Expression of the $\text{Na}^+\text{-HCO}_3^-$ cotransporter and its role in pHi regulation in guinea pig salivary glands

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The regulation of intracellular pH (pHi) in epithelial cells is critical for fluid and electrolyte absorption and secretion. Therefore, epithelial cells are equipped with diverse pHi regulatory mechanisms. However, there are some differences in the pHi regulatory mechanisms among species and gland types. In salivary acinar cells, the initial response to a fluid secretion stimulus is an acidification of the cytosol resulting from a HCO$_3^-$ efflux into the lumen (10, 13, 19, 37), and the secreted HCO$_3^-$ plays a role in buffering the acid produced by oral bacteria and in providing an optimal pH for salivary amylase activity (6).

It is known that intracellular HCO$_3^-$ is generated by carbonic anhydrase, which converts the CO$_2$ that has freely diffused into the cells from the blood into H$^+$ and HCO$_3^-$ (21). The H$^+$ generated during this HCO$_3^-$ production is then extruded out-
containing solution [HCO₃⁻-buffered solution (BBS)]. 25 mM NaCl was replaced with an equal concentration of NaHCO₃. BBS was gassed with 5% CO₂-95% O₂. Na⁺-free solutions were prepared by replacing Na⁺ with N-methyl-d-glucamine (NMDG). NH₄⁺-containing solutions were prepared by replacing 20 mM Na⁺ with 20 mM NH₄⁺. Cl⁻-free solutions were prepared by replacing Cl⁻ with an equimolar amount of gluconic acid. Additional Ca²⁺ was added to compensate for chelation by gluconate. NBC1 and NBC3 antibodies were purchased from Chemicon (Temecula, CA).

Saliva collection and determination of HCO₃⁻ concentrations. All animals were used according to the protocol of the Animal Care and Use Committee of Seoul National University. Male guinea pigs weighing 400–500 g were anesthetized intraperitoneally using pentobarbital sodium (50 mg/kg). Resting whole saliva of the guinea pig was collected over two 5-min intervals by aspiration. The collection of stimulated saliva was done by a subcutaneous injection of pilocarpine (80 mg/kg). Resting and stimulated human whole saliva were obtained from five healthy volunteers. In human subjects, stimulated saliva was collected using 3% citric acid to cannulate the interlobular excretory duct under the stereomicroscope. Isolated interlobular ducts were then microdissected under the stereomicroscope. Dissected interlobular ducts were placed in storage buffer on ice until the pH was measured. We constructed the cannula using a yellow tip to make its diameter small enough to cannulate the interlobular excretory duct under the stereomicroscope. The microdissected interlobular duct was first cannulated by a modified yellow tip, and its tip was then connected again with the polyethylene cannula (inner diameter 0.28 mm and outer diameter 0.61 mm) for the microperfusion study. A DAD-12 (ALA Scientific Instruments, Westbury, NY) system with a controlled pressure of N₂ gas (30–80 psi) was used for microperfusion at a flow rate of 4 μl/10 min.

