Targeted Disruption of the Nhe1 Gene Prevents Muscarinic Agonist-induced Up-regulation of Na\(^+\)/H\(^+\) Exchange in Mouse Parotid Acinar Cells*

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The onset of salivary gland fluid secretion in response to muscarinic stimulation is accompanied by up-regulation of Na\(^+\)/H\(^+\) exchanger (NHE) activity. Although multiple NHE isoforms (NHE1, NHE2, and NHE3) have been identified in salivary glands, little is known about their specific function(s) in resting and secreting acinar cells. Mice with targeted disruptions of the Nhe1, Nhe2, and Nhe3 genes were used to investigate the contribution of these proteins to the stimulation-induced up-regulation of NHE activity in mouse parotid acinar cells. The lack of NHE1, but not NHE2 or NHE3, prevented intracellular pH recovery from an acid load in resting acinar cells, in acini stimulated to secrete with the muscarinic agonist carbachol, and in acini shrunken by hypertonic addition of sucrose. In HCO\(_3\)^−-containing solution, the rate of intracellular pH recovery from a muscarinic agonist-stimulated acid load was significantly inhibited in acinar cells from mice lacking NHE1, but not in cells from NHE2- or NHE3-deficient mice. These data demonstrate that NHE1 is the major regulator of intracellular pH in both resting and muscarinic agonist-stimulated acinar cells and suggest that up-regulation of NHE1 activity has an important role in modulating saliva production in vivo.

The initial response of salivary gland acinar cells to a fluid secretion stimulus is an acidification of the cytosol resulting from the efflux of HCO\(_3\)^− into the lumen and the generation of acid equivalents via metabolic pathways linked to increased membrane transporter activity (1–3). It is clear that this intracellular acid load is buffered by an increase in Na\(^+\)/H\(^+\) exchanger (NHE)\(^1\) activity, which by alkalining the cytosol, promotes both the secretion of HCO\(_3\)^− via the apical anion channel and the uptake of Cl\(^−\) mediated by the basolateral Cl\(^−\)/HCO\(_3\)^− exchanger (1, 2). Accordingly, specific inhibitors of the Na\(^+\)/H\(^+\) exchanger such as amiloride and its analogue dimethyl amiloride inhibit muscarinic agonist-stimulated salivary flow in perfused glands (4–6) and Na\(^+\) influx in isolated acini (7–9). Thus, up-regulation of Na\(^+\)/H\(^+\) exchanger activity plays a key role in the secretory process by enhancing HCO\(_3\)^− and transepithelial Cl\(^−\) movement.

The Na\(^+\)/H\(^+\) exchanger gene family currently comprises six known isoforms. NHE1–NHE4 have been described in epithelial cells (10, 11); NHE5 is highly expressed in brain (12, 13); and NHE6 is a mitochondrial exchanger (14). NHE1 is ubiquitously expressed, and its main functions appear to be maintenance of intracellular pH and cell volume recovery (see Refs. 11, 16, and 17 for reviews). In contrast, NHE3 is primarily expressed in kidney and gastrointestinal epithelia, where it mediates NaCl reabsorption (18, 19). NHE2 has a similar distribution to NHE3; however, disruption of this gene does not appear to affect NaCl reabsorption. Rather, it appears to be involved in regulating the long-term survivability of gastric parietal cells (20). NHE4 is found in stomach and kidney, where it may be involved in cell volume regulation (21).

Recently, a combination of immunocytochemical, pharmacological, and molecular biological studies have shown that rat parotid and submandibular acini express NHE1 in their basolateral membrane (22–25). In contrast, the subcellular distribution of NHE2 and NHE3 appears to be species- and gland-specific (22, 24, 25). In addition to these uncertainties concerning isoform localization, very little is known about the specific functions of the individual NHE isoforms in acinar cells during resting conditions or when stimulated to secrete fluid. Available evidence suggests that the most highly expressed Na\(^+\)/H\(^+\) exchanger in acinar cells is probably the NHE1 isoform (23–25). However, the mechanisms involved in the up-regulation of Na\(^+\)/H\(^+\) exchanger activity in salivary acinar cells are inconsistent with the known properties of expressed NHE1. Up-regulation in acinar cells is insensitive to protein kinase A activation and is maintained by the agonist-induced increase in intracellular Ca\(^2+\) concentration, independently of protein kinase C and calmodulin (26–29). In contrast, recombinant NHE1 is regulated by protein kinases A and C as well as calmodulin (30–33). To confirm the molecular identity of the Na\(^+\)/H\(^+\) exchanger activity in salivary gland acinar cells, we studied the functional consequences of disrupting the murine Nhe1, Nhe2, and Nhe3 genes on intracellular pH regulation in this cell type. Our results demonstrate that activation of the Na\(^+\)/H\(^+\) exchanger NHE1 is responsible for the up-regulation observed during muscarinic stimulation and thus is the major regulator of intracellular pH in both resting and secreting cells. Some aspects of this work have been previously reported in preliminary form (34).
physiological salt solution (PSS) containing 135 mM NaCl, 5.4 mM KCl, 1 and 4 months.

