transcriptional regulation. To test this hypothesis, EGFP was attached to the N-terminus of BGT1. This fusion protein (EGFP-BGT) had normal Na^+ and Cl^- dependence and inhibitor specificity, compared to BGT1. Transport and trafficking were studied in MDCK cells stably expressing EGFP-BGT. Na^+-dep GABA uptake was increased from 675±69 (isotonic) to 1921±81 pmol/mg/10min after 24h in Hyp medium (500 mOsm). Corresponding uptakes in normal MDCK were 20±10 (isotonic) and 500±80 (Hyp). Confocal microscopy of transfectants confirmed increased plasma membrane fluorescence in Hyp cells compared to isotonics. However, western blotting of cell lysates with anti-EGFP antibody showed no increase in total cellular EGFP content in response to Hyp stress. We conclude that redistribution of pre-existing EGFP-BGT protein from cytoplasm to plasma membrane may be an important step in the overall cell response to Hyp stress.

108.8

Role of Reactive Oxygen Species (ROSs) in the Osmotic Stimulation of COX-2 Expression in mIMCD-K2 Cells

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Our previous studies have shown that hypertonicity stimulates COX-2 expression via MAP kinases, and that COX-2 is cytoprotective in osmotic stress. We examined the role of ROSs in mediation of the tonicity-stimulated COX-2 expression in mIMCD-K2 cells. Exposure to hypertonicity (525 mOsm by NaCl) for 16 hrs markedly induced COX-2 protein expression and PGE2 release. Both effects were abolished by the NADPH oxidase inhibitor diphenyleneiodonium chloride, DPI (25-50 μM). N-acetyl-L-cysteine (NAC), tempo, and tempol had similar effects. DPI abolished and NAC significantly reduced the tonicity stimulation of COX-2 promoter activity. In contrast, the HSP70 protein expression was not affected. DPI and NAC significantly reduced the tonicity-induced phosphorylation of p44/42 but not p38. The osmotic stress caused a transient and significant increase in superoxide release with a peak at 1 h as determined by cytochrome c assay. Superoxide generated by xanthine/xanthine oxidase or H202 markedly stimulated COX-2 protein expression and PGE2 release. While hypertonicity at 525 mOsm for 48 hours did not induce cell death, in the presence of DPI (50 μM) or apocynin (1 mM) or NAC (6 mM), the same stress induced massive cell death, determined by caspase assay. Under isosmotic condition, none of the antioxidant agents exhibited obvious cytotoxicities. Our studies suggest a novel survival pathway, ROSs/MAPK/COX-2, in IMCD cells exposed to hyperosmolarity.

108.9

INTEGRATED CFTR EXPRESSION AND AUTOCRINE ATP SIGNALING GOVERN HUMAN AIRWAY EPITHELIAL CELL VOLUME REGULATION

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CFTR and mdr control cell volume regulation in heterologous cell systems via autocrine ATP signaling {Braunstein et al., *JBC* 276(9): 6621, 2001; Roman et al., *JMB* In Press, 2001}. To address the role of CFTR expression and ATP agonist signaling in human airway epithelial cell volume regulation, wild-type (WT) and mutant forms of CFTR were transfected transiently (80% efficient) into IB3-1 CF human airway epithelial cells null for functional or detectable CFTR. Regulatory volume decrease (RVD) was assessed in cells studied in suspension in real-time via a Coulter Counter Multisizer III system and was measured indirectly via SPQ halide fluorescence assay on adherent cells with hypotonicity-induced Cl- efflux. Results consistent to both assays: (1) parental and empty vector (mock)-transfected cells did not RVD; (2) WT-CFTR and T1478X-CFTR transfected cells had a full RVD response; (3) delF508-CFTR, G551D-CFTR, and S1455X-CFTR failed to support RVD; (4) RVD in mock-transfected IB3-1 cells was rescued by agonists to P2Y G protein-coupled receptors and/or P2X purinergic receptor channels; and (5) RVD in WT-CFTR-expressing IB3-1 cells was attenuated by ATP receptor antagonist (suramin) or ATP scavengers (apyrase or hexokinase). Taken together, these data show that CFTR and autocrine ATP signaling govern human airway epithelial cell volume regulation.

