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Sodium-Coupled Bicarbonate Transporters

Walter F Boron

Department of Cell and Molecular Physiology, Yale University School of Medicine, New Haven, CT. USA

Summary

Together, the Na^+-coupled HCO_3^- transporters and the AE family of anion exchangers (i.e., Cl-HCO_3 exchangers) comprise the bicarbonate transporter (BT) superfamily. Virtually all BTs are important for the regulation of intracellular pH (pH_i) in cells throughout the body. Specific BTs also play roles in cell-volume regulation, as well as for the transport of salt and/or acid-base equivalents across many epithelia. Electrogenic Na/HCO_3 cotransporters (NBCe's) play key roles in HCO_3^- reabsorption by the renal proximal tubule, and HCO_3^- secretion by the pancreatic duct. Electroneutral NBC's (NBCn's) regulate pH_i in vascular smooth muscle and are present in/near axons in the brain. Finally, the Na^+-driven Cl-HCO_3 exchanger (NDCBE's) appear to be the major pH_i regulators in CNS neurons. A characteristic of most, but not all, BT's is that they are inhibited rather effectively by 4,4'-diisothiocyanostilbene-4,4'-disulfonate (DIDS).

Anion Exchangers (AEs)

The founding BT-superfamily member is the Cl-HCO_3 exchanger (Figure 1A), described decades ago in RBCs, and cloned 16 years ago by Kopito and Lodish [1] as the anion exchanger AE1. In other cells, Cl-HCO_3 exchangers (AE2, AE3) normally functions as acid loaders. Recently, AE4 (more closely related to the NBCs than the AEs) was cloned from kidney, and may represent the apical Cl-HCO_3 exchanger in beta-intercalated cells [2]. AE1 has long been thought to exist as a dimer, a view supported by cryo-EM studies to a 20-angstrom resolution [3].

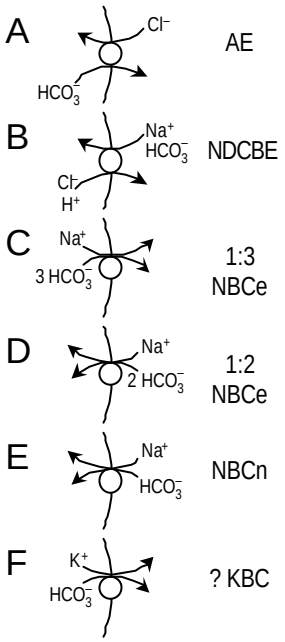

Figure 1. HCO_3^- transporters

Na^+-Driven Cl^--HCO_3^- Exchanger (NDCBE)

The Na^+-driven Cl-HCO_3 exchanger (Figure 1B) was first described by Roger Thomas and by De Weer, Russell and Boron, working on snail neurons [4, 5, 6], squid axons [7, 8, 9] and barnacle muscle [10]. This was the first transporter shown to be involved in pH_i

regulation. We have now cloned the Na^+-driven Cl-HCO_3 exchanger from human brain (NDCBE1) and also from squid neurons (SF1). Mike Romero cloned from *Drosophila* a related cDNA (NDAE) that appears to encode a Na^+-driven Cl-anion (i.e., OH^-) exchanger [11]. Wang *et al.* has cloned a related mouse cDNA, not yet well characterized physiologically [12].

NDCBE1 appears to be the major pH_i regulator in many neurons. It is highly expressed in brain and testis [13]. At the amino-acid level, NDCBE1 is about 50% identical to NBCe1 and about 75% identical to NBCn1. NDCBE requires Na^+, HCO_3^- and Cl^-. It is electroneutral, not associated with any channel activity (see NBCn1 below), and is highly sensitive to DIDS. ^{36}Cl-flux measurements show that the unidirectional Cl^- efflux requires external Na^+ and HCO_3^-, and blocked by DIDS. Using Na^+ electrodes to measure the DIDS-sensitive net Na^+ efflux, we found that the HCO_3^- to Na^+ stoichiometry is 2:1, as expected for a Na^+-driven Cl-HCO_3 exchanger.

Electrogenic Na/HCO_3 Cotransporter (NBCe) with 1:3 Stoichiometry

The electrogenic Na/HCO_3 cotransporter was first described by Boulpaep and Boron in the salamander renal proximal tubule [14]. This transporter mediates the basolateral step of HCO_3^- reabsorption in the proximal tubule. Because this transporter has a Na^+:HCO_3^- stoichiometry of 1:3 (Figure 1C), it mediates net HCO_3^- *efflux* at the resting membrane voltage (V_m). We expression cloned this transporter from the salamander kidney (Figure 2: NBCe1-A), the first of the cation-coupled HCO_3^-transporters to be cloned [15]. Soleimani and coworkers later cloned it from human kidney [16], and we, from rat kidney [17].