Solutions—Microfluorometric experiments were carried out in a
physiological salt solution (PSS) containing 135 mM NaCl, 5.4 mM KCl, 1.2 mM CaCl₂, 0.8 mM MgSO₄, 0.33 mM NaHPO₄, 0.4 mM KH₂PO₄, 10 mM glucose, 20 mM Heps (pH 7.4 with NaOH), and 2 mM glutamine. Some experiments were performed in HCO₃⁻-free PSS containing an additional 10 mM NH₄Cl for 120 s and then switching back to NH₄⁺-free PSS. Na⁺/H⁺ exchanger activity was determined using the NH₄⁺-induced intracellular acid load. Recovery consisted of a rapid, initial near-linear increase in intracellular pH, followed by a slower recovery; thus, the recovery that comprised the linear portion was used to calculate the initial rate of pH recovery (pH units/min). The duration of the linear increase in intracellular pH, followed by a slower recovery of intracellular pH toward the initial resting intracellular pH (first prepulse in trace). Preincubation of acinar cells with the Na⁺/H⁺ exchanger inhibitor EIPA (5 µM) significantly reduced the initial rate of intracellular pH recovery (second prepulse). B, recovery from an NH₄⁺-induced intracellular acid load was measured at 530 nm. Intracellular pH was estimated by

EXPERIMENTAL PROCEDURES

Materials and Null Mutant Animals—Collagenase P was purchased from Roche Molecular Biochemicals, and BCECF/AM and EIPA were from Molecular Probes, Inc. (Eugene, OR). All other chemicals were obtained from Sigma. Targeted disruption of murine Na⁺/H⁺ exchanger isoforms Nhe1, Nhe2, and Nhe3 was carried out as described by Bell et al. (35), Schultheis et al. (20), and Schultheis et al. (19), respectively. Heterozygous offspring were used to establish breeding colonies in the University of Rochester vivarium. All animals were housed in micro-isolator cages with access to laboratory chow and water ad libitum with a 12-h light/dark cycle. Offspring were tail-clipped post-weaning, and genotypes were determined by polymerase chain reaction or by Southern blotting. All experiments were carried out on animals aged between 1 and 4 months.

Solutions—Microfluorometric experiments were carried out in a physiological salt solution (PSS) containing 135 mM NaCl, 5.4 mM KCl, 1.2 mM CaCl₂, 0.8 mM MgSO₄, 0.33 mM NaHPO₄, 0.4 mM KH₂PO₄, 10 mM glucose, 20 mM Heps (pH 7.4 with NaOH), and 2 mM glutamine. Some experiments were performed in HCO₃⁻-free PSS in which 25 mM NaCl was substituted with 25 mM NaHCO₃.

Acinar Cell Preparation and Measurement of Na⁺/H⁺ Exchanger Activity—Parotid acini (5–20 cells) were prepared from Nhe1, Nhe2, and Nhe3 littersmates of wild-type (+/+), heterozygous (+/−), and knockout (−/−) genotypes by collagenase digestion using the method described recently for mouse lacrimal gland (35). In brief, glands removed from male and female animals were minced in ice-cold Earle’s minimal essential medium (Biofluids, Inc., Rockville, MD) supplemented with 0.075 units/ml collagenase P, 2 mM glutamine, and 0.1% bovine serum albumin; incubated in the same medium at 37 °C for 75 min with continuous agitation (80 cycles/min); and periodically dispersed by trituration at 30, 45, 60, and 75 min. The final acinar preparation was resuspended in PSS containing 0.1% bovine serum albumin at 30 °C, top-gassed with 100% O₂, and loaded with the pH-sensitive fluorescent indicator BCECF by incubation with the membrane-permeant acetoxymethyl ester form of the probe (BCECF/FAM, 2 µM) for 30 min. Intracellular BCECF fluorescence was monitored in ratio mode from single acinar clumps adhering to the base of a superfusion chamber mounted on a Nikon Diaphot microscope interfaced with a Spex ARC Microfluorometer. Cells were excited at 495 and 433 nm using monochromators (0.5-µm slit width), and emitted fluorescence was measured at 530 nm. Intracellular pH was estimated by in situ calibration of the excitation ratio using the high K⁺/nigericin protocol as described previously (36).

