Bicarbonate-regulated Adenylyl Cyclase (sAC) Is a Sensor That Regulates pH-dependent V-ATPase Recycling*

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Modulation of environmental pH is critical for the function of many biological systems. However, the molecular identity of the pH sensor and its interaction with downstream effector proteins remain poorly understood. Using the male reproductive tract as a model system in which luminal acidification is critical for sperm maturation and storage, we now report a novel pathway for pH regulation linking the bicarbonate activated soluble adenylyl cyclase (sAC) to the vacuolar H⁺-ATPase (V-ATPase). Clear cells of the epididymis and vas deferens contain abundant V-ATPase in their apical pole and are responsible for acidifying the lumen. Proton secretion is regulated via active recycling of V-ATPase. Here we demonstrate that this recycling is regulated by luminal pH and bicarbonate. sAC is highly expressed in clear cells, and apical membrane accumulation of V-ATPase is triggered by a sAC-dependent rise in cAMP in response to alkaline luminal pH. As sAC is expressed in other acidos/base transporting epithelia, including kidney and choroid plexus, this sAC-dependent signal transduction pathway may be a widespread mechanism that allows cells to sense and modulate extracellular pH.

We recently identified bicarbonate-activated soluble adenylyl cyclase (sAC) as a chemosensor mediating bicarbonate-dependent elevation of cAMP (1), defining a potential transduction pathway for cells to sense variations in bicarbonate, as well as the closely related parameters, pCO₂ and pH (1–3). sAC is distinct from transmembrane adenylyl cyclases. It is insensitive to regulation by forskolin or heterotrimeric G proteins (2) and is directly activated by bicarbonate ions. It does not have the molecular identity of the pH sensor and its interaction with downstream effector proteins remain poorly understood. The male reproductive tract is a model system in which luminal acidification is critical for sperm maturation and storage, and we now report a novel pathway for pH regulation linking the bicarbonate activated soluble adenylyl cyclase (sAC) to the vacuolar H⁺-ATPase (V-ATPase). Clear cells of the epididymis and vas deferens contain abundant V-ATPase in their apical pole and are responsible for acidifying the lumen. Proton secretion is regulated via active recycling of V-ATPase. Here we demonstrate that this recycling is regulated by luminal pH and bicarbonate. sAC is highly expressed in clear cells, and apical membrane accumulation of V-ATPase is triggered by a sAC-dependent rise in cAMP in response to alkaline luminal pH. As sAC is expressed in other acid/base transporting epithelia, including kidney and choroid plexus, this sAC-dependent signal transduction pathway may be a widespread mechanism that allows cells to sense and modulate extracellular pH.

sAC is highly expressed in spermatozoa (7) where it is proposed to mediate the bicarbonate-dependent cAMP elevation that precedes capacitation, hyperactivated motility, and acrosome reaction needed for fertilization (1). While spermatozoa mature and are stored along the epididymal lumen, they are kept in a quiescent state by an acidic pH of 6.5–6.8 and a low bicarbonate concentration of 2–7 mM (8). We have previously shown (9, 10) that a sub-population of epithelial cells, the so-called clear cells, are important players in the acidification capacity of the epididymis. Clear cells express high levels of the V-ATPase in their apical pole, and are responsible for the bulk of proton secretion in the vas deferens. Proton secretion by clear cells occurs in a chloride-independent but bicarbonate-dependent manner (11). Similarly to kidney intercalated cells, epididymal clear cells regulate their rate of proton secretion via V-ATPase recycling between intracellular vesicles and the apical plasma membrane (12). In these cells, as well as proton-secreting cells in the turtle bladder, an increase in V-ATPase surface expression and in apical surface area (including microvilli) closely correlates with an increase in proton secretion (13–15). Proton-secreting epithelial cells actively regulate their rate of proton secretion in response to variations in the pH of their immediate environment (15). However, the molecular entities underlying this response still remain unknown. In the present study, we tested whether bicarbonate-regulated sAC might play a role in the dynamic V-ATPase recycling that occurs in these cells.

EXPERIMENTAL PROCEDURES

Laser Capture Microdissection and RT-PCR—Epithelial cells from rat cauda epididymis were harvested by laser capture microdissection, and mRNA was extracted and amplified in vitro following a T7-based amplification procedure, as we recently described (16). For RT-PCR, oligonucleotide primer pairs were designed to amplify a short sequence in the 3′ end of the cDNA. Primers were synthesized by Sigma-Genosys (The Woodlands, TX) and are listed in Table I. The identity of PCR products was confirmed by direct sequencing (MGH, Molecular Biology DNA Sequencing Core Facility).

