JOP. J. Pancreas (Online) 2001; 2(4 Suppl):182-191.

The Electrogenic Na^+/HCO_3^- Cotransporter, NBC

Michael F Romero

Department of Physiology and Biophysics, Department of Pharmacology, Case Western Reserve University School of Medicine. Cleveland, OH, USA

Summary

Electrogenic Na^+/HCO_3^- (NBC) function has been characterized in many mammalian tissues including, kidney, pancreas, and brain. Cloning efforts identified a single cDNA, NBC/NBC1, that possesses all the functional attributes of the electrogenic Na^+/HCO_3^- cotransporter. This NBC clone is related to the anion exchangers and thus forms a bicarbonate transporter superfamily. Presently two N-terminal and one C-terminal isoforms are known. All three isoforms appear to arise from the same gene and seem to have identical function. NBC antibodies have localized NBC isoforms in kidney, pancreas, brain, small intestine, colon, epididymis, eye, heart, liver, salivary glands, stomach, and testis. Functionally, NBC appears HCO_3^- and Na^+ selective. NBC stoichiometry in *Xenopus* oocytes is 1 Na^+ : 2 HCO_3^-, implicating a possible accessory protein interaction.

Background of the Electrogenic Na^+/HCO_3^- Cotransporters

HCO_3^-, like other ions and nutrients in the blood, is filtered in the kidney at the glomerulus, then absorbed by transport processes in the renal nephron. The proximal tubule is responsible for 80-90% of renal HCO_3^- absorption. HCO_3^- in the luminal fluid combines with secreted H^+ (mostly by Na^+-H^+ exchange [1]) to form CO_2 and H_2O, both of which easily enter the proximal tubule cell. Prior to the 1980's the mode of HCO_3^- movement from the proximal tubule cells back into the blood was elusive. A basolateral HCO_3^- conductance pathway was hypothesized.

Boron and Boulpaep made the astonishing discovery that this HCO_3^- absorption process was coupled asymmetrically to Na^+ transport [2]. This transport activity was called the "electrogenic Na^+/HCO_3^- cotransporter." This electrogenic Na^+/HCO_3^- cotransporter mediated a "fingerprint" transport [2]: Na^+ transport, HCO_3^- transport, electrogenic (1 Na^+ : at least 2 HCO_3^-), no Cl^- transport/dependence, and stilbene inhibition. Later, a functionally similar cotransport activity was reported in mammals: bovine corneal endothelial cells [3], the basolateral membrane of rat proximal convoluted tubule [4], the basolateral membrane of rabbit proximal straight tubule [5], and many other preparations (for review see [6]).

Yet until 1995, the molecular nature of this protein(s) was unknown. This kidney of the salamander *Ambystoma tigrinum* was used to expression clone a renal electrogenic Na^+/HCO_3^- cotransporter (NBC) [7, 8]. As the cotransport activity originally characterized in this tissue, NBC

