Acid-Base Transport by the Renal Proximal Tubule

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One of the major tasks of the renal proximal tubule is to secrete acid into the tubule lumen, thereby reabsorbing approximately 80% of the filtered HCO$_3^-$ as well as generating new HCO$_3^-$ for regulating blood pH. This review summarizes the cellular and molecular events that underlie four major processes in HCO$_3^-$ reabsorption. The first is CO$_2$ entry across the apical membrane, which in large part occurs via a gas channel (aquaporin 1) and acidifies the cell. The second process is apical H$^+$ secretion via Na-H exchange and H$^+$ pumping, processes that can be studied using the NH$_4^+$ prepulse technique. The third process is the basolateral exit of HCO$_3^-$ via the electrogenic Na/HCO$_3$ co-transporter, which is the subject of at least 10 mutations that cause severe proximal renal tubule acidosis in humans. The final process is the regulation of overall HCO$_3^-$ reabsorption by CO$_2$ and HCO$_3^-$ sensors at the basolateral membrane. Together, these processes ensure that the proximal tubule responds appropriately to acute acid-base disturbances and thereby contributes to the regulation of blood pH.

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the formation of titratable acid (14). The tiny amount of HCO₃⁻ that, in parallel, moves into the blood is the “new HCO₃⁻.”

In this review, for the sake of simplicity, I refer to PT trans-epithelial acid-base transport as “HCO₃⁻/H₂CO₃ reabsorption” (JHCO₃). In fact, I mean the sum of JHCO₃ and the creation of new HCO₃⁻. We will see that these processes are under the powerful control of a system that we are only beginning to understand, one that acutely monitors plasma [CO₂] and [HCO₃⁻/H₂CO₃] but not plasma pH.

Apical CO₂ Entry

Effect of CO₂ on pHᵢ

Since the work of Jacobs on Symphytum flower petals (the pigment of which is a pH indicator) in 1920 (15), it has been appreciated that CO₂ can rapidly cross cell membranes and acidify the cytoplasm (reviewed in reference [16]). Thomas (17) and Boron and De Weer (18), both working with pH-sensitive microelectrodes, did the first work with physiologic levels of CO₂ on animal cells. Both studies confirmed that CO₂ does indeed cause a rapid fall in pHᵢ (Figure 2A), as shown for the squid axon by the initial part of the record in Figure 2B. If CO₂ simply equilibrated across the cell membrane, as shown in Figure 2A, then pHᵢ would have fallen—by an amount determined by the initial pHᵢ, [CO₂], and intracellular buffering power (16,19,20)—and stabilized. However, pHᵢ recovers from this acute acid load via a mechanism that requires the input of energy. The experiment in Figure 2B was the first to demonstrate such a pHᵢ recovery, that is, dynamic pHᵢ regulation. We now know that a Na⁺-driven Cl⁻/HCO₃⁻ exchanger (discussed below) is responsible for this pHᵢ recovery—a metabolic compensation (i.e., HCO₃⁻ uptake) to a respiratory acidosis (i.e., CO₂ influx). The withdrawal of CO₂ causes pHᵢ to rise substantially above its initial level, this overshoot being a direct reflection of the preceding metabolic compensation. Subsequent work by scores of authors on countless cell types, including the PT and other renal cells, shows that the response of virtually all cells to CO₂/HCO₃⁻ is some variation on the theme first demonstrated with the squid giant axon (for review, see reference [21]).

Membranes with Negligible Gas Permeability

One of the core dogmas of physiology has been that all gases rapidly cross all membranes by dissolving in the membrane lipid. In the case of CO₂, the gas produces the effects that are shown in Figure 2B. For example, perfusing the lumen of a PT with CO₂/HCO₃⁻ causes pHᵢ to fall rapidly. Thus, when perfusing single gastric glands as in Figure 3A (22), using the...
technique that was introduced originally by Burg et al. (23), we were surprised by the parietal cell data in Figure 3B (and chief cell data; data not shown), which demonstrate that adding CO$_2$/HCO$_3^-$ to the luminal perfusate causes no detectable pH$_i$ change, even though far lower [CO$_2$] levels—when presented to the basolateral surface—evoke easily detectable pH$_i$ decreases (24). The perfusate with 100% CO$_2$ maintained its acidity along the entire length of the lumen, ruling out the possibility that the CO$_2$ had totally escaped in the first few milliseconds of its journey. Furthermore, luminal CO$_2$/HCO$_3^-$ failed to alter pH$_i$ over a wide range of [CO$_2$/HCO$_3^-$] ratios, ruling out the possibility that the separate influxes of CO$_2$ and HCO$_3^-$ might have precisely compensated for one another and yielded a null pH$_i$ shift. These were the first membranes shown to have negligible gas permeability. We also found that lowering luminal pH$_i$ to 1 failed to alter pH$_i$ (22).

We similarly observed null pH$_i$ shifts, rather than the expected pH$_i$ increase, when perfusing lumens of gastric gland (24) or colonic crypts (25) with NH$_3$/NH$_4^+$. Kikeri et al. (26), when perfusing the lumen of a mouse mucosal thick ascending limb with NH$_3$/NH$_4^+$, previously observed a substantial and paradoxical pH$_i$ decline, which proves that the pH$_i$ effects of the NH$_4^+$ influx overwhelmed those (if any) of an NH$_3$ influx. In fact, it would be interesting to see whether the apical membranes of the medullary thick ascending limb, like those of the gastric gland, indeed are impermeable to CO$_2$ and NH$_3$.

