HYPOXIA REDUCES NASAL EPITHELIAL POTENTIAL DIFFERENCES IN RATS
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Ascent to high altitude leads to pulmonary edema formation in some individuals. Recent laboratory evidence supports the hypothesis that hypoxia may impair the function of the alveolar epithelium and thus augment edema accumulation via reduced clearance of lung liquid. We investigated the effect of hypoxia on epithelial sodium transport in adult Sprague-Dawley rats, measuring the nasal transepithelial potential difference (nasal PD) as an index of airway sodium transport. Baseline potential differences were similar to those previously reported in other species (-24.0 * 0.7 mV), and administration of amiloride, an inhibitor of apical sodium channels, decreased the nasal PD by 42% (n = 24, p = 0.001). Exposure to hypobaric hypoxia simulating an altitude of 18,000 feet for 24 hours caused a significant fall in nasal PD (-23.7 vs. –18.8 mV, n = 15, p = 0.001). Hypoxia caused no further drop in nasal PD in amiloride-treated animals, suggesting that hypoxia acts by inhibiting sodium transport. Administration of ouabain, an inhibitor of Na-K-ATPases, for 24 hours (1 mg/kg every 12 hours, intraperitoneal) also caused a significant fall in nasal PD (-27.8 vs. –16.8 mV, n = 5, p = 0.001). Hypoxia caused no further drop in nasal PD in ouabain-treated animals (n = 5, p = 0.06), suggesting that hypoxia acts by inhibiting Na-K-ATPases. To test the hypothesis that hypoxia acts via endogenous ouabain-like factors, hypoxic animals were treated with anti-digoxin Fab fragments, which reversed the hypoxia-induced fall in nasal PD (-18.0 vs –24.2 mV, n = 3, p = 0.05). We conclude that subacute exposure to moderate hypoxia can inhibit sodium transport by the airway epithelium in rats and suggest that endogenous ouabain-like factors may contribute to those effects.

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EFFECTS OF LIPOPOLYSACCHARIDE ON TRACHEAL EPITHELIAL BIOELECTRIC RESPONSES TO SEROSALLY- AND MUCOSALLY-APPLIED METHACHOLINE. R.A. Johnston and J.S. Fedan. Dept. of Pharmacol. & Toxicol., West Virginia University, and PPRB, HELD, NIOSH, Morgantown, WV 26505 USA.

We examined whether lipopolysaccharide (LPS) affects transepithelial potential difference (V_{ms}) of guinea-pig tracheal epithelium and bioelectric responses to serosally- and mucosally-applied methacholine (MCh). V_{ms} was measured *in vitro* using the isolated perfused trachea apparatus, which allows addition of MCh separately to the mucosal or serosal surface. Eighteen hours after LPS (4 mg/kg, i.p.) basal V_{ms} was increased (saline: -14.9±1.6 mV; LPS: -27.7±2.5 mV; $p<0.05$). In both saline and LPS-treated animals, the dose-response curves for serosally-added MCh were biphasic (hyperpolarization at [MCh] $<10^{-6}$ M; depolarization at [MCh] $>10^{-6}$ M). After LPS-treatment the EC_{50} and maximum response for hyperpolarization were decreased and increased, respectively ($p<0.05$), but the EC_{50} and maximum response for depolarization were not changed. Mucosal MCh was less potent and dose-response curves also were biphasic (hyperpolarization $<10^{-3}$ M; depolarization $>10^{-3}$ M). The maximum hyperpolarization was increased ($p<0.05$) after LPS-treatment; the depolarization was unaffected. The results suggest that high and low affinity muscarinic receptors initiate changes in V_{ms}. The mechanism involved in high affinity receptor-induced hyperpolarization is potentiated after LPS-treatment.

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NEUROPEPTIDE Y (NPY) REDUCES NOREPINEPHRINE (NE) INDUCED INCREASES IN VECTORIAL WATER FLUXES ACROSS TRACHEAL EPITHELIA. Jonathan E. Phillips and Donovan B. Yeates. VA Chicago Health Care System and Depts of Medicine and Chemical Engr., University of Illinois, Chicago, IL.

