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Muscarinic receptor-induced acidification in sublingual mucous acinar cells: loss of pH recovery in Na^+–H^+ exchanger-1 deficient mice

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1. Intracellular pH (pH_i) plays an important role in regulating fluid and electrolyte secretion by salivary gland acinar cells. The pH-sensitive, fluorescent dye 2′,7′-bis(carboxyethyl)-5(6)-carboxylfluorescein (BCECF) was used to characterize the mechanisms involved in regulating pH_i during muscarinic stimulation in mouse sublingual mucous acinar cells.

2. In the presence of HCO_3^-, muscarinic stimulation caused a rapid decrease in pH_i ($0{\cdot}24 \pm 0{\cdot}02$ pH units) followed by a slow recovery rate ($0{\cdot}042 \pm 0{\cdot}002$ pH units min^{-1}) to the initial resting pH_i in sublingual acinar cells. The muscarinic receptor-induced acidification in parotid acinar cells was of a similar magnitude ($0{\cdot}25 \pm 0{\cdot}02$ pH units), but in contrast, the recovery rate was ~4-fold faster ($0{\cdot}181 \pm 0{\cdot}005$ pH units min^{-1}).

3. The agonist-induced intracellular acidification was inhibited by the anion channel blocker niflumate, and was prevented in the absence of HCO_3^- by treatment with the carbonic anhydrase inhibitor methazolamide. These results indicate that the muscarinic-induced acidification is due to HCO_3^- loss, probably mediated by an anion conductive pathway.

4. The Na^+–H^+ exchange inhibitor 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA) amplified the magnitude of the agonist-induced acidification and completely blocked the Na^+-dependent pH_i recovery.

5. To examine the molecular nature of the Na^+–H^+ exchange mechanism in sublingual acinar cells, pH regulation was investigated in mice lacking Na^+–H^+ exchanger isoforms 1 and 2 (NHE1 and NHE2, respectively). The magnitude and the rate of pH_i recovery in response to an acid load in acinar cells isolated from mice lacking NHE2 were comparable to that observed in cells from wild-type animals. In contrast, targeted disruption of the *Nhe1* gene completely abolished pH_i recovery from an acid load. These results demonstrate that NHE1 is critical for regulating pH_i during a muscarinic agonist-stimulated acid challenge and probably plays an important role in regulating fluid secretion in the sublingual exocrine gland.

6. In NHE1-deficient mice, sublingual acinar cells failed to recover from an acid load in the presence of bicarbonate. These results confirm that the major regulatory mechanism involved in pH_i recovery from an acid load is not Na^+–HCO_3^- cotransport, but amiloride-sensitive Na^+–H^+ exchange via isoform 1.

The regulation of intracellular pH (pH_i) in epithelial cells is critical for maintaining normal enzyme activity as well as for modulating fluid and electrolyte absorption and secretion (Aronson, 1985). There are several ion transport pathways involved in epithelial pH_i regulation including Na^+–H^+ exchangers, Cl^-–HCO_3^- exchangers and Na^+–HCO_3^- cotransporters (Geibel *et al.* 1990; Kopito, 1990; Steward *et al.* 1996). In salivary acinar cells, Na^+–H^+ exchange plays a significant role in regulating Cl^-- and HCO_3^--dependent fluid secretion during muscarinic stimulation via at least two mechanisms. Upregulation of Na^+–H^+ exchanger activity maintains a neutral intracellular pH, thereby enhancing the production of HCO_3^- (Turner, 1993) and the activity of the intracellular pH-sensitive anion channel (Arreola *et al.* 1995). Moreover, Na^+–H^+ and Cl^-–HCO_3^- exchangers act in concert to drive NaCl uptake in exchange for H^+ and HCO_3^- loss across the basolateral membrane, thereby, increasing the intracellular [Cl^-] and enhancing Cl^- efflux through apical anion channels (Case *et al.* 1984; Melvin *et al.* 1988; Brown *et al.* 1989; Lau *et al.* 1989).