**AQP1: A Bifunctional Water/Gas Channel**

In a seminar at the University of Pennsylvania, in which I presented the data in Figure 3B, I suggested that the unknown specialization of gastric-gland apical membranes that allows them to resist luminal pH values below 1 also renders these membranes impermeable to CO$_2$ and NH$_3$. Paul De Weer asked me whether I had considered the possibility that the difference between apical and basolateral gastric-gland membranes might be that the basolateral membranes possess “gas channels” that the apical membranes lack. At first blush, I thought that the gas-channel suggestion was outlandish. De Weer soon forgot his comment, but I did not. After returning home, I began to imagine where gas channels might exist, if they existed at all. As luck would have it, Peter Agre, after a seminar at Yale, had generously sent us the cDNA encoding AQP1—discovered in red blood cells (RBC)—so that we might confirm the observation (27) that the water channel AQP1 is not permeable to H$^+$ (see also reference [28]). The combination of De Weer’s comment and Agre’s seminar provoked us to think about why RBCs—which transport gas, not water, for a living—should be such a rich reservoir of AQP1. Although there is no doubt that AQP1 is a water channel, might its physiologic role in RBCs be as a gas channel?

To test this hypothesis, Nazih Nakhoul injected cRNA encoding human AQP1 (or water as a control) into Xenopus oocytes, which he later injected with CA II protein. The purpose of the CA II was to catalyze the intracellular reaction CO$_2$ + H$_2$O $\rightarrow$ HCO$_3^-$ + H$^+$ and thereby keep [CO$_2$] low near the inner surface of the cell membrane, maximizing the CO$_2$ influx. Nakhoul found that when he exposed oocytes to CO$_2$/HCO$_3^-$, those that expressed AQP1 exhibited a CO$_2$-induced fall in pH$_i$ that was 40% faster than the control cells (29). This was the first evidence that the presence of a protein could enhance the movement of a dissolved gas across a cell membrane.

Gordon Cooper extended Nakhoul’s observations in experiments such as those shown in Figure 4. An important difference in Cooper’s experiments is that, rather than inject CA II, he enhanced CO$_2$ influx by removing the oocyte’s vitelline membrane (30). Focusing on the purple record in Figure 4, we see that switching the extracellular solution from one buffered with HEPES to one buffered with 1.5% CO$_2$/10 mM HCO$_3^-$ caused pH$_i$ to fall slowly, at a rate of $-9.6 \times 10^{-4}$ pH units/s. When Cooper subsequently transferred the oocyte to deionized water, osmosis caused it to lyse in 180 s. This oocyte had a relatively low level of AQP1 expression. The oocyte that is represented by the orange record had a much higher rate of acidification and a more rapid lysis. Finally, the oocyte that is represented by the green record had an even higher acidification rate and an even quicker lysis. These results showed that CO$_2$ entry paralleled AQP1 expression. Cooper additionally demonstrated that p-chloromercuribenzene sulfonate (pCMBS), an organic mercury that blocks AQP1’s water permeability, also eliminates the statistical difference between AQP1 and control oocytes. Finally, Cooper found that the C189S mutant, which Preston et al. (31) showed to be mercury insensitive in terms of water permeability, also is pCMBS insensitive in terms of CO$_2$ permeability.

Prasad et al. (32) confirmed that AQP1 enhances CO$_2$ permeability, studying purified AQP1 protein that was reconstituted into vesicle that was made from Escherichia coli membranes. Yang et al. (33) published experiments that seem to disprove the hypothesis that AQP1 acts as a conduit for CO$_2$. However, as discussed by Cooper et al. (34), these experiments were designed in a way that precluded detection of any enhancement of CO$_2$ permeability by AQP1.

Using a mass-spectroscopy technique to measure the permeability of RBC to $^{12}$C$^{18}$O$^{16}$O, Forster et al. (35) in 1998 made the
intriguing observation that 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS)—which, among other things, blocks the Cl-HCO$_3^−$/HCO$_3^−$-exchanger AE1—greatly reduces CO$_2$ permeability. In 2003, Blank and Ehmke (36), using the fluorescent dye BCECF to monitor pH in RBC ghosts, showed that HgCl$_2$ (presumably by blocking AQP1) and DIDS (presumably by blocking AE1) greatly reduced CO$_2$ permeability. Also in 2003, Uehlein et al. (37) demonstrated that a homologue of AQP1 enhances CO$_2$ permeability in plants, enhancing photosynthesis (the rate-limiting step for which is the availability of CO$_2$) and the growth of leaves. More recently, Gros et al. (38), using the mass-spectroscopy approach with normal and AQP1-null human RBCs, found that AQP1 is responsible for approximately 60% of the cells’ CO$_2$ permeability. An unidentified RBC protein, possibly the Rh complex or AE1, is responsible for an additional approximately 30%. It is interesting that parallel experiments on oocytes showed that DIDS blocks approximately 50% of the CO$_2$ flux through AQP1.

