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REGULATION OF THE EPITHELIAL SODIUM CHANNELS BY PROTEIN KINASES

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Epithelial sodium channels (ENaC) play a critical role in the regulation of blood pressure, extracellular fluid volume and the thickness of the fluid layer coating the respiratory passages. They are regulated by hormones such as aldosterone and insulin, as well as by intracellular feedback systems which inhibit the channel in response to increases in intracellular Na^+ and Cl^- concentrations. Recent studies have shown that Sgk, a kinase activated by insulin, phosphorylates the ubiquitin-protein ligase, Nedd4-2, and in so doing prevents it from binding to and inhibiting epithelial Na^+ channels. Since Nedd4-2 mediates the inhibition of epithelial Na^+ channels in response to increased intracellular Na^+, this observation suggests that Sgk should inhibit Na^+ feedback regulation of the channels. Sgk, however, is only one of a number of kinases activated by insulin which also raises the question of whether these other kinases, which include Akt and the S6 kinase, are also able to modulate the activity of epithelial Na^+ channels. In addition, epithelial Na^+ channels have been reported to be regulated by phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol 3,4,5-trisphosphate (PIP3). Taken together with recent reports that insulin and purinergic agonists such as ATP may be exerting their effects on epithelial Na^+ channels, this raises the question of the extent to which the effects of insulin on epithelial Na^+ channels are mediated by kinases and the extent to which they are mediated directly by phospholipids. We have been undertaking patch-clamp studies on salivary duct cells and Ussing chamber studies to investigate the roles of kinases and of phospholipids in regulating epithelial Na^+ channels. In particular, we have focused on the mechanisms by which PIP2 regulates epithelial Na^+ channel activity and the roles of kinases and phospholipids in mediating the effects of insulin and ATP on the channels.

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ENHANCED Cl^- AND HCO_3^- TRANSPORT IN THE PAROTID GLANDS OF NHE1-DEFICIENT MICE

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Fluid secretion is inhibited 20~30% in both *Nhe1*$^{-/-}$ and *Nhe2*$^{-/-}$ mice. A limiting step in saliva production is Cl^- influx across the basolateral membrane. This Cl^- uptake is dependent on $Na^+/K^+/2Cl^-$ cotransport and Cl^-/HCO_3^- exchange mechanisms. The dependence of these two major Cl^- uptake mechanisms on Na^+/H^+ exchanger (Nhe) expression was examined in parotid acinar cells of Nhe-deficient mice. $Na^+/K^+/2Cl^-$ cotransporter activity significantly increased in acinar cells from Nhe1-deficient mice, whereas, no changes were detected in mice lacking Nhe2. In agreement with these observations, northern and western blot analyses demonstrated that expression of the "secretory" $Na^+/K^+/2Cl^-$ co-transporter Nkcc1 was enhanced in glands from *Nhe1*$^{-/-}$ but not *Nhe2*$^{-/-}$ mice. In contrast, Cl^-/HCO_3^- exchanger activity increased dramatically, however northern and western blot analyses detected only subtle changes in the expression of Cl^-/HCO_3^- exchanger Ae2 transcript and protein. The increased Cl^-/HCO_3^- exchanger activity was completely blocked by the carbonic anhydrase inhibitor acetazolamide. An increase in carbonic anhydrase II (CaII) expression correlated with the increased Cl^-/HCO_3^- exchanger activity in *Nhe1*$^{-/-}$ mice. Moreover; CaII-deficient mice expressed significantly less acetazolamide-sensitive anion exchanger activity. A CaII-specific antibody co-immunoprecipitated Ae2 suggesting an *in vivo* association between these two proteins. Moreover, there was an increase in the formation of such complexes in Nhe1-deficient mice, consistent with CaII being involved in the observed up-regulation of anion exchanger activity. Together, these results demonstrate that Cl^- and HCO_3^- metabolism and transport mechanisms undergo compensatory changes in mice lacking *Nhe1* gene expression to limit the extent of electrolyte and acid-base balance perturbations.