pH measurements. Isolated acini or intralobular ducts were incubated with 2 μM BCECF-AM for 30 min at room temperature and washed once with PSS containing 0.1% BSA. They were kept on ice until use. For the microperfusion study, 5 μM BCECF-AM was loaded via the ductal lumen. The fluorescence of isolated acini or developed for isolating interlobular ducts from the guinea pig parotid gland (PG) and submandibular gland (SMG) of the guinea pig. A: NBC1 transcripts with a predicted molecular size of 437 (NH₂ terminal) and 182 bp (middle portion) were generated using degenerative oligonucleotide primers derived from conserved NBC sequences of the rat (GenBank Accession No. AF011390, mouse (GenBank Accession No. AF141934), and human (GenBank Accession No. AF011390) were used to amplify cDNA clones of two different NBC isoforms (kNBC1 and pNBC1). Guinea pig β-actin (GenBank Accession No. AF508792) was amplified to assess the cDNA yield. Primers were designed to amplify, specifically, the following transcripts of guinea pig β-actin (435 bp): 5'-TGGGCTGACATCAAGGAGAAG-3' (sense) and 5'-GCTGGAGGAGTGAGGAG-3' (antisense). The degenerate primers for the NBC1s (kNBC1 and pNBC1) were 5'-GAGAAAACAGGTCCGCTATG-3', 5'-AGCATGACAGGCTGCTGTA-3', 5'-TGTCGGCCCACAAGTCTCTGTG-3', and 5'-TTCTCTACCTGAGTACATATT-3'. The degenerative primers for NBC3 (GenBank Accession No. AF047033) were 5'-AGGAAACAATGTGGAGAGAG-3' and 5'-ACTCCCCATATAAGGAAAGCCCA-3'. The cycling parameters were as follows: 38 cycles of 94°C for 55 s, 55°C for 55 s, and 72°C for 2 min. Expected PCR products of ~437, 182, and 316 bp were generated, and DNA sequencing was analyzed using dye terminator methods with BaseStation (MJ Research). These PCR fragments showed a 95% homology to human NBC1 (GenBank Accession No. AF011390) and NBC3 (GenBank Accession No. AF047033). Subsequently, we designed the following specific primers for guinea pig NBC1: 5'-GACAGGTAGGAGTGAGTCTCAA-3' and 5'-ATCCATGGTACAGCTGTTCTT-3'. The expected PCR product was ~320 bp.

Acinar cell and intralobular duct preparations. The guinea pig PG and SMG were dispersed into individual ducts (intralobular) and acini by a modification of the methods described previously (10). Briefly, guinea pigs were anesthetized by an injection of diethyl ether and killed by cervical dislocation. The PG or SMG was quickly removed and finely minced in PSS supplemented with 0.1% sodium pyruvate, 0.02% trypsin inhibitor, and 0.1% BSA. The tissues were then digested in the same solution containing collagenase (100 U/ml) at 37°C for 60 min with continuous agitation. During the incubation, cells were periodically dispersed by trituration at 20, 40, and 60 min.

Isolation of interlobular ducts and microperfusion via the ductal lumen. Microdissection of the ducts was performed under a stereomicroscope by a previously described method, which was originally
ducts was measured by photon counting using a Photon Technology system (South Brunswick, NJ). BCECF fluorescence was recorded at excitation wavelengths of 440 and 490 nm with an emission wavelength of 530 nm. The 490-to-440-nm fluorescence ratios were calibrated using the high-potassium nigericin procedure described previously (34). Values are reported means ± SE of the numbers of acinar aggregates or ducts examined. Acidification of the cells was induced by an exposure to 20 mM NH₄Cl for 1 min. We then observed the pHᵢ recovery for the two different types of salivary glands. During the pHᵢ measurements, bath solution was superfused at a flow rate of 3 ml/min.

**Immunohistochemistry of NBCs.** Male guinea pigs were anesthetized via inhalation of diethyl ether. The PG and SMG were removed and postfixed in cold fixative (3% paraformaldehyde in 0.01 M phosphate buffer, pH 7.4) for 3 h. The tissues were rinsed and dehydrated in ethanol followed by xylene, embedded in paraffin, and cut at a thickness of 2 μm. For immunohistochemistry, sections were dewaxed, rehydrated, and incubated for 30 min with 3% H₂O₂ in 100% methanol to remove endogenous peroxidase activity. For antigen retrieval, sections were put in Tris-EGTA buffer (1 mM Tris solution supplemented with 0.5 mM EGTA, pH 9.0) and heated using a microwave for 10 min. Nonspecific binding of immunoglobulin was prevented by incubating sections in 0.01M PBS containing 0.1% BSA, 0.05% saponin, and 0.2% gelatin for 1 h at room temperature. Sections were then incubated overnight at 4°C with primary antibodies diluted in 0.01 M PBS containing 0.1% BSA and 0.3% Triton X-100. After being washed three times in washing buffer (0.01 M PBS containing 0.1% BSA, 0.05% saponin, and 0.2% gelatin), sections were incubated with a biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) at a dilution of 1:200 for 1 h at room temperature followed by an incubation with avidin and biotinylated horseradish peroxidase complex (Vector Laboratories) at 1:100 for 1 h at room temperature. Sections were then visualized with 3,3′-diaminobenzidine (Sigma). The rat kidney was used as a positive control. After being counterstained with hematoxylin, sections were dehydrated and coverslipped. Slides were examined and photographed with a light microscope. Polyclonal antibodies to the subunits of NBC1/pNBC and NBC3 were obtained from Chemicon. The immunogens of the guinea pig anti-NBC1 (NBC1/pNBC) and rabbit anti-NBC3 were amino acids 928–1035 of the COOH terminus of human NBC1 and a 19-amino acid peptide sequence with the cytoplasmic COOH terminus of human NBC3, respectively.