A final example of a physiologic role for AQP1 as a gas channel is provided by Zhou et al. (39), who worked with PT from wt versus AQP1-null mice (generously provided by Alan Verkman). Zhou et al. found that lack of AQP1 leads to a substantial deficit in HCO$_3^−$ reabsorption, as would be predicted by the model in Figure 1. He also performed another set of experiments that rely on a novel rapid-mixing technique that was developed by Zhao et al. (40) to create out-of-equilibrium (OOE) CO$_2$/HCO$_3^−$ solutions. We describe this approach in more detail below. When Zhou perfused the PT lumen with CO$_2$-free solution and exposed the basolateral surface to a “pure” HCO$_3^−$ solution (i.e., one with a physiologic [HCO$_3^−$] and pH but virtually no CO$_2$), it made no difference whether the PT was from a wt or AQP1-null mouse: The backflux of “carbon” was the same. However, when he exposed the basolateral surface to a “pure” CO$_2$ solution (i.e., one with a physiologic [CO$_2$] and pH but virtually no HCO$_3^−$), the backflux, compared with wt tubules, was 60% lower in AQP1-null than in wild-type tubules. These data are consistent with the hypothesis that the CO$_2$ permeability of AQP1 plays a major role in the reabsorption of HCO$_3^−$.

A lingering issue has been whether it is reasonable to expect a dissolved gas such as CO$_2$ to pass through AQP1. Recent molecular dynamics simulations by Wang et al. (41) suggested that CO$_2$ could pass (1) through each of the four aquapores of an AQP1 tetramer, single file with water and (2) through the central pore formed by the four monomers. This central pore seems to be a vacuum through which CO$_2$ and O$_2$ can move with great speed.

**Apical H$^+$ Secretion**

*The NH$_4^+$ Prepulse*

From his original work on the membrane-permeability properties of *Spirogyra* in the late 19th century, Overton understood that NH$_3$ in a solution that contains NH$_4^+$ (NH$_3$ + H$^+$ ⇌ NH$_4^+$)—as well as various neutral amines (R-NH$_2$ + H$^+$ ⇌ R-NH$_3^+$) in solutions that contain their charged ammonium ions—crosses cell membranes predominantly in their uncharged form. Working with *Rhododendron* flower petals (the pigment of which shifts from red to blue in response to alkalinity) and starfish eggs that contain neutral red, Jacobs (42) in 1922 recognized that the influx of NH$_3$—even in acidic solutions of NH$_4$Cl—causes a rise in pH (Figure 5A). Many decades later, Thomas (17) used a pH-sensitive microelectrode to monitor the rise in pH that was caused by exposing a snail neuron to a solution that contained NH$_4$Cl. The left side of Figure 5B shows an experiment from 1976 in which Boron and De Weer (18) exposed a squid giant axon for a relatively brief period to a solution that contained 10 mM NH$_4$Cl. Although the NH$_3$ in the solution was present at very low levels compared with the NH$_4^+$, the influx of this NH$_4^+$ nevertheless led to a consumption of cytoplasmic H$^+$ and thus an increase in pH. Removing the NH$_4$Cl caused the pH to changes to reverse. However, pH always fell to a value slightly below the initial one.

The explanation for the pH undershoot in the first part of the experiment becomes clear with a more protracted exposure to NH$_4$Cl. The rapid initial NH$_3$-induced pH increase was followed by a much slower but sustained pH decrease, which reflects the influx of the weak acid NH$_4^+$ into the axon, which lowers the pH. Subsequently removing the NH$_4$Cl, after this long exposure, caused pH to undershoot the pre-NH$_4$Cl value by an exaggerated amount. The magnitude of this undershoot reflects the degree of NH$_4^+$ influx during the previous NH$_4$Cl exposure. This was the first example of what has come to be known as the “ammonium prepulse” technique for acid loading cells. In fact, the axon will use its pH-regulatory machinery to recover from the intracellular acid load in Figure 5B but only in the presence of CO$_2$/HCO$_3^−$, as Boron and De Weer (43) subsequently demonstrated. This later work was the first practical use of an NH$_4^+$ prepulse as a tool to acid load a cell and the first demonstration of what has come to be known as the “ammonium prepulse” technique for acid loading cells.

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**Figure 5.** Effect of NH$_3$/NH$_4^+$ on pH$_i$, the NH$_4^+$ prepulse. (A) Model of NH$_3$ equilibration across the cell membrane. (B) Experimental record from a squid giant axon. The axon was cannulated at either end, and a glass, pH-sensitive microelectrode was inserted from one end, and a KCl-filled microelectrode was inserted from the other. Data from reference (18).
that cells can use HCO₃⁻ uptake to neutralize an acid load and thereby regulate pHᵢ.

**Apical Na-H Exchange**

One of the first vertebrate cells to be the focus of a study that exploited the NH₄⁺ prepulse technique was the PT of the salamander. Figure 6 shows the results of an experiment by Boron and Boulpaep (44), performed on an isolated, perfused tubule, cells of which were impaled with microelectrodes for monitoring pHᵢ and basolateral membrane potential (basolateral Vₘ). At first, both the lumen and the “bath” (i.e., the basolateral solution) contained a Na⁺-free solution to block Na⁺-dependent processes for regulating pHᵢ. In addition, the bath contained 4-acetamido, 4'-isothiocyanato-2,2'-stilbene disulfonate (SITS; an analog of DIDS) to block the electrogenic Na/HCO₃ co-transporter on the basolateral membrane. Applying NH₄⁺ caused a rapid rise in pHᵢ, followed by a slower fall. Washing away the NH₄⁺ caused pHᵢ to fall substantially and then recover only very slightly. Subsequently returning 100 mM Na⁺ to the lumen caused pHᵢ to return to its initial value. Other experiments showed that this pHᵢ recovery is inhibited by amiloride, demonstrating that it was an apical Na-H exchanger that was responsible for the pHᵢ recovery. Murer et al. (45), in their studies of brush border membrane vesicles, had been the first to observe Na-H exchange. The groups of Aronson (46) and Saktor (47) also made seminal contributions to our understanding of Na-H exchange by working with brush border membrane vesicles. The experiment shown Figure 6 was the first demonstration of apical Na-H exchange by a living epithelial cell.