An amino-acid sequence alignment of the various AEs led to a proposed consensus motif at which DIDS covalently reacts with the AEs: KLXK (X=I,Y) [18], where the first K is K539. The predicted location of this site is at the extracellular face of the fifth membrane-spanning segment. At the homologous site, NBCe1 has KMIK, suggesting a motif of KZXK (Z=M,L).

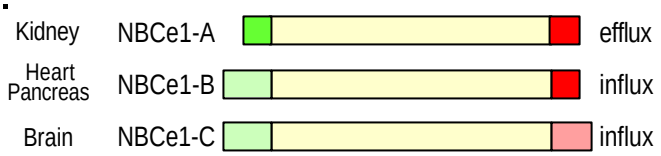

Figure 2. Alternative splicing of NBCe

Electrogenic Na/HCO_3 Cotransporter (NBCe) with 1:2 Stoichiometry

In 1989, Deitmer and Schlue described an *inwardly* directed electrogenic Na/HCO_3 cotransporter in leech glial cells [19]. Based on known values of $[Na^+]_i$, pH_i and V_m, they concluded that the stoichiometry must be 1:2. Others subsequently described inwardly directed electrogenic Na/HCO_3 cotransporters in several mammalian cells, including pancreatic duct cells [20, 21]; we functionally identified it in rat astrocytes [22]. Kurtz [23], as well as our group [24], cloned the "pancreatic" NBCe (Figure 2: NBCe1-B), which is a splice variant of NBCe1-A. We also cloned a "brain" NBCe (Figure 2: NBCe1-C) [25], and showed that an NBCe protein is present in astrocytes [25] and the basolateral membrane of pancreatic duct [26]. NBCe1-B plays a critical role in HCO_3^- secretion by the pancreatic duct.

A key issue is what determines whether NBCe1 operates with a 1:3 or 1:2 stoichiometry? Is it a difference in amino-acid sequence, posttranslational modification, formation of a heterodimer with an as-yet unidentified NBC, or an additional subunit? Alternative splicing (Figure 2) makes the N terminus of renal NBCe (NBCe1-A, which mediates HCO_3^- efflux *in situ*) quite different from that of either pancreatic or brain NBCe (NBCe1-B and -C, which mediate HCO_3^-influx *in situ*).

The *ionic* mechanism of NBCe1 has not been investigated. Figure 3 summarizes potential mechanisms. If the same protein (i.e., one of the NBCe1 isoforms) must function with two

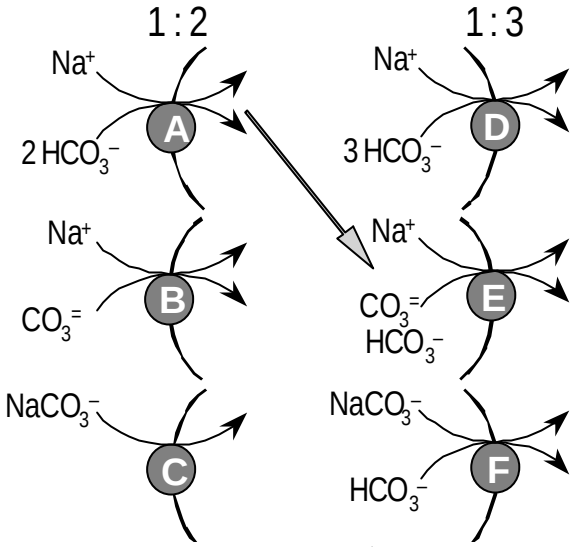

Figure 3. Models of electrogenic Na^+/HCO_3^- cotransport

different stoichiometries, then the simplest way to accomplish this feat would be to shift from binding HCO_3^- to binding CO_3^{2-} at one site (Figure 3: A→E). Other transitions would require introducing a new binding site (Figure 3: A→D, B→E, C→F).

Electroneutral Na/HCO_3 Cotransporter (NBCn)

In the late 1980s and early 1990s, several groups described an apparently electroneutral Na/HCO_3 cotransporter (1:1 stoichiometry) that extrudes acid from oligodendrocytes [27], vascular smooth muscle [28], cardiac Purkinje fibers [29] and cardiac myocytes [30]. Because of problems depleting cells of Cl^- or measuring very small electrical changes, it was not entirely clear that an electroneutral Na/HCO_3 cotransporter even existed. We cloned NBCn1 ("n"=neutral) from rat aorta [31] and demonstrated, in oocytes, that it is indeed electroneutral and independent of Cl^-.