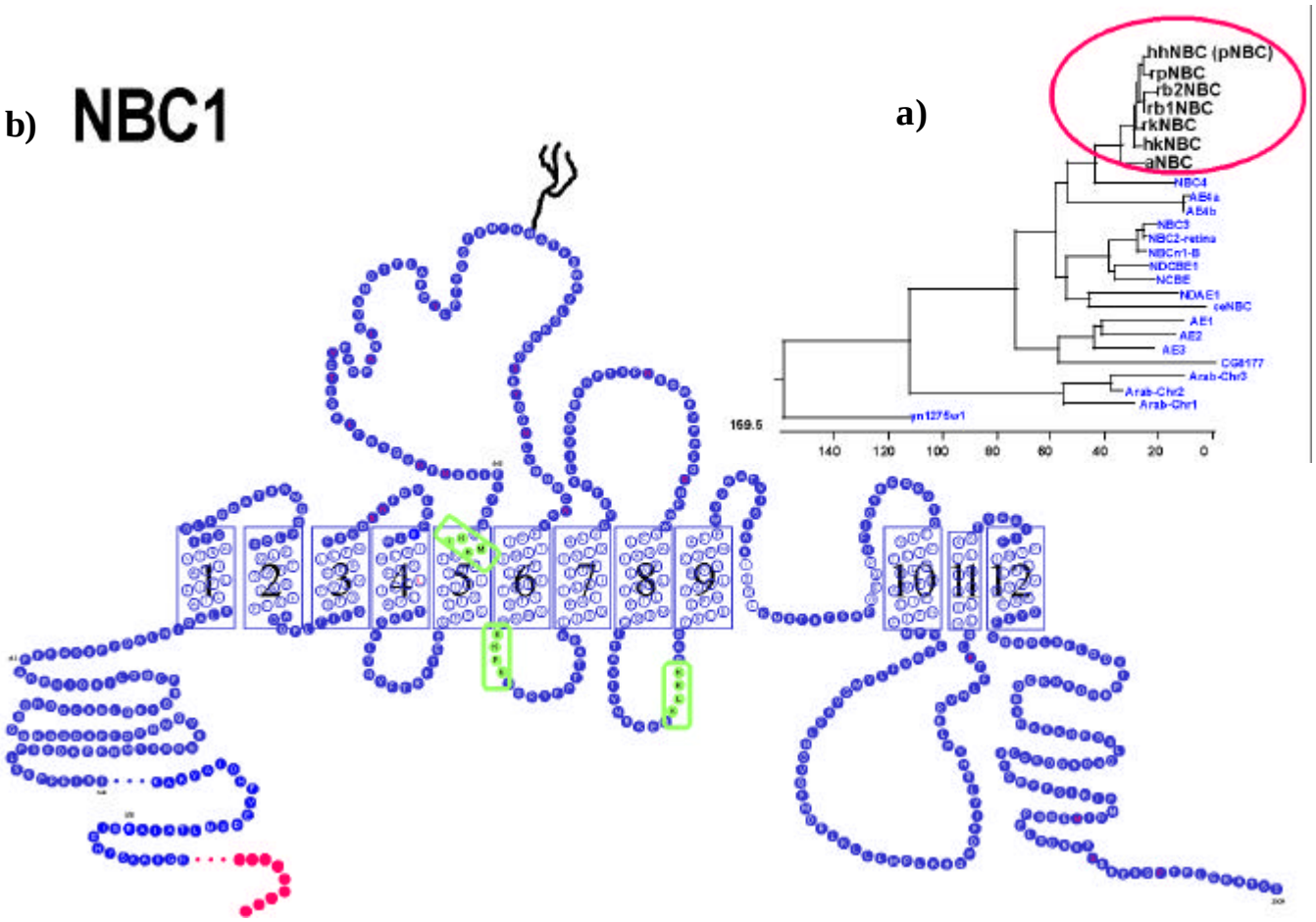

Figure 1. Predicted bicarbonate transporter superfamily relationships.
The a) panel is a dendrogram for representative member of the bicarbonate transporter superfamily (BTS) [54]. The open circle indicates the electrogenic Na^+/HCO_3^- cotransporter isoforms collectively known as NBC or NBC1. AE1-3 are the mammalian anion exchangers. Other BTS members have been identified and cloned: ceNBC (AF004926), NDAE1 (AF47468), NBC3 (AF47033), NBCn1-D (AF80106), NCBE (AB033759), NDCBE1 (AF069512), AE4a and AE4b (AB038264), NBC4 (AF207661), and yeast yn1275w1. A second *Drosophila* protein (CG8177) and three *Arabidopsis* proteins (Arab-Chr1, Arab-Chr2, and Arab-Chr3) have been predicted from genome sequencing projects but not functionally evaluated. The b) panel is a working membrane topology model of NBC based largely on structural results of AE1 (see [55]). Both N- and C-termini are predicted as intracellular. A large, central extracellular loop uses one N-linked glycosylation site N617 [56] though seven are predicted. Three predicted DIDS-binding motifs are indicated by boxes.

transported Na^+ and HCO_3^-, and was electrogenic (1 Na^+ : at least 2 HCO_3^-), Cl^- independent, and inhibited by stilbenes (such as 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid: DIDS). Interestingly using amphibian kidney rather than mammalian tissue to clone NBC was the key to success [8, 9]. Surprisingly, this electrogenic NBC sequence was molecularly related to the electroneutral band-3 like proteins, i.e., the anion exchangers AE1, AE2, and AE3 [8, 9]. This homology revealed a probable bicarbonate transporter superfamily (BTS) [8] that now has many seemingly topologically related members (Figure 1). This relationship and NBC's cloning has renewed interest in HCO_3^- transporters.

NBC Clones, Proteins and Gene

The renal or "kidney" NBC ORF (open reading frame) (kNBC) encodes 1035 amino

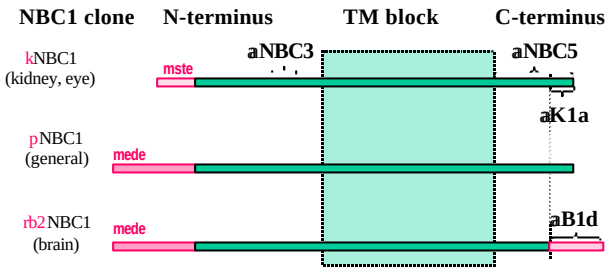

Figure 2. Schematic of electrogenic Na^+/HCO_3^- cotransporter isoforms and antibodies.
Diagram of the three NBC1 isoforms: two N-terminal (kNBC and pNBC) and one additional C-terminal (rb2NBC). All other sequence between clones is identical within a species. Sequence regions recognized by NBC antibodies are indicated by brackets and annotated with "α".

acids (Figure 2, top) and predicts a protein of 116 kDa [8, 9, 10, 11]. The NBC-protein is predicted to have both the N- and C-termini intracellular (Figure 1), many potential phosphorylation sites, as well as several N-linked glycosylation sites.

A second N-terminal NBC isoform was cloned from pancreas (pNBC) [12] and heart (hhNBC) [13]. This clone has the first 41 amino acids replaced by a different 85 amino acids (Figure 2, middle). This pNBC encodes 1079 amino acids and predicts a protein of 120 kDa [12, 13, 14]. The longer NBC protein also encodes similar transport [12, 13] and is electrogenic [13].

Table 1. Tissue distribution of the electrogenic Na^+/HCO_3^- cotransporters NBC (NBC-1).

Tissue	NBC Isoform	Identification Method	Reference
Brain	rb1NBC (pNBC)	Cloning	[14, 39]
Brain	rb2NBC	Cloning	[14]
		IF localization [(a)]	[40, 41]
Colon	pNBC	Northern blot	[12]
	kNBC/pNBC	Western blot	[14]
Duodenum	kNBC and pNBC	RT-PCR / IF localization	[42]
(Small Intestine)	kNBC/pNBC	Western blot	[14]
Epididymis	NBC	IF localization	[43]
Eye	kNBC and pNBC	RT-PCR	[44]
	pNBC	Cloning / RT-PCR	[45]
	pNBC	Western blot	[45]
Heart	hhNBC/pNBC	Cloning	[13]
	kNBC/pNBC	Western blot	[14]
Kidney	kNBC	Cloning	[8, 11]
		IF localization	[46, 47]
Kidney	pNBC	Cloning	[48]
Kidney	rb2NBC	Western blot	[14]
Liver	NBC	Northern blot	[9, 12]
	rb2NBC	Western blot	[14]
Lung	NBC	Northern blot	[9]
	kNBC/pNBC	Western blot	[14]
Pancreas	pNBC	Cloning	[12, 49]
		IF localization	[49, 50]
Prostate	pNBC	RT-PCR / cloning	[51]
		Northern	[12]
Salivary Glands	NBC	IF localization	[52]
Stomach	NBC	Cloning / RT-PCR	[53]
		Northern	[11, 12]
Testis	kNBC/pNBC	Western blot	[14]
Thyroid	pNBC	Northern blot	[12]

IF is immunofluorescence. NBC-1 is SLC4A4. Note that "NBC" as an isoform designation indicates that the exact isoform of NBC is currently unknown or unpublished.
(a) The study by Bevensee *et al.* identifying rb2NBC in the brain [14] is the only study to date identifying NBC isoforms by immunohistochemistry.