Western Blotting—Adult rats were anesthetized and perfused through the left ventricle with PBS (10 mM phosphate buffer containing 0.9% NaCl), pH 7.4, containing protease inhibitors (Complete, Roche). The epididymis was removed and the cauda region was dissected and homogenized. Electrophoresis and immunoblotting were performed as described previously (16), using a monoclonal antibody (R21) raised against the catalytic regions (C1 and C2) of sAC (6). Protein was added at 20 μg/lane, and the antibody was used at a concentration of 1:250.

Tissue Fixation and Immunofluorescence—Adult male Sprague-Dawley rats were anesthetized with sodium pentobarbital (65 mg/kg body weight, intraperitoneally) and perfused via the left ventricle with

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PBS (pH 7.4) followed by a fixative containing 4% paraformaldehyde, 10 mM sodium periodate, 70 mM lysine, and 5% sucrose (PLP) as described previously (11, 12). Kidney and male reproductive tract organs were harvested and further fixed by immersion in the same fixative overnight. Immunofluorescence was performed on 4 μm cryostat sections. An affinity-purified chicken polyclonal antibody against the E subunit of the V-ATPase was used. This antibody has been characterized previously (17, 18). To localize sAC, two antibodies were used. An affinity-purified polyclonal rabbit antiserum described previously (1) and monoclonal antibody 2G1 (6), both against the catalytic regions (C1 and C2) of sAC.

In Vivo Perfusion of the Distal Cauda Epididymidis—Adult male Sprague-Dawley rats were anesthetized with sodium pentobarbital as described above. The vas deferens was cannulated through the lumen with a micro cannula (0.4 mm OD, 0.2 mm ID; Kent Scientific Corporation, Torrington, CT) connected to a 10-ml syringe. A small incision was made in the distal cauda epididymal region to allow the perfusate to exit the tube. Perfusion was performed retrogradely at a rate of 45 μl/min using a syringe pump (Model 341B, Fisher). The lumen was initially washed free of sperm with PBS (10 mM sodium phosphate, 2 mM potassium phosphate, 137 mM NaCl, 2.7 mM KCl) adjusted to different pH values (6.5, 6.8, 7.1, or 7.8), as indicated under “Results.” Horseradish peroxidase (HRP, Sigma) was added to the perfusate at a concentration of 5 mg/ml to detect endocytosis in the absence or presence of inhibitors and/or cpt-cAMP as indicated under “Results.” At the end of the experimental period, the luminal solution was changed for HRP-free ice-cold PBS for 3 min (in the continued presence of agonists or inhibitors, if applicable) to wash the lumen free of HRP. The vas deferens and cauda epididymis were harvested and fixed by immersion in a solution containing 4% paraformaldehyde, 10 mM sodium periodate, 70 mM lysine, and 5% sucrose (PLP) as described previously (11, 12). Tissues were then washed in PBS, pH 7.4, and stored in PBS containing 0.02% sodium azide. At least three vas deferens and cauda epididymis were perfused for each condition described. A minimum of 10 cells per tissue were examined by confocal microscopy for a total of at least 30 cells per condition.

Immunogold Electron Microscopy—Small pieces of PLP-fixed epididymis were post-fixed by immersion in phosphate buffer containing 4% paraformaldehyde and 0.5% glutaraldehyde for 4 h at room temperature. For V-ATPase immunogold staining, pieces of tissues were embedded at low temperature with HM20 resin as described previously (10). Double labeling for V-ATPase and HRP was performed on LR White Resin embedded sections.

Quantification of Gold Particle Labeling—The relative amount of V-ATPase present in the apical microvilli of clear cells was determined from the number of V-ATPase-associated gold particles per unit length of apical membrane. Gold particles present in the apical membrane including microvilli were counted for each cell, and the number was divided by the total length of apical membrane and microvilli for that cell. Using a Wacom graphics tablet and NIH Image software, at least ten cells were analyzed in each group. Data are presented as mean ± S.E.

Cyclase Assay—Cyclase assays were performed in 100 μl reaction volume with 100 ng of His6-tagged rat sAC, purified over a nickel-nitrilotriacetic acid affinity column as described (1) in the presence of 50 mM Tris-HCl buffer, pH 7.5, 20 mM creatinephosphate, 100 units/ml creatine phosphokinase, 1 mM ATP, 2.5 mM MgCl₂, and 50 mM NaHCO₃, for 30 min at 30 °C. Estrogen compounds were purchased from Steraloids, Inc. (Newport, RI).