![Fig. 2. Labeling of NBC1 (indicated by arrows). A: rat kidney used as a positive control, which revealed NBC1 labeling at the basolateral membrane (BLM) side of proximal tubules. B: NBC1 antibody-stained BLM of PG acinar cells. C and D: strong labelings of NBC1 in the luminal membrane (LM) of intralobular ducts (C) and interlobular excretory ducts (D). E: strong labelings of BLM were observed in SMG intralobular duct cells, but there was no NBC1 staining in SMG acinar cells. F: strong labelings of NBC1 in the BLM of interlobular ducts of the SMG. Magnification: ×400 in A–F.](http://www.ajpgi.org/)
RESULTS

HCO₃⁻ concentrations in resting or stimulated whole saliva of guinea pigs and humans. The saliva collected from guinea pigs and humans was analyzed for HCO₃⁻ concentrations. We used whole saliva, which is a mixture of salivas from three sets of major glands and many minor glands. Resting and stimulated whole saliva of the guinea pigs contained 4.0 ± 1.0 (n = 5) and 16.2 ± 0.8 mM (n = 5) HCO₃⁻, respectively, whereas, in humans, resting and stimulated saliva contained 2.4 ± 0.3 (n = 5) and 16.4 ± 3.1 mM (n = 5) HCO₃⁻, respectively. The results indicate that HCO₃⁻ concentrations of guinea pig saliva, in either a resting or stimulated state, are very similar to those of humans.

RT-PCR analysis for NBC1 and NBC3 expression in guinea pig salivary glands. RT-PCR was performed to determine if electronegenic NBC1 and electroneutral NBC3 mRNA are expressed in the PG and SMG of guinea pigs. NBC1 transcripts with a predicted molecular size of 437 bp (NH₂ terminal) and of 182 bp (middle portion) were observed from whole PG tissue (Fig. 1A). We confirmed that our RT-PCR product was NBC1 by comparing its partial sequence with the sequence of human NBC1 (1) and the guinea pig NBC1 gene (11), the results of which are shown in our companion study. It was found that NBC1 in our experiments is the pancreatic type, pNBC1 (11), which showed a 95% sequence homology with human pNBC1 (GenBank Accession No. AF011390). Subsequently, using the specific pNBC1 primer for the guinea pig (with an expected product size of 320 bp), we then characterized NBC1 mRNA expression in acini and ducts from two different glands, the PG and SMG (Fig. 1B). Figure 1B, top, shows that NBC1 transcripts are expressed in both acini and ducts of the PG but only in ducts of the SMG. Figure 1B, middle, shows NBC3 transcripts with a predicted molecular size of 316 bp. In PGs, the mRNA of NBC3 was detected only in ducts, but NBC3 mRNA was expressed both in acinar and ducts in SMGs.

