The SLC4 family of transporters

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The ABC of Solute Carriers

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The SLC4 family of HCO_3^- transporters

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Abstract The SLC4 family consists of ten genes. All appear to encode integral membrane proteins with very similar hydropathy plots—consistent with the presence of 10–14 transmembrane segments. At least eight SLC4 members encode proteins that transport HCO_3^- (or a related species, such as CO_3^{2-}) across the plasma membrane. Functionally, these eight proteins fall into two major groups: three Cl-HCO_3 exchangers (AE1–3) and five Na^+-coupled HCO_3^- transporters (NBCe1, NBCe2, NBCn1, NDCBE, NCBE). Two of the Na^+-coupled HCO_3^- transporters (NBCe1, NBCe2) are electrogenic; the other three Na^+-coupled HCO_3^- transporters and all three AEs are electroneutral. At least NDCBE transports Cl^- in addition to Na^+ and HCO_3^-. Whether NCBE transports Cl^-—in addition to Na^+ and HCO_3^-—is unsettled. In addition, two other SLC4 members (AE4 and BTR1) do not yet have a firmly established function; on the basis of homology, they fall between the two major groups. A characteristic of many, though not all, SLC4 members is inhibition by 4,4′-diisothiocyanatostilbene-2,2′-disulfonate (DIDS). SLC4 gene products play important roles in the carriage of CO_2 by erythrocytes, the absorption or secretion of H^+ or HCO_3^- by several epithelia, as well as the regulation of cell volume and intracellular pH.

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Overview

The SLC4 family of transporters—also known as the bicarbonate-transporter family—includes the products of ten human genes. Figure 1 summarizes the interrelatedness of the ten proteins, based on a computerized phylogenetic analysis (http://www.ebi.ac.uk/clustalw/). Table 1 summarizes the gene nomenclature (column 1), preferred protein names (column 2), other (sometimes conflicting) names that have been used for the proteins (column 3), functional information (columns 4 and 5), tissue distribution of the proteins (column 6), associated

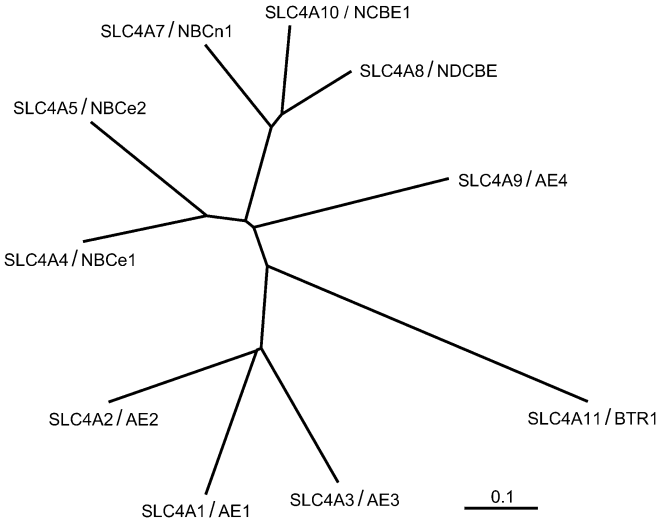

Fig. 1 SLC4 family tree. The computerized phylogenetic analysis was performed using the deduced amino acid sequence of one representative human splice variant for each of the ten SLC4 genes (available at http://www.ebi.ac.uk/clustalw/). Using sequences from only one species (i.e., human) simplifies the conceptualization and quantitation of sequence identity

Table 1 Characteristics of the SLC4 family members

Human gene symbol	Protein name	Aliases	Predominant substrates	Transport type*	Tissue distribution and cellular/subcellular expression	Link to disease†	Human gene locus	Sequence Accession ID	Splice variants and their specific features
SLC4A1	AE1	Band 3	Chloride, bicarbonate	E	Erythrocytes, intercalated cells of renal collecting duct, heart and colon	Hemolytic anemia, distal renal tubular acidosis	17q21–q22	NM_000342	2
SLC4A2	AE2		Chloride, bicarbonate	E	Widely distributed; basolateral in most epithelial cells		7q35–q36	NM_003040	5
SLC4A3	AE3		Chloride, bicarbonate	E	Brain, retina, heart and smooth muscle. Also epithelial cells of kidney and GI tract		2q36	NM_005070	≥2
SLC4A4	NBCe1	NBC, NBC1	Sodium bicarbonate (and/or carbonate)	C	NBCe1-A: renal proximal tubule, eye; NBCe1-B: widely distributed, pancreas, heart, eye; NBCe1-C: brain	Severe proximal renal tubular acidosis, ocular abnormalities, short stature	4q21	NM_003759	3
SLC4A5	NBCe2	NBC4	Sodium bicarbonate (and/or Carbonate)	C	Liver, testes, spleen		2p13	NM_133478	≥1
SLC4A6 (not used)	–	–	–	–	–	–	–	–	–
SLC4A7	NBCn1	NBC2, NBC3	Sodium bicarbonate	C	Widely distributed, spleen, testes, brain, heart, lung, liver and kidney; not skeletal muscle	Blindness, auditory impairment	3p22	NM_003615	≥3
SLC4A8	NDCBE	kNBC3	Sodium bicarbonate, chloride	C and E	Brain, testes, kidney and ovary		12q13	NM_004858	2
SLC4A9	–	AE4	Inconclusive	?	At least kidney		5q31	NM_031467	1
SLC4A10	–	NCBE	Inconclusive	?	Brain		2q23–q24	NM_022058	2
SLC4A11	–	BTR1	Unknown	O	Kidney, salivary gland testis, thyroid, trachea		20p12	NM_032034	1

* C: Cotransporter
E: Exchanger
O: Orphan transporter
† All examples are genetic defects

diseases (column 7), gene localization (column 8), and representative sequence accession numbers (column 9). Note that human genes are capitalized (e.g., SLC4A1), whereas rodent genes are in lower case (e.g., slc4a1). The preferred protein names reflect known transport functions, of which there are three: (1) Cl-HCO_3 (or "anion") exchanger (the AEs); (2) Na/HCO_3 cotransport (the NBCs), which may be either electrogenic ("e" suffix) or neutral ("n" suffix); and (3) Na^+-driven Cl-HCO_3 exchange (NDCBE). Because the functions of three other gene products are not conclusively established (AE4, NCBE, and BTR1), their protein names should be regarded as provisional until they are eventually assigned permanent, preferred names such as NBCn2 or NDCBE2.

Table 2 summarizes the percent identity of the deduced amino-acid sequences among the family members. The eight family members whose physiological function is more-or-less well defined fall into two major phylogenetic subfamilies—one of which can be further subdivided for a total of three groups—that correlate reasonably well with function:

- The Cl-HCO_3 exchangers AE1–3, which are about 53–56% identical to one another at the amino-acid level.
- The electrogenic Na/HCO_3 cotransporters NBCe1 and NBCe2, which are about 53% identical to each other, and about 28–34% identical to the AEs.
- The electroneutral Na^+-coupled HCO_3^- transporters NBCn1, NDCBE, and NCBE, which are about 71–76% identical to each other, about 30–34% identical to the AEs, and about 39–50% identical to the electrogenic NBCs. It is not clear whether NCBE is a Na^+-

Table 2 Percent identity among SLC4 family members*

	SLC4A1 AE1	SLC4A2 AE2	SLC4A3 AE3	SLC4A4 NBCe1	SLC4A5 NBCe2	SLC4A7 NBCn1	SLC4A8 NDCBE	SLC4A9 AE4	SLC4A10 NCBE	SLC4A11 BTR1
AE1		55%	53%	34%	29%	33%	30%	29%	32%	19%
AE2			56%	34%	28%	30%	34%	34%	33%	19%
AE3				34%	30%	31%	33%	33%	33%	20%
NBCe1					53%	50%	50%	50%	50%	20%
NBCe2						39%	39%	39%	41%	14%
NBCn1							72%	40%	71%	19%
NDCBE								42%	76%	20%
AE4									42%	19%
NCBE										20%
BTR1										

* Numbers represent "% identity" of the deduced amino-acid sequences of representative, human splice variants. The comparisons were made using LaserGene's DNAStar software and Clustal W with gap inclusions

driven Cl-HCO_3 exchanger or an electroneutral Na/HCO_3 cotransporter.