The magnitude and duration of the resulting stimulation-induced cytosolic acidification are thus regulated by Na^+–H^+ exchanger activity. Four distinct isoforms of Na^+–H^+ exchangers (NHE1–NHE4) with different kinetics and pharmacological properties have been identified in epithelial tissues (Orlowski *et al.* 1992; Wang *et al.* 1993; Bookstein *et al.* 1994*b*). NHE1 is ubiquitously expressed and is thought to be involved in maintaining the intracellular pH homeostasis and cell volume (Noel & Pouyssegur, 1995), whereas NHE2–NHE4 show a more limited tissue distribution and are thought to be involved in organ-specific functions such as NaCl absorption (Biemesderfer *et al.* 1993; Bookstein *et al.* 1994*a*; Schultheis *et al.* 1998*a*,*b*). More recently, the cloning and expression of NHE5 (Attaphitaya *et al.* 1999; Baird *et al.* 1999) and NHE6 (Numata *et al.* 1998) have also been described. High level expression of NHE5 is restricted to the brain, whereas NHE6 expression appears to be restricted to mitochondria.

Multiple NHE isoforms are expressed in a salivary gland-specific manner (He *et al.* 1997; Lee *et al.* 1998; Park *et al.* 1999). It appears that NHE1 is the major isoform mediating recovery from an intracellular acid challenge in both rat (Robertson *et al.* 1997; Park *et al.* 1999) and mouse parotid serous acinar cells (Evans *et al.* 1999). Subsequent to the muscarinic agonist-induced acidification, a rapid NHE-dependent pH_i recovery occurs in parotid and submandibular acinar cells (Lau *et al.* 1989; Soltoff *et al.* 1989; Steward *et al.* 1989), and in mouse parotid this recovery has been directly linked to NHE1 expression (Evans *et al.* 1999). In contrast, rat sublingual mucous acini show little pH_i recovery in response to an agonist-induced acidification (Zhang *et al.* 1992).

The mechanism for the observed variability in response to stimulation in different salivary glands is not known, but it probably reflects either a different acidification mechanism or the expression of different Na^+–H^+ exchanger isoforms. To address this issue, we investigated the pH_i regulatory mechanisms activated during stimulation in mouse sublingual mucous acinar cells. We show that the intracellular acidification induced by muscarinic stimulation is due to HCO_3^- loss mediated by an anion conductive pathway. The magnitude and the duration of this acidification correlate with the activity of an EIPA-sensitive Na^+–H^+ exchanger, consistent with NHE1 or NHE2 expression (Park *et al.* 1999), but not NHE3 or NHE4 expression (Chambrey *et al.* 1997; Park *et al.* 1999). Targeted disruption of the *Nhe1* and *Nhe2* genes demonstrate that NHE1, but not NHE2, is essential for regulating pH_i during muscarinic stimulation and, therefore, is important for regulating fluid secretion in the mouse sublingual gland. Furthermore, the lack of pH_i recovery in HCO_3^--containing medium demonstrates the absence of Na^+–HCO_3^- contransporter activity. Some aspects of this work have been previously reported in abstract form (Nguyen & Melvin, 1999).

METHODS

Materials and solutions

The acetoxymethyl ester form of 2′-7′-bis(carboxyethyl)-5-carboxyfluorescein (BCECF-AM) and 5-(*N*-ethyl-*N*-isopropyl) amiloride (EIPA) were purchased from Molecular Probes (Eugene, OR, USA). Collagenase P was from Boehringer-Mannheim GmbH, (Penzberg, Germany), and all other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA).

HCO_3^--free solutions contained (mM): 135 NaCl, 5·4 KCl, 0·4 KH_2PO_4, 0·33 NaH_2PO_4, 0·8 $MgSO_4$, 1·2 $CaCl_2$, 10 glucose, 20 Hepes, pH 7·4 with Tris-Base. HCO_3^--containing solutions contained (mM): 110 NaCl, 5·4 KCl, 0·4 KH_2PO_4, 0·33 NaH_2PO_4, 0·8 $MgSO_4$, 1·2 $CaCl_2$, 10 glucose, 20 Hepes and 25 $NaHCO_3$ (pH 7·4 with NaOH). When NH_4Cl was used to induce an acid load, 30 mM NaCl was replaced with NH_4Cl. Solutions containing HCO_3^- were equilibrated with 5% CO_2 and 95% O_2, whereas HCO_3^--free solutions were gassed with 100% O_2. The high K^+ solution used to calibrate the fluorescence signals contained (mM): 120 KCl, 20 NaCl, 0·8 $MgCl_2$, 20 Hepes and 0·005 nigericin, and the pH adjusted to the required value between 5·6 and 8.