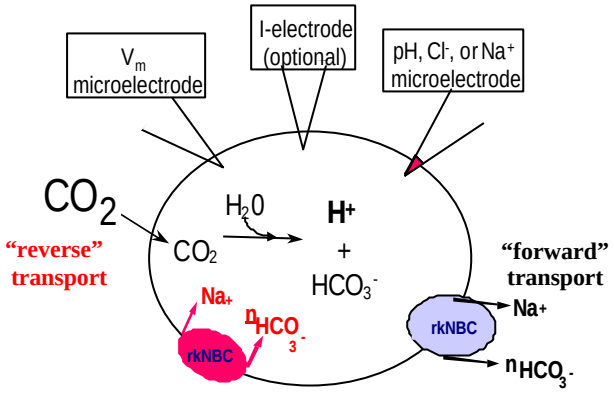

Figure 3. Experimental arrangement-*Xenopus* oocyte electrophysiology.

A unique C-terminus accounts for the third NBC isoform (rb2NBC) (Figure 2, bottom). This rb2NBC was recently cloned and characterized from the rat brain [14]. The rb2NBC clone results from 61 unique COOH-terminal amino acids, the result of a 97-bp deletion and frame shift near the end of the open-reading. The encoded rat protein is 1094 amino acids and predicts a protein of about 130 kDa [14]. This C-terminal NBC isoform has not yet been identified in human. Again, rb2NBC was found to mediate apparently identical transport activity as rat kidney NBC (rkNBC) and human pancreatic/heart NBC (hpNBC/hhNBC) [14].

The human NBC1 gene (SLC4A4) resides at 4q21 [12, 15]. More recent data indicates that SLC4A4 is about 400-450 kb [16]. Both pNBC and kNBC are transcribed from the same gene, but kNBC is transcribed from an alternative internal promoter [16].

NBC clones and their corresponding proteins have been identified in several tissues other than the kidney, pancreas, and brain (Table 1). Interestingly, the kidney seems to express all of the identified NBC mRNAs and proteins. In keeping with this observation, renal disease is one of the major phenotypes of human NBC mutations [17, 18, 19]. That is, these affected patients have a permanent proximal renal tubular acidosis (type 2 RTA) manifest as blood pH about 7.05 and blood $[HCO_3^-]$ about 5-8 mM, rather than the normal 7.4 and about 23-29 mM, respectively. The eye is also effected by these NBC point mutations, manifest as bilateral glaucoma, bilateral cataracts, and bandkeratopathy [18]. The effects on other tissues where NBC isoforms are expressed (Table 1) are not obvious. Whether the mutations cause a biophysical change in cotransport activity or result in a cellular protein processing problem, is not well understood.

NBC Expression in Oocytes

Xenopus oocytes were used to expression clone kNBC [7, 8]. Figure 3 illustrates the experimental arrangement with two or more microelectrodes. The experimental assay uses a bath perfusion

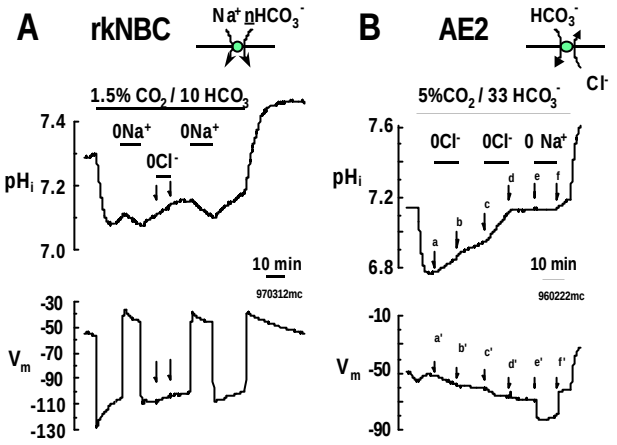

Figure 4. Oocyte expression of NBC and AE2

A comparison of NBC expression (A) and AE2 expression (B) in *Xenopus* oocytes.

A. Rat kidney NBC expressed in a *Xenopus* oocyte. Addition of CO_2/HCO_3^- elicits an immediate hyperpolarization (HCO_3^- influx) and a decrease of pH_i which begins to recover as a result of the HCO_3^- influx. Na^+ removal in CO_2/HCO_3^-, depolarizes and acidifies the oocyte. However, bath Cl^- removal does not change pH_i or V_m.