As a HCO_3^- cotransporter, NBCn1 is only very weakly inhibited by DIDS. This observation is not surprising, inasmuch as the consensus DIDS-reaction motif is disrupted in NBCn1. At the position homologous to the KLXK in AE1, NBCn1 has KLFH. We hypothesized that we could increase NBCn1's DIDS sensitivity by serially converting NBCn1's KLFH to KLXK. Our first NBCn1 mutant (H751K, which yields KLFK) is 100% blocked by 500 μM DIDS. Thus, our data suggests that a more general DIDS-reaction consensus sequence is KZYK (Z=L,M,T,V, and X=F,I,M,T,V,Y).

A major surprise was that, even though NBCn1 is electroneutral in its transport mode, expression of NBCn1 is associated with a Na^+-channel activity. This Na^+ current is that unlike any described elsewhere, and that is *stimulated* by DIDS. The NBCn1-associated Na^+-channel property could represent a channel native *Xenopus* oocyte (e.g., its expression could be triggered by expressing NBCn1). Alternatively, the Na^+ channel could represent "slippage" of NBCn1, a view supported by the DIDS result. We have attempted to eliminate this Na^+-channel property by generating chimeras between NBCe1 and NBCn1. Replacing the putative cytoplasmic N or C termini of NBCn1 has no effect on either the electroneutrality of NBCn1 in its transport mode, nor on the Na^+-channel property of NBCn1.

Our rat NBCn1 clone is closely related (about 90% identical at the amino-acid level), though not identical to a human clone described by Pushkin *et al.* [32], and that maps to chromosome 3 [33]. Because they did not measure V_m or current, Pushkin *et al.* could not come to any firm conclusions about electrogenicity/neutrality. Curiously, their clone (unlike ours) in oocytes is inhibited by ethylisopropylamiloride (EIPA, an inhibitor of Na-H exchange) and functions without HCO_3^-. Preliminary data from Kurtz suggests that the potential PDZ-binding domain at the C terminus of NBCn1 interacts with the vacuolar ATPase [34].

K/HCO_3 Cotransporter

$Cl\text{-}HCO_3$ exchangers are present in many mammalian cells, and were long thought to be the sole acid loader for non-epithelial cells.

However, we found that the pH_i recovery (i.e., a decrease in pH_i) from alkaline loads by rat neurons or astrocytes is neither Cl^- dependent nor DIDS sensitive. The molecular substrate of this pH_i recovery may be the K/HCO_3 cotransporter, in squid axons [35, 36, 37]. K/HCO_3 cotransporter is not blocked by DIDS, but by quaternary amines. Preliminary data show that phenyl-propyltetraethylammonium ($PPTEA^{+)}$ blocks recovery from alkaline loads in astrocytes.

Other HCO_3^- Transporters

Perhaps related, very distantly to the BT superfamily is the sulfate anion transporter (SAT) family. SAT, which can exchange sulfate for CO_3^{2-}, is present at the basolateral membranes of small intestine and renal proximal tubule. Another SAT-family member, DRA (down-regulated in adenoma) encodes a $Cl\text{-}HCO_3$ exchanger present in apical membranes of certain small-intestine cells [38] and pancreatic ducts [39]. Along with cystic fibrosis transmembrane conductance regulator (CFTR) and NBCe1-B, DRA may play a critical role in the secretion of HCO_3^- by the pancreatic duct. Pendrin, which is related to DRA and functions as a Cl-formate exchanger [40], may also transport HCO_3^- [41].

Key words Bicarbonates; Cloning, Organism; Hydrogen-Ion Concentration; Ion Transport; Kidney Tubules, Proximal; Oocytes; Pancreatic Ducts

Abbreviations AE: anion exchanger; BT: bicarbonate transporter; DIDS: 4,4'-diisothiocyanostilbene-4,4'-disulfonate; DRA: down-regulated in adenoma: EIPA: ethylisopropylamiloride; KBC: K/HCO_3 cotransporter; NBCe: electrogenic Na/HCO_3 cotransporter; NBCn: electroneutral Na/HCO_3 cotransporter; NDAE: Na^+-driven anion exchanger; NDCBE: Na^+-driven $Cl\text{-}HCO_3$ exchanger; pH_i: intracellular pH; PPTEA: phenyl-propyltetraethylammonium; SAT: sulfate anion transporter; V_m: membrane voltage

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Correspondence
Walter F Boron
Department of Cellular and Molecular Physiology
333 Cedar St
New Haven, CT 06520-8026
USA
Phone: +1-203.785.4070
Fax: +1-203.785.4951
E-mail address: walter.boron@yale.edu

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