Cation-Coupled Bicarbonate Transporters

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Abstract

Cation-coupled HCO_3^- transport was initially identified in the mid-1970s when pioneering studies showed that acid extrusion from cells is stimulated by CO_2/HCO_3^- and associated with Na^+ and Cl^- movement. The first Na^+-coupled bicarbonate transporter (NCBT) was expression-cloned in the late 1990s. There are currently five mammalian NCBTs in the SLC4-family: the electrogenic Na,HCO$_3$-cotransporters NBCe1 and NBCe2 (*SLC4A4* and *SLC4A5* gene products); the electroneutral Na,HCO$_3$-cotransporter NBCn1 (*SLC4A7* gene product); the Na^+-driven Cl,HCO$_3$-exchanger NDCBE (*SLC4A8* gene product); and NBCn2/NCBE (*SLC4A10* gene product), which has been characterized as an electroneutral Na,HCO$_3$-cotransporter or a Na^+-driven Cl,HCO$_3$-exchanger. Despite the similarity in amino acid sequence and predicted structure among the NCBTs of the SLC4-family, they exhibit distinct differences in ion dependency, transport function, pharmacological properties, and interactions with other proteins. In epithelia, NCBTs are involved in transcellular movement of acid-base equivalents and intracellular pH control. In nonepithelial tissues, NCBTs contribute to intracellular pH regulation; and hence, they are crucial for diverse tissue functions including neuronal discharge, sensory neuron development, performance of the heart, and vascular tone regulation. The function and expression levels of the NCBTs are generally sensitive to intracellular and systemic pH. Animal models have revealed pathophysiological roles of the transporters in disease states including metabolic acidosis, hypertension, visual defects, and epileptic seizures. Studies are being conducted to understand the physiological consequences of genetic polymorphisms in the *SLC4*-members, which are associated with cancer, hypertension, and drug addiction. Here, we describe the current knowledge regarding the function, structure, and regulation of the mammalian cation-coupled HCO_3^- transporters of the SLC4-family.

Introduction

CO_2 and HCO_3^- constitute the most important buffer system in the body with a number of unique features: CO_2 reacts with H_2O to form carbonic acid (H_2CO_3), which is a weak acid that partially dissociates into H^+ and the conjugate base HCO_3^-. The plasma concentration of H_2CO_3 is very low (~3 μmol/L), but any H_2CO_3 consumed is replenished from existing CO_2. The partial pressure of arterial CO_2 is normally maintained at 40 mmHg (equivalent to

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1.2 mmol/L CO_2) and is regulated by the respiratory system. Given that the respiratory system has a normal function, the CO_2/HCO_3^- buffer system is "open," and adding H^+ to the body or removing H^+ from the body will have minimal effects on the partial pressure of CO_2 but affect the concentration of HCO_3^- ([HCO_3^-]): adding H^+ reduces [HCO_3^-], whereas removing H^+ raises [HCO_3^-]. Thus, the mechanism of pH regulation in the body becomes the mechanism of maintaining [HCO_3^-] in the body.

The regulation of intracellular pH (pH_i) and extracellular pH (pH_o) is biologically very important since the structure and function of virtually all proteins are influenced by pH. A change in pH may for instance affect activities of enzymes and the structure and function of membrane proteins and signaling molecules, resulting in abnormal cell functions (268). Cells are equipped with several acid-base transport pathways that enable them to exert control of pH_i. These pathways include primary active transporters such as the H-ATPase (28) and the H,K-ATPase (258); secondary active transporters such as the Na,H-exchangers (39, 227, 329), proton-coupled carboxylate, nitrate, and oligopeptide transporters and the Na^+-dependent and -independent HCO_3 transporters (255, 295) and channels with conductance for H^+ or HCO_3^- (87, 303). This article focuses on Na^+-coupled bicarbonate transporters (NCBTs) that comprise electrogenic Na,HCO_3-cotransporters, electroneutral Na,HCO_3-cotransporters, and the Na^+-driven Cl,HCO_3-exchanger (Fig. 1). NCBTs move HCO_3^- across cell membranes and this movement is equivalent to the movement of H^+ in the opposite direction because of the ubiquitous carbonic anhydrase (CA) reaction. NCBTs belong to the *SLC4* gene products that also include Cl,HCO_3-exchangers (11, 77) and a distantly related Na,borate-transporter (233).

Since the first cloning of the Na,HCO_3-cotransporter NBCe1 (266), our understanding of the molecular and cellular physiology of the NCBTs in the body has been significantly advanced. *In vitro* and *in vivo* studies have demonstrated the physiological importance of the NCBTs and also the pathological implications of disturbed transporter function are gradually being appreciated. NCBTs are widely expressed in the body and serve many functions in addition to the housekeeping role of maintaining the intracellular H^+ concentration at a fairly constant level. In epithelial tissues, such as the kidney, intestine, pancreatic duct, and choroid plexus, NCBTs contribute to transcellular movement of HCO_3^- and thus to the secretion and excretion of acid or base. In nonepithelial tissues such as the brain, the heart, vascular smooth muscle, and the endothelium, NCBTs primarily regulate pH_i. This has distinct physiological consequences depending upon which tissue is affected. Knockout mice with targeted disruption of *slc4* genes exhibit abnormalities in pH-related physiology ultimately leading to defects in neuronal activity, sensory function, or the regulation of blood pressure or cerebrospinal fluid pressure and volume. In humans, genome-wide association studies have revealed a significant association between genetic polymorphisms in *SLC4* genes and susceptibility to pathological conditions or diseases such as hypertension, cancer, and drug addiction. Furthermore, mutations in NBCe1 have been identified in patients with renal tubular acidosis, ocular defects, and aberrant dentition. Studies have also provided information on biochemical and biophysical properties of the transporters. Each of the known NCBTs exhibits distinct properties of ion dependence, electrogenicity, stoichiometry, and interaction with other proteins although NCBT proteins have

considerable sequence homology (>30%) and possibly similarities in protein structures (40). The underlying molecular mechanism of HCO_3^- transport is likely at least partially conserved among the NCBTs.

