37 Effects of hyperosmotic stress on cultured airway epithelial cells

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Studies on the effect of hypertonic NaCl or mannitol on mucociliary clearance (MCC) in patients with asthma or cystic fibrosis have shown improved MCC, but the mechanism behind this effect is unclear. It also has been shown that hypertonic saline opens tight junctions in airway epithelia. Thus, the effect of hypertonic solutions on MCC may be due to increased water transport across the epithelium resulting in more diluted mucus. We examined the relation between osmolarity and permeability changes in airway cell cultures of 16HBE14o- and Calu-3 cells by adding NaCl, NaBr, LiCl, mannitol or xylitol (295–700 mOsm). The transepithelial resistance (TEER) was measured as indicator of the tightness of the cultures. Morphology was studied by transmission electron microscopy, with lanthanum nitrate added to the luminal side of the epithelium to investigate tight junction permeability. In 16HBE14o- cells electrolytes caused a significant decrease in TEER above 450 mOsm, whereas non-electrolytes caused a decrease in TEER above 590 mOsm. In Calu-3 cells hyperosmolar NaCl did not significantly change TEER, while exposure to xylitol caused increased TEER. Morphological studies revealed a substantial, but reversible, damage in both cell lines after exposure to hypertonic solutions at 590 mOsm and higher. This was paralleled by an increased number of open tight junctions in 16HBE14o- cells but not in Calu-3 cells. Opening of the tight junctions may lead to transiently increased paracellular water transport during inhalation of hypertonic solutions. Hyperosmolar solutions cause reversible damage to the cultured cells, but cell lines may show characteristics different from the luminal airway lining because they lack the influence of multiple cell types typically found in vivo.

38 PUFA-mediated Cl^- transport regulation in model airway cells

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Aim: to examine whether polyunsaturated fatty acid (PUFA) application would affect Cl^- transport in model airway epithelial and submucosal cells.
Methods: 16-HBE, CFBE (homozygous for deltaF508), Calu-3 and CFSME (deltaF508/2QX) cells were co-cultured with the following PUFAs: linoleic (LA), arachidonic (AA), α-linolenic (ALA), ecosopentanoic (EPA) and docosahexanoic (DHA) acids during 72 hours. Upon cessation of PUFA incubation with cells, the parameters of Cl^- transport were estimated using the fluorescent chloride indicator MQAE and digital imaging technique.
Results: In 16-HBE and Calu-3 cells, which are model counterparts of bronchial epithelium and glandular submucosal cells expressing wtCFTR, all PUFAs used in this study attenuated the rate of CFTR-mediated Cl^- efflux. In contrast, LA increased basal Cl^- transport in both cell lines whereas the other PUFAs accelerated the rate of basal Cl^- efflux only in 16-HBE but not in Calu-3 cells. In CFBE cells expressing ΔF508 CFTR, the function of CFTR was not affected by any PUFAs whereas AA, LA and DHA similarly to 16-HBE and Calu-3 cells were able to accelerate the rate of basal Cl^- efflux.
Conclusion: PUFAs increased the basal chloride efflux in CF cells, the most efficient fatty acid being DHA. This chloride efflux is not mediated by CFTR but may be mediated by Ca-dependent chloride channels, which is being investigated further.

39 Alpha-melanocyte stimulating hormone modulates respiratory function in guinea-pigs

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Aim: Alpha-melanocyte stimulating hormone (alpha-MSH), a 13 amino acid neuropeptide, exhibits anti-inflammatory, antipyretic and antibacterial properties. We investigated the *in vivo* effect of alpha-MSH on changes in pulmonary function provoked by histamine.
Methods: Bronchoconstriction was induced by 10 μg/kg histamine i.v. in Hartley guinea-pigs (6 animals per group, male-female 1:1, weight 500–700 g). Changes in the respiratory rate, type of respirations or amplitude of respirations were continuously monitored (classic Konzett and Rössler's method of whole body plethysmography modified by Gjuris). Bronchoconstriction following pre-treatment with 3 doses of alpha-MSH (0.01 mg/kg, 0.1 mg/kg and 1 mg/kg) was compared to histamine response without pre-treatment.
Results: The lowest dose of alpha-MSH was ineffective, but pre-treatment with 0.1 mg/kg and 1 mg/kg α-MSH exhibited strong and pharmacologically relevant reduction of bronchoconstriction ($p < 0.05$ and $p < 0.001$ respectively). We found a dose-related modulatory effect of alpha-MSH on histamine induced changes in pulmonary function.
Conclusion: Our results show that exogenous alpha-MSH modulates processes in the lungs of guinea pigs. This neuropeptide is found in human bronchoalveloar lavage fluid; it binds to melanocortin MC_5 receptor identified in the lung tissue and also to melanocortin MC_1 and MC_3 receptors expressed on different inflammatory cells present in the airway. Several chronic inflammatory diseases have already been identified as potential therapeutical targets of alpha-MSH. Therefore, beneficial effects beyond blocking bronchoconstriction in CF are possible and we feel further studies on this matter are justified.

40 The trefoil factor TFF3 promotes human airway epithelial differentiation

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Previously, we have shown that in CF there is a delay in the airway epithelium regeneration. Molecules that could promote the airway epithelial differentiation could therefore be of major therapeutic interest.
The predominant trefoil factor, TFF3, is secreted with mucins by goblet cells and is able to increase cell migration but its role in the epithelial differentiation remains unknown.
We analyzed the spatio-temporal expression of TFF3 and mucins during human airway epithelial differentiation using an in vivo open tracheal xenograft model and an in vitro air-liquid interface (ALI) cell culture model. By immunohistochemistry, RT-PCR and western blot analysis, we observed that TFF3, MUC5AC and MUC5B were expressed when epithelia were completely differentiated. Using MUC5AC and β-tubulin markers, we analyzed the effects of exogenous recombinant human TFF3 (2 μM) added at day 0 in ALI cultures. We observed an increase in the MUC5AC positive cell number and in the β-tubulin positive ciliated cell number ($p < 0.01$). These data demonstrate that the trefoil factor TFF3 promotes airway epithelial differentiation and might help novel therapeutic strategy design in CF airway epithelial differentiation.
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