The two family members whose function is inconclusive (AE4) or unknown (BTR1) are less closely related phylogenetically to any of the others, including themselves.

Similarities among the SLC4 family members

Of the eight family members with more-or-less well-established functions, all are integral membrane proteins that carry bicarbonate (HCO_3^-) and/or carbonate (CO_3^{2-})—in addition to at least one monoatomic ion—across the plasma membrane. They have at least three other structural or functional similarities.

Perhaps the most striking similarity is the hydropathy analysis and—presumably—membrane topology. Figure 2A is a hydropathy plot of NBCe1, and Fig. 2B is a topological model of the renal splice variant of NBCe1 that is based on a recent AE1 model proposed by Zhu et al. [113]. Like all members of the SLC4 family, NBCe1 has a long N-terminal hydrophilic domain and a much shorter C-terminal hydrophilic domain, both of which are intracellular. An X-ray crystallographic analysis of the N-terminal domain of AE1 shows that it is a dimer [111], consistent with the conventional view that AE1 itself—and perhaps each SLC4 family member—exists in a dimeric state. Separating the hydrophilic N and C termini are 10–14 transmembrane (TM) segments. Authors generally agree on the assignments of the first six TMs. However, various experimental approaches have yielded conflicting results for the last four to eight TMs, perhaps because these TMs are more flexible and/or more easily shifted into unnatural conformations. In addition to 13 α-helical TMs, the model shown in Fig. 2B includes two re-entrant loops, one between TMs 9 and 10, and one just

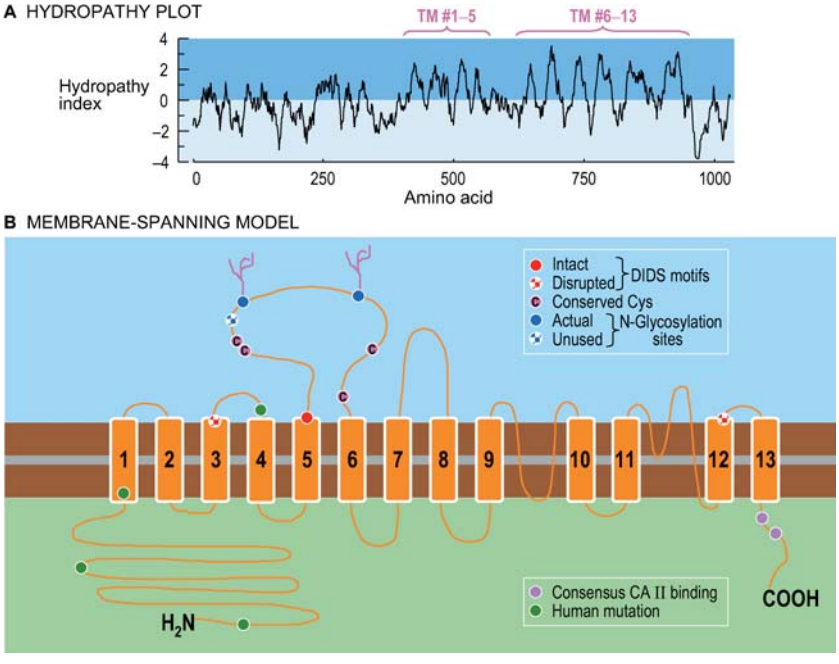

Fig. 2A, B Structural analysis. **A** Hydropathy plot of the renal splice variant of human NBCe1. **B** Topology model of NBCe1 based on the model of AE1 proposed by Zhu et al. [113]

after TM 11. In addition, it also includes an extended structure that crosses from the extra- to the intracellular fluid just before TM 12. A note of caution: one should regard the topology model in Fig. 2B—as well as any other such model—as one of several educated guesses. In the case of the SLC4 family, other authors have proposed simpler models that include 10–14 TMs. The value of these models is that they help us to envision relationships among parts of the molecule, and challenge us to design experiments to test the models.

A second similarity among some—though not all—members of the SLC4 family is inhibition by disulfonic stilbene derivatives such as DIDS. At least for AE1 and NBCe1, this drug interacts with residues near the extracellular end of TM 5 and perhaps elsewhere (see Fig. 2B).

A third similarity among SLC4 family members is glycosylation. Four of the SLC4 family members are known to be N-glycosylated: AE1 is N-glycosylated on only its fourth extracellular loop, whereas AE2, AE3, and NBCe1 are all N-glycosylated on only their third extracellular loops. The rest of the SLC4 family members have at least two consensus N-glycosylation sites on the third, but none on the fourth, extracellular loop.

Differences among the SLC4 family members

The above similarities notwithstanding, subgroups of the SLC4 family members differ from one another in important ways. At least four structural or functional characteristics distinguish the transporters.

Perhaps the most striking distinguishing property among the SLC4 members is the very nature of transport activity. For example, AE1, AE2, and AE3 exchange monovalent anions from opposite sides of the membrane. The Na/HCO_3 cotransporters move Na^+ and a HCO_3^--related species from one side of the membrane to the other. Finally, the Na^+-driven Cl-HCO_3 exchanger NDCBE appears to be a hybrid cotransporter/exchanger that cotransports Na^+ and two HCO_3^- (or one Na^+ plus one CO_3^{2-}, or one $NaCO_3^-$ ion pair) into the cell in exchange for a single Cl^-. Understanding how such closely related proteins can mediate such fundamentally different functions is one of the major challenges—and one of the major opportunities—in transporter research.

A second major distinguishing factor among SLC4 family members is whether, in addition to HCO_3^- and/or CO_3^{2-}, the transporter carries an anion or a cation. For some family members, the additional ion is Cl^- (i.e., AE1, AE2, and AE3), for some it is Na^+ (e.g., NBCe1, NBCe2, NBCn1), and for at least one, it is both Cl^- and Na^+ (NDCBE).

A third major distinguishing factor is whether or not the transporter is electrogenic. At least two of the members of the SLC4 family are electrogenic (i.e., NBCe1 and NBCe2); that is, one complete cycle of transport activity results in the movement of one or two net negative charges across the membrane, carrying electrical current and causing a shift in membrane potential (V_m). The other six SLC4 family members that have been more-or-less well characterized are electroneutral; that is, a complete transport cycle results in no net movement of electrical charge (i.e., current) across the membrane, and thus no change in V_m.

A fourth distinguishing characteristic among the SLC4 family is the third extracellular loop. A characteristic of the Na^+-coupled HCO_3^- transporters is that, compared to AE1–3, their third extracellular loops (i.e., between putative TM 5 and TM 6) are unusually long—consistently the longest TM-to-TM loop in the protein. Moreover, these long third extracellular loops have four highly conserved cysteine residues (see Fig. 2B). Among AE1–3, only AE2 has a single cysteine residue in its third extracellular loop. It is notable that AE4, whose function is still controversial, has the four conserved cysteine residues in its third extracellular loop. BTR1, whose function is unknown, has no cysteine residues in the third extracellular loop.