B. Intracellular pH experiment on an oocyte expressing the AE2 anion exchanger. Addition of CO_2/HCO_3^- acidifies the oocyte but does not elicit a hyperpolarization as with NBC. Removal of bath Cl^- elicits an increase in pH_i (ab and cd). However, Na^+ removal in CO_2/HCO_3^- does not change pH_i in an oocyte expressing AE2.

system. Addition of CO_2/HCO_3^- to the solutions causes a decrease of intracellular pH (pH_i) because CO_2 may traverse the oocyte plasma membrane, be hydrated intracellularly to form HCO_3^- and H^+. If an oocyte is expressing NBC, this CO_2/HCO_3^- addition will elicit an immediate hyperpolarization (Figure 4a) due to the coupled entrance of Na^+ with multiple HCO_3^- ions ("reverse transport" in Figure 3). Once pH_i achieves a steady-state, extracellular removal of Na^+ (replacement by an impermeant cation such as choline or N-methyl-D-glucamine), depolarizes the oocyte and decreases pH_i (Figure 4a) ("forward transport" as in the proximal tubule, Figure 3). Figure 4a illustrates that this electrogenic HCO_3^- transport activity is unaffected by extracellular Cl^- removal. By contrast, Figure 4b shows that an oocyte expressing AE2 does not mediate electrogenic transport and increases pH_i after extracellular Cl^- removal, yet is unaffected by extracellular Na^+ replacement.

Anions transported

The NBC protein in the renal proximal tubule is the major, perhaps exclusive, mode of "HCO_3^-" exit from the cell into the blood [18, 19, 20]. However, the chemical form of "HCO_3^-" (i.e., HCO_3^-, CO_3^{2-} or the $NaCO_3^-$ ion pair) transported by the NBC protein is still under investigation. Anions transported are indicated in Table 2**.**

Grichtchenko and coworkers have determined the extracellular [HCO_3^-] dependence of *Ambystoma* NBC (aNBC) and rkNBC expressed in *Xenopus* oocytes [21]. Exposing oocytes briefly to pH 7.5 solutions containing a range of HCO_3^- concentrations (also varying [CO_2] to keep extracellular/outside pH (pH_o) constant), they measured transport either from the hyperpolarization or outward current mediated by NBC. The apparent K_m for external HCO_3^-, with the cotransporter running in the inward direction, was about 6-7 mM for both NBCs [21, 22, 23]. This same study revealed that SO_4^{2-}, SO_3^{2-}, and HSO_3^- are not transported by NBC [21].

Our initial expression experiments with NBC, indicated that organic anions could not substitute for the HCO_3^- ion [8, 9]. Similarly, total removal of Cl^- does not effect the activity of NBC [9, 12, 13, 21]. In contrast to oocyte experiments, NBC

Table 2. Ion specificity of the electrogenic Na^+/HCO_3^- cotransporter NBC.

	Transported	Apparent $K_{0.5}$	V_m dependence	Reference
Cation				
Na^+	Yes	about 30 mM	Yes	[25]
$Choline^+$	No	-	Yes, as $0Na^+$	[8, 9, 25]
$NMDG^+$	No	-	Yes, as $0Na^+$	Romero and Boron, unpublished
Li^+	Minor	-	Yes, as $0Na^+$	[25]
K^+	No	-	ND	[25]
Anion				
HCO_3^-	Yes	about 6.5 mM	Yes	[8, 9, 13, 14, 21, 25]
Cl^-	No	-	No	[9, 14, 21, 25]
$Butyrate^-$	No	-	No	[8, 9]
$Propionate^-$	No	-	No	Sciortino and Romero, unpublished
SO_4^{2-}	No	-	No	[21]
SO_3^{2-}	No	-	No	[21]
CO_3^{2-}	Unlikely	-	No	[21]

V_m is membrane voltage, apparent $K_{0.5}$ is the ion concentration at the apparent half maximal transport rate, and ND is not determined. "Yes, as $0Na^+$" indicates that NBC exhibits V_m dependence when non-transported test ions are used for a Na^+ removal maneuver (i.e., reverse transport).

activity assayed by 2'7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) pH measurements in transfected HEK-293 cells does not appear to require HCO_3^- presence, i.e., a $Na^+/(OH^-)_n$ cotransport mode [24]. HCO_3^- is absolutely required for electrogenic Na^+/HCO_3^- cotransport in oocyte experiments [25].

Cations transported

In experiments using ^{22}Na uptake on basolateral membrane vesicles of rabbit kidney cortex, Li^+, K^+, and choline each appeared to partially support Na/HCO_3 cotransporter activity [26]. Studying ^{22}Na uptake, Jentsch and coworkers [27] determined electrogenic, DIDS-inhibitable Na^+/HCO_3^- cotransporter activity in BSC-1 cells. They found an apparent K_m for Na^+ of 20-40 mM

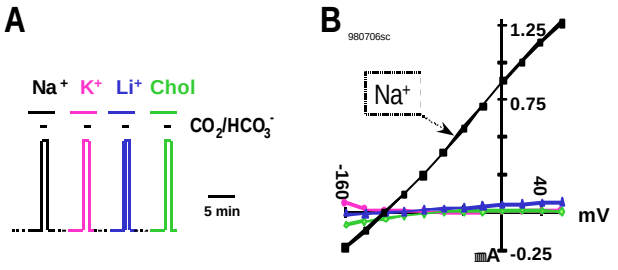

Figure 5. Cation dependence of NBC

A. Solution pulse protocol. This solution protocol is used to test whether K^+, Li^+, or choline$^+$ are capable of stimulating a HCO_3^- -dependent current from rkNBC oocytes. Oocytes were voltage clamped at –60 mV and bathed in ND96 for 5 min before switching to test cation/non-HCO_3^- ringer for 5 min. The Bath solution was then switched to the corresponding 1.5% CO_2/10 mM HCO_3^- solution for 2 min, e.g., Li^+-ND96 to 1.5% CO_2/10 mM HCO_3^-/96 mM Li^+) and returned to non-HCO_3^- Ringer for 2 min. An *I-V* relation was recorded before and after each solution change. HCO_3^- -stimulated current for each cation was taken as the difference between the non-HCO_3^- and HCO_3^- *I-V* responses. Cation solutions order was randomized.