**Basolateral Na/HCO₃ Co-Transport**

*Initial Description*

In the late 1970s and early 1980s, the mechanism of HCO₃⁻ exit across the PT basolateral membrane was a subject of considerable interest (reviewed in references [2,3]). One suggestion was that HCO₃⁻ exited via Cl-HCO₃ exchange, although the dominant view seems to have been that HCO₃⁻ exited via a conductive pathway, the simplest explanation for which would be a HCO₃⁻ channel. While exploring these options in an isolated, perfused salamander PT, Boulpaep and Boron (48) found that lowering basolateral [HCO₃⁻] ([HCO₃⁻]₀) at a fixed [CO₂]₀ causes pHᵢ to fall rapidly—an observation that was consistent with both models. In addition, they found that this maneuver causes basolateral Vₘ to shift rapidly in the positive direction, consistent with the HCO₃⁻ conductance model. However, when they removed basolateral Na⁺, they made a startling observation (Figure 7): Although pHᵢ fell as expected (e.g., reversing the basolateral Na-H exchanger would have caused an internal acidification), the basolateral Vₘ shifted rapidly in the positive direction. Lowering the concentration of an extracellular cation should have caused basolateral Vₘ to shift in the negative direction. The observation that lowering [HCO₃⁻]₀ and removing basolateral Na⁺ each caused basolateral Vₘ to shift in the positive direction—together with other data—pointed to a new kind of transporter, an electrogenic Na/HCO₃ co-transporter that moves more negative charge as HCO₃⁻ (or CO₃⁻) ions than positive charge as Na⁺. Like the previously discovered Cl-HCO₃ exchanger of RBCs and the Na⁺-driven Cl-HCO₃ exchanger of invertebrates, the Na-/HCO₃ co-transporter is blocked by the disulfonic stilbenes SITS and DIDS. However, in contrast to the other two transporters, the electrogenic Na/HCO₃ co-transporter is independent of Cl⁻. Later work by Soleimani and Aronson on basolateral membrane vesicles from rabbit renal cortex pointed to a Na⁺:HCO₃⁻ stoichiometry of 1:3 (49), which ensures that the transporter normally mediates the exit of Na⁺ and HCO₃⁻ across the basolateral membrane—the basolateral step of HCO₃⁻ reabsorption. Later work by Frömter and his colleagues (50–52) showed that the electrogenic Na/HCO₃ co-transporter is the dominant basolateral pathway for HCO₃⁻ in the S1 and S2 segments of the PT, where the vast majority of the HCO₃⁻ reabsorption occurs, but that a basolateral Cl-HCO₃ exchanger also is important in the S3 segment.

![Figure 6](image_url)

**Figure 6.** Use of an NH₄⁺ prepulse to acid-load an isolated, perfused salamander PT cell. pHᵢ was measured with a recessed-tip glass pH microelectrode (17), in conjunction with a KCl-filled microelectrode. The tubule was exposed throughout the experiment, at both the luminal and the basolateral sides, to HEPES-buffered solution that lacked CO₂/HCO₃⁻. During the indicated time, the tubule was exposed from both the luminal and the basolateral (i.e., "bath") surfaces to a solution in which 20 mM NH₄⁺ replaced 20 mM Na⁺, an NH₄⁺ prepulse designed to acid-load the cell. Extracellular [Na⁺] was either 0 or 100 mM, as indicated. Vₘ, basolateral membrane potential; SITS, 4-acetamido, 4'-isothiocyanato-2,2'-stilbene disulfonate. Data from reference (44).

![Figure 7](image_url)

**Figure 7.** The effect of basolateral Na⁺ removal in an isolated, perfused salamander PT, the electrogenic Na/HCO₃ co-transporter. pHᵢ was measured with a recessed-tip glass pH microelectrode (17), in conjunction with a KCl-filled microelectrode. The tubule was exposed throughout the experiment, at both the luminal and the basolateral sides, to a solution buffered with 1.5% CO₂/10 mM HCO₃⁻. During the indicated times, basolateral Na⁺ was replaced with an organic cation. Dotted red lines indicate expected Vₘ response in the absence of an electrogenic Na/HCO₃ co-transporter. Data from reference (48).
The absence of a naturally abundant and highly enriched source of the electroneutral Na+/HCO₃⁻ co-transporter—unlike the Cl-HCO₃⁻ exchanger AE1, which is highly expressed in RBCs—frustrated early attempts to obtain the cDNA that encodes the electroneutral Na+/HCO₃⁻ co-transporter. Michael Romero joined our laboratory in 1992 with the goal of expression-cloning the transporter in Xenopus oocytes. Matthias Hediger in the laboratory of Ernest Wright had perfected this approach in cloning the Na/glucose transporter (53–55). Later, as an independent investigator, Hediger had already expression-cloned a wide range of membrane proteins, including a glutamate transporter (56), a subunit of heteromeric amino-acid transporters (57), a urea transporter (58), and an H-driven peptide transporter (59). We were most fortunate that Hediger was interested in collaborating with Romero.