Physiological roles of the SLC4 family members

In mammals, the SLC4 membrane proteins are critical for several physiological processes, including the carriage of carbon dioxide (CO_2) from the systemic capillaries to the pulmonary capillaries, the secretion or resorption of acid-base equivalents by numerous epithelia (e.g., $NaHCO_3$ reabsorption by the kidney, HCl secretion by the stomach, $NaHCO_3$ secretion by the pancreas), NaCl reabsorption by certain epithelia (e.g., ileum, proximal colon), the regulation of cell volume in multiple cell types, and the regulation of intracellular pH (pH_i) in nearly every cell of the body.

Focus of this review

In this review, we focus almost exclusively on vertebrate—especially human—members of the bicarbonate-transporter family. However, it is possible to trace SLC4-related genes to invertebrates [78, 102], yeast [112] and plants [38, 94]. Given the rich variety of SLC4 transport activity in vertebrates (i.e., exchange vs. cotransport vs. exchange/cotransport hybrids), we should not be surprised if the SLC4-related gene products from these other life forms are unique either in terms of mechanism or ions transported. Indeed, a plant SLC4 homologue that has been dubbed BOR1 [94] mediates the transport of boron—not the human version!—perhaps as borate.

The Cl-HCO_3 (anion) exchangers

The three identified anion exchangers—AE1, AE2, and AE3—mediate the electroneutral exchange of one monovalent anion for another across the plasma membrane. The preferred substrates are HCO_3^- and Cl^-, although the

AEs can also transport OH^-, and AE1 can cotransport SO_4^{2-} plus H^+ in exchange for Cl^-, but at a very low rate [51]. In living cells, the physiologically relevant transport activity is the exchange of Cl^- for HCO_3^-, and the transmembrane chemical gradients for these two ions determine the direction of net transport. For most cell types, the inward Cl^- chemical gradient dominates, driving the exchange of extracellular Cl^- for intracellular HCO_3^-. As we shall see, the direction of net Cl-HCO_3 exchange in erythrocytes depends on whether the cells are in the systemic or pulmonary capillaries. The disulfonic stilbenes SITS and DIDS block the transport activity of all three AEs.

For a recent summary of the AE literature, refer to the excellent review by Alper et al. [5].

AE1 (SLC4A1)

AE1, the Cl-HCO_3 exchanger of erythrocytes, was one of the first transporters of any sort to be physiologically identified. Among the members of the SLC4 family, AE1 was also the first to be cloned [59]. It is also the family member most intensively studied, and about which the most is known.

AE1—also known as the Band 3 protein for its position in SDS-polyacrylamide gel electrophoresis of erythrocyte membrane proteins—is the most abundant membrane protein in red blood cells, accounting for about a quarter of all membrane protein. Kopito and Lodish in 1985 reported the cloning of murine AE1 [59], which paved the way to the cloning of other examples of AE1 as well as AE2 and AE3. Human AE1 consists of 911 amino acids. According to the model of Zhu et al. [113], the cytoplasmic N-terminal domain of human AE1 is 404 amino acids long; this domain serves as an anchorage site for several proteins, including components of the cytoskeleton, some glycolytic enzymes, and hemoglobin.

The membrane-spanning domain of human AE1 consists of ~475 amino acids [113], and is responsible for the anion-exchange function of the protein. The final 40 amino acids of human AE1, the cytoplasmic C-terminal portion, includes an anchorage site for the soluble enzyme carbonic anhydrase II (CA II) [101].

In erythrocytes, AE1 plays a key role in CO_2 carriage from the systemic tissues to the lungs. CO_2 that is generated by metabolism moves from the mitochondria to the blood plasma and, as depicted in Fig. 3, this CO_2 enters the erythrocyte. There, CA II converts CO_2 plus H_2O into HCO_3^- and H^+. AE1 disposes of the newly generated HCO_3^- in exchange for Cl^- (the so-called "chloride" or "Hamburger" shift), while de-oxygenated hemoglobin (which has a higher H^+ affinity than fully oxygenated hemoglobin) buffers the H^+. These two processes permit the erythrocyte to take up additional CO_2. The entire process is called the Jacobs-Stewart cycle. In the lungs, this cycle reverses itself, releasing CO_2 into the blood plasma for diffusion into the alveoli and excretion from the body via ventilation. As noted above, CA II binds to a C-terminal domain of AE1. In addition, erythrocytes express a high level of the water channel aquaporin 1 [72], which is also permeable to CO_2 [34, 66]. A cluster or metabolon [90] of four proteins—AE1, CA II, AQP1, and hemoglobin—might promote CO_2 carriage by juxtaposing CA II to the site of CO_2 entry, by juxtaposing AE1 to the point of HCO_3^- formation, and by juxtaposing hemoglobin to the point of H^+ formation.

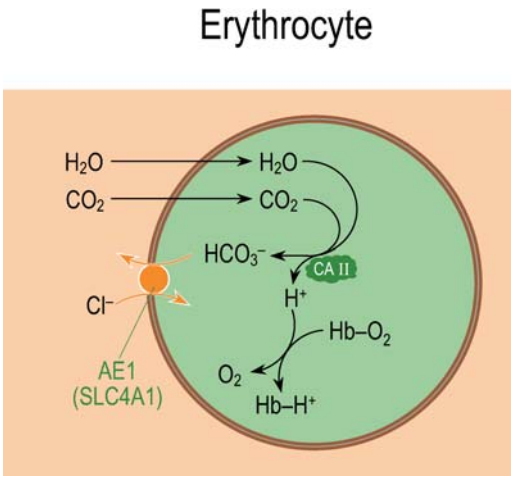

Fig. 3 Role of AE1 in the uptake of CO_2 by erythrocytes in systemic capillaries. *CA II* is carbonic anhydrase II, a soluble enzyme that also can bind to the C terminus of AE1. *Hb* is hemoglobin, *Hb-O_2* is hemoglobin fully saturated with four O_2 molecules, and *Hb–H^+* is protonated hemoglobin that binds three O_2 molecules. CO_2 may, in part, enter the cell via the water channel AQP1. In the pulmonary capillaries, all of the reactions reverse, releasing CO_2 into the blood plasma

After erythrocytes, the cells with the highest AE1 expression are type A (or α) intercalated cells of the collecting duct of the kidney. Here, AE1 is present in the basolateral membrane [100] and plays a key role in the reabsorption of HCO_3^- from the lumen to the blood. An alternate promoter site produces a unique renal transcript that—compared to the erythroid transcript (eAE1)—results in an N-terminal variant (kAE1) that lacks the first 65 amino acids in humans [22, 81]. AE1 mRNA is also expressed at lower levels in heart [80] and colon [68].

Naturally occurring mutations of AE1 can cause human disease affecting the function of erythrocytes and/or intercalated cells of the distal nephron [4, 89]. The deletion Δ400–408 (encompassing the end of the cytoplasmic N terminus and beginning of TM 1) produces a transporter that, by itself, is nonfunctional. However, the heterodimer consisting of the mutant and wild-type transporters is functional. Moreover, the presence of this mutant AE1 polypeptide increases the rigidity of the erythrocyte membrane and may confer protection against cerebral malaria. The erythrocytes have an altered shape (Southeast Asian ovalocytosis, SAO), but exhibit no clinical pathology.

AE1—as well as AE2 and AE3, which we shall discuss later—is inhibited by the stilbene derivatives SITS and

DIDS [14, 27, 28], both of which have two negatively charged sulfonate groups. In addition, SITS has one isothiocyano group that is capable of reacting covalently with free amines; DIDS has two such groups. SITS interacts with AE1 in two steps: (1) a rapid, reversible electrostatic ionic interaction, and (2) a slower irreversible covalent reaction with an AE1 lysine. In human AE1, the target of the covalent reaction is the lysine at position 539 (K539), near the extracellular end of TM 5 [13, 61, 67, 86, 107]. On the basis of homologies among AE1–3, Kopito et al. [58] proposed a potential DIDS-reaction motif at the putative extracellular end of TM 5: KLXK (where the first K is K539 in human AE1, and X is I or Y).