This article provides an outline of our current understanding of the mammalian NCBTs. The first section describes studies of Na,HCO_3-transport physiology in the precloning era. The second section focuses on postcloning studies of NCBT proteins and describes current progresses in HCO_3-transporter physiology, ion transport, structure, and disease.

The Precloning Era

The first Na$^+$-coupled HCO_3^- described

The idea that cation-coupled HCO_3^- transport is important for regulation of pH_i was developed in the mid-1970s when Boron and Thomas first realized that the energetically uphill extrusion of acid, or influx of base, during recovery from an intracellular acid load in squid and snail neurons was stimulated by CO_2/HCO_3^- (41, 314). The pH_i-recovery was furthermore [HCO_3^-]-dependent (42, 315) and was inhibited by the anion transport inhibitors DIDS and SITS (275, 313). They next realized that during recovery from the acid load, intracellular Cl^- was required and the recovery of pH_i was associated with Cl^- efflux (275). It was further demonstrated that the intracellular Cl^- concentration ([Cl^-]$_i$) fell while the intracellular Na^+ concentration ([Na^+]$_i$) increased (315). The latter was consistent with the pH_i recovery from an acid load in snail neurons being completely blocked under conditions where extracellular Na^+ was removed (315). This observation was substantiated in squid giant axons (276) and further elaborated by the demonstration that HCO_3^--dependent pH_i recovery from intracellular acidosis in the barnacle muscle fiber was dependent on the extracellular [Na^+] with a K_m for Na^+ higher than 50 mmol/L (42). In this latter study, it was concluded, based on measurements of the kinetics of the transport, that likely transport modes were cotransport of Na^+ and CO_3^{2-} or Na^+ and 2 HCO_3^- in exchange for Cl^-, while the data seemed to exclude that the ion pair $NaCO_3^-$ was the transported species. This is interesting in relation to a later study in squid axons, where transport of the ion pair $NaCO_3^-$ could not be ruled out (37). As further discussed below, the exact mode of transport and the ionic species being translocated (Fig. 2) are still matters of debate.

In the 1980s, several groups reported the presence of Na^+-driven Cl/HCO_3-exchange in crayfish neurons (217, 218) and skeletal muscle (103), among others. It was also demonstrated that the Na^+-dependent HCO_3^- influx operated in parallel with the Na,H-exchanger to protect against intracellular acidification in leech neurons (282) and in a hamster lung fibroblast line (168) where the transport was shown to be important for DNA synthesis and cell growth. The Na^+-driven Cl/HCO_3-exchange was also found in several other cell lines derived from, for example, the kidney and tumors although not all cell lines exhibited the activity (172, 260). In some cells, for example, Vero cells, it seems that the transport was the dominant acid extrusion pathway in the near-physiological pH_i range (318, 319). It should be appreciated that these initial discoveries, which have proven very important for our understanding of mammalian physiology, were made in nonmammalian cells.

The second Na$^+$-coupled HCO$_3^-$ described

The demonstration—In 1983, Boron and Boulpaep (39) concluded based on a series of elegant measurements of pH$_i$, [Na$^+$]$_i$, [Cl$^-$]$_i$, and membrane potential in salamander proximal tubules that a basolateral, electrogenic Na,HCO$_3$-cotransporter was present and responsible for the reabsorption of filtered HCO$_3^-$ across the basolateral membrane of the proximal tubule. This transport amounts to 3.5 to 4 moles of HCO$_3^-$ in the human proximal tubule every day and is crucial for normal acid-base homeostasis in the body. Importantly, the transport was reported to be independent of Cl$^-$ and thus different from the Na$^+$-dependent Cl,HCO$_3$-exchange, which was already known (see above). In a figure from this important paper (39), the authors depicted how different substrates in different stoichiometries can provide a net transport of HCO$_3^-$ which is the result of the transporter activity (Fig. 2). The novel mechanism of HCO$_3$-cotransport was confirmed *in vivo* by Frömter's group through demonstration of an electrogenic HCO$_3^-$ transport over the basolateral membrane of rat proximal tubules (48, 352) and in 1985, it was demonstrated that the transport was coupled to Na$^+$ efflux (353). In the latter study, it was further argued based on thermodynamic arguments that the stoichiometry of the transport to result in net efflux of HCO$_3^-$ would have to be 1 Na$^+$ to 3 HCO$_3^-$. Using pH-sensitive fluorophores (13) and electrophysiological measurements (29), the presence of electrogenic Na,HCO$_3$-cotransport was also demonstrated in rat and rabbit proximal tubules. In a cell line derived from monkey kidney epithelium, the kinetics of the Na,HCO$_3$-cotransporter was investigated (146). Based on these findings, it was suggested that the apparent K_m for HCO$_3^-$ was about 10 mmol/L at 151 mmol/L Na$^+$, but 35 mmol/L at 20 mmol/L Na$^+$ while the apparent K_m for Na$^+$ was 19 and 28 mmol/L in the presence of 56 mmol/L and 17 mmol/L HCO$_3^-$, respectively. These findings indicate that the apparent K_m for Na$^+$ may be in the range of 20 to 25 mmol/L at physiological [HCO$_3^-$] (147). In vesicles from basolateral membranes of rabbit proximal tubules, the apparent K_m for Na$^+$ was only 10 mmol/L at 21 mmol/L HCO$_3^-$ (10).