Preparation of sublingual acinar cells

C57Bl/6 male and transgenic mice were fed *ad libitum* on a standard diet and water. Targeted disruptions of the murine *Nhe1* and *Nhe2* genes were previously performed as described by Bell *et al.* (1999) and Schultheis *et al.* (1998*a*), respectively. Heterozygous offspring were used to establish breeding colonies in the University of Rochester vivarium. Mice were rendered unconscious by exposure to a rising concentration of CO_2 gas and killed by exsanguination. The sublingual glands were quickly removed, trimmed of connective tissues, and finely minced in digestion medium (Eagle's modified essential medium, Biofluids, Inc., Rockville, MD, USA) containing collagenase P (0·3 mg per 7·5 ml per animal). The minced glands were incubated at 37 °C in a shaker with continuous agitation (100 cycles min^{-1}). After the first 20 min interval the minced sublingual glands were dispersed by gentle pipetting (10 times) with a 10 ml plastic pipette and centrifuged (210 *g* for 15 s). The supernatant was discarded and the pellet was re-suspended in 7·5 ml collagenase digestion medium for 40 min, at the end of which time the acinar cells were rinsed and harvested by centrifugation. The resulting sublingual acinar cell preparation was loaded with pH-sensitive fluoroprobe by incubation for 30 min at room temperature with BCECF-AM at a concentration of 2 μM. The BCECF-loaded acinar cells were continuously gassed with 100% O_2.

Fluorescence measurement of pH_i

BCECF-loaded acinar cells were allowed to adhere to the base of a superfusion chamber mounted on a Nikon Diaphot 200 microscope interfaced with an Axon Imaging Workbench system (Foster City, CA, USA). Cells were excited at 490 and 440 nm and emitted fluorescence was measured at 530 nm. Intracellular pH was estimated by *in situ* calibration of the ratio of fluorescence at 490nm to that at 440 nm (F_{490}/F_{440}) performed using the nigericin-high K^+ method of Thomas *et al.* (1979). The relationship between F_{490}/F_{440} and pH_i was linear over the pH range 6·4–7·6 ($n = 5$). Data presented in the figures are from single representative experiments. Values quoted are the means ± S.E.M. for the number of acinar aggregates examined. All experiments were performed with three or more separate preparations.

RESULTS

Muscarinic agonist stimulation induces HCO_3^--dependent intracellular acidification in sublingual acinar cells

The data presented in Fig. 1*A* show the effects of stimulation with the muscarinic agonist carbachol (Cch) on the pH_i of mouse mucous sublingual and serous parotid acini in HCO_3^--containing medium. Stimulation with 10 μM Cch resulted in a rapid (half-time, ~40 s, $n = 7$) decrease in pH_i ($-0{\cdot}24 \pm 0{\cdot}02$ pH units), which slowly recovered towards the pre-stimulation level in sublingual acinar cells. This is considerably slower ($0{\cdot}042 \pm 0{\cdot}002$ pH units min^{-1}) than the recovery rate seen in acinar cells from other types of salivary glands (Lau *et al.* 1989; Soltoff *et al.* 1989; Steward *et al.* 1989), including mouse parotid acinar cells (Fig. 1*A*; $0{\cdot}181 \pm 0{\cdot}005$ pH units min^{-1}, $n = 7$). This transient decrease in pH_i in response to muscarinic stimulation has been attributed to HCO_3^- efflux in the rat parotid, rat sublingual and rabbit mandibular glands (Melvin *et al.* 1988; Nauntofte & Dissing, 1988; Lau *et al.* 1989; Steward *et al.* 1989; Zhang *et al.* 1992). To investigate the HCO_3^- dependence of the muscarinic agonist-induced acidification, mouse sublingual acinar cells were stimulated in a HCO_3^--free medium. Figure 1*B* shows that the acidification was not completely abolished; however, the magnitude of the carbachol-induced acidification was significantly reduced in HCO_3^--free medium (> 50 % less compared with that observed in the presence of HCO_3^-; pH_i, $-0{\cdot}10 \pm 0{\cdot}04$ pH units, $n = 5$). The residual

