Albuterol modulates its own transepithelial flux via changes in paracellular permeability

Hoshang J. Unwalla, PhD; Gabor Horvath, MD; Felix D. Roth; Gregory E. Conner, PhD; and Matthias Salathe, MD

ONLINE DATA SUPPLEMENT

Methodology:

Cell culture: All experiments were performed with ALI cultures grown on 12-well plates using collagen coated Transwell filters. Cells were grown in the absence of epinephrine for at least two days prior to any experiments. Airway epithelial cells from cystic fibrosis patients were obtained at time of transplant with IRB approval and informed consents. The cells used for this study were homozygous for ΔF508.

Transport and uptake studies: ALI cultures were preincubated for 30 min at 37˚C in transport buffer (modified HBSS; in mM: 138 NaCl, 5.3 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.4 Mg_2SO_4, 0.4KH_2PO_4, 0.3 Na_2HPO_4, 5.6 glucose, 10mM HEPES, pH 7.4). Experiments were initiated by adding ^{3}H-albuterol. Appearance of substrate in the receiver compartment (basolateral for absorptive transport, apical for secretory transport) was evaluated as a function of time.

For uptake kinetics, cultures were preincubated for 30 min at 37˚C in transport buffer. Experiments were initiated by replacing the apical solution with 0.4 mL of transport buffer containing different concentrations of H^3-albuterol. After 5, 10 and 15 minutes, apical and basolateral solutions were aspirated and cells were washed three times with ice-cold transport buffer. Transport buffer was completely aspirated. Monolayers were excised from the Transwell, and dissolved in 500 µL 0.11% Triton X-100 in Tris-HCl by incubating for 4 h with shaking. Isotope was quantified by scintillation counting.

Effect of extracellular pH on transport: ALI cultures were preincubated with transport buffer of various pH (5.7, 7.4, 8.2) on the apical and basolateral side for 30 min at 37˚C. Experiments were initiated by replacing the donor solution with transport buffer containing 10 µM ^{3}H -albuterol. Appearance of albuterol in the

receiver compartment was evaluated as a function of time. Intracellular albuterol concentrations were assayed as described above.

Sodium dependence of transport: ALI cultures were preincubated with transport buffer in the absence or presence of Na^+ ions for 30 min at 37˚C. Where appropriate, NaCl (137 mM) was iso-osmotically replaced with choline chloride. Uptake was determined over 10 min after which H^3-albuterol was quantified as described above.

Tight junction perturbation: For calcium depletion experiments, ALI cultures were treated apically in transport buffer with 6 mM EGTA or transport buffer alone for 1 hr and TEER was measured using an EVOM Epithelial Tissue Volt-ohmmeter (World Precision Instruments, Sarasota, FL). Transport experiments were initiated by replacing the apical solution with 0.4 ml of transport buffer containing 10 μM H^3-albuterol and 500 nM ^{14}C mannitol. Appearance of both substrates in the receiver compartment was evaluated as a function of time. For luminal hypertonicity experiments, cultures were pre-treated with 100 mM glucose or transport buffer for 1 hr and TEER was measured. Experiments were initiated by replacing the apical solution with 0.4 ml of transport buffer containing 10 μM ^3H- albuterol. Appearance of albuterol in the receiver compartment was evaluated as a function of time.

Confocal microscopy: NHBE cells differentiated at the ALI were washed with transport buffer, and treated with 10 μM albuterol or vehicle (as control) for 30 minutes. Following albuterol treatment, cells were washed with PBS, fixed in 4% PFA and permeabilized with PBS 1% triton X 100. Cells were blocked with 3% BSA, 3% goat serum in PBS and double stained with antibodies to β_2-AR (SantaCruz Biotechnologies) or non-immune IgG (control) and acetylated tubulin. Cells were

counterstained with secondary antibodies for one hour, washed with blocking buffer and stained with DAPI. After mounting with Prolong Gold (with DAPI), images were captured using a Lieca SP5 confocal laser scanning microscope, provided by the University of Miami Imaging Core Facility.