pH Sensitive Changes in Nasal Potential Difference in Healthy Adults

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RATIONALE: Defects in chloride channel expression and function are hallmarks of cystic fibrosis, however alternative chloride channels have been detected by nasal potential difference (NPD) and may offer new therapeutic strategies to explore. CLC-2, a member of the voltage and volume regulated family of chloride channels is expressed predominantly on the apical surface of developing lung and is downregulated after birth. CLC-2 like chloride secretion occurs in response to acidic pH in fetal airway cells and in transfected mature human airway cells. To investigate alternative chloride channel pathways in mature respiratory epithelia, we examined pH-sensitive NPD in a cohort of heathy adult volunteers. METHODS: CLC-2 function was evaluated by measuring pH stimulated changes on transepithelial NPD. Transepithelial voltage changes were measured by perfusing the nasal epithelia with basal Ringers solution, then Ringers with Amiloride 0.1mM to block the ENaC channel, finally chloride secretion was enhanced using chloride replaced by gluconate (NOCl) solutions of pH 7.4, 6.2 and 5.0. The change in voltage between the NOCl solution 7.4 and 5.0 was used as a measure of pH sensitive chloride secretion. 0.1mM ATP was perfused to check the integrity of the system. RESULTS: NPD was measured in 14 healthy, non CF adults. Mean age of subjects was 36.9 years. Average Basal PD was -16.7 mV. Average depolarization with perfusion of Amiloride was 8.9mV. NOCl solution at a pH of 7.4 produced a mean hyperpolarization of -5.6mV. Average change in PD with acidic pH/NOCl was -1.34 mV. Of the 14 subjects, 4 showed pH sensitive hyperpolarization during NPD of >-4 mV. The average change in PD with ATP was -14.4 mV. CONCLUSIONS:Hyperpolariszation of NPD can be induced by use of low pH/NOCl solutions in certain adults. This may be due to persistent expression of CLC-2 channels. A correlation between function and protein expression is being sought.

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The Involvement of Conventional Protein Kinase C in the down Regulation of Epithelial Sodium Channel Subunit by Tumor Necrosis Factor-alpha in Alveolar Type II Epithelial Cells

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BACKGROUND: We previously reported that one of the major pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) inhibited the mRNA expression of alpha-subunit of ENaC (α-ENaC) in alveolar type II cells. The objectives of this study is to determine whether the other two ENaC subunits, β-, and γ-ENaC would be also regulated by TNF-α and whether protein kinase C (PKC) would be involved in this TNF-α effect. METHODS: Freshly isolated rat alveolar type II cells were treated with TNF-α at a concentration of 100 ng/ml. After 24h stimulation, mRNA expression of each ENaC subunit was measured by RT-PCR technique. Next, the cells were pretreated with GF109203X (wide range PKC inhibitor) and Go6976 (conventional PKC inhibitor) for 30 minutes, then stimulated with TNF-α. mRNA expression of each ENaC subunit was also measured. RESULTS: The expression of α- and γ-ENaC was inhibited by stimulation with TNF-α, whereas β-ENaC was not (α-ENaC = 64.0%, γ-ENaC = 73.9%). This inhibition of α- and γ-ENaC was blocked by pretreatment with both GF109203X (α-ENaC = 94.7%, p = 0.0046; γ-ENaC = 83.7%, p = 0.4251) and Go6976 (α-ENaC = 94.6%, p = 0.0131; γ-ENaC = 95.3%, p = 0.0362). CONCLUSION: These results suggest that TNF-α could regulate not only α-ENaC but also γ-ENaC expression. Because β-ENaC was inhibited, each ENaC is thought to be differentially regulated by TNF-α. In addition, it is considered that the impact of TNF-α on ENaC expression could be due to the activation of conventional PKC.

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Sodium/Iodide Symporter and Thiocyanate Transport in Airway Epithelial Cells

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Lactoperoxidase oxidizes thiocyanate (SCN) to hypothiocyanite (OSCN), a biocidal compound that is important in airway host defense against infection. Characterization of the basolateral (BL) SCN transporter will provide insight into regulation of this antibacterial system. Normal Human Bronchial Epithelial cells (NHBE), grown and differentiated at an air-liquid interface (ALI), were rinsed apically with PBS and membrane resistance measured to assess the monolayer's integrity. Cultures were incubated with either 14C-SCN or unlabeled iodide at different concentrations with or without perchlorate (100uM), furosemide (100uM) or ouabain (100uM) and transport to the apical surface was assessed by washing the cultures at 2 min. intervals. Some washes contained SCN or I anions. A colorimetric assay was used to measure iodide efflux. Transport of both anions depended on the BL concentration and SCN transport was inhibited by iodide in the media. The SCN transport rate was 2-3 nmoles/hr/0.3cm2 and was observed in the presence of a transepithelial concentration gradient. The iodide transport rate was similar to SCN. BL perchlorate and ouabain inhibited transport but furosemide did not suggesting that transport was a secondary active process, ruling out the Na/K/2Cl transporter and implicating the NaI symporter (NIS). RT-PCR using RNA from ALI cultures and NIS oligonucleotide primers showed a fragment of the expected size suggesting that NIS was expressed by NHBEs. In conclusion, SCN and iodide transport were shown to be a concentration dependent, saturable and active process. The iodide transport characteristics and NIS mRNA expression suggested that NIS may be responsible for SCN transport and that NIS might have an role in host defense against infection.