Na⁺/H⁺ exchanger activity was determined using the NH₄⁺/NH₃ prepulse technique (37). Acidloading was accomplished by exposing cells to Na⁺-replete PSS containing an additional 10 mM NH₄Cl for 120 s and then switching back to NH₄⁺-free PSS. Na⁺/H⁺ exchanger activity was determined in unstimulated and stimulated cells by measuring the initial rate of intracellular pH recovery from an NH₄⁺-induced acid load. Recovery consisted of a rapid, initial near-linear increase in intracellular pH, followed by a slower recovery; thus, the recovery that comprised the linear portion was used to calculate the initial rate of pH recovery (pH units/min). The duration of the linear portion varied with genotype and experimental conditions. To compare Na⁺/H⁺ exchanger activities directly in stimulated and unstimulated cells, we used a paired NH₄⁺ prepulse protocol in which the same acinus was first acid-loaded in the absence of stimulation and then, following recovery, acid-loaded a second time in the presence of an agonist. In these experiments, the initial intracellular pH values for recovery rate calculations were normalized by the peak acidification value from the first pulse.

RESULTS

Loss of Intracellular pH Regulation in Acini Isolated from Nhe1 Null Mutants—Nhe1, Nhe2, and Nhe3 null mutant animals were used to determine the functional significance of each isoform to intracellular pH regulation in parotid acinar cells. Since inhibition of Na⁺/H⁺ exchange does not alter resting intracellular pH (data not shown), Na⁺/H⁺ exchanger activity must be low in unstimulated acinar cells. Thus, Na⁺/H⁺ exchanger activity was revealed by acid loading cells using an NH₄⁺ prepulse as described under “Experimental Procedures.” A representative intracellular pH trace obtained from an acinus isolated from a wild-type animal (+/+) is shown in Fig. 1A. In HCO₃⁻-free PSS, removal of 10 mM NH₄Cl led to an intracellular acidification, followed by an intracellular pH recovery at an initial rate of 0.57 pH units/min (n = 41). When intracellular pH recovery was complete, this maneuver was repeated, except that the Na⁺/H⁺ exchange inhibitor EIPA (5 µM)
was added 30 s prior to NH$_4^+$ removal. This concentration of EIPA is known to induce near-maximal inhibition of NHE activity in PS120 cells expressing rat NHE1 or NHE2, but not NHE3 (25), NHE4 (38), or NHE5 (12, 13). Consistent with these observations, 5 μM EIPA reduced the rate of intracellular pH recovery to 0.09 pH units/min, an inhibition of >80% (n = 14), thus indicating the absence of NHE3, NHE4, or NHE5 and, most likely, the presence of NHE1 and/or NHE2 activity in mouse parotid acinar cells. Acini isolated from heterozygous (Nhe1$^{+/−}$) animals also completely recovered their intracellular pH, at a comparable initial rate (0.59 pH units/min, n = 11), and this recovery was also markedly inhibited by 5 μM EIPA (~90%; data not shown). In contrast, virtually no recovery from an acid load was observed in acini from Nhe1$^{−/−}$ mutant mice (Fig. 1B), i.e. disrupting the Nhe1 locus mimicked the inhibitory effect of EIPA. Fig. 1 also shows that acini isolated from both Nhe2 (panel C) and Nhe3 (panel D) null mutant mice possessed intracellular pH recovery rates comparable to those from +/- animals (a summary of the above results is given in Fig. 2). These results demonstrate that NHE1 is the dominant regulator of intracellular pH in unstimulated mouse parotid acinar cells.