Electrogenic Na,HCO$_3$-cotransport has subsequently been demonstrated in other tissues: in 1984, electrogenic Na,HCO$_3$-cotransport was reported in corneal epithelial cells (145, 148) and later in many other tissues including leech glia (89, 281). In the basolateral membranes of gastric parietal cells (78), hepatocytes (100), and pancreatic ducts (141, 323, 357), Na,HCO$_3$-cotransport was also reported, although the electrogenicity of the transport was not tested in all of these studies. Electrogenic Na,HCO$_3$-cotransport has also been reported in the heart: Vaughan-Jones and his colleagues using guinea pig hearts suggested that the transport might be electroneutral but could not rule out a 1 Na$^+$ to 2 HCO$_3^-$ stoichiometry (173, 185), while the electrogenic nature of the transport was more apparent in cat and rat hearts (9,53). Electrogenic Na,HCO$_3$-cotransport was also characterized in rat astrocytes (24, 27). The transport in astrocytes had a stoichiometry of 1 Na$^+$ to 2 HCO$_3^-$ and induced intracellular alkalinization in gliotic hippocampal slices. The Na,HCO$_3$-cotransport was stimulated by membrane depolarization, which occurs due to increased extracellular K$^+$ during neuronal firing (115, 116). This activity, called depolarization-induced alkalinization, helps compensate pH$_o$ changes caused by neuronal firing (269).

CO_3^{2-} or HCO_3^-?—Already in the first paper (39) describing the electrogenic Na,HCO_3-cotransport, Boron and Boulpaep addressed the possibility that the transported anion could be either CO_3^{2-} or HCO_3^- (Fig. 2 and Fig. 3). They also pointed out that, if CO_3^{2-} was the transported anion, it might be in the form of the ion pair $NaCO_3^-$. Clearly, CO_3^{2-} is present in much lower concentrations than HCO_3^- (although considerably higher than the concentration of free H^+, which is transported by the Na,H-exchangers). It is certainly possible that the affinity for CO_3^{2-} could be much higher than the affinity for HCO_3^-. This issue was further discussed by Jentsch and co-workers (150), who argued, based on modeling considerations, that the ion pair $NaCO_3^-$ was an attractive substrate for the electrogenic Na,HCO_3-cotransporter, although separate transport of Na^+ and CO_3^{2-} or of HCO_3^- could not be ruled out. Based on the ability of SO_3^{2-} to support the transport (although this was not confirmed in the study of Jentsch (147)) and the poor ability of Li^+ to support the transport, Soleimani and Aronson concluded that the transport likely involved 1 Na^+, 1 CO_3^{2-}, and 1 HCO_3^- (297). Frömter and co-workers (292) suggested a very elegant approach to the question which is illustrated in Figure 3. The basis for this approach is that no matter whether HCO_3^- or CO_3^{2-} is the transported ion pH near the membrane is expected to decrease when the transport is activated. However, if HCO_3^- is the transported species, then inhibition of the CA will reduce the activity induced acidification while if CO_3^{2-} is the transported species the acidification will be enhanced (see Fig. 3). It is, therefore, possible to distinguish between CO_3^{2-} and HCO_3^- transport via the transporter by measurements of pH near the membrane in the absence and presence of a CA inhibitor. Frömter and co-workers applied this idea to their previous data (292) and also exploited the idea in later papers (222). Based on these findings, they suggested that in situations with a stoichiometry of 1 Na^+ to 3 HCO_3^-, 1 CO_3^{2-} is transported with 1 HCO_3^-, while in situations with a stoichiometry of 1 Na^+ to 2 HCO_3^- it is likely that 2 HCO_3^- are transported (221).

The question of the kinetic model of the electrogenic Na,HCO_3-cotransporter was investigated in a detailed study by Gross and Hopfer (124). Based on a comparison of experimental data and a model for ordered binding of Na^+ and HCO_3^-, it was concluded that an ordered model is possible and that the major voltage dependence of the transporter is associated with the binding and translocation of HCO_3^-, whereas the binding of Na^+ is voltage independent.