B. rkNBC *I-V* response of cations. HCO_3^- subtracted *I-V* response curves from the current sweeps show that only Na^+ (black) stimulates a strong HCO_3^- -dependent current. Extracellular K^+ (red) and choline$^+$ (green) have *I-V* relations that lie on the voltage axis, indicating no transport. Li^+ (blue) shows only a slight current response of a maximal about 3% of the Na^+ response over the voltage range tested. Modified from [25].

at 28 mM HCO_3^-. These investigators also found that Na^+/HCO_3^- cotransporter activity was specific for Na^+; neither Li^+ or K^+ could substitute. Amlal *et al.* have reported that after transfecting hkNBC into HEK-293 cells, a low affinity for Li^+ and lesser affinity for K^+ is measured when monitoring pH_i using BCECF [24]. When expressed in *Xenopus* oocytes and studied electrophysiologically, Na^+ transport is observed [25]. Neither aNBC nor rkNBC seem to be able to transport Li^+ [25, 28, 29].

Voltage clamp experiments using rkNBC show that neither choline$^+$, Li^+, nor K^+ could substitute for Na^+ (Figure 5) [25, 30]. Cation transport by NBC is summarized in Table 2. Moreover, both influx (outward current) and efflux (inward current) of $NaHCO_3$ depend on extracellular Na^+ and voltage [25]. Regardless of extracellular [Na^+], influx (outward I increasing with depolarization) is always measured for V_m more positive than –40 mV; and efflux (inward I increasing with hyperpolarization) is always measured for V_m more negative than –100 mV. The apparent affinity ($K_{0.5}$) for extracellular Na^+ is about 30 mM for all voltages between –160 and +60 mV [25]. In general, reducing $[Na^+]_o$ in this physiologic V_m range enables NBC to mediate predominantly efflux of $NaHCO_3$ from the cell.

Stoichiometry

In their original work on the electrogenic Na^+/HCO_3^- cotransporter of the salamander proximal tubule, Boron and Boulpaep demonstrated that the cotransporter moves more HCO_3^- than Na^+ [2]. Based on measurements of pH_i, V_m and intracellular Na^+ activity, they made a thermodynamic argument that the Na^+:HCO_3^- stoichiometry had to be at least 1:2. However, they could not rule out the possibility that it is higher (e.g., 1:3). Subsequent work by Lopes *et al.* [31] on proximal tubule suggested, again on thermodynamic grounds, that the Na^+:HCO_3^- stoichiometry was at least 1:3.

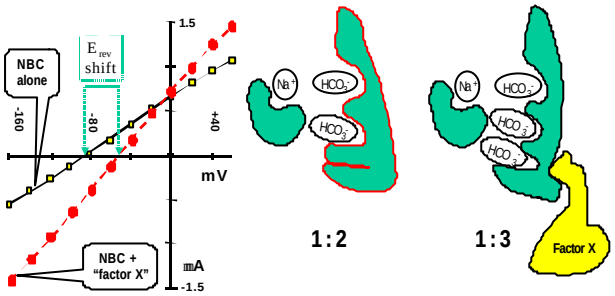

Figure 6. Biophysical modification of NBC-model
Left. Model voltage clamp experiment illustrating the predicted change in the NBC I-V plot after modification by "factor X" in the proximal tubule or eye. Changing from a 1:2 (solid line with squares) to 1:3 (dotted line with circles) Na^+:HCO_3^- coupling will not only shift E_{Rev}, but would likely increase the current measured at the voltage extremes. The basal current at –60mV for 1:3 is also predicted to be much less than we typically measure for NBC, i.e., +200 nA to slight inward current (-50 to -100 nA).
Right. Diagram indicating "how" mechanistically a NBC-interacting protein could change ionic coupling. We envision a conformational change in NBC which exposes/opens an additional HCO_3^- binding pocket.

Using rabbit renal basolateral membrane vesicles (BLMV), Soleimani and Aronson reasoned that the net transport direction depends on both the Na^+:HCO_3^- coupling ratio and the electrochemical gradients for Na^+ and HCO_3^- [32]. By altering these gradients and measuring the direction of net transport in rabbit BLMV, these workers concluded that the renal electrogenic Na^+/HCO_3^- cotransporter must have a stoichiometry of 1:3. Any of three models could account for this apparent 1:3 stoichiometry of the cotransporter: (*i*) Na^+ plus 3 HCO_3^-, *(ii)* Na^+ plus HCO_3^- plus CO_3^{2-}, or *(iii)* the $NaCO_3^-$ ion pair and HCO_3^-. Two groups working with isolated proximal tubules have suggested that, under special conditions, the renal electrogenic Na^+/HCO_3^- cotransporter can alter its stoichiometry from 1:3 to 1:2, and thus change the net direction of net HCO_3^- transport [33, 34].
Even though the data, obtained under "physiological" conditions, on native renal cells or native cell-derived materials points to a stoichiometry of 1:3, it should be pointed out that the Na^+:HCO_3^- coupling ratio has not been measured directly. Recently, by permeabilizing the apical membrane of monolayers of proximal tubule cell-lines, Gross and Hopfer found a linear voltage dependence on the 4,4'-dinitrostilben-2,2'-disulfonic acid- (DNDS)-inhibitable short-circuit current across the epithelia basolateral membrane [35]. When expressed in *Xenopus* oocytes, both giant patch [36] and 2-electrode voltage clamp experiments [25] of rkNBC, show not only a voltage dependence of both inward and outward NBC transport (i.e., larger outward I with depolarization, or larger inward I with hyperpolarization), but also a Na^+:HCO_3^- stoichiometry of 1 Na^+ : 2 HCO_3^-. This result is surprising, given that the human NBC mutations [18] imply that NBC is the major HCO_3^- exit pathway back to the blood for the proximal tubule and the kidney in general. That is, a putative accessory protein (Figure 6) or modification factor must modify NBC stoichiometry in the renal proximal tubule.