Regulation of Intracellular pH during Muscarinic Stimulation and Cell Shrinkage—Na$^+/H^+$ exchanger activity is up-regulated in salivary gland acinar cells in two distinct phases during muscarinic agonist-induced fluid secretion: (i) an initial rapid increase in activity induced by the cell shrinkage associated with salt loss via the apical Cl$^−$ and basolateral K$^+$ channels and (ii) a secondary, slower Ca$^{2+}$-dependent up-regulation that is independent of protein kinase C and calmodulin (26–29). To determine which NHE isoform(s) regulates intracellular pH during fluid secretion, we first investigated the effect of the muscarinic agonist carbachol on Na$^+/H^+$ exchanger activity in wild-type and null mutant mice. Fig. 3A shows that application of 10$^{-5}$ M carbachol (a near-maximal concentration) 30 s prior to acid loading in acinar cells from +/- mice induced a significant up-regulation of Na$^+/H^+$ exchanger activity (n = 17). Up-regulation reflected both a 2.3-fold increase in the initial rate of exchanger-mediated intracellular pH recovery (compare rates shown in Figs. 2 and 4A) and an alkaline shift in the intracellular pH sensitivity (Fig. 4B). The muscarinic-induced alkaline shift generated a subsequent intracellular alkalinization (0.13-pH unit increase) relative to the initial resting intracellular pH and is consistent with previous observations for rat submandibular (28) and parotid (23) acini. However, both of these up-regulatory events were completely abolished in acini...
from Nhe1<sup>−/−</sup> mice (Fig. 3B). In contrast, up-regulation remained intact in acinar cells from NHE2-deficient mice (Fig. 3C) and mice lacking NHE3 (Fig. 3D). These data directly demonstrate that NHE1 is the major Na<sup>+</sup>/H<sup>+</sup> exchanger isoform up-regulated during muscarinic agonist-induced fluid secretion. A summary of the effects of carbachol stimulation on the increase in the initial rate of exchanger-mediated intracellular pH recovery (panel A) and the alkalinization of the intracellular pH (panel B) is given in Fig. 4.

Intracellular Cl<sup>−</sup> concentration falls by 20–30% during muscarinic agonist-stimulated fluid secretion, thereby changing the extra- to intracellular ion gradients and causing a concomitant decrease in cell volume (39). Cell shrinkage activates Na<sup>+</sup>/H<sup>+</sup> exchanger activity in salivary acinar cells (27). To determine which NHE isoform(s) are up-regulated in response to hypertonic-induced cell shrinkage, acini were shrunken by the addition of sucrose (60 mM; 180 s prior to NH<sub>4</sub><sup>+</sup> removal). Fig. 5A shows that sucrose, like carbachol, up-regulated both phases of intracellular pH recovery in +/+ acini (rate of recovery = 0.92 pH units/min; alkalinization relative to original resting intracellular pH = 0.14 pH units, n = 13). This cell shrinkage-induced response was completely inhibited in acinar cells lacking NHE1 (Fig. 5B), but was intact in acinar cells from Nhe2<sup>−/−</sup> and Nhe3<sup>−/−</sup> mice (Fig. 5, C and D). These results show that NHE1 is responsible for the cell shrinkage-induced component of intracellular pH regulation associated with fluid secretion.

Role of NHE1 in Buffering the Intracellular Acid Load Resulting from HCO<sub>3</sub><sup>−</sup> Secretion—The results described above demonstrate that Na<sup>+</sup>/H<sup>+</sup> exchanger isoform NHE1 directly regulates the recovery from an acid load in unstimulated and stimulated acinar cells. However, the Na<sup>+</sup>/H<sup>+</sup> exchanger in salivary gland acinar cells is known, under physiological conditions, to buffer the intracellular acid load that results from increased metabolic activity and secretion of HCO<sub>3</sub><sup>−</sup> through the nonselective apical anion channel (1–3). To test whether NHE1 mediates this intracellular pH recovery, we stimulated wild-type (+/+ ) and null mutant (−/−) acini with 10<sup>−5</sup> M carbachol in HCO<sub>3</sub><sup>−</sup>-replete PSS. As shown in Fig. 6A (+/+ , upper trace), application of carbachol induced a rapid acidification of 0.25 pH units (n = 16), followed by a progressive recovery of intracellular pH to the original value within 200 s in a +/+ acinus. In contrast, Nhe1 null mutant acini (−/−, lower trace) exhibited a significantly enhanced acidification (0.40 pH units, n = 11) and a dramatically reduced rate of intracellular pH recovery and magnitude of intracellular alkalinization in parotid gland acini isolated from Nhe1, Nhe2, and Nhe3 null mutant mice during muscarinic stimulation. Acinar cells were isolated and treated as described in the legend to Fig. 3. Data are summarized and presented as mean ± S.E. A, initial rate of pH recovery from intracellular acidification. The muscarinic agonist-stimulated rate of pH recovery from an acid load for acinar cells from Nhe1<sup>−/−</sup> mice was significantly less than the rate observed in acinar cells from wild-type controls (p < 0.0013). B, magnitude of the alkalinization beyond the original resting pH. +/+ , n = 17; Nhe1<sup>−/−</sup>, n = 11; Nhe2<sup>−/−</sup>, n = 5; and Nhe3<sup>−/−</sup>, n = 6.