Immunohistochemical localization of NBC1 and NBC3. Immunohistochemistry was performed to examine the cellular and subcellular localization of NBCs in guinea pig salivary glands. The rat kidney (32) was used as a positive control, which revealed NBC1 expression (indicated by arrows) at the basolateral membrane (BLM) of proximal tubules (Fig. 2A). Figure 2, B–D, shows staining of NBC1 in guinea pig PGs. Strong labelings were observed in the BLM (Fig. 2B) of acinar

Fig.3. Immunoperoxidase labeling of NBC3 (indicated by arrows) in the rat kidney (A), SMG (B–D), and PG (E and F) from the guinea pig. A: NBC3 immunolabeling was seen at intercalated cells of the cortical collecting duct in the rat kidney, which was used as a positive control. B–D: in SMGs, NBC3 labeling was associated with the apical plasma membrane of acini (B–D), intralobular ducts (C), and interlobular ducts (D). E and F: in PGs, NBC3 labeling was associated with the basolateral plasma membrane of intralobular ducts (E) and interlobular ducts (F), whereas no labeling was seen in acini (E). Magnification: ×630 in A–F.
cells. In intralobular (Fig. 2C) and interlobular ducts (Fig. 2D), strong stainings of NBC1 were shown in the luminal membrane (LM). In SMGs, NBC1 staining was evident in the BLM of intralobular (Fig. 2E) and interlobular ducts (Fig. 2F) but not in acini. Figure 3 shows NBC3 expression (indicated by arrows). Staining in intercalated cells of the cortical collecting duct in the rat kidney was used as a positive control (12) (Fig. 3A). In the SMG, NBC3 staining was strong in the LM of acinar cells (Fig. 3, B–D) and the LM of intralobular (Fig. 3, B and C) and interlobular ducts (Fig. 3D). In PGs, NBC3 staining was observed in the BLM of intralobular (Fig. 3E) and interlobular ducts (Fig. 3F) but not in acini.

**pH** recovery in **PG** and **SMG** acinar cells. Throughout the experiments, we used a NH4Cl prepulse technique to acidify the cells. When cells are exposed to NH4Cl for 1 min (indicated by the solid bars), they are transiently alkalized and then become acidified later. Using this technique, we measured the pH recovery rate from cell acidosis and investigated the involved transporters using specific blockers. Figure 4 shows pH recovery patterns of acinar cells from two different types of guinea pig salivary glands, the SMG (A and B) and PG (C and D). Figure 4, A and C, shows the pH recovery in HBS, whereas Fig. 4, B and D, shows the pH recovery in BBS. The pH recovery of acinar cells in both salivary glands did not recover after the NH4Cl pulse in the Na+-free solution (indicated by the open bars), suggesting that the pH recovery was Na+-dependent. We first examined the effect of an amiloride derivative, EIPA, which is an NHE inhibitor, on pH recovery. As shown in Fig. 4, A and C, 1 μM EIPA completely inhibited pH recovery in both types of acinar cells in HBS. The experiment was repeated in a different bath solution, BBS (Fig. 4, B and D). Although the pH recovery was also completely inhibited by 1 μM EIPA in SMG acinar cells (Fig. 4B), 1 μM EIPA only partly inhibited the pH recovery in PG acinar cells (Fig. 4D).

*Functional expression of NBC1 in PG acinar cells.* Figure 5A summarizes the results of the experiments shown in Fig. 4. The pH recovery rate of SMG acinar cells was not much different between the two bath solutions, HBS and BBS. Furthermore, the complete inhibition of pH recovery in SMG acinar cells in the presence of 1 μM EIPA in both HBS and BBS suggests that NHE1 is the exclusive pH regulator in this tissue. In PG acinar cells, the rate of pH recovery in BBS (0.687 ± 0.091 pH units/min, n = 5) was much faster (P < 0.01) than in HBS (0.288 ± 0.028 pH units/min, n = 12). In the presence of HCO3-, EIPA did not completely inhibit the pH recovery (0.151 ± 0.034 pH units/min, n = 11), suggesting that in PG acinar cells, an EIPA-insensitive, HCO3-/Na+-dependent mechanism, in addition to NHE, is involved in pH recovery. Thus, we further examined whether the residual component of pH recovery is inhibited by DIDS, a specific NBC1 blocker. Figure 5B shows a representation of four experiments in BBS. The EIPA-insensitive pH recovery component in PG acinar cells was completely inhibited by 1 mM DIDS.