Several mutations scattered throughout the AE1 protein can lead to a form of autosomal-dominant hereditary spherocytosis (HS) that is characterized by increased erythrocyte fragility and thus a hemolytic anemia. However, HS is not associated with a renal defect. Conversely, several mutations in the membrane-spanning domain of AE1—several mutations at R589 in TM 6 [24, 50, 53], S613F in TM 7 [24], ΔV850 [25], and an 11-amino-acid deletion in the cytoplasmic C terminus [53]—can produce an autosomal-dominant distal renal tubular acidosis (dRTA) that has no associated erythrocyte pathology. When heterologously expressed in *Xenopus* oocytes, the transport activity of the dominant mutants is only moderately affected. The properties of recessive dRTA-associated AE1 mutants heterologously expressed in *Xenopus* oocytes suggest that these recessive mutants produce renal pathology because they do not traffic properly to the intercalated cell plasma membrane. However, heterologous co-expression of glycophorin A—a membrane protein that binds to AE1 and is found only in erythrocytes—rescues the surface delivery of recessive mutant AE1 polypeptides in the oocyte [95, 110]. Presumably native glycophorin A does the same in erythrocytes, explaining the lack of erythroid pathology in recessive dRTA.

AE2 (SLC4A2)

A fragment of AE2 was initially cloned in 1986 by Demuth et al. [36] from human kidney and lymphoma cells, and described as a nonerythroid band 3 [36]. Alper et al. [6] later cloned a full-length mouse ortholog by low-stringency probing of a mouse-kidney library.

The three AEs are most similar in their membrane-spanning domains; they diverge most markedly in the cytoplasmic N terminus. Human and mouse AE2 have three alternative promoters (a, b, and c) that yield a total of five splice variants in mouse: a, b1, b2, c1, and c2 (it is not clear whether humans have distinct c1 and c2 variants). The physiological significance of these variants remains under study. All AE2 variants have cytoplasmic N termini that are longer than that of AE1. For example, the longer cytoplasmic N terminus of human AE2a is almost entirely responsible for the substantially greater overall length of AE2a (1240 residues) compared to human erythrocyte AE1 (911 residues).

AE2 is the most widely distributed of the AEs. It is expressed at the basolateral membranes of most epithelial cells. Expression levels are especially high in gastric parietal cells [92], choroid-plexus epithelial cells [8], surface enterocytes in colon [7], and renal collecting duct [9, 93]. In gastric parietal cells, AE2 presumably plays a key role in H^+ secretion, exporting into the blood at least some of the HCO_3^- that balances the H^+ pumped into the lumen of the gastric gland. AE2 may contribute to H^+ secretion by the TAL. When arranged in parallel with a Na-H exchanger, AE2 contributes to net NaCl uptake and—in an epithelial cell such as in the choroid plexus—may contribute to the transepithelial movement of Na^+ and Cl^-. It is reasonable to postulate that, in most cells in which it is expressed, AE2 contributes to the regulation of pH_i (by exporting HCO_3^- in response to intracellular alkali loads) and/or the regulation of cell volume (by contributing to volume-regulatory increases in cell volume by taking up Cl^-). Indeed, the activity of AE2—heterologously expressed in *Xenopus* oocytes and assessed as ^{36}Cl efflux—is stimulated by: (1) increases in either intracellular or extracellular pH [91]; (2) hypertonicity, a response that requires the pH_i increase that is produced by the shrinkage-induced activation of the native *Xenopus* Na-H exchanger [46]; and (3) NH_4^+ [45], the application of which paradoxically causes oocyte pH_i to fall. All of these responses can be modified or inactivated by engineered mutations in the highly conserved WRETARWIKFEE motif [77] in the cytoplasmic N terminus [29, 91].

AE3 (SLC4A3)

AE3 was first cloned by Kopito et al. [58] in 1989. Human AE3 has two alternative promoters that yield two splice variants, the cardiac form (cAE3) and the brain form (bAE3). These naming conventions are of historical significance only—both splice variants may be present in heart, brain and elsewhere. We already noted that the three AEs are most similar in their membrane-spanning domains. The cytoplasmic N terminus of human cAE3, like that of AE2c, is somewhat longer than that of AE1 (1034 total residues in human cAE3 vs. 911 in eAE1). The cytoplasmic N terminus of human bAE3, like that of AE2a and AE2b, is substantially longer (1232 total residues for human bAE3).

AE3 is found mostly in excitable tissues: brain [58, 60], retina [57], heart [62, 109] and smooth muscle [23]. However, AE3 variants are also expressed in epithelial cells, including those of the kidney and GI tract. The tissue-distribution data are mainly the result of Northern blots and RT-PCR, and the relative distribution of AE3 splice variants as determined by these techniques varies with developmental stage and among species.

Like AE2, AE3 is stimulated by increases in pH_i. Thus, it is reasonable to postulate that AE3 contributes to the

regulation of pH_i by exporting HCO_3^- in response to intracellular alkali loads. The substitution polymorphism Ala867Asp, which occurs in the extracellular loop between TM 5 and TM 6, has been suggested to confer a small degree of susceptibility to idiopathic generalized epilepsy [82].

The electrogenic Na/HCO_3 cotransporters

The two identified electrogenic Na/HCO_3 cotransporters—NBCe1 and NBCe2—mediate the movement in the same direction of one Na^+ and what appears to be either two or three HCO_3^-. As a result, one or two negative charges cross the plasma membrane with each Na^+. In most cases, the electrogenic NBCs appear to move the equivalent of one Na^+ and two HCO_3^- ions in one complete cycle of transport activity. Given such a 1:2 stoichiometry, as well as typical values of membrane potential (V_m) and ion gradients for Na^+ and HCO_3^-, thermodynamics predicts that NBCe1 and NBCe2 should move Na^+ and HCO_3^- into the cell, thereby raising $[Na^+]_i$ and pH_i as well as shifting V_m to more negative values. A notable exception is the basolateral membrane of the renal proximal tubule, where NBCe1 appears to have a 1:3 stoichiometry. As predicted by thermodynamics, renal NBCe1 mediates the net movement of Na^+, HCO_3^-, and net negative charge out of the cell.

For all of the Na^+-coupled HCO_3^- transporters—the two electrogenic NBCs and the three electroneutral transporters that we will discuss below—a fundamental uncertainty exists concerning the nature of the HCO_3^--related ion that the protein carries. For example, the electrogenic NBCs with a 1:2 stoichiometry might carry one Na^+ and two HCO_3^- ions, one Na^+ and one CO_3^{2-}, or a single $NaCO_3^-$ ion pair ($Na^+ + CO_3^{2-} \leftrightarrows NaCO_3^-$). Preliminary data suggest that, for both NBCe1 and NBCe2, the HCO_3^--related substrate is either CO_3^{2-} or $NaCO_3^-$ [40].

The disulfonic stilbenes SITS and/or DIDS block the transport activities of both NBCe1 and NBCe2.