NPY, an inhibitory neuromodulator, has been found to colocalize with NE in the sympathetic neurons within the airway mucosa. We hypothesized that NPY reduces NE-induced increases in the vectorial water fluxes across native ovine tracheal epithelia. Unidirectional water fluxes were measured using a novel fluorescence technique which exploited the three fold higher quantum yield of ANTS dissolved in D_2O compared to ANTS dissolved in H_2O. Luminal to basolateral ($J_w^{L\rightarrow B}$) and basolateral to luminal ($J_w^{B\rightarrow L}$) transepithelial water fluxes were measured under open circuit conditions. The vectorial water fluxes were derived from the temporal gradients of the fluorescent photons emitted by 1mM ANTS present in the luminal and basolateral HBSS, which were prepared in H_2O and D_2O, respectively. In these absorptive epithelia the baseline $J_w^{L\rightarrow B}$ (6.4±0.4 µl/min/cm^2) was greater than $J_w^{B\rightarrow L}$ (4.9±0.5 µl/min/cm^2, $p<0.05$, n=6). The measured epithelial potential difference (-9±1 mV), short-circuit current (Isc = 64±4µA/cm^2), and resistance (130±20 Ω•cm^2) are characteristic of a leaky biological membrane. Exogenous NPY (0.1 to 10 µM) alone had no effect on the vectorial water fluxes. NE (10^{-4} M, basolateral) alone increased $J_w^{L\rightarrow B}$ (6.4 to 7.0 µl/min/cm^2, $p<0.05$, n=6), $J_w^{B\rightarrow L}$ (4.9 to 6.0 µl/min/cm^2, $p<0.05$, n=6), and slightly increased Isc (62 to 64 µA/cm^2). NPY (1 µM, basolateral) in the presence of NE (10^{-4} M, basolateral) decreased the baseline $J_w^{L\rightarrow B}$ (6.4 to 5.2 µl/min/cm^2, $p<0.05$, n=6) with no effect on $J_w^{B\rightarrow L}$. These novel data suggest that NPY inhibited the NE-induced increases in transepithelial water transport, reducing the net respiratory water absorption. This provides the first evidence for an inhibitory neural mechanism to decrease airway hydration.

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DEXTRAN TRANSPORT AS AN INDEX OF FUNCTIONAL TIG JUNCTIONS IN A CALU-3 LUNG EPITHELIAL CELL MOD
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Cell culture models of lung epithelium are useful to study the mechanis of drug transport when grown as confluent sheets with functional ti junctions and high transepithelial electrical resistance (TEER). Howe even when TEER is consistently high, transepithelial permeability to solu can be variable, suggesting that TEER measurements may not de transient changes in junctional integrity. We evaluated dext permeability as a marker of intact, functional tight junctions to calib inter and intra-experimental transport variability of molecules being te within our system. Dextrans (Texas-Red®, 0.5 mg/mL) of vari molecular weights (3000, 10000, 40000, and 70000) were added to ei the apical or basolateral surface of confluent monolayers of Calu-3 c grown on collagen-coated Snapwell filters at an air-liquid interf Transport was measured over 2 hrs. using a fluorescent plate reader. apparent permeability (P_{app}) was similar for all MW (4.2 ± 0.3 x 10^{-5} cm) at day 3 when Calu-3 cell monolayers had not developed tight junct (TEER=0). At day 11 when TEER was >250 Ω•cm^2, P_{app} was up to 1 fold less for all MWs and highest for the smallest MW (P_{app} = 3.8 ± 0. 1.0 ± 0.1 x 10^{-8} cm/sec for 3000 and 70000 MW respectively). In summ TEER confirmed the presence or absence of tight junctions. Once T was established, dextran permeability was size dependent and indicati intact, functional tight junctions over time.