Future Directions and Summary

With the cloning of several genomes, one wonders the direction science will take. Recent emphasis on protein interactions, will undoubtedly lead to a better understanding of cellular processes and integrated cellular function. NBC is found throughout mammalian tissues. NBC like all of our "favorite proteins" will likely be found to have several protein partners mediating specialized cellular functions. For example, NBC is postulated to have an accessory role in facilitating CFTR's role as a Cl^- and HCO_3^- channel in CaLu-3 cells [37]. Another study implicated Na^+-H^+ exchange regulatory factor (NHERF) might also regulate NBC activity [38].
Molecular and immunologic reagents will enable investigators to study HCO_3^- transport processes more easily. Localization will be necessary to

generate new cellular models for ion transport and acid-base homeostasis. And, the physiology of several tissues should be revisited to integrate the role of NBC.

Key words Electrophysiology; Epithelium; Hydrogen-Ion Concentration; Ion Transport; Microelectrodes; Nervous System; Oocytes; Xenopus

Abbreviations AE: anion exchanger; aNBC: *Ambystoma* NBC; BCECF: 2'7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; BLMV: basolateral membrane vesicles; BTS: bicarbonate transporter superfamily; DIDS: 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid; DNDS: 4,4'-dinitrostilben-2,2'-disulfonic acid; hhNBC: human heart NBC: hpNBC: human pancreas NBC; kNBC: kidney NBC; NBC: electrogenic Na^+/HCO_3^- cotransport; NHERF: Na^+-H^+ exchange regulatory factor; ORF: open reading frame; pH_i: intracellular pH; pH_o: extracellular/outside pH; rb2NBC: C-terminal isoform of rat brain NBC; rkNBC electrogenic rat kidney NBC; RTA: renal tubular acidosis

Acknowledgements The author would like to thank collaborators and colleagues whose work was summarized here: Walter F. Boron, Emile L Boulpaep, Matthias A Hediger, Mark O. Bevensee, Urs V. Berger, Inyoung Choi, Bruce A. Davis, Peying Fong, Irina I. Grichtchenko, Nazih L. Nakhoul, Eleni Roussa, Chris M. Sciortino, Caroline R Sussman, Bernhard M Schmitt, Frank Thévenod, Patricia Bray-Ward, David Ward, and Duncan Wong This work was supported by a grant from the American Heart Association and a Howard Hughes Medical Institute grant to CWRU.

Correspondence
Michael F Romero

Department Physiology and Biophysics
Case Western Reserve University
School of Medicine
2119 Abington Rd, SOM-E545
Cleveland, OH 44106-4970
USA
Phone: +1-216.368.3180
Fax: +1-216.368.4952
E-mail address: mfr2@po.cwru.edu

References

1. Alpern RJ. Renal acidification mechanisms. In: Brenner BM, ed. Brenner and Rector's - The Kidney. 6th ed. Philadelphia, PA, USA: W.B. WB Saunders Co., 2000: 455-519.

2. Boron WF, Boulpaep EL. Intracellular pH regulation in the renal proximal tubule of the salamander. Basolateral HCO_3^- transport. J Gen Physiol 1983; 81:53-94. [83163104]

3. Jentsch TJ, Keller SK, Koch M, Wiederholt M. Evidence for coupled transport of bicarbonate and sodium in cultured bovine corneal endothelial cells. J Membr Biol 1984; 81:189-204 [85058146]

4. Alpern RJ. Mechanism of basolateral membrane $H^+/OH^-/HCO_3^-$ transport in the rat proximal convoluted tubule. A sodium-coupled electrogenic process. J Gen Physiol 1985; 86:613-36. [86061554]

5. Sasaki S, Shiigai T, Yoshiyama N, Takeuchi J. Mechanism of bicarbonate exit across basolateral membrane of rabbit proximal straight tubule. Am J Physiol 1987; 252:F11-8. [87125188]

6. Boron WF, Boulpaep EL. The electrogenic Na/HCO_3 cotransporter. Kidney Int 1989; 36:392-402. [90080600]

7. Romero MF, Hediger MA, Boulpaep EL, Boron WF. Expression cloning of the renal electrogenic Na/HCO_3 cotransporter (NBC-1) from *Ambystoma tigrinum*. FASEB J 1996; 10:A89.

8. Romero MF, Hediger MA, Boulpaep EL, Boron WF. Expression cloning and characterization of a renal electrogenic Na^+/HCO_3^- cotransporter. Nature 1997; 387:409-13. [97305959]

9. Romero MF, Fong P, Berger UV, Hediger MA, Boron WF. Cloning and functional expression of rNBC, an electrogenic Na^+-HCO_3^- cotransporter from rat kidney. Am J Physiol 1998; 274:F425-32. [98147192]

10. Burnham CE, Amlal H, Wang Z, Shull GE, Soleimani M. Cloning and functional expression of a human kidney Na^+:HCO_3^- cotransporter. J Biol Chem 1997; 272:19111-4. [97382229]

11. Burnham CE, Flagella M, Wang Z, Amlal H, Shull GE, Soleimani M. Cloning, renal distribution, and regulation of the rat Na^+-HCO^-_3 cotransporter. Am J Physiol 1998; 274:F1119-26. [99020870]

12. Abuladze N, Lee I, Newman D, Hwang J, Boorer K, Pushkin A, Kurtz I. Molecular cloning, chromosomal localization, tissue distribution, and functional expression of the human pancreatic sodium bicarbonate cotransporter. J Biol Chem 1998; 273:17689-95. [98316338]

13. Choi I, Romero MF, Khandoudi N, Bril A, Boron WF. Cloning and characterization of a human electrogenic Na^+-HCO^-_3 cotransporter isoform (hhNBC). Am J Physiol 1999; 276:C576-84. [99170502]

14. Bevensee MO, Schmitt BM, Choi I, Romero MF, Boron WF. An electrogenic Na/HCO_3 cotransporter (NBC) with a novel C terminus, cloned from rat brain. Am J Physiol Cell Physiol 2000; 278:C1200-C11. [20299445]

15. Romero MF, BA Davis, CR Sussman, P Bray-Ward, D Ward, and WF Boron. Identification of multiple genes for human electrogenic Na/HCO_3 cotransporters (NBC) on 4q. J Am Soc Nephrol 1998; 9:11A.