NBCe1 (SLC4A4)

In 1983 and working on the salamander renal proximal tubule, Boron and Boulpaep described the first Na/HCO_3 cotransporter [18], the electrogenic Na/HCO_3 cotransporter. In 1997, Romero et al. [77] used an expression-cloning approach to obtain the first cDNA that encodes a Na^+-coupled HCO_3^- transporter, the renal electrogenic Na/HCO_3 cotransporter. They named this clone NBC for **N**a/**B**icarbonate **C**otransporter. We shall refer to this clone as aNBCe1-A –"a" for *Ambystoma* (i.e., salamander), "e" for electrogenic, "1" because it represents the first of two genes encoding electrogenic NBCs, and "A" for the first known splice variant of that gene. Subsequent work led to the cloning of NBCe1-A homologues in human [26] and rat [76]. The renal variant arises from an alternate promoter in intron 3 [2]. Two other NBCe1 splice variants are known. In NBCe1-B, which was first cloned from pancreas [1] and heart [33] and is the most widespread splice variant, the N-terminal 85 amino acids of NBCe1-B replace the N-terminal 41 amino acids of NBCe1-A. In NBCe1-C, which is found almost exclusively in the brain [15], a 97-bp deletion near the C terminus causes a unique 61 C-terminal amino acids to replace the 46 C-terminal amino acids in NBCe1-B. All three NBCe1 splice variants have two motifs similar to the one in AE1 that binds CA II, and data suggest that the common C terminus of NBCe1-A/B can bind CA II in vitro [35, 44].

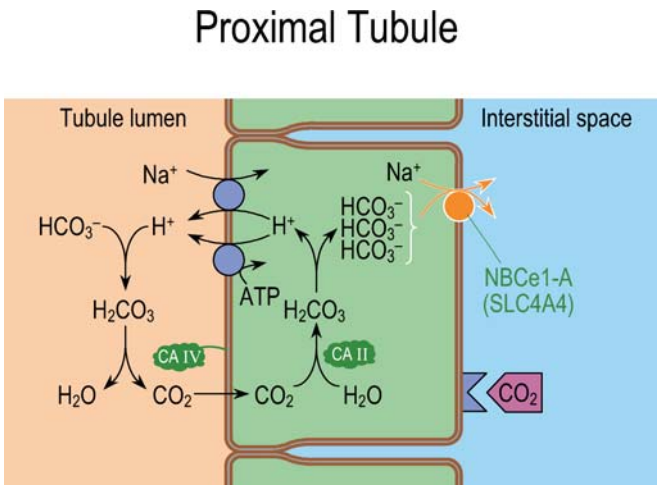

Fig. 4 Role of NBCe1-A in HCO_3^- reabsorption by the renal proximal tubule. Two acid extruders in the apical membrane, the Na-H exchanger NHE3 and a vacuolar-type proton pump, transfer H^+ to the tubule lumen. There, the GPI-linked enzyme carbonic anhydrase IV (*CA IV*) uses the H^+ to titrate the filtered HCO_3^- to CO_2 and H_2O. These two products enter the cell across the apical membrane; the water channel AQP1 assists in the uptake of H_2O and may also play a role in CO_2 uptake. Inside the cell, CA II converts CO_2 and H_2O to H^+ (which is extruded into the lumen) and HCO_3^-. NBCe1-A, operating with a Na^+:HCO_3^- stoichiometry of 1:3, exports HCO_3^- into the interstitial space. The HCO_3^- eventually diffuses into the blood. A CO_2-sensing mechanism responds to high levels of $[CO_2]$ by increasing the rate of HCO_3^- reabsorption

Protein-localization studies have shown that NBCe1 is present in the basolateral membranes of renal proximal tubule [85], pancreatic ducts [64], and epididymis [52], as well as in astrocytes and neurons in several regions of the brain [84], several tissues within the eye [17], and blood vessels and intercalated disks within the heart [106].

In the kidney, NBCe1-A mediates the movement of HCO_3^- equivalents from the proximal-tubule cell to the blood, thereby completing the reabsorption of HCO_3^- from lumen to blood (Fig. 4). In the pancreas, NBCe1-B plays a major role in the accumulation of intracellular HCO_3^-; the basolateral Na-H exchanger assists in this task by extruding H^+ across the basolateral membrane and driving the cytoplasmic CO_2/HCO_3^- equilibrium toward the formation of HCO_3^-. The accumulation of cytoplasmic HCO_3^- is the first step in the secretion of HCO_3^- from

the blood to the lumen of the exocrine ducts (Fig. 5). In nonepithelial cells, NBCe1-B and NBCe1-C contribute to pH_i regulation by moving alkali into cells in response to intracellular acid loads.

An intriguing and still unsettled question is how the Na^+:HCO_3^- stoichiometry of NBCe1 apparently shifts from 1:2 to 1:3. Measurements of transporter currents, intracellular pH and Na^+ in *Xenopus* oocytes heterologously expressing NBCe1-A revealed a stoichiometry of 1:2 rather than the expected 1:3 [88]. Muller-Berger et al. [65] computed the Na^+:HCO_3^- stoichiometry from reversal potentials and slope conductances measured using inside-out giant patches from *Xenopus* oocytes heterologously expressing NBCe1-A. They found that the stoichiometry was 1:2 when the cytosol-side [Ca^{2+}] was 100 nM or less, but was usually 1:3 when the cytosol-side [Ca^{2+}] was 500 nM. Gross and colleagues [42] have expressed NBCe1-A and NBCe1-B in various epithelial cell lines, mounted confluent monolayers in an Ussing chamber, permeabilized the apical membranes with Amphotericin B, obtained *I–V* plots in the presence and absence of the reversible DIDS analog 4,4′-dinitro-2,2′-disulfonate (DNDS), and then computed the Na^+:HCO_3^- stoichiometry from the reversal potential of the difference current. They found that, when expressed in a mouse renal-proximal-tubule cell line, both NBCe1-A or NBCe1-B had stoichiometries of 1:3, whereas when expressed in a renal-collecting-duct cell line, both had stoichiometries of 1:2 [42]. Moreover, it appears that, when NBCe1-A is expressed in a proximal-tubule cell line, phosphorylating Ser-982 (in the second of two putative CA-II binding sites in the cytoplasmic C terminus; see Fig. 2B) shifts the stoichiometry from 1:3 to 1:2 [43]. Finally, two of the aspartate residues near Ser-982 appear to be necessary for the phosphorylation-induced shift in stoichiometry [44]. The simplest explanation for the results of Gross et al. [42] is that the proximal tubule contains unique components required for a 1:3 stoichiometry. A concern with these experiments is that DNDS produces a relatively small change against rather large background currents [42].

Like AE1–3 (see discussion of AE1, above), NBCe1 is blocked by the stilbene derivative DIDS [77]. Moreover, at the putative end of TM 5, NBCe1 has a sequence (KMIK) that is very similar to the consensus DIDS-reaction motif for the AEs (KLXK, X = I or Y). Thus, the actual DIDS-reaction motif might be KXXK, where X is any of several hydrophobic residues. Preliminary work on human NBCe1-A suggests that KXXK plays a critical role in reversible DIDS blockade [63]. A lysine-to-asparagine mutation at either position increases the apparent DIDS-binding constant; mutating both lysines produces the greatest effects.

Investigators have reported four naturally occurring mutations of NBCe1, all of which cause a severe and persistent recessive proximal renal tubular acidosis—with the pH of arterial blood being as low as 7.10—and ocular abnormalities such as bilateral glaucoma, bilateral cataracts, band keratopathy, and blindness [37, 47, 48]. Two missense mutations target Arg residues that are highly conserved among SLC4 family members [47], one in the middle of the cytoplasmic N terminus (R298S in NBCe1-A—see Fig. 2B), and one near the extracellular end of proposed TM 3 (R510H in NBCe1-A—see Fig. 2B). Another missense mutation [37] targets a Ser residue near the proposed intracellular end of TM 1 (S427L in NBCe1-A—see Fig. 2B). At the residue homologous to S427 in NBCe1-A, NBCe2 also has a Ser; the three AEs and the three electroneutral Na^+-coupled HCO_3^- transporters (NBCn1, NCBE, and NDCBE) all have an Ala. The final mutation [48] is a nonsense mutation (Q29) in the portion of the cytoplasmic N terminus that is unique to NBCe1-A, and produces the same renal phenotype as the other mutations.