RESULTS

sAC Expression and Localization in the Epididymis and Kidney—Epididymal epithelial cells were harvested by laser capture microdissection, and mRNA was amplified in vitro. RT-PCR detection of mRNA transcripts specific for sAC and markers specific for clear cells in epididymal epithelial cells isolated by laser capture microdissection. B1, B1 subunit of the V-ATPase; E, E subunit of the V-ATPase; CAII, carbonic anhydrase II.

![FIG. 1. sAC detection and localization in the epididymis. A, RT-PCR detection of mRNA transcripts specific for sAC and markers specific for clear cells in epididymal epithelial cells isolated by laser capture microdissection. B1, B1 subunit of the V-ATPase; E, E subunit of the V-ATPase; CAII, carbonic anhydrase II, bp, 20-bp DNA ladder. B, detection of sAC protein by Western blot in total homogenates from rat cauda epididymidis using a monoclonal anti-sAC antibody. A strong band at around 48 kDa was detected indicating the presence of the active form of sAC. C, double immunofluorescence staining showing V-ATPase (green) and sAC (red) in rat cauda epididymidis. A strong sAC staining is observed in clear cells, identified by their positive staining for V-ATPase. sAC immunoreactivity is also observed in sperm, consistent with its previously described localization. Sub-epithelial muscle cells are also stained for sAC. Bar, 15 μm.](https://example.com/fig1.png)
PCR detected sAC mRNA, and the presence of other clear cell markers confirmed the validity of this preparation (Fig. 1A). Western blot analysis using a specific monoclonal antibody, R21, against the N-terminal portion of sAC (6) detected a major band at around 48 kDa, the predicted molecular mass of the sACₜ splice variant (2, 19), in the cauda epididymidis (Fig. 1B). sAC was localized by immunofluorescence using two different anti-sAC antibodies. Monoclonal antibody R21 gave a strong staining in clear cells, which were identified by their positive staining for the V-ATPase (Fig. 1C). No specific immunoreactivity was detected in adjacent principal cells, but surrounding muscle tissue and, as previously described (1, 7), spermatozoa were positive. The same pattern of staining was obtained using an affinity-purified anti-sAC rabbit polyclonal antiserum, but no staining was detected using control pre-immune serum (data not shown). sAC is also expressed in the kidney, where it is located in epithelial cells of distal tubules (Fig. 2A), thick ascending limb of Henle (Fig. 2, A and B) and collecting ducts (Fig. 2, B and C), consistent with previous results showing bicarbonate-stimulated adenylyl cyclase activity in rat kidney (20). Proton secretion is a common function of epididymal clear cells and of renal distal tubules, thick ascending limb of Henle, and collecting duct intercalated cells. Thus, sAC is highly expressed in specialized proton-secreting cells of epididymal and renal epithelia, where it might play a role in regulating acid/base transport in these tissues. To test for a potential role of sAC in modulating proton secretion, we used the epididymis as a model system.

**Fig. 2.** sAC localization in the kidney. Immunolocalization of sAC in kidney cortex (A), inner stripe of the outer medulla (B) and inner medulla (C). Cortical (cTAL) and medullary (mTAL) thick ascending limb of Henle, and distal tubules (DT) show significant expression of sAC. Collecting ducts (CD), which were identified by positive double labeling using V-ATPase antibodies (not shown), also show significant sAC immunolabeling. G identifies glomeruli, and PT identifies proximal tubules. Bars, 25 μm.