**pH** recovery and **NBC1** expression in intralobular ducts. We next examined pH regulation in intralobular striated ducts from two different types of salivary glands, the PG and SMG. Figure 6, A and B, shows superimposed raw traces for pH recovery from NH4Cl-induced acidosis at various EIPA concentrations in HBS. The degree of inhibition by EIPA was in proportion to its concentration in both ducts. The 1 μM EIPA concentration that had completely inhibited pH recovery in acinar cells (see Fig. 4, A and C)
partly inhibited the pH\textsubscript{r} recovery of PG and SMG ducts by 59 ± 9% (n = 4) and 73 ± 11% (n = 3), respectively, compared with their full recovery rates. Only the higher concentrations of EIPA, 100 and 10 \(\mu\)M, could almost completely inhibit pH\textsubscript{r} recovery by 97 ± 8% (n = 3) and 93 ± 7% (n = 4), respectively, in PG and SMG ducts. The IC\textsubscript{50} was estimated as 0.33 ± 0.12 (n = 4) and 0.18 ± 0.17 \(\mu\)M (n = 3) in PG and SMG ducts, respectively (Fig. 6C). By contrast, in BBS (Fig. 6, D and E), the higher concentrations (100 and 10 \(\mu\)M) of EIPA inhibited pH\textsubscript{r} recovery by only 42 ± 6% (n = 4) and 49 ± 9% (n = 3) in PG and SMG ducts, respectively, compared with the normal recovery. These residual pH\textsubscript{r} recoveries were further inhibited by 93 ± 4% (n = 3) and 91 ± 8% (n = 3) in PG and SMG ducts by 1 mM DIDS, suggesting that, in addition to NHEs, NBC1 is functionally expressed in the two intralobular duct types. On the basis of the immunohistochemistry data, intralobular NBC1 appears to be expressed in the LM of PG ducts and the BLM of SMG ducts. However, the immunohistochemical data alone may not be sufficient to verify the functional expression of NBC1. Therefore, we went on further to perform a microperfusion study in interlobular excretory ducts, which enabled separated perfusions, one via the bath and the other via the ductal lumen.

Subcellular localization and NBC1 expression in interlobular ducts. Figure 7 shows a series of microperfusion studies in interlobular ducts of the PG and SMG. When we studied the pH\textsubscript{r} recovery in the LM of the ducts, the bath was superfused with Na\textsuperscript{+}-free solution to inhibit the pH\textsubscript{r} recovery that may occur in the BLM of ducts. The addition of Na\textsuperscript{+} (140 mM) into the ductal lumen caused pH\textsubscript{r} recovery from the NH\textsubscript{4}Cl-induced acidification, and 1 \(\mu\)M EIPA completely inhibited the pH\textsubscript{r} recovery in HBS in interlobular ducts (Fig. 7A). However, the same concentration of EIPA did not block the pH\textsubscript{r} recovery in BBS, and the residual pH\textsubscript{r} recovery component was completely blocked by the addition of 1 mM DIDS (Fig. 7B). This result suggests that NBC1 is functionally expressed in the LM of the interlobular duct. We also wanted to know whether Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange is expressed in the LM. We then added 1 mM DIDS into the ductal lumen to examine whether NBC1 functions in a resting state. The application of DIDS for 5 min slowly decreased the pH\textsubscript{r} from 7.45 to 7.2, suggesting that HCO\textsubscript{3}\textsuperscript{-} in the ductal lumen is reabsorbed in the resting state. However, the pH\textsubscript{r} recovery pattern in the LM of SMG interlobular ducts was different. Figure 7D shows superimposed traces of pH\textsubscript{r} recovery under different EIPA concentrations in HBS. We found that 5 \(\mu\)M EIPA only partly (71 ± 4%, n = 3) inhibited pH\textsubscript{r} recovery and that 50 \(\mu\)M EIPA inhibited pH\textsubscript{r} recovery by 94 ± 6% (n = 3) compared with normal recovery. This result indicates that the LM of SMG interlobular ducts expresses EIPA-resistant NHE isoforms, probably NHE3. Furthermore, Fig. 7E shows the inhibitory effect of the highest concentration of EIPA, 50 \(\mu\)M, on the pH\textsubscript{r} recovery was not significantly different (\(P > 0.1\)) between HBS (94 ± 6%) and BBS (92 ± 5%, n = 3), suggesting that NBC1 is not functionally expressed in the LM of SMGs.

