675.15

Intracellular Calcium Chelation Decreases Time of Rapid Liquid Clearance from Saline-Filled Alveoli in the Rat Lung

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Previously we determined by lung micropuncture, that saline microinfusions in single alveoli clear rapidly and that the clearance time (Ct) decreases during lung hyperinflation (Faseb J, 1999). We now determined the extent to which cellular processes regulate Ct. We established the isolated blood-perfused rat lung preparation at constant pulmonary artery, left atrial and airway pressures of 10, 5 and 5 cmH_2O, respectively. By videomicroscopy, we micropunctured the lung surface to microinfuse saline in ~6 alveoli. Alveolar margins were not visible in saline-filled alveoli, but became progressively evident as the alveoli cleared the microinjected liquid and refilled with air. We microinjected colored solution (Evans Blue) to confirm absence of fluid leak at the micropuncture site. In video replays, we viewed the microinjected region to determine Ct as the time taken for the alveolar margin to become visible in a single, non-micropunctured alveolus. In separate experiments, we gave alveolar microinfusions of the active transport blockers ouabain (1 mM, 15 min, n=3) and amiloride (10 μM, 15 min, n=3), and of the intracellular calcium (Ca^{2+}) chelator, BAPTA-AM (40 μM, 15 min, n=3). At baseline, Ct was 4.1±0.6 s (mean±SE, n=3). The active transport blockers had no effect on Ct. However, BAPTA-AM increased Ct by 223±47 % above baseline ($P<0.05$). We interpret that the rapid removal of microinjected alveolar liquid occurred by Ca^{2+}-dependent processes, but not by active ion transport. When relatively few alveoli are liquid filled as in early pulmonary edema Ca^{2+}-dependent processes, such as surfactant secretion may provide surface forces for rapid removal of alveolar liquid towards proximal airways (HL64896, HL10142).

675.16

EFFECT OF ION TRANSPORT INHIBITORS ON THE BIOELECTRIC RESPONSES OF GUINEA-PIG TRACHEAL EPITHELIUM TO HYPERTONIC SODIUM CHLORIDE SOLUTION

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Previously, our laboratory has shown that in the presence of serosally-added methacholine (MCh; 3×10^{-7} M), the application of hypertonic D-mannitol solution (120 mOsm) to the mucosal surface of guinea-pig tracheal segments mounted in Ussing chambers decreased the transepithelial short-circuit current (I_{sc}). The purpose of this study was to determine if I_{sc} responses to elevated tonicity are independent of the osmolyte used and to elucidate which epithelial ion channels and/or transporters are involved in the epithelial bioelectric response to elevated mucosal tonicity. In the presence of serosally-added MCh (3×10^{-7} M), exposure of the mucosal surface to modified Krebs-Henseleit solution made hypertonic with added NaCl (120 mOsm) decreased the I_{sc}. Both amiloride (3×10^{-4} M; mucosal) and bumetanide (10^{-4} M; serosal) attenuated the decrease in I_{sc}. Iberiotoxin (10^{-7} M; serosal and mucosal), a Ca^{2+}-activated K^+ channel blocker, had no effect on I_{sc}. The I_{sc} increased rather than decreased in the presence of the Cl channel blocker, NPPB (10^{-4} M; mucosal). The effect of ouabain (10^{-3} M; serosal), a Na^+-K^+-ATPase inhibitor, was also examined; however, it caused a progressive reduction in I_{sc} which prevented assessment of the response to NaCl. These results suggest that apical Na^+ channels and the basolateral Na^+-K^+-2Cl cotransporter are important in the epithelial bioelectric response to elevated mucosal tonicity while the role of apical Cl channels in this response is unclear.

675.17

BASOLATERAL KCl COTRANSPORT IN ADULT ALVEOLAR EPITHELIAL CELLS IS ACTIVATED BY TERBUTALINE AND PARTICIPATES IN Cl ABSORPTION.