Stoichiometry—To determine the stoichiometry of the electrogenic Na,HCO_3-cotransporter, a number of elegant experiments have been performed. In their original observation (39), Boron and Boulpaep depicted several possible modes of ion movement for the HCO_3-transporters (Fig. 2) and suggested a stoichiometry of 1 Na^+ to 2 HCO_3^-. As pointed out above, Frömter's group reported a stoichiometry of 1 Na^+ to 3 HCO_3^- in the rat kidney *in situ* (353) and also demonstrated that a 1 Na^+ to 3 HCO_3^- stoichiometry was necessary to mediate net efflux of HCO_3^- through that pathway (353). The same stoichiometry was found in vesicles from the basolateral membranes of rabbit proximal tubules (298). However, in the isolated rabbit proximal tubule, the stoichiometry of the Na,HCO_3-cotransporter was found to be 1 Na^+ to 2 HCO_3^- (291), which might suffice to cause net efflux of HCO_3^- (291) given the intracellular $[Na^+]$ and $[HCO_3^-]$ in this

preparation. The observation was provocative as the same group found that in the rat proximal tubules *in situ* the stoichiometry was 1 Na^+ to 3 HCO_3^- (353) as discussed above. It was concluded that this was not due to species differences but rather reflected that the stoichiometry of the Na,HCO_3-cotransporter could vary (291, 292). This statement was strongly supported by another observation from the same year (245) that isohydric hypercapnia of the bath solution in addition to changing the net direction of HCO_3^- transport from efflux to influx in isolated salamander proximal tubules also changed the stoichiometry of the Na,HCO_3-cotransport from 1:3 to 1:2. Frömter's group also demonstrated that "improved" incubation conditions (i.e., cell culture medium and norepinephrine instead of physiological salt solutions) led to a shift in stoichiometry from 1:2 to 1:3 in isolated rabbit proximal tubules (220) and an increased rate of HCO_3^- transport, which was approaching the *in vivo* values under these conditions (165). Further progress was made when the electrogenic Na,HCO_3-cotransporter NBCe1 was expressed in *Xenopus laevis* oocytes. Here, the stoichiometry was found to be 1 Na^+ to 2 HCO_3^- (129) and unaffected by the ATP concentration (129) and the temperature (95). However, it was demonstrated that the stoichiometry was changed from 1:2 to 1:3 when the Ca^{2+} concentration in the oocytes was increased from <100 to 500 nmol/L (221). This strongly suggests that intracellular Ca^{2+} can modify the transport function. It was also suggested by the authors that this stoichiometry change was most likely occurring via a Ca^{2+}-dependent protein kinase. In this respect, it may be of interest that an increased intracellular Ca^{2+} concentration via calmodulin inhibits the HCO_3^--dependent uptake of $^{22}Na^+$ into vesicles of basolateral membranes from rabbit proximal tubules (272).

The regulation by pH—The regulation of the electrogenic Na,HCO_3-cotransporter by pH, $[CO_2]$, and $[HCO_3^-]$ has been investigated by several investigators and turns out to be complex. Based on $_{22}Na^+$ fluxes in vesicles from the basolateral membrane of rabbit proximal tubules, it was suggested that pH_i through an allosteric effect modulates the activity of the electrogenic Na,HCO_3-cotransporter (299). This concept was further developed in a more physiological condition (125), and it was concluded that the binding constant for the substrates of the transporter decreased with increasing pH_i. An inherent problem in these experiments was that change in pH to a new steady state is associated with changes in $[CO_2]$ and/or $[HCO_3^-]$. To overcome this problem, Boron's group developed a technique by which the independent effects of H^+, HCO_3^-, and CO_2 can be assessed using out-of-equilibrium solutions (358). Using this technique on rabbit proximal tubules, it was somewhat surprisingly shown that an increase in basolateral $[HCO_3^-]$ inhibited the HCO_3^- transport and an increase of $[CO_2]$ accelerated the HCO_3^- transport (Fig. 4), while H^+ apparently did not affect transport activity (359, 363). In further studies, it was demonstrated that the signaling pathway for the stimulating effect of CO_2 on HCO_3^- transport in the proximal tubules involves the local renin angiotensin system acting on the angiotensin 1A receptor presumably in an autocrine manner (360, 362) as well as tyrosine kinases presumably in the ErbB family of receptor tyrosine kinases (361). These findings are consistent with a well-known stimulatory effect of angiotensin II on HCO_3^- reabsorption in the proximal tubules (106). But also nonreceptor tyrosine kinases of the src family and MAP kinases have been suggested to be important for the stimulatory response to both angiotensin II and hypercapnic acidosis (264, 273).

The third Na$^+$-coupled HCO$_3^-$ described

Na$^+$-coupled HCO$_3^-$ influx in smooth muscle cells was demonstrated in small arteries (1) where it was DIDS-sensitive, and in the guinea pig ureter (8) where it was DIDS-insensitive and associated with hyperpolarization. The presence of Na$^+$-coupled HCO$_3^-$ influx was also confirmed in a smooth muscle cell line (257). Here, it was suggested that, in addition to an electroneutral Na$^+$-coupled Cl,HCO$_3$-exchange activity, cells exposed to Cl$^-$ free conditions demonstrated Na$^+$-dependent HCO$_3^-$ uptake. The uptake rate was insensitive to presumed depolarization by increasing extracellular [K$^+$] and it was suggested that this could reflect an electroneutral Na,HCO$_3$-cotransport. As pointed out by Gross and Hopfer (124), the rate of an electrogenic transport is not necessarily strongly dependent on the membrane potential because the effect of the membrane potential depends on how the transport molecule operates, i.e. whether the rate limiting step of the translocation is voltage sensitive. It was, therefore, important to obtain measurements of the membrane potential during activation of the transport and to test the possible involvement of Cl$^-$. This was achieved in small arteries (2) where activation of the Na,HCO$_3$-cotransport had no effect on membrane potential and was not associated with any change in ^{36}Cl$^-$ efflux. Based on these findings, it was suggested that an electroneutral Na,HCO$_3$-cotransporter was present in smooth muscle cells. The presence of an electroneutral Na,HCO$_3$-cotransport was also strongly suggested from work in guinea pig heart muscle (174), although the authors did not exclude the possibility that the transport was in fact electrogenic. In smooth muscle, the electroneutral Na,HCO$_3$-cotransport was shown to be activated by norepinephrine and suggested (3) to be important for extrusion of the acid load associated with smooth muscle contraction, that is, a housekeeping role. In the heart the Na,HCO$_3$-cotransport activity was shown to be affected by epinephrine and ATP (174), although for the reasons outlined above it is difficult to know whether this reflects effects on the electroneutral Na,HCO$_3$-cotransporter. Remarkably, little work was done on electroneutral Na,HCO$_3$-cotransport until the transport molecule was cloned.