We next examined the pH\textsubscript{r} recovery mechanisms in the BLM of interlobular ducts. The ductal lumen was perfused with Na\textsuperscript{+}-free solution to inhibit the pH\textsubscript{r} recovery that may occur in the LM of ducts. Figure 8, A and B, shows pH\textsubscript{r} recovery patterns for PGs. The addition of Na\textsuperscript{+} to the bath solutions neither induced pH\textsubscript{r} recovery in HBS (Fig. 8A) nor BBS (Fig. 8B), although the addition of Na\textsuperscript{+} into the ductal lumen rapidly restored pH\textsubscript{r}. This result strongly suggests that any Na\textsuperscript{+}-dependent pH\textsubscript{r} recovery mechanisms, such as NHE1, do not exist in the BLM of PG ducts. In contrast to the PG, the addition of Na\textsuperscript{+} to the bath solution rapidly restored pH\textsubscript{r} in SMG ducts, and the pH\textsubscript{r} recovery rate was further increased by the addition of Na\textsuperscript{+} into the ductal lumen in HBS from a rate of 0.094 ± 0.016 (n = 3) to 0.502 ± 0.023 pH units/min (n = 3; Fig. 8C). Figure 8D shows that 1 \(\mu\)M EIPA only partly inhibited pH\textsubscript{r} recovery by 38 ± 12% (n = 3) compared with the normal recovery in HBS.
appropriate experimental model for studying HCO$_3^-$ whole saliva in guinea pigs was similar with that of humans, HCO$_3^-$ blocker for NHE1 or NHE2, completely inhibited pHi recovery in salivary acinar cells, because 1 mM DIDS, probably NHE1 and/or NHE2, played a putative role in pHi regulation. Throughout the experiments, 1 mM DIDS completely inhibited all of the residual EIPA-insensitive pHi recovery components in BBS. The absence of pHi recovery in the BLM of PG ducts suggests that NBC3, with its location confirmed by immunohistochemistry, does not play any role in pHi regulation. However, in the SMG duct, we cannot rule out the possibility that high concentrations of EIPA to different NHE isoforms (17), another type of NHE isoform, probably NHE3 located in the LM (24), appears to act together with NHE1 to regulate pHi in ducts. We did not further examine the molecular identities of the NHE isoforms expressed in these ducts, because our main aim in the present study was to elucidate a HCO$_3^-$ transport mechanism in relation with the expression of NBCs. Thus, after determining the maximal concentration of EIPA needed to inhibit NHEs, we studied the activity of NBCs in the presence of the maximal concentration of EIPA.

Functional expression of NBCs in guinea pig salivary glands. Table 1 summarizes our major findings for NBC1 and NBC3 expression, including all the RT-PCR, immunohistochemistry, and functional studies. Our results provided evidence for a heterogeneous distribution of NBCs in guinea pig salivary glands. NBC1 showed distinct membrane domain localization; it was expressed in the BLM of PG acinar cells and the LM of intralobular and interlobular PG ducts. Particularly, it is noteworthy that NBC1 was functionally expressed in the LM of PG ducts. Roussa (30) also reported NBC1 expression by immunohistochemistry in the LM of human PG striated ducts. We not only verified its localization by immunohistochemistry but also confirmed its function by pH$_i$ measurements using microperfusion. Although immunolabeling of NBC3 was also detected in PG and SMG ducts, we did not find any evidence of NBC3 involvement in pH$_i$ regulation. Throughout the experiments, 1 mM DIDS completely inhibited all of the residual EIPA-insensitive pH$_i$ recovery components in BBS. The absence of pH$_i$ recovery in the BLM of PG ducts suggests that NBC3, with its location confirmed by immunohistochemistry, does not play any role in pH$_i$ regulation. However, in the SMG duct, we cannot rule out the possibility that high concentrations of