**Fig. 5.** Hypertonic-induced cell shrinkage up-regulates NHE1. Experiments were performed as described in the legend to Fig. 1. Acini were shrunken by the addition of 60 mM sucrose during the time periods indicated (open bars). A, addition of sucrose up-regulates Na<sup>+</sup>/H<sup>+</sup> exchanger activity in +/+ acini (as evidenced by the two phases described in the legend to Fig. 3). B, sucrose-induced up-regulation is abolished in Nhe1<sup>−/−</sup> parotid acini. C, the initial rate of Na<sup>+</sup>/H<sup>+</sup> exchanger-dependent, intracellular pH recovery in parotid gland acini isolated from Nhe1, Nhe2, and Nhe3 null mutant mice following hypertonic-induced cell shrinkage. Data are presented as mean ± S.E. The initial rate of pH recovery from an acid load for acinar cells from wild-type controls was significantly greater than the rate observed in acinar cells from Nhe1<sup>−/−</sup> mice (p < 0.002) following cell shrinkage. D, magnitude of the alkalinization of the intracellular pH recovery in parotid gland acini isolated from Nhe1, Nhe2, and Nhe3 null mutant mice following hypertonic-induced cell shrinkage. Data are presented as the magnitude of the alkalinization beyond the original resting pH (represented by the dashed line; mean ± S.E.). +/+ , n = 13; Nhe1<sup>−/−</sup>, n = 8; Nhe2<sup>−/−</sup>, n = 4; and Nhe3<sup>−/−</sup>, n = 4.
Lack of Na\(^+\)/H\(^+\) Exchanger Up-regulation in NHE1-deficient Mice

**Fig. 6.** NHE1 buffers the intracellular acid load resulting from the muscarinic agonist-stimulated efflux of HCO\(_3^-\). A, BCECF-loaded parotid acini were perfused with PSS containing 25 mM NaHCO\(_3\) and stimulated with 10\(^{-5}\) M carbachol by the open bar. Upon stimulation, acini from +/- glands (upper trace) rapidly acidified and then recovered their intracellular pH due to up-regulation of Na\(^+\)/H\(^+\) exchanger activity (see “Results”). The magnitude of the initial acidification was enhanced in Nhe1\(^{-/-}\) acini, and the subsequent rate of intracellular pH recovery was significantly attenuated (lower trace). B, shown is the initial rate of Na\(^+\)/H\(^+\) exchanger-dependent, intracellular pH recovery in parotid gland acini isolated from wild-type and Nhe1 null mutant mice and from Nhe2 and Nhe3 null mutant mice following muscarinic-induced acidification. Data are presented as mean ± S.E. The initial rate of pH recovery from the muscarinic-induced acid load in acinar cells from wild-type controls was significantly greater than the rate observed in acinar cells from Nhe1\(^{-/-}\) mice (p < 0.008). C, shown is the magnitude of the muscarinic-induced acidification in the presence of HCO\(_3^-\) in parotid gland acini isolated from wild-type and null mutant mice and from Nhe2 and Nhe3 null mutant mice following hypertonic-induced cell shrinkage. Data are presented as the magnitude of the acidification below the original resting pH (mean ± S.E.): +/-: +/+; n = 16; Nhe1\(^{-/-}\): n = 11; Nhe2\(^{-/-}\): n = 4; and Nhe3\(^{-/-}\): n = 3.