The first step in expression cloning is to inject size-selected mRNA from a tissue source (rabbit kidneys in our case) into Xenopus oocytes and then assay for expression of the desired function. Unfortunately, repeated attempts with rabbit mRNA were fruitless. Before giving up, we decided to inject mRNA from salamander kidneys, reasoning that amphibian oocytes might better express an amphibian mRNA. To our delight, this approach was successful. Another key element in this study was collaboration with Romero. It is interesting to note that even the pure rat clone failed to express robustly in Xenopus oocytes until subcloned into a Xenopus expression vector that flanks both the 5' and 3’ ends of the rat NBC with the corresponding untranslated regions of the Xenopus β-globin mRNA. This result probably explains why we and others failed in our attempts to expression clone NBCe1 using mammalian mRNA. Bernhard Schmitt raised the first antibodies to the electronegenic NBC, and he and his colleagues demonstrated that the protein is expressed heavily at the basolateral membranes of the S1 and likely the early S2 segments of the renal PT (70).

A second electroneutral Na+/HCO₃⁻ co-transporter was discovered during the cloning of the Na/glucose transporter (53–55). The Na-glucose transporter was expressed by NCBE, cloned by Wang et al. (71) and characterized by Grichtchenko et al. (80), is a Na⁺-driven Cl-HCO₃⁻ exchanger that is expressed heavily in brain but also in other tissues, including kidney. NCBE, cloned by Wang et al. (81), is electroneutral, but whether it transports Cl⁻ still is controversial. Form follows function among mammalian transporters

**Figure 8.** Dendrogram of the SLC4 family of HCO₃⁻ transporters. All of the transporters are members of the SLC4 family.

**Other Members of the SLC4 Family**

With the discovery of at least one electroneutral NBC—the clone was identified by Pushkin et al. (71), and the encoded protein was characterized as an electroneutral NBC by Choi et al. (72)—we appended the suffix “e” to designate electronegenic and “n” to designate electroneutral. Moreover, Virkki et al. (73) and Sassani et al. (74) demonstrated that one of four cDNA clones that previously were isolated by Pushkin et al. (75,76) actually encodes a second electroneutral NBC. Thus, the original, renal electroneutral NBC is known as NBCe1-A. A second splice variant of the same gene—identified by Abuladze et al. (77) in pancreas and by Choi et al. (78) in heart—is known as NBCe1-B. This splice variant seems to be the most widely expressed NBCe1 variant. A brain-specific splice variant that was identified by Bevensee et al. (79) is known as NBCe1-C. The second electroneutral NBC gene is NBCe2, and the electroneutral NBC is NBCn1.

In addition to NBCn1, at least two other electroneutral Na⁺-coupled HCO₃⁻ transporters exist. NDCBE, cloned and characterized by Grichtchenko et al. (80), is a Na⁺-driven Cl-HCO₃⁻ exchanger that is expressed heavily in brain but also in other tissues, including kidney. NCBE, cloned by Wang et al. (81), is electroneutral, but whether it transports Cl⁻ still is controversial. Form follows function among mammalian transporters

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Na\(^{+}\)-coupled HCO\(_3\)^– transporters, with the electrogenic NBC grouping together in the peach-colored subregion in the dendrogram in Figure 8 and the electroneutral Na\(^{+}\)-coupled HCO\(_3\)^– transporters grouping together in the green subregion.

Two members of the SLC4 family have deduced amino acid sequences that lie apart from the rest. AE4 originally was named as a CI-HCO\(_3\) exchanger (82). However, its function is unsettled, and key areas of its amino acid sequence are more reminiscent of a Na\(^{+}\)-coupled HCO\(_3\)^– transporter. BTR1, originally cloned by Parker et al. (83), seems to be a Na\(^{+}\)-coupled borate (i.e., boron!) transporter (84), renamed NaBC1. Human mutations in NaBC1 cause a corneal condition that is known as congenital hereditary endothelial dystrophy (85).

**Structure**

Figure 9 is a model of the topology of NBCe1-A, based on studies of the Cl-HCO\(_3\) exchanger AE1 (86). All members of the family have a large cytoplasmic N terminus (Nt) and a much smaller cytoplasmic C terminus (Ct). The crystal structure of most of the Nt of AE1 has been solved (87) and shows that the Nt is a dimer. We now have crystallized the Nt of NBCe1-A, and a preliminary x-ray diffraction analysis indicates that this Nt, too, is a dimer (88).

Various approaches (e.g., see references [89,90]) generally have yielded similar conclusions about the amino acid residues that contribute to transmembrane segments 1 through 5 (TM1 through TM5). Therefore, investigators feel reasonably confident about these assignments. However, investigators are far less comfortable about the topology of the remainder of the molecule because the data sometimes are conflicting, presumably because the later TMs are more flexible.

One characteristic of the Na\(^{+}\)-coupled HCO\(_3\)^– transporters is that they all have a long extracellular loop between TM5 and TM6 (11). Moreover, this loop is the site of consensus glycosylation sites—proved in the case of NBCe1-A (91). Another curiosity is the four heavily conserved cysteine residues in these loops but not those of other SLC4 family members. (It is interesting that AE4 has these for Cys residues.) Work on AE1 shows that lysine residues near the end of TM5 and TM12 can bind covalently the inhibitor DIDS. Preliminary work from our laboratory (92) indicates that these same regions are important for the reversible, noncovalent binding of DIDS.