NBCe2 (SLC4A5)

The nomenclature of the NBCe2 protein (also called NBC4) is confusing because five of the reported six variants (named a–f) are likely to be amplification products of incompletely spliced pre-mRNA. We shall refer to the questionable variants by their originally published names: NBC4, followed by a letter (e.g., NBC4a). We shall restrict use of the term NBCe2 for clones known to function, of which there is currently only one, NBCe2-C (equivalent to NBC4c). Eventually the field will have to adopt a standard nomenclature that eliminates reference to the questionable clones.

Pushkin et al. [74] reported two variants of an NBC-related clone. Both NBC4a and NBC4b contain a unique putative exon just after the beginning of proposed TM 11. In addition, NBC4b contains a unique 16-bp insert in proposed TM 13 that causes a frameshift and premature stop codon. Pushkin et al. [74] deposited two other variants in GenBank, one of which (NBC4c) yields a functional protein. The other is missing two exons, resulting in the elimination of all amino acids between the proposed end of TM 10 and near the proposed beginning of TM 13. Subsequent attempts by Virkki et al. [103] to obtain the four variants by PCR yielded only NBC4c, consistent with the hypothesis that the others are cloning artifacts. Both Virkki et al. [103] and Sassani et al. [83] have now characterized NBC4c (i.e., NBCe2-C)—which consists of 1121 amino acids—as an electrogenic NBC. As studied in *Xenopus* oocytes NBCe2-C has an apparent stoichiometry of 1:2 [103]; as expressed in the mPCT renal cell line NBCe2-C has an apparent stoichiometry of 1:3 [83].

Xu et al. [108] have described two additional variants. In NBC4e, a single nucleotide substitution corresponding to the middle of exon 24 (out of 26 total exons) converts what would be Trp-1052 (TGG) in NBCe2-C to a stop codon (TAG) in NBC4e. Thus, NBC4e would terminate at Ala-1051 (approximately the 13th residue after the cytoplasmic end of TM 13), leading to the deletion of the 70 most C-terminal residues of NBCe2-C. In NBC4f, a single-nucleotide deletion corresponding to the middle of

exon 16 converts what would be Leu-759 (CTG), Thr-760 (ACC) and Leu-761 (CTGa) in NBCe2-C to Arg-759 (CGA), Pro-760 (CCC) and a stop codon (TGA). Thus, NBCf would terminate after the novel Pro-760 (approximately two residues before the cytoplasmic end of TM 6), leading to the deletion of the 361 most C-terminal amino acids of NBCe2-C. Because NBC4e and NBC4f arise as the result of single-nucleotide substitutions in the middle of exons, both may represent cloning artifacts. Nevertheless, using fluorescence-based measurements of pH_i and microelectrode recordings of V_m in oocytes injected with NBC4e cRNA, Xu et al. [108] concluded that the transporter mediates electroneutral Na/HCO_3 cotransport. If validated, this observation would be the first example in which a defined change in amino-acid sequence (i.e., NBCe2-C vs. NBC4e) causes a change in $Na^+:HCO_3^-$ stoichiometry.

Based on Northern analysis of mouse, NBCe2 is expressed most abundantly in liver, testes, and spleen; lower levels are present in heart, kidney, stomach, lung, and brain [74].

The electroneutral Na^+-couple HCO_3^- transporters

At least three genes encode membrane proteins that mediate the electroneutral movement of Na^+, HCO_3^- (or a related species) across the plasma membrane. One, NBCn1, normally mediates the uptake of one Na^+ and one HCO_3^-, but no movement of Cl^-. A second, NDCBE, appears to mediate the uptake of one Na^+ and two HCO_3^-, as well as the coupled exit of one Cl^- (i.e., it is a Na^+-driven Cl-HCO_3 exchanger). The third, NCBE, was originally described as a Na^+-driven Cl-HCO_3 exchanger, although the data supporting the involvement of Cl^- are inconclusive.

Nomenclature

As discussed earlier, column 1 in Table 1 summarizes the nomenclature of the SLC4 proteins, which is based on homology and the chronological order of discovery. Column 2 summarizes protein nomenclature, which is based on transport function. The alternate protein names of the electroneutral Na/HCO_3 cotransporter proteins, listed in column 3 of Table 1, are extremely confusing because various authors assigned names—sometimes conflicting names—before determining with confidence the key functional attributes of three of the transport proteins. The protein name of an SLC4 family member should be regarded as provisional until the following minimal functional characteristics (or lack thereof) have been determined experimentally: (1) HCO_3^- dependence (at fixed pH_i and pH_o), (2) Na^+ dependence, (3) Cl^- dependence, and (4) electrogenicity. Regarding the last point, it should be noted that electrogenicity cannot be determined by examining the effect of a presumed or even a known effect of a V_m change on transport activity—thermodynamics cannot predict kinetics! Only measurements of V_m and/or current can provide the necessary evidence for distinguishing electrogenicity from electroneutrality. In summary, before assigning protein name, one must be certain that the transporter has precisely the function associated with one of the following candidate names: (1) a Cl-HCO_3 exchanger (AE), (2) an electrogenic Na/HCO_3 cotransporter (NBCe), (3) an electroneutral Na/HCO_3 cotransporter (NBCn), or a Na^+-driven Cl-HCO_3 exchanger (NDCBE).

The names "SBC2" and NBC2 [49] refer to a cloning artifact that consists mainly of the authentic sequence of the human electroneutral NBC (NBCn1).[1] However, the N-terminal portion is missing the amino acids encoded by the first three exons, and instead begins with the amino acids that would be encoded by an inverted exon 4. Additionally, the C terminus of NBC2 lacks the 26 amino acids encoded by the final exon. Thus, the name NBC2 is no longer in use.

The authentic cDNA sequence for the human electroneutral NBC (NBCn1) was named NBC3 [73] before experiments were performed to test its electrogenicity. The name NBC3 [10] was also used simultaneously for a fragment of the authentic sequence for the Na^+-driven Cl-HCO_3 exchanger (NDCBE). Thus, the name NBC3 is degenerate—referring both to SLC4A7 and SLC4A8.

When Romero et al. [77] originally cloned the electrogenic Na/HCO_3 cotransporter from salamander, they proposed that the nomenclature of that clone and future clones should follow function. Mindful of that suggestion, and of the confusion that has surrounded numerical names such as NBC2 and NBC3, we recommend that the electrogenic Na/HCO_3 cotransporters be referred to as NBCe1 and NBCe2, that electroneutral Na/HCO_3 cotransporter be referred to as NBCn or NBCn1 (if another clone should prove to be NBCn2), and the Na^+-driven Cl-HCO_3 exchanger should be referred to as NDCBE. Under no circumstances should a Na^+-driven Cl-HCO_3 exchanger be referred to as an "NBC," a name that implies lack of exchange activity.

NBCn1 (SLC4A7)

In 1999, Pushkin et al. [73] reported a new NBC-related cDNA, cloned from human skeletal muscle. They concluded that the cDNA encoded a Na/HCO_3 cotransporter, which they named NBC3, but did not study the electrophysiology of the transporter [73]. Later, Choi et al. [30] cloned from rat aorta three cDNAs (variants B, C and D) that are homologous to NBC3 [30] and pointed out that the C terminus has a putative PDZ-binding domain. Using microelectrodes to monitor pH_i and V_m in oocytes injected with cRNA, they demonstrated that the B variant

[1] NBC2 lacks the amino acids encoded by exon 7. As discussed later, some authentic splice variants of NBCn1 can probably also lack exon 7. NBC2 includes both the "A" and "B" cassettes that may be present in authentic NBCn1 splice variants (see Table 3).