DISCUSSION

Guinea pig salivary glands as a HCO$_3^-$ transport model. HCO$_3^-$ is the most important buffering system in saliva. Although its concentration is very low in unstimulated saliva, it can be increased up to 60 mM at high flow rates in humans (2). However, it is still unclear what mechanisms mediate HCO$_3^-$ secretion and absorption in salivary glands. The marked differences in salivary HCO$_3^-$ secretion among species and among glands within species suggests that the expression patterns of the NBC may well differ both among species and glands. For example, with increasing salivary flow rates, HCO$_3^-$ concentrations rise in human PGs but fall in rat PGs (33). Interestingly, we found that the value of the HCO$_3^-$ concentration of whole saliva in guinea pigs was similar with that of humans, suggesting that guinea pig salivary glands may be used as an appropriate experimental model for studying HCO$_3^-$ transport in humans.

Functional expression of NHEs in guinea pig salivary glands. We found that there was a significant difference in the threshold concentrations of EIPA required to inhibit NHE activity between two tissues, acini and ducts from two salivary glands (the PG and SMG). Amiloride-sensitive NHEs, probably NHE1 and/or NHE2, played a putative role in pH$_i$ regulation in salivary acinar cells, because 1 mM of EIPA, a specific blocker for NHE1 or NHE2, completely inhibited pH$_i$ recovery in HBS. However, the inhibition threshold of EIPA in duct cells was more than one order of magnitude higher than that in acinar cells. On the basis of the inhibitory efficiency of the

BBS, and the residual component was further inhibited by 90 ± 2% (n = 3) by the addition of 1 mM DIDS, suggesting that NBC1 is functionally expressed in the BLM. All our major findings are summarized in Table 1.
EIPA (>50 \mu M) inhibited NBC3 activity. Whether high concentrations of EIPA inhibit NBC3 activity (27) or not (25) is still controversial.

Physiological role of NBCs in guinea pig salivary glands.
We found that when guinea pig PG acinar cells were acidified, the pHi recovered to the prestimulus level. In this process, in addition to NHE1 and/or NHE2, NBC1 in the BLM also contributed to pHi recovery by taking up HCO$_3^-$.