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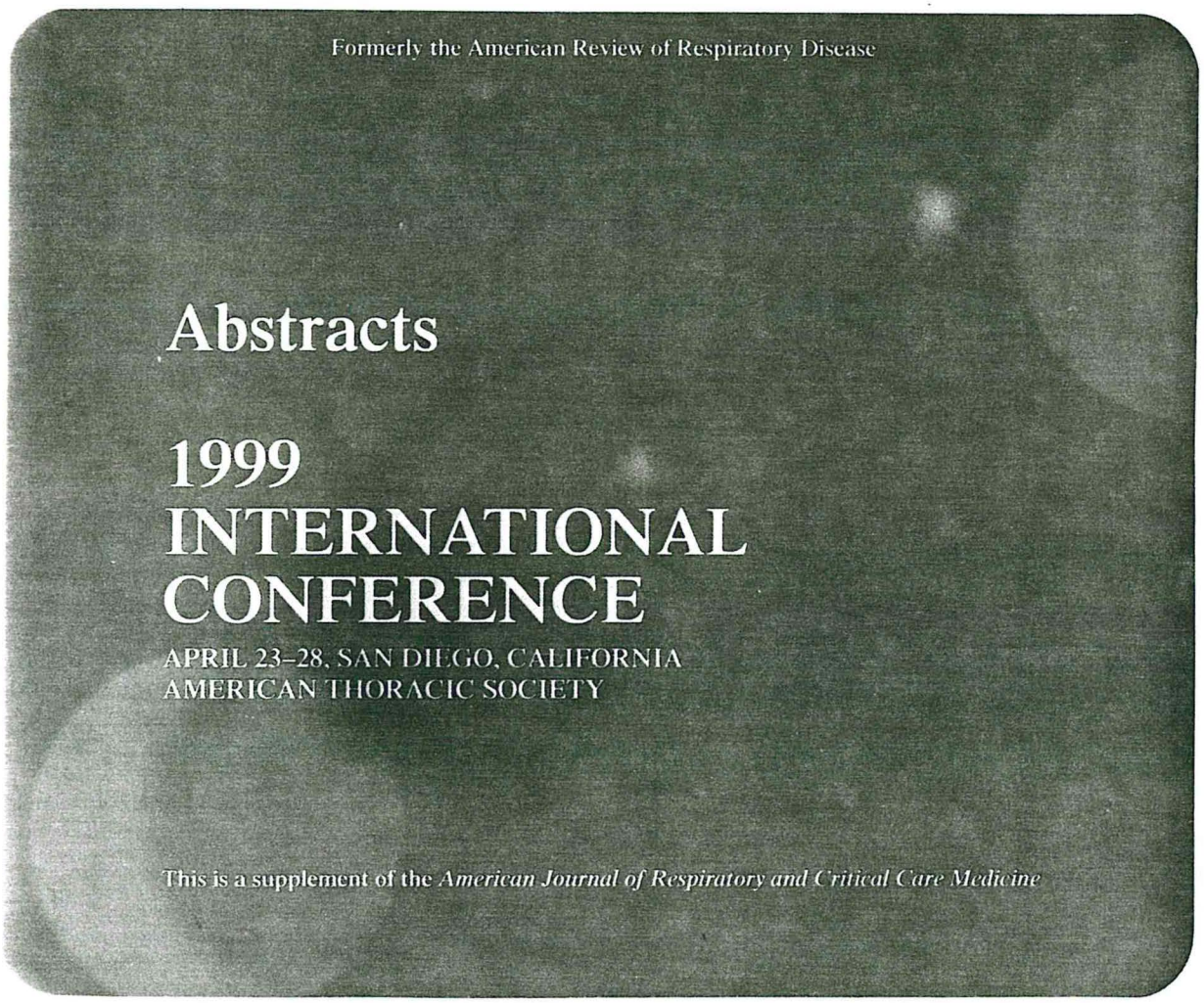

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