108.10

Bioelectric response of guinea-pig (GP) tracheal epithelium (E) to hypotonic solution

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Hypotonic solution causes cell swelling. Hypotonic solution in the lumen of the GP trachea depolarizes the E and contracts the airway smooth muscle. This study examined the effects of mucosal hypotonic solution on the bioelectric properties of GP tracheal E mounted in Ussing chambers. Stepwise dilution of the mucosal modified Krebs-Henseleit (MKH) solution (1% to 50% with water) progressively decreased transepithelial short circuit current (I_{sc}) and increased resistance (R_t). Responses to hypotonicity were unaffected by 1 μM MK-571, 5 μM piriprost, or 10 U/ml apyrase added bilaterally, indicating that LTD_4 and ATP were not involved. Mucosal amiloride (30 μM) decreased basal I_{sc} (10%) but did not affect responses to hypotonicity. Mucosal NPPB (100 μM) decreased basal I_{sc} (88%). Hypotonicity in the presence of NPPB reversed the polarity of I_{sc} and inhibited the increase in R_t, but the ΔI_{sc} was unaffected. I_{sc} also was decreased after reducing [NaCl] in the mucosal bath (113→85 mM). These results indicate that basal I_{sc} across GP tracheal E is mainly due to Cl^- secretion. The ΔI_{sc} induced by hypotonicity is consistent with passive transepithelial flux of Na (S→M). Funded by NIOSH.

108.11

Regulation of the Na-K-2Cl cotransporter NKCC1 by a volume-sensitive MKK7-JNK1 MAP kinase cascade.

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The mechanism by which changes in cell volume activate volume-regulatory transporters is unknown. NKCC1 is a ubiquitous volume-regulatory transporter that is activated by cell shrinkage. We have previously shown that NKCC1 is phosphorylated in vitro by c-jun N-terminal kinase (JNK), a member of the MAP kinase family, and that JNK is activated by cell shrinkage. To determine whether JNK is responsible for activating NKCC1, bovine aortic endothelial cells were transfected with antisense oligodeoxynuceotides (ODN) against JNK isoforms followed by determination of JNK abundance and NKCC1 activity. Transfection of either antisense or control oligonucleotides reduced cell growth and JNK protein abundance, indicating a toxic effect of ODN on the cells. However, there was further reduction in JNK1 abundance with antisense ODN. In cells transfected with JNK1 antisense ODN, hypertonic NKCC1 activity was reduced 45% vs. cells transfected with control ODN. Basal NKCC1 activity was reduced 35%. In contrast, JNK2 antisense ODN had no effect on NKCC1 activity. To identify the upstream kinase responsible for activating JNK in shrunken cells, MKK4 and MKK7 were immunoprecipitated and tested for activation of JNK. Hypertonic shrinkage activated MKK7 but not MKK4. We conclude that, in aortic endothelial cells, NKCC1 is phosphorylated and activated by JNK1 which in turn is regulated by volume-sensitive activation MKK7. This defines a MAP kinase cascade involved in the regulation of cell volume.

108.12

Characterization of the interaction between cation-chloride cotransporters and the stress-activated serine-threonine-kinases SPAK and OSR1

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Cation-chloride cotransport is involved in ion homeostasis, as well as regulation of cell volume and neuronal excitability. In the brain, five cotransporters have been identified so far: NKCC1 and KCC1-4. Using the N' terminus of KCC3a as a bait in a yeast 2-hybrid (Y2H) screen, we have recently identified the Ste20-related proline-alanine rich kinase, SPAK, as an interactor. Here we show that NKCC1 and NKCC2 also interact with SPAK in Y2H assays. We suggest a 10 AA sequence as a preliminary binding motif, with an initial "RFXV" sequence, followed by six uncharacterized AAs that are essential for the interaction. In addition, we narrowed down the binding site of SPAK to 97 AAs at the C' terminus of the protein. Sequence alignment with the oxidative stress-response kinase, OSR1, shows 80% sequence homology in this region. In fact, mouse OSR1 interacts with the N' terminus of KCC3. Using a SPAK antibody, we show that SPAK colocalizes with NKCC1 in the apical membrane of choroid plexus cells of wild-type mice, but it is retargeted to the cytoplasm in NKCC1 knockout mice. We suggest that the stress-activated kinases SPAK

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