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Alveolar epithelial cells were isolated from adult rats and maintained in monolayer culture under defined media conditions following previously published procedures (Jiang et al., Am. J. Physiol.275:C1610-C1620, 1998). Transepithelial resistances ranged between 1500-1800 Ω cm^2. Treatment with terbutaline was previously shown to activate Cl channels in the apical membrane and to increase net Cl absorption. In this study we show that terbutaline stimulation increases net Cl efflux from 0.31 to 1.56 mEq/cm^2h^1 across the basolateral membrane under conditions where an outward [K] gradient exists and the membrane voltage is clamped at zero mV. When the [K] gradient is eliminated, the effect of terbutaline on net Cl efflux is inhibited to the extent that no significant Cl efflux can be detected across the basolateral membrane. Western blot analysis using antibodies to the four cloned isoforms of KCl cotransporters revealed the presence of KCC1 and KCC4 isoforms in monolayer cultures of these cells. The estimated molecular weights were 90 KDa for KCC1 and 70 KDa for KCC4. These results provide additional evidence that supports a novel mechanism for Cl absorption that involves uptake of Cl across the apical membrane by Cl channels and efflux across the basolateral membrane by an electroneutral cotransport mechanism that is coupled to the [K] concentration gradient.

LUNG VENTILATION/GAS EXCHANGE/AIRWAY REACTIVITY (676.1-676.2)

676.1

Ventilation-perfusion distributions during pursed-lips breathing in emphysema and chronic bronchitis.

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The multiple inert gas elimination technique (MIGET) is a robust tool to assess both ventilation-perfusion (V'A/Q') distributions and the role of extrapulmonary factors determining arterial oxygenation during spontaneous breathing. The pursed lips breathing (PLB) is often used in the management of patients with chronic obstructive lung disease. Previous clinical studies have demonstrated that PLB improves arterial oxygen saturation and CO2 removal as well as relieving dyspnea. PLB increases the expiratory resistance and thus increases the expiratory resistive work of breathing, promoting greater expiratory rib cage and abdominal muscle recruitment in response to the expiratory loads.

The aim of this study was to examine the effects of PLB on breathing pattern and V'A/Q' distributions in patients with emphysema and chronic bronchitis.

Three patients with stable emphysema and 3 with chronic bronchitis were asked to breathe successively through the nose and with PLB during 30 minutes. This period was necessary to perfuse the solution of the 6 inert gases and to reach the steady state for the MIGET. Both types of breathing were used in each subject in a random order. All the measurements were done within 3 hours.

We have found that PLB reduced the dead space (from 76.3% to 51.7%) in emphysematous subjects only. Ventilation to regions with a normal V'A/Q' ratio increased from 25.3 ± 13.8 % to 51.1 ± 10.8 %.

We conclude that PLB improves V'A/Q' distributions in emphysematous patients but not in patients with chronic broochitis.

676.2

Effects Of Optison® on Pulmonary Hemodynamics and Gas Exchange in the Dog

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Optison®, a suspension of albumin microspheres containing octafluoropropane gas, provides ventricular contrast during cardiac ultrasound imaging. We asked whether Optison® produced deleterious effects on pulmonary hemodynamics or gas exchange after intravenous administration, and if so for how long. Twenty-four mongrel dogs were assigned randomly to one of four groups: (A) Optison®, (B) albumin control, (C) Optison® following creation of acute pulmonary microvascular embolism raising pulmonary artery pressure to 33 mmHg, and (D) albumin after similar embolism. Respiratory and inert gas exchange were followed at 1-3 minute intervals for 30 minutes after 3 sequential doses of 1, 3 and 5 ml of Optison® or 1% albumin. Optison® had no effect on arterial PO_2, PCO_2, VA/Q mismatch or cardiac output at any dose or at any time. Minor (5 mmHg), transient increases in pulmonary artery pressure were seen over the first 10 minutes after the 5 ml dose in normal dogs, but not in dogs with prior microembolism. We conclude that even after a 5 ml dose (25 times the single clinical dose), the effects of Optison® on pulmonary hemodynamics and gas exchange are transient and insignificant in the dog, even in the presence of pre-existing pulmonary hypertension. Supported by Mallinckrodt, Inc., St. Louis, MO.

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ABSTRACTS

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