K$^+$-coupled bicarbonate transport

The K$^+$-coupled HCO$_3^-$-transport involves movement of K$^+$ and HCO$_3^-$ out of cells and thus mediates cellular acid loading. The transport was found in squid axons where it has been suggested to serve a major component of base efflux following intracellular alkali loads (131). Measurements of axonal pH$_i$ revealed that the K$^+$-dependent base efflux from the axons is unrelated to those observed with Li$^+$, Na$^+$, or Cs$^+$. This base efflux is not inhibited by DIDS or by inhibitors of the H,K-ATPase or the NHEs but by quaternary ammonium ions (86). The K$^+$-coupled HCO$_3^-$ transport also appears to exist in mammalian tissues. Electroneutral K,HCO$_3$-cotransport has been observed in the medullary thick ascending limb of the kidney (186). The cotransport is DIDS-sensitive and can be inhibited either by raising extracellular [K$^+$] or by depleting intracellular K$^+$. The mechanism may be mediated either by K$^+$-coupled HCO$_3^-$ transport or by K,Cl,HCO$_3$ cotransport (43). In rat hippocampal neurons, the pH$_i$ recovery from an alkaline load is neither Cl$^-$-dependent nor DIDS sensitive (Bevensee and Boron, personal communication). The protein responsible for K$^+$-coupled HCO$_3^-$ transport is presently not identified.

The Cloning Era

With the descriptions of the Na,HCO$_3$-transport first in non-mammalian cells and later mammalian cells it was obvious that the genes encoding the proteins mediating the transport should be cloned. Several unsuccessful attempts were done to clone the relevant genes. The first successful cloning was the cloning from the salamander kidney of the gene encoding the electrogenic Na,HCO$_3$-cotransporter NBCe1 in 1997 (266). Mammalian NBCe1 orthologs were then cloned by many researchers, followed by successful cloning and characterization of other mammalian NCBTs.

Also NCBT genes from invertebrates were cloned (243, 267, 325). The *Drosophila* gene encodes the transporter responsible for Na$^+$-driven Cl/HCO$_3$ exchange (267). The two genes cloned from squid giant axons encode an electrogenic Na,HCO$_3$-cotransporter (243) and a Na$^+$-driven Cl,HCO$_3$ exchanger (325). Apart from providing important information on base transport proteins in vertebrates, these papers also paid tribute to the invaluable information obtained from invertebrate model systems when sodium coupled bicarbonate transport was first described in the cloning era.

Currently, there are five mammalian NCBT genes which exhibit >50% amino acid sequence homology with one another. They are proposed to share a similar topology with 14 transmembrane segments (Fig. 5). One additional gene Slc4a9 codes for a related protein, which has been suggested to be an anion exchanger (159, 320, 341). Interestingly the Slc4a9 gene product has also been suggested to be an electroneutral Na,HCO$_3$-cotransporter (234). This suggestion has recently been supported by the finding that a sodium-dependent bicarbonate transport in the basolateral membrane of β-intercalated cells in mouse collecting ducts disappears in Slc4a9 knockout mice (56). Therefore, the *Slc4a9* gene product AE4 could have been discussed here but is probably equally well discussed together with the other anion-exchangers. The current article describes the cloning and functional characterization of the five well-characterized NCBTs. Human canonical proteins, to which all positional information and amino acid variation refer according to the UniProtKB/Swiss-Prot, are summarized in Table 1.

SLC4A4 (NBCe1)

NBCe1 was isolated by an expression cloning method using the salamander kidney cDNA library (266). NBCe1 contains 1,035 amino acids and has a high sequence homology with previously cloned Cl,HCO$_3$-exchangers (AE1-3). The predicted structure of NBCe1 contains a cytoplasmic N-terminal domain, a transmembrane (TM) domain with 12–14 membrane-spanning segments, and a cytoplasmic C-terminal domain. The mammalian NBCe1 orthologs with similar amino acid sequences (later named NBCe1-A) were subsequently isolated (50, 265), and their variants NBCe1-B and -C were identified (5, 70). NBCe1-A and -B differ in their N-terminal domains in which the first 41 amino acids in NBCe1-A are replaced with 85 alternative amino acids in NBCe1-B. Human NBCe1-B (1079 amino acids) is the canonical protein. NBCe1-C is identical to NBCe1-B except for the C-terminus in which the last 61 amino acids are replaced with 46 alternative amino acids (26). In addition, two additional N-terminal variations were reported by Liu et al. (197). The extracellular loop between TM5 and TM6 has two sites for glycosylation that produces negligible effect on

transport function in *Xenopus* oocytes (69). Similar to the AEs, NBCe1 forms a homodimer comprised of two individually functional subunits (109, 153).

