.

The sequestration of metal within intracellular ferritin confers an antioxidant function to this protein. Such storage requires that the metal be transported across a cell membrane. We tested the hypothesis that, in response to *in vitro* exposures to catalytically active metal, human bronchial epithelial (HBE) cells increase the expression of NRAMP-2. HBE cell cultures were exposed to either 0 - 500 μM ferric ammonium citrate (FAC) or 0 - 200 μg/ml oil fly ash for 24 hours. Concentrations of mRNA for NRAMP-2 both with and without an IRE were estimated by reverse transcription-polymerase chain reaction and westerns for proteins were quantified in parallel. mRNA for NRAMP2 with an IRE did not change while that without an IRE increased in a dose-dependent manner after exposures to either FAC or the oil fly ash. Similarly, western blots confirmed no change in NRAMP2 with an IRE while that without an IRE increased with exposure to either FAC or oil fly ash. Deferoxamine, a metal chelator, inhibited the response to oil fly ash. We conclude that increases in NRAMP2 without an IRE in HBE cells expression can be metal-dependent. This response can function to diminish the oxidative stress a metal chelate presents to a living system. *This does not necessarily reflect EPA policy.*

This abstract is funded by:

CFTR Cl^- Channels with R Domain Deletions and Translocations Show Phosphorylation-Dependent and Independent Activity.

Olafur Baldursson, Lynda S. Ostedgaard, Tatiana Rokhlina, Joseph F. Cotton and Michael J. Welsh. Howard Hughes Medical Institute, Departments of Internal Medicine and Physiology and Biophysics University of Iowa College of Medicine Iowa City, Iowa 52242.

Phosphorylation of the R domain regulates CFTR Cl^- channel activity. Earlier studies suggested that the R domain controls activity via more than one mechanism; a phosphorylated R domain may stimulate activity, and an unphosphorylated R domain may prevent constitutive activity, i.e., opening with ATP alone. However, the mechanisms responsible for these two regulatory properties are not understood. In this study we asked whether the two effects are dependent on its position in the protein and whether smaller regions from the R domain mediate the effects. We found that several portions of the R domain conferred phosphorylation-stimulated activity. This was true whether the R domain sequences were present in their normal location or were translocated to the C-terminus. We also found that some parts of the R domain could be deleted without inducing constitutive activity. However, when residues 760-783 were deleted, channels opened without phosphorylation. Translocation of the R domain to the C-terminus did not prevent constitutive activity. These results suggest that different parts of the phosphorylated R domain can stimulate activity, and that their location within the protein is not critical. In contrast, prevention of constitutive activity required a short specific sequence that could not be moved to the C-terminus. These results are consistent with a recent model of an R domain composed primarily of random coil in which more than one phosphorylation site is capable of stimulating channel activity, and net activity reflects interactions between multiple sites in the R domain and the rest of the channel.

NHLBI#HL61234
This abstract is funded by:

PROTEASE-ACTIVATED RECEPTOR (PAR-2) ACTIVATION INDUCES CHLORIDE SECRETION IN BOVINE TRACHEAL EPITHELIAL CELLS

K. Kawatani, M. Kondo, J. Tamaoki, M. Taira, K.Isono, K. Takevama, E. Tagaya, A. Nagai. First Department of Medicine, Tokyo Women's Medical University, School of Medicine, Tokyo 162-8666, Japan

PAR-2 is activated by trypsin and is known to immunohistologically co-localize with trypsin in airway epithelium. We investigated the effect of PAR-2 agonists on Cl secretion in cultured bovine tracheal epithelium. Cl secretion was assessed by short circuit current (Isc) in the presence of amiloride in Ussing chamber. Intracellular calcium concetration ($[Ca^{2+}]i$) was measured by fura-2 method. SLIGRL-NH_2, a selective PAR-2 activating peptide (10^{-7} to 3×10^{-5} M) added to basolateral but not apical side, induced a concentration-dependent biphasic increase in Cl secretion (10^{-5} M; ΔIsc 5.7 ± 1.1 $\mu A/cm^2$, EC_{50} 1×10^{-6} M), and this effect was strongly inhibited by pretreatment with indomethacin. The control peptide, LSIGRL-NH_2 (10^{-6} M) had little effect on Cl secretion. The response to SLIGRL-NH_2 were abolished by prior desensitization to trypsin but not thrombin (PAR-1, 3, 4 agonist). Furthermore, both trypsin and SLIRGL-NH_2 transiently increased $[Ca^{2+}]i$. These data suggest that basolateral PAR-2 activation increases Cl secretion via calcium- and cycloxygenase-dependent pathway in airway epithelial cells.

This abstract is funded by:

AIRWAY EPITHELIAL CELLS EXPRESS ROMK CHANNELS

G. K. Fyfe, C. A. Ambrose, R. M. Douglas & M. E. Egan, Depts of Peds. and Cell. & Mol. Physiol., Yale University Sch. of Med., New Haven, CT.

The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is believed to play an important role in modulating airway surface fluid (ASF). Its effects on ASF are thought to be due to its ability to function as a cAMP-stimulated Cl^- channel and to act as a regulator of other ion channels such as the epithelial Na^+ channel (ENaC) and the outwardly rectifying Cl^- channel (ORCC). Previously, we have demonstrated that CFTR can also interact with a renally-derived K^+ channel, ROMK. In airway epithelia the interactions between CFTR/ENaC and CFTR/ORCC have been demonstrated, but the CFTR/ROMK interaction has only been studied in heterologous expression systems. To examine if the CFTR/ROMK interaction is important in airway epithelia, crude cell membranes were prepared from several primary and immortalized airway cell lines (normal & CF). Cellular proteins were resolved using SDS-PAGE, transferred to polyvinylidene fluoride membranes, and western blotting was performed. Primary anti-ROMK antibody was used at a 1:100 dilution and secondary anti-rabbit IgG-horse radish peroxidase conjugate used at 1:2000. Upon exposure to enzyme chemiluminescence (ECL) reagents, signal was detected in all airway cells, as well as T84 cells and kidney membranes ($n \geq 3$ per cell line). ECL signal was lost upon competition of the primary antibody with a ROMK epitope/GST fusion protein. These data suggest that ROMK channel proteins are expressed in airway cells. Electrophysiologic studies are underway to confirm functional expression of ROMK. In addition, further studies of CFTR/ROMK interactions in airway epithelial cells are ongoing. This interaction may provide a therapeutic target for manipulation of ion and fluid content in ASF.

This abstract is funded by: N.I.H. and M.O.D.

AMERICAN JOURNAL OF

Respiratory and Critical Care Medicine

Volume 163 • Number 5 • April 2001

AN OFFICIAL JOURNAL OF THE AMERICAN THORACIC SOCIETY