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Comparison of Bioelectric Responses of Fresh Tracheal Epithelium (FE) and Air-Liquid Interface Epithelial Cell Cultures (CE) from Guinea Pigs

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To determine whether primary air-interface cultured guinea-pig tracheal epithelial cells (CE) retain the bioelectric response profile of fresh epithelial cells (FE), we compared short circuit current (Isc) and transepithelial resistance (Rt) responses to ion transport blockers, methacholine (MCh), and hyperosmolar challenge, using Ussing chambers. In FE, baseline values were: Isc: 49±4 μA/cm², Rt: 97±6 Ω-cm²; in CE, Isc: 9.3±0.4 μA/cm², Rt: 787±98 Ω-cm² (FE vs. CE, p<0.05). Amiloride (30 μM), bumetanide (10 μM) and ouabain (10 μM) reduced Isc in FE and CE, and there were no differences (% change in Isc) in the effects seen in FE and CE. Iberiotoxin (0.1 μM) had no effect on Isc in FE or CE. NPPB (10 μM) decreased Isc by 11% in FE and 71% in CE (p<0.05). Serosal MCh (0.3 μM) elicited a monotonic increase in Isc. In FE, MCh caused a transient increase in Isc, which was followed by a plateau; the former response was inhibited by NPPB. The enhanced effect of NPPB on CE and responses to MCh suggests that a relative shift in Cl^- secretion had occurred in CE. In the presence of MCh, the addition of D-mannitol (D-M; 0.27-266.8 mosM) to elevate osmolarity elicited concentration-dependent, progressive decreases in Isc in FE. In CE, D-M increased Isc over the range of 0.27-84.3 mosM, but decreased Isc and Rt over the range of 84.3-266.8 mosM. The results suggest that CE causes marked changes in the bioelectric response to NPPB, MCh and hyperosmolarity, some of which are attributable to changes in Cl^- secretion. These changes might reflect a phenotypic modification occurring in CE or the absence of modulators originating in the airway wall.

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Insulin Increases Plasma Membrane Na,K-ATPase Protein Abundance in Alveolar Epithelial Cells

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Tight control of blood glucose levels with insulin infusion in patients with sepsis resulted in improved outcomes by yet undescribed mechanisms. Regulation of the Na^+ pump by insulin occurs trough diverse, tissue and isoform specific mechanisms, which include reversible, covalent modification of catalytic subunits, increased intracellular Na^+ concentration and effects on the protein abundance. We sought to investigate whether insulin regulates Na,K-ATPase protein abundance at the plasma membrane of A549 cells. The plasma membrane abundance of the Na,K-ATPase α1-subunit was quantitated by labeling the cell surface with biotin and pulling down the labeled proteins with streptavidin and Western blotting with an antibody against the Na^+ pump. Insulin elicited a concentration dependent increase in the number of Na,K-ATPase α1-subunit at the plasma membrane. The effect of insulin was apparent within minutes and peaked around 15 min to achieve a ≈2.2 fold increase above basal levels. The insulin-mediated increase was sensitive to genistein, an inhibitor of tyrosine kinase activity, suggesting that the regulation of Na^+ pump may involve receptor autophosphorylation. Pretreatment with wortmannin, a specific inhibitor of phosphatidylinositol 3-kinase and with the protein kinase C inhibitor bisindolylmaleimide blocked the insulin-stimulated increase in Na^+ pump at the plasma membrane level. Accordingly, we provide here evidence that insulin regulates the activity of Na,K-ATPase by promoting its translocation to the plasma membrane via PI3K and PKC pathway.

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Cyclic AMP-Stimulated Na^+ Transport in Fetal Lung Explants and in Fetal Lung Epithelial Cells: Role of PKA, PI-3K and sgk1

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In rodents and amphibians, the serum and glucocorticoid-regulated protein kinase, sgk1, regulates the activity of the epithelial Na^+ channel (ENaC) in the collecting duct but little information is available on the role of sgk1 in ENaC-mediated Na^+ transport in the mammalian lung. We find that, in the presence of amiloride, both dibutyryl cAMP (cAMP) and glucocorticoids (GC) increase the lumen volume of pre-alveolar ducts in human fetal lung (HFL) explants, suggesting that these agents independently increase Na^+ transport in HFL. Consistent with these findings, fetal distal lung epithelial (FDLE) cells isolated from HFL develop a tight resistance on permeable supports and demonstrate cAMP-stimulated benzamil-sensitive short-circuit current, indicative of Na^+ transport and this effect is potentiated in the presence of GC. The cAMP-stimulated Na^+ conductance is abolished by either LY294002, an inhibitor of PI3-Kinase (PI3-K) or by H89, an inhibitor of protein kinase A (PKA). In addition, we find that cAMP-stimulation increase the abundance of sgk1, a PI3-K activated protein, in HFL.

These results demonstrate that cAMP stimulates Na^+ transport in FDLE via a PI3-K and PKA-dependent pathway. cAMP also increase sgk1 expression; collectively, these findings are consistent with a model where an increase in sgk1 abundance increases the number of active Na^+ channels at the apical cell surface thus leading to an increase in Na^+ transport.

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