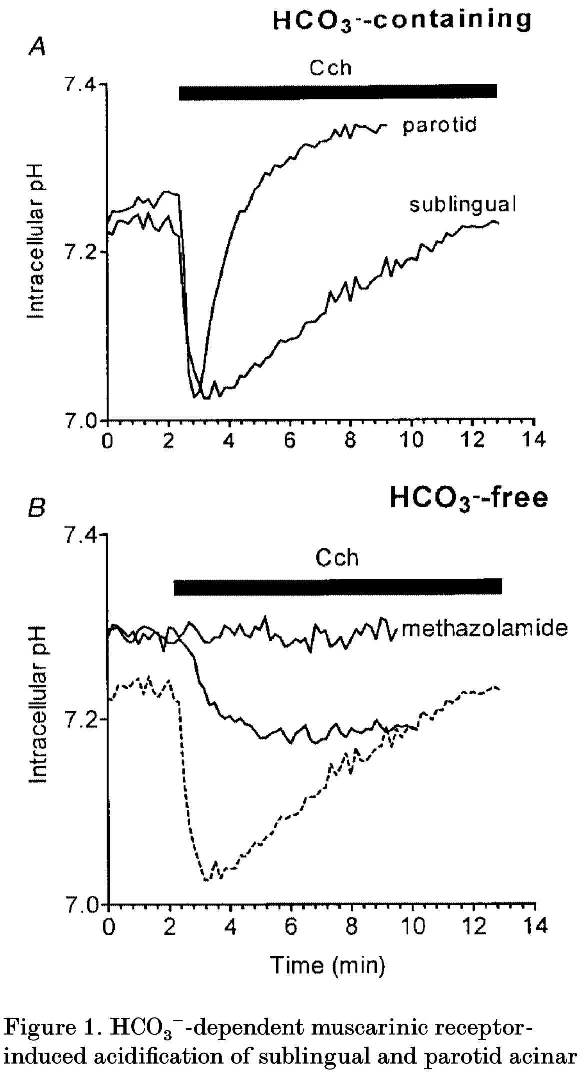

Figure 1. HCO_3^--dependent muscarinic receptor-induced acidification of sublingual and parotid acinar cells

Acinar cells were isolated and loaded with BCECF as described in Methods. *A*, the pH_i response of sublingual and parotid acinar cells in the presence of 25 mM HCO_3^- to 10 μM carbachol (Cch) during the time period indicated by the filled bar. *B*, the effect of Cch on the pH_i in sublingual acini perfused with HCO_3^--free medium in the absence or presence of the carbonic anhydrase inhibitor methazolamide (1 mM). For comparison to the response of acinar cells in HCO_3^--containing medium, the sublingual acini trace from *A* is also shown (dashed line).

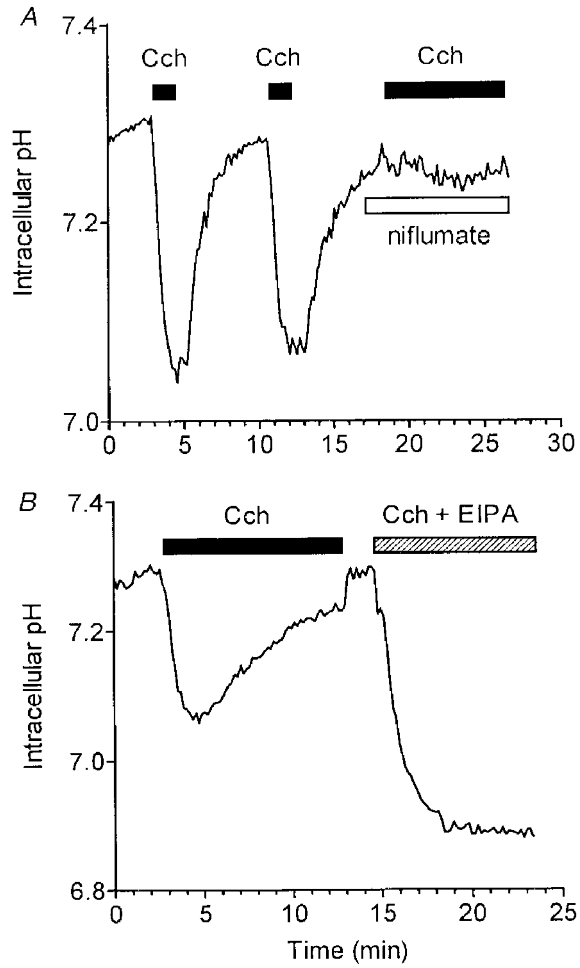

Figure 2. Inhibitory effects of niflumate on agonist-induced HCO_3^- efflux and EIPA-sensitive pH_i recovery

BCECF-loaded acini were perfused with HCO_3^--containing medium. *A*, acini were stimulated with 10 μM Cch for two 30 s periods as indicated by the filled bars, and then with Cch after the addition of 50 μM niflumate during the period indicated by the open bar. *B*, acini were stimulated with 10 μM Cch as indicated by the filled bar. After removal of Cch for 2 min, acini were again stimulated by Cch in the presence of 10 μM EIPA during the interval indicated by the hatched bar.

acidification in the absence of extracellular HCO_3^- may be due to the increased production of metabolic acid or to the efflux of HCO_3^- generated by intracellular carbonic anhydrase. To test this latter possibility, the effect of the carbonic anhydrase inhibitor methazolamide was examined. Figure 1*B* demonstrates that the acidification induced by carbachol was totally abolished when acini ($n = 7$) were perfused in HCO_3^--free medium containing 1 mM methazolamide in all cases.