**Fig. 3.** V-ATPase recycling at physiological luminal pH. A and B, cauda epididymis tubule perfused with PBS containing HRP at the physiological luminal pH of 6.8. Anti-HRP staining revealed high endocytic activity of clear cells (A, arrows) compared with adjacent principal cells. Clear cells were identified by their positive immunofluorescence staining for the V-ATPase (B, arrows). The box indicates the apical region of clear cells, which is examined in more detail in panel C. C, Electron microscopy double immunogold labeling for HRP and V-ATPase showed that a sub-population of HRP-containing endosomes located in the sub-apical region (15 nm gold, arrowheads) also contain V-ATPase (8 nm gold, arrows). Bars, 50 μm (A and B), 120 nm (C).
V-ATPase Recycling Is Modulated by Luminal pH—Cauda epididymis was perfused in vivo through the lumen with HRP, a marker of endocytosis, in PBS adjusted to different pH values. Double immunofluorescence labeling for HRP and V-ATPase was performed on PLP-fixed cryostat sections. At the physiological luminal pH of 6.8, clear cells, identified by their positive immunoreactivity for V-ATPase, show a high endocytic activity compared with adjacent principal cells (Fig. 3, A and B, arrows). Electron microscopy double immunogold labeling for HRP and V-ATPase showed that a sub-population of HRP-containing endosomes clearly contain V-ATPase, indicating that the proton pump is actively recycled at physiological luminal pH (Fig. 3C). The effect of luminal pH variations on V-ATPase recycling was examined using confocal microscopy. At the acidic pH of 6.5, V-ATPase is distributed between the apical microvilli and intracellular sub-apical vesicles (Fig. 4, A, A’, and A”). Double labeling for HRP indicated that the V-ATPase-containing vesicles partially co-localize with HRP-containing endosomes (yellow staining), indicating that a significant amount of V-ATPase is located in the endocytic compartment. In contrast, at the alkaline luminal pH of 7.8, V-ATPase is mainly located in apical microvilli (Fig. 4, B, B’, and B”, green) and not in HRP-containing endosomes (red), which are present in the sub-apical region. The absence of yellow staining indicates no co-localization of V-ATPase and HRP. The arrows indicate the frontier (often visible as a dark line) between apical microvilli and the sub-apical region. C and D, immunogold electron microscope images showing V-ATPase staining in clear cells at pH 6.5 (C) and pH 7.8 (D). At luminal pH 6.5, V-ATPase is present largely in intracellular vesicles, and few gold particles are seen in apical microvilli. At luminal pH 7.8, V-ATPase is located mainly in well developed apical microvilli and fewer particles are seen inside the cell. E, histogram showing the mean effect of luminal pH on the number of V-ATPase-associated gold particles per unit length of apical membrane. At least 10 clear cells were analyzed for each perfusion condition. Data are expressed as means ± S.E. (*, p < 0.005). Bars, 5 μm (A and B), 720 nm (C and D).
in luminal pH by inducing a rapid (within 15 min), pH-dependent
shutting of V-ATPase between the intracellular HRP-posit-
itive endocytic compartment and apical microvilli. This alk-
line-induced apical membrane V-ATPase accumulation is a
potential mechanism to restore luminal pH to its physiolog-
ic acid value.

cAMP Induces the Apical Translocation of V-ATPase—Previ-
ous studies have shown that cAMP stimulates proton secretion
in a variety of acidifying epithelial cells (21–23) and induces
the exocytic insertion of many actively recycling membrane
proteins, including AQP2, CFTR, and GLUT4 (24–26). To
determine whether the pH-related translocation of V-ATPase was
modulated by cAMP, cauda epididymis were perfused at lu-
minal pH of 6.5 with PBS containing the cAMP permeant
analogue, 8-(4-chlorophenylthio)-cAMP, (cpt-cAMP, 1 mM;
Sigma) for 15 min. Under these conditions, V-ATPase was
present in the apical microvilli and no co-localization of V-
ATPase and HRP was observed, indicating that cAMP induced a
translocation of V-ATPase from intracellular vesicles into the
apical membrane, even at acidic luminal pH (Fig. 5A).

Intracellular Bicarbonate Is Essential for the Alkaline pH-
induced V-ATPase Translocation—Because sAC is directly
activated by bicarbonate ions, we examined the potential con-
tribution of intracellular HCO₃⁻ in the pH- and cAMP-dependent
V-ATPase recycling. CO₂/HCO₃⁻ is the main buffer in biolog-
ic systems, and the equilibrium between HCO₃⁻ and H⁺ with
CO₂ and H₂O is catalyzed by carbonic anhydrases. Clear cells
express very high levels of the cytosolic carbonic anhydrase
type II (11, 27), and we have shown that the carbonic anhy-
drase inhibitor, acetazolamide, markedly decreases the rate of
proton secretion in isolated vas deferens (11). We, therefore,
hypothesized that intracellular bicarbonate production via car-
bonic anhydrase type II in response to an alkaline luminal pH
(subsequent increase in intracellular pH) could activate
sAC. Elevation of cAMP in response to sAC stimulation would
then induce an accumulation of V-ATPase in the apical mem-
brane. Cauda epididymis were perfused in vivo with PBS at
pH 7.8, in the presence of acetazolamide and excess (15 mM)
MgCl₂ in the absence of inhibitor, or in the presence of 3, 6, or 12 μM 2-hy-
droxysteradiol. Values shown in inset represent single deter-
minations.