Abuladze et al. (93) have mutated a large number of charged residues that are conserved among SLC4 members and have identified several mutations that interfere with function. Choi et al. (unpublished observations) have constructed a series of chimeras between NBCe1-A and one splice variant of NBCn1 (NBCn1-B) in which they exchanged the cytoplasmic Nt domain, third extracellular loop, and/or the cytoplasmic Ct domain. They found that none of these three domains is important for determining the electrogenicity of the transporter but that both TM1 through 5 and TM6 through 13 of NBCe1 must be present for the chimera to function as an electrogenic co-transporter.

**Naturally Human Mutations of NBCe1 (SLC4A4)**

Investigators thus far have identified 10 mutations (94–100) in the human SLC4A4 gene that are associated with a variety of defects that may include severe autosomal recessive, pRTA, ocular abnormalities, growth and mental retardation, and abnormal dentition. The sites of these mutations are indicated by green circles in Figure 9. Three of these are nonsense mutations that lead to premature truncation of the protein, and seven are missense mutations.

Of the three missense mutations, the first is Q29X (95), numbered for the renal splice variant NBCe1-A, which results in a truncation very early in the cytoplasmic Nt. The second is the deletion of nucleotide 2311 (97), which presumably causes a frameshift in codon 721 and, after 27 anomalous amino acids, a premature stop in the extracellular loop between TM7 and TM8. The third missense mutation is a 67-nucleotide deletion at the boundary of exon 23 and the following intron (100); it would lead to a truncated cytoplasmic Ct.

Of the seven missense mutations, R298S in the cytoplasmic Nt, S427L in TM1, and R510H near the extracellular end of TM4 all seem to reduce targeting of the co-transporter to the basolateral membrane in polarized renal epithelial cells in culture (101). All three also exhibit decreased functional expression in *Xenopus* oocytes, although it is not clear to what extent this effect reflects decreased protein delivery to the plasma membrane (94,96,98,101,102).

Work from our laboratory suggests that R881C most likely causes a trafficking defect with normal intrinsic function (103), and A799V seems to cause defects in both trafficking and intrinsic function (104). The most recently discovered NBCe1 mutation is L522P in TM4, which does not traffic to the plasma membrane in oocytes (99).

**Basolateral CO\(_2\) and HCO\(_3\)^– Sensors**

**Initial Hints**

More than a half century ago, Brazeau and Gilman (105) and Pitts and his colleagues (106) published classical experiments demonstrating that respiratory acidosis elicits a rapid, compen-
satory increase in renal H⁺ secretion. However, the problem of determining how the kidney senses acid-base disturbances had been refractory to further dissection, mainly because the equilibrium CO₂ + H₂O ⇌ HCO₃⁻ + H⁺ makes it difficult to attribute effects to changes in [CO₂] or to [HCO₃⁻] or to [H⁺]. We became interested in this problem in a very indirect way.

Figure 10 shows the arrangement of an isolated, perfused PT. Figure 11 shows two experiments in which Nakhoul et al. (107) used the absorbance spectrum of a fluorescein dye to monitor pHᵢ in the cells of an isolated, perfused S3 segment of a rabbit PT. In Figure 11A, switching only the luminal buffer from HEPES to CO₂/HCO₃⁻ caused the expected decrease in pHᵢ. The influx of CO₂ produced the initial pHᵢ decline, and the efflux of HCO₃⁻ across the basolateral membrane sustained the intracellular acidification. In Figure 11B, we see that making the same solution change on the basolateral side has a very different effect. For a few seconds, pHᵢ declined rapidly, as it did when we added CO₂/HCO₃⁻ to the lumen. However, within 5 s, pHᵢ began a rapid increase to a value that is far higher than the initial pHᵢ. One might argue that the sustained pHᵢ increase could have been due to HCO₃⁻ uptake. However, adding basolateral CO₂/HCO₃⁻ triggers a similar pHᵢ increase even when CO₂/HCO₃⁻ is already present in the lumen, and under these conditions, the tubule actively reabsorbs HCO₃⁻. That is, the net traffic of HCO₃⁻ across the basolateral membrane is outward. Therefore, as unlikely as it seems, CO₂ and/or HCO₃⁻ on the basolateral side of the cell somehow must trigger the secretion of H⁺ across the membrane on the apical side.

Further experiments by Chen and Boron (108,109) showed that adding CO₂/HCO₃⁻ to the bath or to the bath and the lumen markedly stimulated both apical Na-H exchangers and proton pumps. However, adding CO₂/HCO₃⁻ to the only lumen was without effect. These experiments directly demonstrated that a sensor for CO₂ and/or HCO₃⁻—located at or near the basolateral membrane—can stimulate H⁺ extrusion at the opposite side of the cell. These experiments provided another surprise: Decreases in pHᵢ produce only very small increases in the rates of apical Na-H exchange and H⁺ pumping in a living PT cell. The real regulation of H⁺ transport rates comes not from intracellular protons but from basolateral CO₂ and/or HCO₃⁻. But which?