In other salivary gland acinar cells, the agonist-induced HCO_3^- efflux is thought to occur primarily via an anion conductance channel (Melvin *et al.* 1988; Steward *et al.* 1996). To test this possibility in mouse sublingual acini, the effect of the anion channel inhibitor niflumate on the HCO_3^--dependent acidification was determined. Figure 2*A* shows that repetitive 30 s exposures to Cch produced intracellular acidifications of similar rate and magnitude, whereas 50 μM niflumate inhibited the drop in pH_i by >95% ($n = 7$).

During sustained carbachol stimulation, the niflumate-sensitive, HCO_3^--dependent acidification slowly recovers to the original unstimulated pH_i. Recovery from an acid load in most mammalian cells is mediated by a Na^+–H^+ exchange mechanism (Alpern, 1990). Addition of 10 μM of the Na^+–H^+ exchange inhibitor EIPA (or removal of extracellular Na^+, data not shown) to carbachol-stimulated acini resulted in a nearly 2-fold amplification of the agonist-induced decrease in pH_i, and completely blocked pH_i recovery (Fig. 2*B*). These results suggest that an amiloride-sensitive Na^+–H^+ exchanger, probably NHE1 or NHE2, is the primary transport mechanism involved in the recovery of pH_i during muscarinic stimulation in sublingual mucous acinar cells.

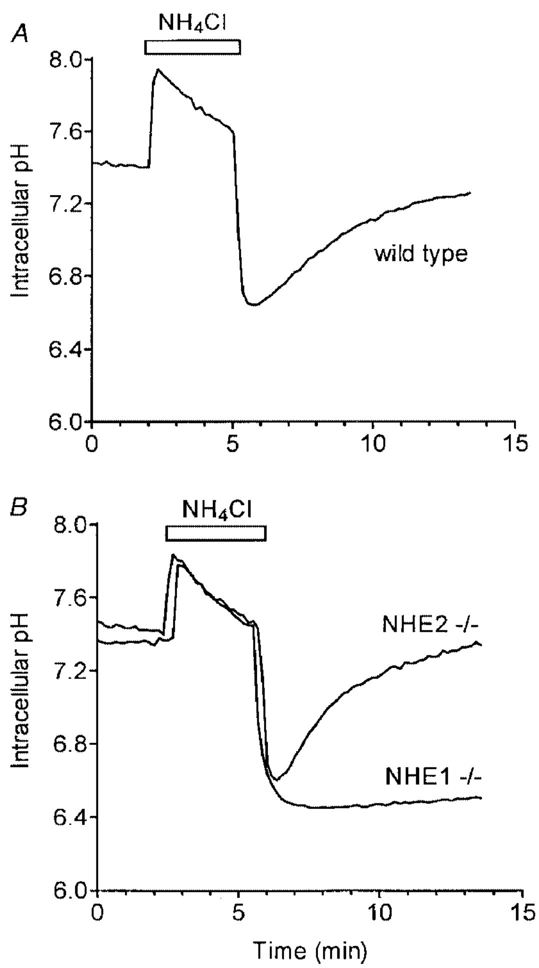

Figure 3. Loss of pH_i recovery in sublingual mucous acinar cells from NHE1-deficient mice

BCECF-loaded acini were perfused with HCO_3^--free, Na^+-containing medium and then acid loaded by a 3 min exposure to NH_4Cl. *A*, pH_i recovery in acini isolated from NHE1 wild-type mice. *B*, loss of pH_i recovery in acini from NHE1-deficient mice (NHE1 −/−); and pH_i recovery in acinar cells from mice lacking NHE2 expression (NHE2 −/−) was comparable to that seen in wild-type mice (*A*).

