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Functional Roles of Na^+/H^+ Exchanger Isoforms in Saliva Secretion

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Na^+/H^+ exchange mediates the electroneutral exchange of extracellular Na^+ for intracellular H^+. In this manner, Na^+/H^+ exchange regulates intracellular pH, cell volume, and transepithelial Na^+ absorption. In salivary acinar cells, upregulation of Na^+/H^+ exchanger activity is thought to buffer the acidification that results from HCO_3^- secretion in response to muscarinic stimulation, thereby enhancing the activity of the intracellular pH-sensitive anion channel. Moreover, Na^+/H^+ and Cl^-/HCO_3^- exchangers act in concert to uptake NaCl in exchange for H^+ and HCO_3^- loss across the basolateral membrane, thus increasing the intracellular $[Cl^-]$ and enhancing Cl^- efflux via apical anion channels. Four members of the Na^+/H^+ exchanger gene family (NHE1, NHE2, NHE3, and NHE4) with different kinetics and pharmacological properties have been identified in rat epithelial tissues.

To address the possibility that multiple NHE isoforms are involved in salivary gland function, Na^+/H^+ exchanger activity in acinar cells was characterized in the presence of the amiloride derivative, 5-(N-ethyl-N-isopropyl) amiloride (EIPA). Low concentrations of EIPA (IC_{50}=0.014±0.005 μM) inhibited intracellular pH recovery from an acid load in acinar cells, suggesting the expression of amiloride-sensitive isoforms NHE1 and/or NHE2 (1). Single cell semiquantitative RT-PCR confirmed that NHE1 transcripts are most abundant in this cell type. In contrast, the intermediate sensitivity of ductal cells to EIPA indicated that inhibitor-sensitive and -resistant Na^+/H^+ exchanger isoforms are coexpressed. Ductal cells were about one order of magnitude more resistant to EIPA (IC_{50}=0.754±0.104 μM) than cell lines expressing NHE1 or NHE2 (IC_{50}=0.076±0.013 μM or 0.055±0.015 μM, respectively). Conversely, ductal cells were nearly one order of magnitude more sensitive to EIPA than a cell line expressing the NHE3 isoform (IC_{50}=6.25±1.89 μM). Semiquantitative RT-PCR demonstrated that both NHE1 and NHE3 transcripts are expressed in ducts. NHE1 was immunolocalized to the basolateral membranes of acinar and ductal cells, whereas NHE3 was exclusively seen in the apical membrane of ductal cells. Immunoblotting, immunolocalization, and semiquantitative RT-PCR experiments failed to detect NHE2 expression in either cell type. Taken together, our results demonstrate that NHE1 is the dominant functional Na^+/H^+ exchanger in the plasma membrane of rat parotid acinar cells, whereas NHE1 and NHE3 act in concert to regulate the intracellular pH of ductal cells (1).

To investigate the unique functional roles of the individual NHE isoforms expressed in parotid acinar and ductal cells, we studied the functional consequences of disrupting the murine *Nhe1*, *Nhe2*, and *Nhe3* genes on intracellular pH regulation and Na^+ absorption in the cells. The absence of NHE1 expression, but not NHE2 or NHE3, abolished intracellular pH recovery from an acid load in resting acinar cells, in muscarinic-stimulated cells, and in acini shrunken by the addition of sucrose (2). Similarly, the rate of intracellular pH recovery from a muscarinic agonist-stimulated acid load in HCO_3^--containing solution was significantly decreased in acinar cells from *Nhe1* null mutant mice, whereas pH recovery in cells from NHE2- or NHE3-deficient mice was comparable to that in acinar cells from wild-type mice. Moreover, the volume of saliva secretion in response to muscarinic stimulation was dramatically reduced in mice lacking of NHE1, and to a lesser extent in NHE2-deficient mice, while secretion was not altered in NHE3-deficient mice (3). In contrast, NaCl content and osmolality of saliva from wild-type animals were comparable to those obtained from *Nhe1*, *Nhe2*, and *Nhe3* null mutant mice. These data demonstrate that NHE1 is the major isoform regulating intracellular pH in resting and muscarinic- and cell shrinkage-stimulated acinar cells. Furthermore, NHE1 plays a critical role in modulating saliva secretion in vivo, apparently by acting in concert with NHE2. Colocalization of NHE2 with the apical anion channel suggests that NHE2 may control the activity of this pH-sensitive channel during muscarinic-

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stimulated fluid secretion.

To determine whether the lack of NHE1, NHE2, or NHE3 might alter expression of mRNAs encoding other transporters involved in salivary gland function, transcripts for several transporter genes in NHE-deficient mice were quantitatively compared to those in the corresponding wild-type mice by northern blot analyses (3). Expression of transcripts for the anion exchanger AE2 and the $Na^+/K^+/2Cl^-$ cotransporter NKCC1 was increased in mice lacking NHE1, whereas expression of these genes in mice deficient in NHE2 or NHE3 was comparable to that seen in wild-type mice. The mRNAs for βENaC and γENaC were increased in the salivary glands of mice lacking NHE3, but the expression of αENaC was not altered. These observations suggest that mRNA expression of related ion transport proteins was regulated to compensate for functional defects in mice lacking individual NHE isoform.

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