sAC Is Involved in the pH-regulated V-ATPase Recycling—To
determine whether sAC was involved in this intracellular bi-
carbonate- and cAMP-dependent response, we examined the
effect of luminal alkaline pH on V-ATPase distribution after
inhibition of sAC. Catechol derivatives of estrogen, but not the
parent estrogen compounds, were thought to inhibit soluble
adenylyl cyclase activity from testis (28), and we confirmed
bicarbonate (PBS) different epididymis (H11005 n of the cells at the apical pole (G) for each cell. Double staining for V-ATPase (green) and HRP (red) was performed. A, V-ATPase is distributed between apical microvilli and sub-apical vesicles. The yellow staining indicates partial co-localization of HRP-containing endosomes with V-ATPase-containing vesicles. B, in the presence of bicarbonate, microvilli are more developed, and the V-ATPase is mainly located in apical microvilli. C, 2-hydroxyestradiol induced a significant reduction of the size of microvilli, and V-ATPase is distributed between apical microvilli and sub-apical vesicles. Thus, inhibition of sAC prevents the bicarbonate-induced apical V-ATPase translocation. D, E, and F, the level of V-ATPase in microvilli was quantified by measuring the area occupied by V-ATPase-labeled microvilli (enclosed by the white line), normalized for the width of the cells at the apical pole (blue line) for each cell. G, histogram showing the mean values obtained from at least 10 cells from three to four different epididymis (n = 30–50 cells per condition). The area occupied by V-ATPase-positive microvilli is significantly higher in the presence of bicarbonate (Bic) than in the absence of bicarbonate at the same pH (PBS). In the presence of the sAC inhibitor 2-hydroxyestradiol (Bic + 2HE), the microvilli area is significantly lower compared with the bicarbonate control (Bic). This result demonstrates that 2-hydroxyestradiol prevented the bicarbonate-mediated V-ATPase translocation into the apical microvilli. Results are expressed as mean ± S.E. *, p < 0.001 versus PBS or Bic + 2HE. Bar, 5 μm.

these observations using recombinant purified rat sAC protein (Fig. 6). Rat sAC activity was inhibited by 1,3,5(10)-estratrien-2,3,17β-diol (2-hydroxyestradiol; 2-OH estradiol) with an IC_{50} of 10 μM, and inhibition was non-competitive with substrate ATP. The parent compound 1,3,5(10)-estratrien-3,17β-diol (estradiol) was essentially inert up to 100 μM. Cauda epididymidis were perfused at pH 7.8 in the presence of the sAC inhibitor, 2-hydroxyestradiol (20 μM) for a period of 20 min. Under these conditions, V-ATPase was distributed between apical microvilli and sub-apical vesicles (Fig. 5D) in a manner similar to that observed at pH 6.5 (compare with Fig. 4A). A significant amount of V-ATPase co-localized with HRP-containing endosomes (Fig. 5D, yellow), indicating that inhibition of sAC prevents the usual alkaline pH-induced response (compare with Fig. 4B). Addition of cpt-cAMP for 10 min at the end of the 2-hydroxyestradiol period and in the continued presence of 2-hydroxyestradiol induced a redistribution of V-ATPase into apical microvilli (Fig. 5E), demonstrating that clear cells had retained their ability to respond to cAMP despite the presence of catechol estrogens. Negative controls were conducted by using estradiol (20 μM), which does not inhibit sAC. Under these conditions, clear cells underwent the same V-ATPase translocation to the apical membrane (Fig. 5F) as that observed normally at pH 7.8 or in the presence of cpt-cAMP. In addition, no effect of estradiol was observed on V-ATPase localization at luminal pH 6.5 (data not shown), demonstrating that estradiol is not an activator of V-ATPase exocytosis. These results demonstrate that blockage of V-ATPase apical translocation by catechol estrogens was specifically attributed to inhibition of sAC.