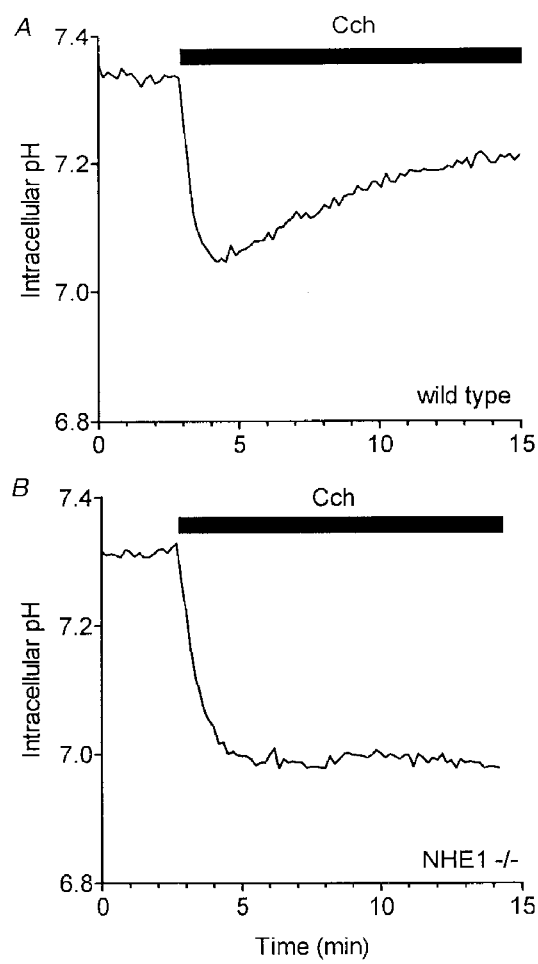

Figure 4. Intracellular pH_i recovery from Cch-stimulated acidification is absent in acini from NHE1-deficient mice

Sublingual acinar cells were loaded with BCECF. *A*, pH_i response of acinar cells isolated from wild-type mice in the presence of 25 mM HCO_3^- to 10 μM Cch during the time period indicated by the filled bar. *B*, loss of the pH_i recovery in acini from mice lacking expression of NHE1 (NHE1 −/−).

Loss of pH_i recovery in NHE1-deficient sublingual mucous acinar cells

The strong EIPA sensitivity (see Fig. 2) of the intracellular pH recovery in sublingual acinar cells indicates that either NHE1 or NHE2 is involved in the alkalinization process (Chambrey *et al.* 1997; Park *et al.* 1999). To directly test the involvement of NHE1 and NHE2 in pH_i regulation, sublingual acinar cells from NHE1- and NHE2-deficient mice were acid loaded by a short exposure to NH_4Cl. Figure 3*A* shows that in cells from wild-type mice, the intracellular pH rapidly recovered towards the initial pH level (initial recovery rate, 0·23 ± 0·01 pH units min^{-1}, comparable to the rate seen in sublingual acinar cells isolated from C57Bl/6 mice, 0·25 ± 0·01 pH units min^{-1}, data not shown). This pH_i recovery was absent under the same experimental conditions in acini from mice lacking NHE1 (Fig. 3*B*), but was present in acini isolated from NHE2 −/− mice, and recovered with an initial rate of 0·22 ± 0·01 pH units min^{-1} (Fig. 3*B*). Furthermore, when acini were stimulated with 10 μM carbachol in the presence of HCO_3^-, an initial rapid cytosolic acidification (pH_i 7·05 ± 0·03) was observed, followed by a slow pH_i recovery (0·039 ± 0·001 pH units min^{-1}) in acini from wild-type animals (Fig. 4*A*; $n = 6$). In contrast, the initial cytosolic acidification was greater (6·98 ± 0·02 pH units), and the pH_i recovery was abolished in acini from NHE1 −/− mice (Fig. 4*B*; $n = 5$). Therefore, these data demonstrate that NHE1 is the major Na^+-H^+ exchanger isoform regulating pH_i in sublingual mucous acinar cells during a muscarinic agonist-induced acid challenge.

Absence of $Na^+-HCO_3^-$ cotransport in mucous sublingual acinar cells

Although Na^+-H^+ exchange is the dominant pH_i regulatory mechanism in mouse sublingual acinar cells, it is not clear whether $Na^+-HCO_3^-$ cotransport activity may also contribute under physiological conditions. To test this possibility, acinar cells were acid loaded in the presence of HCO_3^-. Figure 5*A* shows that acinar cells from wild-type mice displayed an EIPA-sensitive pH_i recovery ($n = 7$), whereas acinar cells from mice lacking NHE1 activity (NHE1 −/−) failed to respond to an acid load (Fig. 5*B*; $n = 8$). These results demonstrate that mouse sublingual acinar cells lack $Na^+-HCO_3^-$ cotransporter activity.