NBCe1-A is predominantly expressed in the renal proximal tubules (283), where it is localized to the basolateral membrane of the tubule cells and mediates HCO_3^- efflux to the peritubular capillaries. In contrast, NBCe1-B is widely distributed in the body (5, 70). In general, NBCe1-B is localized to the basolateral membrane of epithelial cells and mediates cellular HCO_3^- uptake (5, 208, 312), but it is also expressed in the apical membrane of pancreatic ducts (270). NBCe1-B is also expressed in many nonepithelial cells such as cardiomyocytes (70, 155), skeletal muscle (162), corneal endothelia (194), oligodendrocytes (263), neurons, and astrocytes (207, 261). NBCe1-C is exclusively expressed in the nervous system, particularly in astrocytes (207).

SLC4A5 (NBCe2)

NBCe2 is an electrogenic Na,HCO_3-cotransporter first cloned and characterized by Pushkin et al. (253) and later by Virkki et al. (326) and the human canonical NBCe2 has 1137 amino acids with 55% amino acid sequence homology with NBCe1. Currently, eight different variants are reported in the human protein database due to alternative splicing events, but only NBCe2-C appears to be functionally capable of electrogenic Na,HCO_3-cotransport (326). NBCe2 is strongly expressed in the liver, where it is localized to hepatocytes and intrahepatic cholangiocytes in bile ducts (6). The hepatic expression of NBCe2 is consistent with the electrophysiological observation (99, 100) that HCO_3^--dependent acid extrusion in hepatocytes is governed by electrogenic Na,HCO_3-cotransport. Other cells that express NBCe2 include uroepithelial cells and renal collecting duct intercalated cells (6, 81), choroid plexus epithelial cells (46, 81). In skeletal muscle cells, NBCe2 expression has been described in the sarcolemma including the T-tubular membranes (162).

SLC4A7 (NBCn1)

Mammalian cDNAs encoding the electroneutral Na,HCO_3-cotransporter NBCn1 were first cloned from rat aorta (68) and human skeletal muscle (252). NBCn1 has 57% amino acid sequence homology with NBCe1, and its predicted structure is also similar to that of NBCe1. NBCn1 exists as multiple variants due to alternative promoter sites and cytoplasmic N- and C-terminal splicing events. The splicing events result in at least three splice cassettes, cassette I containing 13 amino acids, cassette II containing 123 amino acids (124 in humans), and cassette III containing 36 amino acids. Cassette II is predominantly found in smooth, cardiac, and skeletal muscle tissues (74) and likely reduces protein expression in membranes (349). The human canonical NBCn1 contains 1214 amino acids. NBCn1 is found widely in the body. In most epithelial tissues, the transporter is localized to the basolateral membranes of the cells (113, 230, 248, 249, 327). The transporter is also found in the apical membranes of some epithelial cells such as in pancreatic ducts (205), intercalated cells (256), and salivary glands (113).

NBCn1 moves Na^+ and HCO_3^- into the cells by operating with a stoichiometry of 1 Na^+ to 1 HCO_3^-. Thus, the Na,HCO_3-cotransport by NBCn1 occurs with no direct changes in the membrane potential. In most cell types— although not in all (33)—NBCn1 is only weakly

inhibited by DIDS (68), a hallmark that distinguishes NBCn1 from other bicarbonate transporters of the SLC4-family. In addition, NBCn1 has Na^+-channel-like activity that is uncoupled from the Na,HCO_3-cotransport activity and stimulated by DIDS (68). NBCn1 is widely distributed in many epithelial and nonepithelial cells (34), and plays a role in pH_i regulation and probably transcellular HCO_3^- movement.

SLC4A8 (NDCBE)

NDCBE is a Na^+-driven Cl,HCO_3-exchanger that moves external Na^+ and HCO_3^- into cells in exchange for internal Cl^- which was first cloned from Drosophila by Romero et al. (267) and from human brain by Grichtchenko et al. (117) and Virkki et al. (235). NDCBE is different from the Cl/HCO_3-exchangers (AEs; SLC4A1-3), which move external Cl^- into the cells in exchange for internal HCO_3^-. Thus, NDCBE serves as an acid extruder, whereas the AEs serve as acid loaders. The human canonical NDCBE contains 1093 amino acids and has 50% amino acid sequence homology with NBCe1 and 70% with NBCn1. Similar to other SLC4 transporters, NDCBE exists as multiple variants due to alternative promoter sites and splicing events.

NDCBE transcripts are strongly expressed in the brain and testes, and weakly in the kidney and ovaries (81, 117). In the brain, the NDCBE protein is localized to cell body membranes, cytosol, and processes of neurons (60, 179), thereby supporting the previous observations that the Na^+-driven Cl,HCO_3 exchanger primarily governs HCO_3^--dependent acid extrusion in neurons (22, 287). NDCBE is found in presynaptic nerve terminals, at the electron microscopic level (296), mostly in glutamatergic terminals and some GABAergic terminals (49). The exchanger likely regulates presynaptic pH that affects neurotransmission. Mice with a targeted disruption of *slc4a8* display abolished thiazide-sensitive and amiloride-insensitive Na^+-reabsorption in the cortical collecting ducts of the kidney (187).