**Fig. 7.** NHE1 is required for the muscarinic-stimulated alkalinization in the absence of HCO\(_3^-\). Shown is the effect of 10\(^{-5}\) M carbachol on intracellular pH in the absence of extracellular HCO\(_3^-\). Stimulation induced an intracellular alkalinization in +/- acini (upper trace) due to up-regulation of the Na\(^+\)/H\(^+\) exchanger (see “Results”). This effect was abolished (became an intracellular acidification) in Nhe1\(^{-/-}\) acini (lower trace). In the absence of extracellular HCO\(_3^-\), the resting intracellular pH was reduced in wild-type acinar cells; thus, for illustrative purposes, the +/- trace is offset by +0.3 pH units. Traces are representative of six or more examples in each case.

**DISCUSSION**

Muscarinic stimulation produces copious amounts of parotid saliva, equivalent to ~5% of the whole body water content/h in mice (41). This vigorous rate of secretion is associated with a dramatic up-regulation of Na\(^+\)/H\(^+\) exchanger activity, which alkalinizes the cytoplasm of salivary gland acinar cells (1–3). The NHE-induced intracellular alkalinization contributes to fluid secretion by promoting both Cl\(^-\)/HCO\(_3^-\) exchanger-mediated Cl\(^-\) uptake and HCO\(_3^-\) secretion via the apical anion channel (1, 42). Despite appreciation of the physiological significance of this important transport mechanism, the molecular identity of the NHE isoform(s) involved in regulating acinar cell intracellular pH was unknown. Thus, the major objective of this study was to directly test the functional significance of individual NHE proteins by studying the effects of gene disruption on intracellular pH regulation and muscarinic-induced up-regulation of Na\(^+\)/H\(^+\) exchanger activity in mouse parotid acinar cells.

Unstimulated acini from Nhe1\(^{-/-}\) null mutant animals were incapable of intracellular pH recovery after acid loading. Thus, in the absence of NHE1, other Na\(^+\)/H\(^+\) exchangers present in acinar cells fail to mediate a significant intracellular pH recovery toward original resting values. Consistent with this observation, acinar cells from Nhe2 and Nhe3 null mutant mice recovered like acini from Nhe1 wild-type mice (Fig. 2). The dominance of NHE1 in regulating overall intracellular pH in unstimulated mouse parotid acini is consistent with its well-documented “housekeeping” function (11, 16, 17). Furthermore, the intracellular pH recovery rate observed in acini from Nhe1 heterozygous mice was comparable to that seen in Nhe1 wild-type mice, suggesting that the aberrant transcripts generated by disruption of the Nhe1 gene (35) do not produce a protein that exerts a dominant-negative effect.

The importance of the Na\(^+\)/H\(^+\) exchanger in regulating intracellular pH during active fluid secretion has been well documented in salivary glands (1–3, 26–30). Stimulated fluid secretion is associated with an intracellular alkalinization (1–3). Consistent with previous studies of the mechanisms involved in the alkalinization process (26–30), the current results demonstrate that both muscarinic stimulation and cell shrinkage increased the rate and magnitude of intracellular pH recovery in mouse parotid acinar cells, suggesting increased affinity for intracellular H\(^+\), i.e. an “alkaline shift.” Thus, this study directly establishes that NHE1 is the major Na\(^+\)/H\(^+\) exchanger isoform up-regulated in acinar cells by muscarinic agonists and by cell shrinkage (Figs. 4 and 5). Nevertheless, because NHE2 is localized to the apical membrane in rat submandibular aci-
Cl cellular \[\text{HCO}_3^-\] increases intracellular pH at a higher value (and hence a higher intra-acinar cells during muscarinic stimulation. By maintaining the \[\text{Ca}^{2+}\] concentration elevated \[\text{Ca}^{2+}\] cell shrinkage, and/or \text{Cl}^- loss plays a central role and that this increased activity is independent of protein kinase C, calmodulin, and phosphorylation of NHE1 (8, 23, 26, 28). A null background affords, by expression of a mutated form of the \text{NHE1} gene, a unique opportunity to perform in vivo structure/function analysis to determine the exact mechanism for activation.

In summary, this study provides the first direct documentation that up-regulation of \text{NHE1} is responsible for the enhanced \text{Na}^+/\text{H}^+ exchanger activity observed in salivary gland acinar cells during muscarinic stimulation. By maintaining the intracellular pH at a higher value (and hence a higher intracellular \[\text{HCO}_3^-/\text{Cl}^-\] exchange (42), thereby providing a greater driving force for \text{Cl}^- and \text{HCO}_3^- exit (1–3) through the pH-sensitive apical anion channel (15).

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