DISCUSSION

Saliva formation is dependent upon the coordinated activity of multiple ion transport mechanisms including pH_i regulatory proteins such as Na^+-H^+ and $Cl^--HCO_3^-$ exchangers (Turner, 1993; Cook *et al.* 1994) and $Na^+-HCO_3^-$ cotransporters (Steward *et al.* 1996). Intracellular pH regulatory proteins in most types of exocrine glands are generally similar, although there are distinct differences as well. For example, $Cl^--HCO_3^-$ exchange is expressed in the acinar cells of parotid and submandibular salivary glands of several species (Turner & George, 1988; Lee *et al.* 1999), but not in rat sublingual (Zhang *et al.* 1992) or human labial gland acini (Valdez *et al.* 1994). All exocrine glands appear to acidify in response to muscarinic stimulation (Nauntofte & Dissing, 1988; Lau *et al.* 1989; Soltoff *et al.* 1989; Steward *et al.* 1996), including the mucous sublingual gland (Zhang *et al.* 1992). However, unlike other exocrine glands (Nauntofte & Dissing, 1988; Lau *et al.* 1989; Soltoff *et al.* 1989; Steward *et al.* 1996), the rate and magnitude of pH_i recovery from this acid challenge are substantially less in sublingual gland acinar cells (Zhang *et al.* 1992).

HCO_3^- efflux upon muscarinic stimulation

The intracellular acidification evoked by muscarinic stimulation in salivary gland acinar cells has been attributed to HCO_3^- flux through non-specific anion channels (Melvin

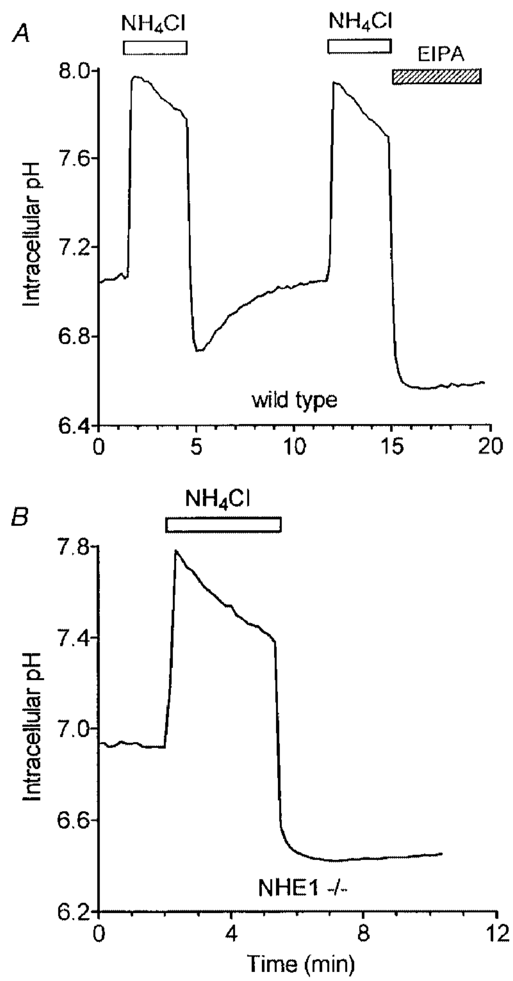

Figure 5. Lack of HCO_3^--dependent pH_i recovery in mucous sublingual acinar cells

BCECF-loaded acini were perfused in HCO_3^--containing solutions (see Methods). Acidification in acini was induced by exposure to 30 mM NH_4Cl for approximately 3 min. *A*, after recovery, acini isolated from NHE1 wild-type mice were acid loaded again in the presence of 10 μM EIPA. *B*, lack of pH_i recovery in acini from NHE1-deficient mice in the presence of HCO_3^-.

et al. 1988; Brown *et al.* 1989; Lau *et al.* 1989). In the present study, the agonist-induced intracellular acidification was significantly reduced in the absence of HCO_3^-, and the residual acidification observed in HCO_3^--free medium was abolished by the carbonic anhydrase inhibitor methazolamide (Fig. 1). These observations indicate, as in other exocrine glands, that HCO_3^- efflux underlies the acidification in sublingual acinar cells. Furthermore, HCO_3^- apparently exits the cells via a non-selective, niflumate-sensitive, anion channel (Fig. 2), consistent with the results found in other exocrine gland acinar cells (Melvin *et al.* 1988; Nauntofte & Dissing, 1988; Lau *et al.* 1989; Steward *et al.* 1996). Thus, it appears that the blunted pH_i recovery observed in sublingual acinar cells does not reflect a unique acidification mechanism, but more probably represents a difference in the mechanism involved in the extrusion of acid equivalents.