Table 3 NBCn1 variants*

Variant	Cassette "A"	Cassette "B"
A	–	✓
B	✓	–
C	–	✓
D	✓	✓

* The A variant (thus far described only for human) and C variant (thus far described only for rat) have identical cassette structure, and are ~91% identical overall at the amino-acid level. The B and D variants have been thus far been described only for rat

encodes a Na/HCO_3 cotransporter that is electroneutral. They named the clone NBCn1-B. The rat clone is poorly sensitive to DIDS and completely insensitive to EIPA, as recently confirmed by Park et al. [69], who expressed human NBCn1 in HEK cells.

Using a two-electrode voltage clamp to study current–voltage relationships in oocytes expressing NBCn1, Choi et al. [32] demonstrated two NBCn1-dependent currents, one of which is a Na^+ current that is paradoxically stimulated by DIDS. It is not yet clear whether one or both currents represent novel oocyte channels or "slippage" of the electroneutral transporter.

The NBCn1 gene has at least two cassettes that may (or may not) be present at two different positions in the mRNA: (1) a 13-residue "A" cassette in the cytoplasmic N-terminal region, and (2) a 36-residue "B" cassette in the cytoplasmic C-terminal region. Table 3 summarizes which cassettes are present in which splice variants. All published electrophysiological data have been obtained on NBCn1-B. Preliminary data suggest that the entirety of exon 7, which corresponds to 124 amino acids in the cytoplasmic N-terminal region, may also be absent from some clones [39].

The molecular basis for the poor sensitivity of NBCn1 to DIDS is apparently the lack of an intact DIDS motif (KXXK) at the extracellular end of putative TM 5 (see discussion of NBCe1). In NBCn1, the sequence is KLFH. Preliminary work indicates that mutating the histidine to lysine (KLFK) renders the transporter DIDS sensitive [31]. NBCn1 appears to interact via its C-terminal PDZ-binding motif to both CFTR [69] and the 56-kDa H^+ ATPase subunit [75].

In human Northern blots probed with a 95-bp oligonucleotide directed only to exon 7, NBCn1 expression is apparent only in heart and skeletal muscle. In rat Northern blots probed with a 836-bp oligonucleotide (which corresponds to part of the extracellular loop between TM 3 and TM 4, extending to about halfway through TM 9), high levels of NBCn1 mRNA are apparent in spleen and testis, and lower levels in heart, brain, lung, liver and kidney but not in skeletal muscle [30].

As depicted in Fig. 5, NBCn1 in the apical membrane of the pancreatic duct may play a housekeeping role by reabsorbing ("salvaging") HCO_3^- from the lumen in between meals [69].

An NBCn1 knockout mouse was recently created by Bok et al. [16]. The mice develop blindness and auditory impairment as the result of the degeneration of sensory receptors in neurons.

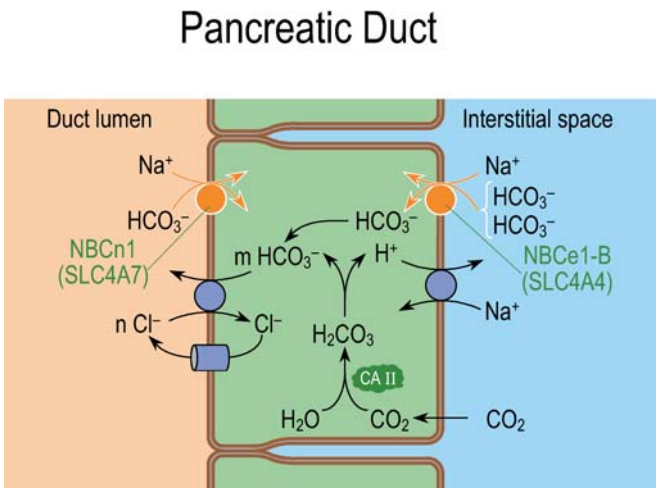

Fig. 5 Role of NBCe1-B in HCO_3^- secretion by pancreatic duct. NBCe1-B at the basolateral membrane appears to operate with a stoichiometry of 1:2, leading to the net uptake of HCO_3^-. A basolateral Na-H exchanger (*NHE1*) contributes to the accumulation of intracellular HCO_3^-, in conjunction with carbonic anhydrase II (*CA II*). At the apical membrane, members of the SLC26a family, which electrogenically exchange Cl^- for HCO_3^- with different stoichiometries (indicated by "n" and "m" in the figure), export HCO_3^- into the lumen [56]. Apical CFTR recycles the Cl^-. During baseline periods, apical NBCn1 may keep luminal [HCO_3^-] relatively low

NDCBE (SLC4A8)

Historically, the Na^+-driven Cl-HCO_3 exchanger was the first acid-base transporter shown to play a role in pH_i regulation—based on microelectrode recordings of pH_i on squid giant axons [19, 21, 79] and snail neurons [96, 97, 98]. This transporter normally mediates the uptake of one Na^+ and the equivalent of two HCO_3^-, and the egress of one Cl^-. In the squid axon, this transporter has an absolute requirement for intracellular ATP, although the ATP is not stoichiometrically hydrolyzed as fuel [20].

The first cDNA encoding activity related to Na^+-driven Cl-HCO_3 exchange was cloned from *Drosophila*, and encodes a Na^+-driven anion exchanger (NDAE1) that can apparently exchange extracellular Na^+ and two HCO_3^- (or two OH^-) for intracellular Cl^- [78]. The Na^+-driven Cl-HCO_3 exchanger (NDCBE) cloned from human brain has an absolute dependence on HCO_3^- as well as for Na^+ and Cl^- [41]. Northern analysis indicates robust expression of a ~12-kb transcript in brain and testis, with weaker expression in kidney and ovary. A mouse ortholog of NDCBE (also called kNBC3) has an alternatively spliced 3′ end, leading to a protein in which the C-terminal 17 amino acids of human NDCBE are replaced by 66 amino acids [105]. Analysis of the genomic sequence suggests that human NDCBE may have a similar C-terminal splice

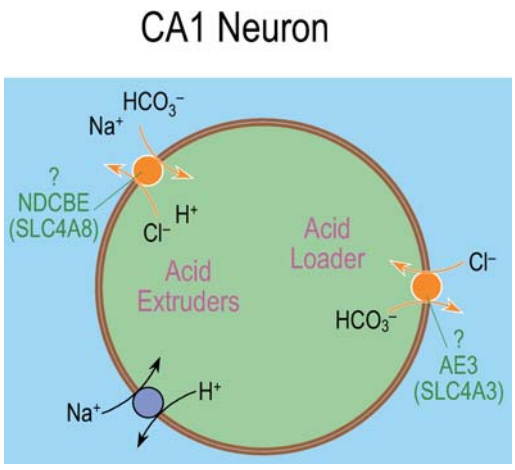

Fig. 6 Proposed role of NDCBE and AE3 in pH_i regulation by neurons. Along with a Na-H exchanger (e.g., NHE5), the Na^+-driven Cl-HCO_3 exchanger NDCBE responds to intracellular acid loads by extruding acid and thereby returning intracellular pH (pH_i) toward normal. A Cl-HCO_3 exchanger (e.g., AE3) responds to intracellular alkali loads by exporting HCO_3^- and thereby returning pH_i toward normal

variant. Recently, a squid NDCBE has been cloned and characterized [102].