SLC4A10 (NBCn2/NCBE)

NBCn2/NCBE was originally cloned from a mouse insulinoma cell line (330). The human canonical NBCn2/NCBE contains 1118 amino acids and its amino acid sequence is 71% and 65% identical to NDCBE and NBCn1, respectively. The human clone exists as two variants depending upon the presence of an N-terminal cassette of 30 amino acids according to the Uniprot database. The rat orthologs vary at the C-terminal end, lacking the last 21 amino acids (107). NBCn2/NCBE is found in the brain with strong expression in neurons particularly in postsynaptic membranes (143), and choroid plexus epithelia (59, 81, 107, 135). The transporter is also found in other tissues such as the stomach and duodenum (81).

NBCn2/NCBE was first reported as a Na^+-dependent Cl,HCO_3-exchanger with a somewhat unusual Cl^- dependency (330). In *Xenopus* oocytes expressing NBCn2/NCBE, the inhibition of $^{36}Cl^-$ efflux by DIDS is far less substantial than inhibition by HCO_3^- removal. Furthermore, Na^+ influx increases linearly with higher external $[Cl^-]$, which would otherwise decrease if the transporter mediated Na^+-driven Cl/HCO_3 exchange. Later, Parker et al. (236) observed that the abnormal Cl^- flux by NBCn2/NCBE is due to Cl^- self-exchange uncoupled from the transport of Na^+ and HCO_3^-. On the other hand, Damkier et

al. (79) observed ^{36}Cl efflux that is DIDS-sensitive and dependent on Na^+ and HCO_3^-. The issue of NBCn2 versus NCBE will be described in detail in the next section.

The Postcloning Era

Following the cloning of the five NCBTs, research has been focused on understanding the cellular, physiological, and pathological roles of NCBT-mediated pH regulation in the body. The results demonstrate that NCBTs play essential roles in HCO_3^- movement across cell membranes to regulate and maintain acid-base homeostasis in many tissues. The physiological importance of NCBTs has been further supported by studies of knockout mice which exhibit abnormalities in distinct physiological processes in tissue-specific manners (Table 2). Studies have also advanced our understanding of biochemical and biophysical properties of the NCBT proteins. The mechanisms for ion translocation, stoichiometry/ electrogenicity, and interactions with other proteins were studied by expressing transporters in heterologous expression systems. This section describes postcloning studies of NCBT physiology, ion transport, structure, and related disease.

SLC4A4 (NBCe1)

Physiology of NBCe1

<u>(i) Renal acid-base regulation:</u> A major task of the kidney is to maintain whole body acid-base homeostasis by reclaiming filtered HCO_3^- and producing new HCO_3^- to neutralize acidic by-products of metabolism (39). The proximal tubule plays a central role in these processes, reabsorbing >80% of filtered HCO_3^-. NBCe1-A is electrogenic (Fig. 6A) have a stoichiometry of 1 Na^+ to 3 HCO_3^- in the basolateral membrane of the proximal tubule (Fig. 7A) and play a major role in reclaiming HCO_3^- (354). Consistent with this functional knockout of NBCe1 (199) leads to reduced uptake of HCO_3^- in the proximal tubules (Fig. 7B) and these mice are growth retarded (Fig. 7C) and die early (Fig. 7D). These phenotypes can be partly rescued by feeding the mice HCO_3^- (Fig. 7C and 7D), which might be expected for a HCO_3^- loosing condition. NBCe1-A is mostly restricted to the S1 and S2 segments of the proximal tubule (209). The kidney makes adaptive changes in HCO_3^- reabsorption and NH_4^+ excretion in response to acid-base disturbances (10, 14, 164, 250), and such adaptive changes involve downregulation or upregulation of NBCe1 in the proximal tubule. NBCe1 in rat proximal tubules is downregulated by metabolic alkalosis (16, 47). This downregulation is expected to blunt HCO_3^- reabsorption and help prevent metabolic alkalosis. In contrast, the effect of metabolic acidosis on NBCe1 appears to vary depending upon experimental procedures. NBCe1 is upregulated by lithium-treated metabolic acidosis (157) and bilateral ureteral obstruction-induced metabolic acidosis (332), consistent with the previous reports that HCO_3^- absorptive capacity in the proximal tubules increases with chronic metabolic acidosis (10,250,279) or respiratory acidosis (73,160). However, Kwon et al. (166) and Brandes et al. (47) observed negligible changes in NBCe1 expression during chronic metabolic acidosis. Wang et al. (333) found that the NBCe1 response to acidosis is time/age-dependent in neonatal unilateral ureter obstruction.

In addition to systemic alkalosis/acidosis, plasma Na^+ affects NBCe1 expression in the kidney. NBCe1 is downregulated in proximal tubules of rats exposed to 280 mmol/L NaCl

in the drinking water for 5 days (16). Proximal tubule NBC activity is possibly altered in hypertension: NBCe1 is more abundant in the renal cortex of spontaneously hypertensive rats (301) compared to control rats; however, NBCe1 expression and function was lower in a proximal tubule cell line from spontaneously hypertensive rats than in control cell lines (239). Finally, NBCe1 is recognized to be one of the Na^+ transporters that are upregulated by chronic exposure to the sympathetic neurotransmitter norepinephrine promoting Na^+ and water retention (300).