16. Abuladze N, Song M, Pushkin A, Newman D, Lee I, Nicholas S, Kurtz I. Structural organization of the human NBC1 gene: kNBC1 is transcribed from an alternative promoter in intron 3. Gene 2000; 251:109-22. [20336635]

17. Igarashi T, Inatomi J, Sekine T, Seki G, Shimadzu M, Tozawa F, et al. Novel nonsense mutation in the Na^+/HCO_3^- cotransporter gene (SLC4A4) in a patient with permanent isolated proximal renal tubular acidosis and bilateral glaucoma. J Am Soc Nephrol 2001; 12:713-8. [21172168]

18. Igarashi T, Inatomi J, Sekine T, Cha SH, Kanai Y, Kunimi M, et al. Mutations in SLC4A4 cause permanent isolated proximal renal tubular acidosis with ocular abnormalities. Nat Genet 1999; 23:264-6. [20014733]

19. Dinour D, Knecht A, Serban I, Holtzman EJ. A novel missense mutation in the sodium bicarbonate cotransporter (NBC-1) causes congenital proximal renal tubular acidosis with ocular defects. J Am Soc Nephrol 2000; 11:A0012.

20. Boron WF, Fong P, Hediger MA, Boulpaep EL, Romero MF. The electrogenic Na/HCO_3 cotransporter. Wien Klin Wochenschr 1997; 109:445-56. [97405315]

21. Grichtchenko II, Romero MF, Boron WF. Extracellular HCO_3 dependence of electrogenic Na/HCO_3 cotransporters (NBC) cloned from salamander and rat kidney. J Gen Physiol 2000 ; 115(5):533-46. [20241938]

22. Grichtchenko II, Romero MF, Boron WF. Extracellular bicarbonate dependence of aNBC, the electrogenic Na/HCO_3 cotransporter cloned from tiger salamander (*Ambystoma tigrinum*). J Am Soc Nephrol 1996; 7:1255.

23. Grichtchenko II, Romero MF, Boron WF. Electrogenic Na/HCO_3 cotransporters (NBC) from rat and salamander kidney have similar external HCO_3^- dependencies. FASEB J 1998; 12:A638.

24. Amlal H, Wang Z, Burnham C, Soleimani M. Functional Characterization of a Cloned Human Kidney Na^+:HCO_3^- Cotransporter. J Biol Chem 1998; 273:16810-5. [98307915]

25. Sciortino CM, Romero MF. Cation and voltage dependence of rat kidney, electrogenic Na^+/HCO_3^- cotransporter, rkNBC, expressed in oocytes. Am J Physiol 1999; 277:F611-23. [99447316]

26. Soleimani M, Lesoine GA, Bergman JA, Aronson PS. Cation specificity and modes of the Na^+:CO_3^{2-}:HCO_3^- cotransporter in renal basolateral membrane vesicles. J Biol Chem 1991; 266:8706-10. [91224960]

27. Jentsch TJ, Schill BS, Schwartz P, Matthes H, Keller SK, Wiederholt M. Kidney epithelial cells of monkey origin (BSC-1) express a sodium bicarbonate cotransport. Characterization by $22Na^+$ flux measurements. J Biol Chem 1985; 260:15554-60. [86059429]

28. Romero MF,Hediger MA, Fong P, Boron WF. Expression of the rat renal electrogenic Na/HCO_3 cotransporter (rNBC). FASEB J; 1997; 11:A25.

29. Romero MF, MA Hediger, EL Boulpaep, Boron WF. Physiology of the cloned *Ambystoma tigrinum* renal electrogenic Na/HCO_3 cotransporter (aNBC): II. Localization and Na^+ dependence. J Am Soc Nephrol 1996; 7:1260.

30. Sciortino CM, Romero MF. Na^+ and voltage dependence of the rat kidney electrogenic Na/HCO_3 cotransporter (rkNBC) expressed in *Xenopus* oocytes. J Am Soc Nephrol 1998; 9:12A.

31. Lopes AG, Siebens AW, Giebisch G, Boron WF. Electrogenic Na/HCO_3 cotransport across basolateral membrane of isolated perfused *Necturus* proximal tubule. Am J Physiol 1987; 253:F340-50. [87296365]

32. Soleimani M, Grassi SM, Aronson PS. Stoichiometry of Na^+-HCO^-_3 cotransport in basolateral membrane vesicles isolated from rabbit renal cortex. J Clin Invest 1987; 79:1276-80. [87166756]

33. Planelles G, Thomas SR, Anagnostopoulos T. Change of apparent stoichiometry of proximal-tubule Na^+-HCO_3^-

cotransport upon experimental reversal of its orientation. Proc Natl Acad Sci USA 1993; 90:7406-10. [93348281]

34. Seki G, Coppola S, Fromter E. The Na^+-HCO_3^- cotransporter operates with a coupling ratio of 2 HCO_3^- to 1 Na^+ in isolated rabbit renal proximal tubule. Pflügers Arch 1993; 425:409-16. [94181423]