**OOE CO₂/HCO₃⁻ Solutions**

In the 1993 paper by Nakhoul et al. (107) is an appendix that analyzes the CO₂/HCO₃⁻-induced alkalization of Figure 11B in the context of the reactions that are responsible for the interconversion of CO₂ and HCO₃⁻. The first of these reactions is very slow, and the second is extremely fast:

\[
CO₂ + H₂O \rightleftharpoons H₂CO₃ \rightleftharpoons HCO₃⁻ + H⁺
\]

Before submitting the paper, I wanted to review my calculations with Robert Berliner, who at the time was Professor Emeritus in our department. After considering the above reaction rates for a couple of hours—and thinking about Figure 11B—Bob wistfully looked up at the ceiling and said that it was too bad that we had to add CO₂ and HCO₃⁻ together, rather than one at a time. In an instant—primed by our discussion of the slow reaction governed by k₁—the thought came to me, “Of course we can!” All we need to do is to take advantage of that slow reaction . . . and thus were born out-of-equilibrium (OOE) solutions.

Figure 12A shows the approach for generating a solution with physiologic levels of HCO₃⁻ and pH but virtually no CO₂ (“pure” HCO₃⁻), and Figure 12B shows the comparable approach for generating a solution with physiologic levels of CO₂ and pH but virtually no HCO₃⁻ (“pure” CO₂). However, implementing OOE solutions proved to be more challenging than conceiving of them. Shortly after my conversation with Bob, Jinhua Zhao arrived as a new postdoctoral fellow. For 2 yr, she: (1) labored with ever more powerful syringe pumps to deliver evenly two streams of liquid, (2) employed various tricks for adequately mixing these two streams, (3) dealt with issues of temperature regulation, and (4) accommodated the chemistry of CO₂ and HCO₃⁻. We tested the system on squid giant axons (40), in which we were able to create “pure” CO₂ solutions that could acidify the cell and increase intracellular buffering power as predicted as well as “pure” HCO₃⁻ solutions that could supply the substrate for a HCO₃⁻ transporter but not increase intracellular buffering power.
Zhao also applied her new technology to the isolated perfused rabbit PT (110) and then introduced Yuehan Zhou to the project. He monitored $J_{\text{HCO}_3}$ as well as the rate of volume absorption ($J_V$) as he systematically used OOE solutions to vary basolateral composition one parameter at a time (Figure 13A).

The results show that raising $[\text{HCO}_3^-]_\text{B}$ (at a fixed $[\text{CO}_2]_\text{B}$ of 5% and a fixed $pH_\text{B}$ of 7.40) causes $J_{\text{HCO}_3}$ to fall (Figure 13B, left)—an appropriate response for the “metabolic” part of metabolic acid-base disturbances. That is, the greater the plasma $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$, the less $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ the kidney ought to reabsorb. Moreover, raising $[\text{CO}_2]_\text{B}$ (at a fixed $[\text{HCO}_3^-]_\text{B}$ of 22 mM and a fixed $pH_\text{B}$ of 7.40) causes $J_{\text{HCO}_3}$ to rise (Figure 13B, middle)—an appropriate compensation for the “respiratory” part of respiratory acid-base disturbances. That is, the greater the plasma $[\text{CO}_2]$, the more $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ the kidney ought to reabsorb. However, we were surprised to find that raising $pH_\text{B}$ (at a fixed $[\text{HCO}_3^-]_\text{B}$ of 22 mM and a fixed $[\text{CO}_2]_\text{B}$ of 5%) elicits no change in $J_{\text{HCO}_3}$ (Figure 13B, right).

In parallel experiments, we measured $pH_\text{B}$ under the conditions of Figure 13B and found that, indeed, $pH_\text{B}$ changes substantially as one increases $pH_\text{B}$ from 6.8 to 8.0. However, these changes in $pH_\text{B}$ and $pH_\text{I}$ do not evoke changes in $J_{\text{HCO}_3}$. Instead, at least in terms of its acute response to acid-base disturbances, the PT regulates plasma pH not by monitoring pH but by monitoring two surrogates: the main buffer components of the body, $\text{HCO}_3^-$ and $\text{CO}_2$ (Figure 14). This tactic of regulating a parameter that the body does not measure directly is not unique to acid-base parameters and the PT. For example, the body uses stretch receptors in vessels to gauge the adequacy of the effective circulating blood volume.

An extremely interesting observation in the above study is that the changes in $J_{\text{HCO}_3}$ occurred without the expected parallel changes in $J_V$. Because the fluid that the PT reabsorbs is approximately isosmotic, an increase in $J_{\text{HCO}_3}$, for example, should have been accompanied by a sizable increase in $J_V$. It seems that the PT cell compensates for changes in NaHCO3 reabsorption by making reciprocal changes in the reabsorption of other solutes, thereby keeping $J_V$ relatively constant (Figure 14). This response would help to maintain a stable BP as the tubule responds to acid-base disturbances.

It is worth noting that in the above experiments, we applied the challenges only to the basolateral surface of the tubule. Therefore, it is possible that PT have a pH sensor at the apical

![Image](https://example.com/image12.png)

*Figure 12. Generating out-of-equilibrium (OOE) CO2/HCO3− solutions. (A) “Pure” HCO3−. (B) “Pure” CO2. In each case, a single syringe pump is used to deliver solutions evenly from two syringes. The two solutions meet at a “T” that is filled with a nylon mesh that promotes mixing. The total flow typically is 7 ml/min. The time from mixing to delivery of the OOE solution to the tubule is approximately 200 ms. Thus, the tubule is exposed continuously to a newly generated solution.*

![Image](https://example.com/image13.png)

*Figure 13. Effect on HCO3− reabsorption of varying—one at a time—basolateral [HCO3−], [CO2] and pH. (A) Model. (B) Experimental data obtained on isolated perfused rabbit S2 PT. The triangles indicate standard equilibrated conditions (5% CO2, 22 mM HCO3−, pH 7.40). All other symbols represent OOE solutions. $J_{\text{HCO}_3}$ rate of HCO3− reabsorption; $J_V$, rate of volume reabsorption; $pH$, basolateral. Data from reference (142).*
Figure 14. Model of the regulation of JHCO3 and JNa by basolateral CO2 and HCO3− in a renal PT. The green arrows represent stimulation, and the red arrows represent inhibition. We propose that basolateral CO2 stimulates the acid-base transporters but reciprocally inhibits the transporters that are responsible for the reabsorption of other solutes. HCO3− would have the opposite effect. Glc, glucose; Lac, lactate; Gln, glutamine; JOther rate of reabsorption of solutes other than NaHCO3.