Na^+–H^+ exchange mediates the agonist-induced pH_i recovery

Recovery from an acid challenge in sublingual acinar cells required extracellular Na^+, was blocked by EIPA, and was independent of HCO_3^-. Our initial interpretation of these results is that Na^+–HCO_3^- cotransport does not play a significant role in pH_i in this gland, but that the predominant alkalinization mechanism is an EIPA-sensitive Na^+–H^+ exchanger. This is in agreement with reports documenting the presence of a Na^+–H^+ exchange mechanism in the acinar cells of other exocrine glands (Melvin *et al.* 1988; Muallem & Loessberg, 1990; Robertson *et al.* 1997). The absence of a Na^+–HCO_3^- cotransporter in mouse sublingual glands is not surprising since the composition of rodent saliva is Cl^- rich and the intracellular HCO_3^- is apparently derived exclusively from the action of carbonic anhydrase on intracellular CO_2 and H_2O (Turner, 1993). This contrasts with the ovine parotid, where a basolateral Na^+–HCO_3^- cotransporter is responsible for the uptake of HCO_3^- and the production of a HCO_3^--rich saliva (Steward *et al.* 1996). Although an unlikely alternative mechanism, an EIPA-sensitive, DIDS-insensitive Na^+–HCO_3^- cotransporter, NBC3, has been recently described (Pushkin *et al.* 1999) that could potentially contribute to the pH_i recovery. However, the lack of pH_i recovery in response to an acid load under physiological conditions (in the presence of HCO_3^-) in sublingual acinar cells isolated from NHE1-deficient mice indicates that Na^+–HCO_3^-cotransport contributes little, if any, to intracellular pH regulation in this cell type (see Fig. 5).

Intracellular pH recovery in sublingual acinar cells isolated from mice lacking NHE1 or NHE2

NHE1 is expressed in the basolateral membrane of both ductal and acinar cells of rat parotid and submandibular glands (Robertson *et al.* 1997; Lee *et al.* 1998; Park *et al.* 1999), whereas NHE2 and NHE3 are seen in the apical membranes of duct cells (Lee *et al.* 1998; Park *et al.* 1999). The localization of the different NHE isoforms has not been reported for mouse salivary glands, nevertheless, the EIPA sensitivity of Na^+–H^+ exchanger activity in sublingual acinar cells (Fig. 2) predicts that either NHE1 or NHE2 is dominant. Therefore, sublingual acinar cells from NHE1- and NHE2-deficient mice were acid loaded to test directly the involvement of NHE1 and NHE2 in pH_i regulation. The pH_i recovery was absent in acini from mice lacking NHE1 but was present in acini isolated from NHE2 −/− mice. These data demonstrate that NHE1 is the major Na^+–H^+ exchanger isoform for regulating pH_i in sublingual mucous acinar cells during a muscarinic agonist-induced acid challenge, an important process required for driving Cl^-- and HCO_3^--dependent secretion via the apical, niflumate-sensitive anion channel.

In conclusion, our results determined that the muscarinic receptor-induced acidification in mouse sublingual acinar cells is mediated by a HCO_3^--dependent, niflumate-sensitive mechanism, most probably an anion channel (Zhang *et al.* 1995). Furthermore, we directly demonstrated that Na^+–HCO_3^- cotransporter activity is absent in these cells, and that the major intracellular pH regulating mechanism responsible for the recovery from an agonist-induced acid challenge is the Na^+–H^+ exchanger isoform NHE1. Thus, both the agonist-induced acidification and the pH_i recovery processes in mouse sublingual acinar cells are comparable to those previously described in other exocrine glands. What then is the mechanism responsible for the marked difference in the pH_i recovery response in sublingual gland acinar cells? The simplest explanation is that less NHE1 protein is expressed in mouse sublingual acinar cells, resulting in the 3- to 5-fold slower pH_i recovery rate ($0{\cdot}042 \pm 0{\cdot}001$ and $0{\cdot}181 \pm 0{\cdot}005$ pH units min^{-1} for sublingual and parotid acinar cells, respectively). Alternatively, a dramatic upregulation of NHE1 activity occurs in mouse (Evans *et al.* 1999) and rat parotid acinar cells (Melvin *et al.* 1988; Lau *et al.* 1989; Soltoff *et al.* 1989) that is absent in sublingual cells. Thus, differential regulation of NHE1 may be involved in the observed differences in rates of pH_i recovery. We are currently investigating such mechanisms.

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