35. Gross E, Hopfer U. Activity and stoichiometry of Na^+:HCO_3^- cotransport in immortalized renal proximal tubule cells. J Membr Biol 1996; 152:245-52. [96327702]

36. Heyer M, Muller-Berger S, Romero MF, Boron WF, Fromter E. Stoichiometry of rat kidney Na-HCO_3 cotransporter (rkNBC) expressed in *Xenopus laevis* oocytes. Pflügers Arch 1999; 438:322-9. [99329282]

37. Devor DC, Singh AK, Lambert LC, DeLuca A, Frizzell RA, Bridges RJ. Bicarbonate and chloride secretion in Calu-3 human airway epithelial cells. J Gen Physiol 1999; 113:743-60. [99246328]

38. Bernardo AA, Kear FT, Santos AV, Ma J, Steplock D, Robey RB, Weinman EJ. Basolateral Na^+/HCO_3^- cotransport activity is regulated by the dissociable Na^+/H^+ exchanger regulatory factor. J Clin Invest 1999; 104:195-201. [99340105]

39. Giffard RG, Papadopoulos MC, van Hooft JA, Xu L, Giuffrida R, Monyer H. The electrogenic sodium bicarbonate cotransporter: developmental expression in rat brain and possible role in acid vulnerability. J Neurosci 2000; 20:1001-8. [20115695]

40. Schmitt BM, Berger UV, Douglas RM, Bevensee MO, Hediger MA, Haddad GG, Boron WF. Na/HCO_3 cotransporters in rat brain: expression in glia, neurons, and choroid plexus. J Neurosci 2000; 20:6839-48. [20453964]

41. Douglas RM, Schmitt BM, Xia Y, Bevensee MO, Biemesderfer D, Boron WF, Haddad GG. Sodium-hydrogen exchangers and sodium-bicarbonate co-transporters: ontogeny of protein expression in the rat brain. Neuroscience 2001; 102:217-28. [21126671

42. Praetorius J, Hager H, Nielsen S, Aalkjaer C, Friis UG, Ainsworth MA, Johansen T. Molecular and functional evidence for electrogenic and electroneutral Na^+-HCO_3^- cotransporters in murine duodenum. Am J Physiol Gastrointest Liver Physiol 2001; 280:G332-43. [21113639]

43. Jensen LJ, Schmitt BM, Berger UV, Nsumu NN, Boron WF, Hediger MA, et al. Localization of sodium bicarbonate cotransporter (NBC) protein and messenger ribonucleic acid in rat epididymis. Biol Reprod 1999; 60:573-9. [99150180]

44. Usui T, Seki G, Amano S, Oshika T, Miyata K, Kunimi M, et al. Functional and molecular evidence for Na^+-HCO_3^- cotransporter in human corneal endothelial cells. Pflügers Arch 1999; 438:458-62. [99448583]

45. Sun XC, Bonanno JA, Jelamskii S, Xie Q. Expression and localization of Na^+-HCO_3^- cotransporter in bovine corneal endothelium. Am J Physiol Cell Physiol 2000; 279:C1648-55. [20485956]

46. Schmitt BM, Biemesderfer D, Romero MF, Boulpaep EL, Boron WF. Immunolocalization of the electrogenic Na^+/HCO_3^- cotransporter in mammalian and amphibian kidney. Am J Physiol 1999; 276:F27-38. [99103987]

47. Maunsbach AB, Vorum H, Kwon TH, Nielsen S, Simonsen B, Choi I, et al. Immunoelectron microscopic localization of the electrogenic Na/HCO_3 cotransporter in rat and *Ambystoma* kidney. J Am Soc Nephrol 2000; 11:2179-89. [20547412]

48. Romero MF, Sussman CR, Choi I, Hediger MA, Boron WF. Cloning of an electrogenic Na/HCO_3 cotransporter (NBC) isoform from human kidney and pancreas. J Am Soc Nephrol 1998; 9:11A.

49. Thevenod F, Roussa E, Schmitt BM, Romero MF. Cloning and immunolocalization of a rat pancreatic Na^+ bicarbonate cotransporter. Biochem Biophys Res Commun 1999; 264:291-8. [99458660]

50. Marino CR, Jeanes V, Boron WF, Schmitt BM. Expression and distribution of the Na^+-HCO_3^- cotransporter in human pancreas. Am J Physiol 1999; 277:G487-94. [99375119]

51. Romero MF, Sciortino CM, Roussa E, Thévenod F. Na^+ /HCO_3^- cotransporters in various species and organs. In: Acid-Base Balance-from Bench to Bedside. Napoli, Italy: DeSanto N, 1999: 45-60.

52. Roussa E, Romero MF, Schmitt BM, Boron WF, Alper SL, Thevenod F. Immunolocalization of AE2 anion exchanger and Na^+-HCO_3^- cotransporter in rat parotid and submandibular glands. Am J Physiol 1999; 277:G1288-96. [20068541]

53. Rossmann H, Bachmann O, Vieillard-Baron D, Gregor M, Seidler U. Na^+/HCO_3^- cotransport and expression of NBC1 and NBC2 in rabbit gastric parietal and mucous cells. Gastroenterology 1999; 116:1389-98. [99278252]

54. Romero MF, Boron WF. Electrogenic Na/HCO_3 cotransporters: Expression cloning and physiology. Annu Rev Physiol 1999; 61:699-723. [99199480]

55. Vince JW, Reithmeier RA. Structure of the band 3 transmembrane domain. Cell Mol Biol (Noisy-le-grand) 1996; 42:1041-51. [97120108]

56. Hu L, Schmitt BM, Boron WF. Glycosylation Analysis of the Na/HCO_3 Cotransporter (NBC). J Am Soc Nephrol 1999; 10:A0023.