WS12.5 The mucus and its behavior in rat and pig explant tissues as models of cystic fibrosis

L. Meiss[1], A. Ermund[1], A. Bähr[2], G.C. Hansson[1]. *[1]University of Gothenburg, Medical Biochemistry, Gothenburg, Sweden; [2]LMU, Molecular Animal Breeding and Biotechnology, Munich, Germany*

The basic defect in cystic fibrosis (CF) is well characterized, but the link between defects in the CF transmembrane conductance regulator (CFTR), the causative gene of CF disease, and the phenomenon of stagnant mucus is not well understood. It has been shown that the ileal mucus in CF mice adheres to the epithelium, is denser, and is less penetrable than that of wild-type mice and that apical addition of 115 mM NaHCO3 to mucus partially normalizes this mucus phenotype. Using this knowledge of effects on ileal mucus, we develop an airway experimental set-up. In the airway, CFTR is localized apically not to MUC5AC-expressing goblet cells, but to the neighboring ciliated epithelial cells. This is similar to the small intestine where the MUC2 secreting goblet cells are localized adjacent to the CFTR expressing enterocytes. Using bead-tracking and mass spectrometry analysis, we study the effects of therapies on the mucociliary transport rate, Muc5ac and Muc5b secretion, and mucus clearance patterns in the bronchotracheal tree of both wild-type and cystic fibrosis porcine models as well as in rat airway explants. Scanning electron microscopy is used to visualize the properties of the mucus layer, and transmission electron microscopy is used to study mucin secretion in detail. Preliminary results suggest the importance of bicarbonate in the proper unpacking and secretion of mucins. We hypothesize that the restoration of bicarbonate to the apical surface of the epithelium in combination with osmolytes may induce proper mucin unpacking in CF epithelia, and therefore could relieve the mucus obstruction that causes clinical problems for cystic fibrosis patients.

WS12.6 CFTR is an essential component in cAMP-dependent exocytosis in human and mouse beta-cell insulin secretion

L. Eliasson[1], A. Edlund[1], J.L. Esguerra[1], A. Wendt[1], M. Flodström-Tullberg[2]. *[1]Lund University Diabetes Centre, Lund University, Dept Clinical Sciences Malmö, Malmö, Sweden; [2]Center for Infectious Medicine (CIM), Karolinska Institute, Department of Medicine Huddinge, Stockholm, Sweden*

Objectives: Patients with Cystic Fibrosis (CF) often have disturbances in glucose metabolism including impaired insulin release. Here we aimed to test the hypothesis that CFTR has a biological function in pancreatic beta-cells.
Methods: The whole-cell configuration of the patch-clamp technique and a ramp protocol was used to measure cAMP-activated currents in single mouse and human beta-cells. Exocytosis was elicited by a train of ten depolarizations from –70 to 0 mV and was measured as an increase in membrane capacitance. Insulin release was measured on mouse-and human islets.
Results: Forskolin evoked an increase in current that was inhibited by CFTR antagonists ($p < 0.001$), CFTRinh-172 or GlyH-101. The cAMP-activated current was also reduced by the Anoctamin1 (ANO1) chloride channel blocker AO1, where the remaining part was inhibited by GlyH-101 ($P < 0.01$). The calculated CFTR conductance was small and we propose that CFTR act as a regulator of ANO1. This was further confirmed by insulin secretion measurements. Glucose- and cAMP-stimulated insulin secretion was reduced by $\geqslant$40% ($p < 0.01$) in the presence of GlyH-101 in human and mouse islets, respectively. When AO1 was added, GlyH had no further inhibitory effect, confirming that CFTR controls ANO1. Moreover, cAMP-amplified exocytosis was reduced by >40% after pre-incubation with CFTR antagonists ($P < 0.05$).
Conclusion: We postulate that CFTR is an essential component in cAMP-dependent insulin secretion and exocytosis in human and mouse beta-cells, and suggest that impaired insulin secretion in patients with CF is caused by defect CFTR mediated beta-cell exocytosis.