Figure 15. Effect of tyrosine-kinase inhibitors on the CO2-induced increase in JHCO3. Data from reference (121).

Role of Tyrosine Kinases

A critical question is how the tubule is able to sense changes in [CO2]l and transduce them into altered cell function. The bacterium Rhizobium meliloti senses O2 (113) and the plant Arabidopsis thaliana senses ethylene (114–119) using membrane proteins that signal through a histidine kinase. Because animal cells lack histidine kinases, we hypothesized that PT cells might use a tyrosine kinase to signal an increase in CO2. Indeed, as shown in Figure 15, 35 nM PD168393, which alkylates a Cys residue in the ATP binding pocket of tyrosine kinases, blocks the ability of the PT to respond to increased [CO2]l. Moreover, this effect, as well as increased expression of NHE3 mRNA, is blocked by overexpressing Csk (112), a natural inhibitor of src kinases.

Role of Angiotensin II

The hormone that has the greatest stimulatory effect on JHCO3 is angiotensin II (AngII), applied at a “low” dosage to either the luminal or basolateral surface (124–127). When applied at a “high” dosage to either surface, however, AngII actually reduces JHCO3 (126,128).

We examined the effect low-dosage (10−11 M) and of high-dosage (10−9 M) AngII on the response of JHCO3 to alterations in [CO2]l (129). We found that low-dosage AngII, added to either the lumen or the bath, tends to shift the JHCO3-versus-[CO2]l relationship to the left, so that lower levels of [CO2]l tend to stimulate HCO3− reabsorption. Conversely, high-dosage AngII, added to either the lumen or the bath, tends to blunt the JHCO3-versus-[CO2]l relationship.

The PT has all of the molecular machinery to generate its own AngII (130–136), which actually appears in the tubule lumen. It is not yet established whether the tubule secretes angiotensinogen, AngI, or AngII. However, preliminary data from our group on Prinivil, an inhibitor of the angiotensin-converting enzyme, suggest that the tubule actually secretes preformed AngII (137). We decided to examine the effect of AngII receptor blockers on the JHCO3-versus-[CO2]l relationship. We were surprised to find that in the absence of added AngII, 10−8 saralasin, a peptide that blocks both AT1 and AT2 receptors, reduces JHCO3 to levels that normally are observed at 0% CO2 (see Figure 13B) and renders the tubule insensitive to changes in [CO2]l between 0 and 20%. The AT1-specific blocker candesartan has a similar effect. Finally, in tubules from AT1a-null mice, we find a moderate depression of JHCO3 at 5% CO2 and a total insensitivity to changes in [CO2]l.

Endogenous luminal AngII plays at least a critical permissive role in the tubule’s response to alterations in [CO2]l. It would be interesting to know whether basolateral CO2 somehow accentuates the endogenous AngII system, perhaps by increasing the secretion of AngII, increasing the density or the sensitivity of apical AT1 receptors, or enhancing downstream signaling from the AT1 receptor to the acid-base transporters.

Conclusions and Outlook

Looking back over the past 60 yr of progress in understanding acid secretion by the PT, it is sobering to realize that the pioneers in the field, working with technologies that are far more primitive than those that are at our disposal today, were able to assemble a good picture of the fundamental processes (138–141): The tubule exchanges luminal Na+ for cytosolic H+, carbonic anhydrase catalyzes the secreted H+ to titrate filtered HCO3− to CO2 and H2O, and these substances enter the cell
and regenerate $\text{HCO}_3^-$ that then moves into the blood. Today, we understand many more of the details, including the dynamics of electrical and chemical gradients and the molecular identities of the transporters. We know their amino acid sequences, and we even know many examples in which specific mutations of these proteins lead to human disease.

These advances notwithstanding, much remains to be learned. We still do not understand how the transporters function at an atomic level or the mechanisms of disease-causing mutations. Such understanding will come eventually through advances in structural biology. However, reading between the lines of this review, one can sense that many mysteries remain even at the cellular and molecular levels. Just how do CO$_2$ and other gases move through aquaporins and other gas channels? Which molecules does the PT cell use to sniff basolateral CO$_2$ and HCO$_3^-$? How does the cell transduce these signals to the acid-base transporters? How does the cell reciprocally regulate the reabsorption of NaHCO$_3$ and of other solutes so as to stabilize $\text{pH}$? What role does locally generated AngII play in these processes? How does the PT cell integrate information from CO$_2$ levels, locally versus systemically generated AngII, and other hormones such as parathyroid hormone and endothelin? With continued support from the National Institutes of Health and other agencies and the enthusiasm of bright young scientists, the renal community can look forward to the day when other winners of the Homer W. Smith Award will answer these questions.

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