Luminal Bicarbonate Induces the Apical Translocation of V-ATPase—Finally, we examined whether clear cells had the ability to respond to variations in luminal bicarbonate in addition to variations in pH. The effect of an increase in luminal bicarbonate at constant pH on V-ATPase localization and recycling was examined. Cauda epididymidis were perfused with either PBS adjusted to pH 7.1 or a solution containing 12 mM bicarbonate and equilibrated with 5% CO_{2} (pH 7.1). Clear cells exposed to luminal bicarbonate exhibited more developed V-ATPase-positive apical microvilli, indicating higher apical membrane translocation of the pump, compared with clear cells exposed to the same pH in absence of bicarbonate (compare Fig. 7, A and B). The sAC inhibitor 2-hydroxyestradiol prevented the bicarbonate-induced V-ATPase apical translocation (Fig. 7C). Quantification of the area occupied by V-ATPase-labeled microvilli, normalized for the width of the cells at the apical membrane border, indicated a significant increase in apical microvilli extension in the presence of bicarbonate (Fig. 7, E and G) compared with the absence of bicarbonate (Fig. 7, D and G), and confirmed that sAC inhibition prevented the bicarbonate-mediated V-ATPase microvilli translocation (Fig. 7, F and G).

DISCUSSION

The present data show that the distribution of the V-ATPase in specialized proton-secreting cells of the epididymis, the so-called clear cells, is closely related to luminal pH. At alkaline luminal pH, the V-ATPase is mainly located in well developed apical microvilli, and at acidic pH, it is actively recycling between sub-apical endosomes and the apical membrane. We showed that intracellular production of bicarbonate is essential for the alkaline pH-induced response and that CAMP induces an apical translocation of V-ATPase identical to that induced by alkaline pH. Catechol estrogens, which are inhibitors of the newly identified soluble adenylyl cyclase, sAC, inhibit the alkalin-dependent V-ATPase redistribution, identifying sAC as the sensor responsible for the pH-dependent V-ATPase recycling. We propose that alkalization of luminal pH, followed by an increase in intracellular pH in clear cells, leads to an elevation of intracellular bicarbonate concentration. Bicarbonate elevation activates sAC and triggers CAMP production, which in turn leads to the accumulation of V-ATPase in apical microvilli. The exact mechanism(s) responsible for the CAMP-induced apical translocation of V-ATPase remain unknown, and further studies will be required to identify the downstream target proteins involved in this process. Clear cells have also the ability to respond to an increase in luminal bicarbonate concentration at constant pH, presumably due to entry of bi-
carbonate across the apical membrane, and subsequent elevation in intracellular bicarbonate concentration followed by sAC activation. A potential candidate for the apical entry of bicarbonate might be NBC3, which is expressed in the apical membrane of clear cells (29).

Previous studies have demonstrated that primary cultures of epididymal principal cells respond to a variety of agonists by secreting bicarbonate into the lumen (30–32). It was then proposed that bicarbonate secretion occurs during sexual arousal to “prime” spermatozoa before ejaculation (32). Although this finding was interesting, early micropuncture measurements had demonstrated that the luminal fluid of the epididymis is acidic and contains a low bicarbonate concentration (8, 33), indicating the presence of acidification mechanisms in this tissue. We have shown that clear cells, which express high levels of the V-ATPase in their apical pole, are key players in luminal acidification (9, 10) and that proton secretion by these cells is abolished upon inhibition of intracellular bicarbonate production by acetazolamide (11). Thus, the marked intracellular redistribution of V-ATPase observed in the present study in the presence of acetazolamide is in agreement with our previous results showing inhibition of proton secretion under these conditions. We propose that clear cells respond to a rise in luminal bicarbonate concentration following agonist-induced principal cell bicarbonate secretion, by increasing their rate of proton secretion. This would re-establish the pH of the lumen to its resting acidic value. The enrichment of sAC in clear cells compared with principal cells provides an exquisite mechanism for such a concerted interaction between principal and clear cells.

The present study also shows high expression of sAC in kidney thick ascending limbs, distal tubules, and collecting ducts. All epithelial cells from the thick ascending limbs and distal tubules, and intercalated cells from the collecting duct also express the V-ATPase (34). The presence of sAC in these segments, as well as the previously reported sAC expression in other acid/base transporting systems including the choroid plexus (1), indicate that the sAC-dependent cAMP signaling pathway may represent a general mechanism allowing these cells to regulate their rate of proton secretion.

This study is the first demonstration of the role of sAC in mediating V-ATPase-recycling in a native epithelium and provides the missing link between extracellular pH (or bicarbonate) signaling and intracellular response. Because sAC is expressed in many biological systems involved in acid/base transport, we propose that this bicarbonate-dependent cAMP pathway may represent a more universal mechanism allowing cells to sense and modulate the pH of their environment. The molecular entities underlying the response of cells to variations in the pH of their immediate environment still remain unknown. Our study unravels key components of a potentially widespread signaling pathway that could be applicable to many biological systems.

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