Human NDCBE, as well as the related transporters from *Drosophila* and squid are very sensitive to inhibition by DIDS even though none of them has a consensus DIDS-interaction motif (KXXK) at the presumed extracellular end of TM 5. However, human NDCBE (as well as NCBE) has such a motif at the presumed extracellular end of TM 3, and squid NDCBE has such a motif at the presumed end of TM 12—as discussed elsewhere [102]. Curiously, the DIDS-sensitive *Drosophila* NDAE1 does not have the consensus motif at either of these locations.

Experiments on freshly dissociated hippocampal CA1 neurons have demonstrated Na^+-driven Cl-HCO_3 exchange activity as a major mode of pH_i regulation (see Fig. 6). In response to acid loads (which produce a fall in pH_i), a Na-H exchanger and a Na^+-driven Cl-HCO_3 exchanger extrude acid [87] and thereby return pH_i toward normal. The term "acid extrusion" refers to an energy-requiring process used to export acid and/or to take up alkali. In response to alkali loads (which produce a rise in pH_i), many neurons use a Cl-HCO_3 exchanger (perhaps AE3) to export HCO_3^-. This acid-loading process also tends to return pH_i toward normal. In the steady state, the actions of the acid extruders and acid loaders oppose each other and thus stabilize pH_i within a narrow range of values. In Fig. 6, we depict NDCBE and AE3 as playing keys roles in a hypothetical "neuron". The extent to which specific acid-base transporters contribute to pH_i regulation in specific neurons and astrocytes is not yet known. NBCe1 has been identified in neurons [15, 84], NCBE (discussed below) is expressed at high levels in the brain [104], and both NBCn1 [30] and NBCe2 [74] also appear to be expressed in the brain. Thus, we should not be surprised if it turns out that different parts of different neurons and glial cells have different complements of acid-base transporters. Nevertheless, the principles underlying Fig. 6 are likely to hold for pH_i regulation in most cells throughout the brain and the rest of the body.

NCBE (SLC4A10)

In 2000, Wang et al. [104] cloned a novel NBC-related cDNA from a mouse insulinoma cell line. Because this clone appeared to encode a Na^+-driven Cl-HCO_3 exchanger, based on a functional characterization carried out partially in HEK cells and partially in *Xenopus* oocytes, they named it NCBE [104] for **N**a-**C**l **b**icarbonate **e**xchanger. Preliminary studies have identified a human ortholog of NCBE (NCBE-A), as well as a second splice variant (NCBE-B) in which 30 amino acids (corresponding to an exon) are inserted in the cytoplasmic C terminus [32]. As expressed in *Xenopus* oocytes, human NCBE-B has absolute requirements for Na^+ and HCO_3^-, is blocked by DIDS, but appears not to require Cl^-. These preliminary data are consistent with the hypothesis that NCBE is an electroneutral Na/HCO_3 cotransporter. If this functional analysis is borne out, we would suggest renaming the clone NBCn2.

In the mouse, NCBE is expressed heavily in brain [104]. In humans, preliminary work indicates the presence of high levels of a ~5.5-kb transcript—and lower levels of a ~9.5-kb transcript—in several regions of human brain [32].

Other SLC4 family members

Genome sequencing and analysis of EST databases have revealed two additional members of the SLC4 family, AE4 and BTR1. Because the sequencing of the human genome is virtually complete, it is unlikely that additional SLC4 members will be found.

AE4 (SLC4A9)

In 2001, Tsuganezawa et al. [99] cloned two splice variants (a and b) of a novel NBC-like cDNA from rabbit kidney. Compared to AE4-a, AE4-b lacks 16 amino acids in the cytoplasmic N terminus. As summarized in Fig. 1, the cDNA is more closely related to the Na^+-coupled HCO_3^- transporters than to the Cl-HCO_3 exchangers. Nevertheless, when expressed in COS cells, AE4-a appeared to behave as a Na^+-independent Cl-HCO_3 exchanger, leading to the name AE4. Moreover, *Xenopus* oocytes expressing AE4-a were reported to mediate a modest level of DIDS-insensitive ^{36}Cl uptake. However, the function of AE4 is still unsettled. Following the cloning of human AE4 by Parker et al. [71], Parker et al. [70] attempted to characterize AE4 in *Xenopus* oocytes. In preliminary electrophysiological experiments, they failed to detect Cl-HCO_3 exchange activity in oocytes

injected with AE4 cRNA. Recently, Ko et al. [55] expressed in HEK and LLC-PK_1 cells an AE4 cDNA that they cloned from rat. These authors found evidence for Cl-HCO_3 exchange that was DIDS sensitive. This last finding is unexpected, inasmuch as AE4 lacks a classical DIDS motif at the presumed extracellular ends of TMs 3, 5 and 12. Thus, additional work will be required to establish the function of AE4.

Immunocytochemical data suggest that the AE4 protein is present in the apical membranes of β-intercalated cells in the rabbit renal cortex [99].

BTR1 (SLC4A11)

In 2001, Parker et al. [71] identified a novel sequence in GenBank and cloned BTR1 (**b**icarbonate-**t**ransporter–**r**elated protein 1) from human kidney [71]. As indicated in Fig. 1, BTR1 is the most unique of the SLC4 members. No functional data on BTR1 are available. Northern analysis indicates that BTR1 is present most abundantly in kidney, salivary gland, testis, thyroid and trachea. [7]

Potential therapeutic/pharmaceutical applications

Myocardial reperfusion injury

Following a period of myocardial ischemia, the very process of reestablishing blood flow—a prerequisite for salvaging the jeopardized myocardium—may paradoxically cause damage itself. At least one contributing factor to this reperfusion injury may be the following. During the period of ischemia, pH_i in affected myocardial cells probably falls appreciably. Nevertheless, during the ischemia, the ability of the cells to respond to the intracellular acidosis may be diminished markedly by relatively low levels of ATP, which may indirectly lead to inhibition of the Na-H exchangers and Na^+-coupled HCO_3^- transporters that would normally be responsible for acid extrusion. The first example in which ATP withdrawal causes blockade of an acid-extruding system was the Na^+-driven Cl-HCO_3 exchanger, which, in squid giant axons, requires ATP [19, 79] but does not hydrolyze it as an energy source [20]. During reperfusion, however, rising levels of ATP now allow acid extruders to respond in a delayed fashion to the intracellular acidosis. The result of a massive acid-extrusion effort can be not only recovery of pH_i from the acid load, but also Na^+ overload. A rise in $[Na^+]_i$ can bias the Na-Ca exchange in favor of net Ca^{2+} uptake, leading to a rise in Ca^{2+} and cell injury. Blocking Na-H exchange can reduce reperfusion injury, at least in clinical models [3, 11, 12].

Khandoudi et al. [54] reported that an antibody targeted to the third extracellular loop of NBCe1 provides substantial protection to rat hearts subjected to ischemia and reperfusion. Thus, it is possible that agents targeting NBCe1 and NBCe2 (or other Na^+-coupled HCO_3^- transporters in the heart) may prove clinically useful for treating reperfusion injury. A similar argument could be made for protecting against reperfusion injury to the brain in stroke. Here, however, the most likely targets would be the major Na^+-coupled HCO_3^- transporters in the brain: NDCBE and NCBE, as well as NBCe1-C and NBCn1.

Hypertension

An agent that reduces Na^+ reabsorption by the kidney will also reduce extracellular fluid volume and thus blood pressure. NBCe1-A is responsible for perhaps ~80% of HCO_3^- reabsorption by the kidney, and a not insignificant fraction of Na^+ reabsorption. Moderately inhibiting NBCe1-A in the renal proximal tubule might reduce Na^+ reabsorption enough to lower blood pressure, and yet not lower HCO_3^- reabsorption beyond the point where the H^+ pumps in the distal nephron could compensate and thereby maintain a stable arterial pH. Thus, NBCe1-A might be a target for treating high blood pressure.

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