The favorable electrical potential due to a membrane depolarization by Cl$^-$/HCO$_3^-$ efflux (9, 10, 26) can further provide a driving force for HCO$_3^-$ influx via NBC1. The concentration of HCO$_3^-$ varies with the flow rate. Unstimulated saliva contains very little HCO$_3^-$, whereas stimulated saliva contains a much higher concentration (2). One possible explanation for the very low HCO$_3^-$ concentration in unstimulated saliva is due to the reabsorption of HCO$_3^-$ in ducts. However, there is no candidate NBC reported, especially in PG ducts, that may mediate HCO$_3^-$ reabsorption. To our knowledge, this is the first report of a functional expression of NBC1 in the LM of PG ducts in mammalian salivary glands. Our results strongly suggest that luminal NBC1 plays a dominant role in HCO$_3^-$ reabsorption. Although NBC3 has been believed to play a HCO$_3^-$ salvage role in mouse SMGs (16), we did not find any evidence of NBC3 expression in the LM of PG ducts. This discrepancy may be due to the fact that NBCs involved in the salvage of HCO$_3^-$ depend on the species and type of salivary gland. As in acinar cells, duct cells are also depolarized by agonists. The depolarization can be induced by Cl$^-$/HCO$_3^-$ exit via the CFTR (7). This favorable electromotive force for the influx mode of NBC1 may further accelerate the reabsorption of HCO$_3^-$ from the ductal lumen. This luminal NBC1 also appears to function in the resting state, because there was a slow decrease of ductal pHi in the presence of 1 mM DIDS. It is unlikely that other DIDS-sensitive NBCs, such as AE1–3 of the SLC4 family (28) and some electrogenic Cl$^-$/HCO$_3^-$ extrangers of the SLC26 family (20), are involved in this process. Although the direction of Cl$^-$/HCO$_3^-$ movement via the Cl$^-$/HCO$_3^-$ exchanger depends on local gradients, it is likely that the Cl$^-$/HCO$_3^-$ exchanger in
our experiment generates net Cl⁻ uptake and HCO₃⁻ efflux, because the Cl⁻ concentration, 115 mM, of the microperfusion solution via the ductal lumen may be much higher than that of duct cells. Although any data for the intracellular Cl⁻ concentration in guinea pig duct cells are not available, studies have reported a Cl⁻ concentration of 38 ± 8 and 57.7 ± 4.1 mM in the rat (36) and rabbit SMG ducts (14), respectively. If we assume that the Cl⁻ concentration of guinea pig duct cells is not much different from that of other rodents such as the rat or rabbit, the Cl⁻/HCO₃⁻ exchanger in our experiments should generate net Cl⁻ uptake and HCO₃⁻ efflux in the resting state. To keep the HCO₃⁻ concentration of the saliva low would be advantageous to facilitate a sour taste in the resting state (18). The high HCO₃⁻ concentration in stimulated PG saliva can be explained partly by the limited time for the duct system to reabsorb HCO₃⁻ at high flow rates and partly by the activation of the luminal Cl⁻/HCO₃⁻ exchanger (15). HCO₃⁻ is also one of the main components in SMG saliva (3). The presence of NBC1 at the BLM of SMG ducts suggests that NBC1 may mediate HCO₃⁻ uptake into duct cells to compensate for the HCO₃⁻ secretion through the CFTR. In this tissue, a strong HCO₃⁻ reabsorption mechanism mediated by NBC1 observed in PG ducts may not be necessary, because the amount of HCO₃⁻ in primary saliva secreted from SMG acinar cells will be much less than that secreted by PGs by the absence of basolateral NBC1.

This study demonstrates that the mechanisms for pHᵢ regulation in the guinea pig PG and SMG are different. NBC1, in addition to NHEs, is involved in regulating the pHᵢ in PG acinar cells and in both ducts of the PG and SMG. We also provide new evidence that NBC1 is expressed in the BLM of PG (A and B) and SMG interlobular ducts (C and D) by the microperfusion technique. It is noteworthy that the addition of Na⁺ into the bath solution did not induce pHᵢ recovery in HBS (A) or BBS (B) until the addition of Na⁺ into the ductal lumen had rapidly restored pHᵢ. C: in SMG interlobular ducts, the addition of Na⁺ into the bath solution induced pHᵢ recovery (0.094 ± 0.016 pH units/min, n = 3), which further increased by the addition of Na⁺ into the ductal lumen (0.502 ± 0.023 pH units/min, n = 3). The concentration of 1 µM EIPA almost completely inhibited this pHᵢ recovery in the BLM (0.021 ± 0.004 pH units/min, n = 3). D: 1 µM EIPA only partly inhibited pHᵢ recovery (0.078 ± 0.005 pH units/min, n = 3) in BBS, and the residual pHᵢ recovery component was almost completely inhibited by 1 mM DIDS (0.013 ± 0.003 pH units/min, n = 3) in the BLM. A–D are representative experiments selected from 3 or 4 experiments.

Table 1. Summary of NBC1 and NBC3 expression patterns in guinea pig salivary glands

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<td>pHᵢ regulation</td>
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<td>NBC3</td>
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NBC, Na⁺ - HCO₃⁻ cotransporter; PG, parotid gland; SMG, submandibular gland; LM, luminal membrane; BLM, basolateral membrane; pHᵢ, intracellular pH; +, presence; −, absence.
the LM of PG ducts and may play a role in HCO$_3^-$ reabsorption.

GRANTS

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REFERENCES