(ii) HCO_3^- secretion in exocrine cells: Fluid and HCO_3^- secretion are vital functions of secretory glands, particularly pancreas and salivary glands. The molecular mechanisms of fluid and HCO_3^- secretion by these exocrine cells have recently been described by Lee et al. (180). In the pancreatic ducts, NBCe1-B is localized to the basolateral membrane, mediates transport with a stoichiometry of 1 Na^+ to 2 HCO_3^-, and mediates HCO_3^- influx (208). Intracellular HCO_3^- becomes a source for luminal HCO_3^- secretion mediated by the SLC26 transporters and CFTR (219). In addition to this basolateral HCO_3^- influx mediated by NBCe1, HCO_3^- is also produced from hydration of CO_2 catalyzed by CA II. A basolateral Na/H-exchanger (NHE1) contributes to the accumulation of intracellular HCO_3^- by extruding the proton produced by hydration of CO_2. Recent studies suggest that basolateral NBCe1 and luminal CFTR are coordinately regulated (347,348). NBCe1 and CFTR interact with IRBIT (IP_3 receptor binding protein released with IP_3), a signaling protein that is released from IP_3 receptors and regulates downstream target molecules (294). The interaction stimulates fluid and HCO_3^- secretion, which can result in a luminal fluid containing up to 150 mmol/L $NaHCO_3$.

NBCe1 is also important for the exocrine function of the salivary glands. Secretion of HCO_3^- to the saliva is essential for oral function and health, as HCO_3^- in saliva buffers acids produced by oral bacteria. HCO_3^--dependent Na^+ influx has been functionally detected in mammalian parotid acinar cells (246, 304). Immunohistochemistry of rat and human parotid glands shows an abundant expression of NBCe1 in the basolateral membranes of acinar and ducts cells (231, 271) and submandibular duct glands (271). By patch clamp recordings of acutely dissociated bovine parotid acinar cells, Yamaguchi et al. (343) have identified a Na^+-dependent and DIDS-sensitive HCO_3^- current that has an apparent coupling ratio of 1 Na^+ and 2 HCO_3^-. RT-PCR analyses recognizes NBCe1-B, but not NBCe1-A, in these cells. It has also been shown that HCO_3^- secretion involves NBCe1 endocytosis and is regulated by acetylcholine, which is responsible for production of fluid and electrolyte secretion in the salivary glands (343). Perry et al. (242) observed in parotid ParC5 cells that NBCe1 is internalized from the basolateral membranes by carbachol and phorbol-12-myristate-13-acetate (PMA) to appear in early endosomes. The internalization of NBCe1 is prevented by PKC inhibitors (240).

(iii) HCO_3^- secretion in the intestine: In the intestine, NBCe1 is abundantly localized to the basolateral membranes of colonic crypts, thereby contributing to intestinal anion secretion (290). cAMP stimulates NBCe1-mediated HCO_3^- transport in crypts (19). Forskolin and carbachol stimulate NBCe1 membrane expression by vesicle trafficking and exocytosis (355). NBCe1 knockout mice exhibit a significant reduction in cAMP-mediated

HCO_3^- secretion in the proximal colon (105). NBCe1 is also expressed in the duodenum, although the role here is unclear (64).

(iv) pH regulation during amelogenesis: NBCe1 is essential for the development of normal enamel. The physiological importance of NBCe1 for enamel development was revealed based on patients with NBCe1 mutations, who have abnormal dentition (91, 140). In addition, the enamel of NBCe1 knockout mice was structurally defective and low in mineral content (170). The incisors of these mice are chalky-white and prone to enamel fracture. Immunohistochemistry shows NBCe1 being localized to the basolateral membrane of ameloblasts and RT-PCR analyses detects NBCe1-B transcripts in ameloblast-like LS8 cells (229). Transcellular HCO_3^- secretion probably regulates extracellular pH to support proton buffering during the growth of hydroxyapatite crystals (169). Many proteins capable of HCO_3^- transport, including NBCe1, are upregulated at the onset of the maturation stage of amelogenesis (171).

(v) Intracellular pH regulation in astrocytes and neurons: Astrocytes represent the most studied nonepithelial cells where NBCe1 contributes to pH regulation (67). NBCe1-B and -C expression has been detected (26,207,261), and electrogenic Na,HCO_3-cotransport has been functionally characterized (24) in astrocytes. The cotransport has a stoichiometry of 1 Na^+ to 2 HCO_3^- and induces alkalinization in gliotic hippocampal slices. Electrogenic Na,HCO_3-cotransport activity is stimulated by membrane depolarization that occurs due to increased extracellular K^+ during neuronal firing (116) and the cotransport activity helps compensate extracellular pH changes under these conditions (269). Interestingly, NBCe1-A is found in some neurons, where electroneutral NCBTs have been known to govern HCO_3^--dependent acid extrusion. The expression of NBCe1-A is supported by recent reports of NBCe1 activity in cultured mammalian neurons (206, 310). Assessed by *in situ* hybridization (108) and immunoblotting (93), NBCe1 mRNA and protein expression begin at the time of birth and persist throughout adulthood.

Early studies have investigated potential pathological implications of disturbed NBCe1 function. Giffard et al. (108) tested whether NBCe1 is associated with vulnerability to acidic injury in ischemia and found that blocking the transporter with DIDS inhibits acidic injury in primary cultures of astrocytes. Jung et al. (149) observed a significant upregulation of Na^+ transporters including NBCe1 and down-regulation of the Na,K-ATPase in ischemic penumbra in rats with permanent middle cerebral artery occlusion. The authors proposed that NBCe1 upregulation contributes to cell damage through intracellular Na^+ accumulation that leads to cell swelling and Ca^{2+} overload. NBCe1 is also upregulated in the left ventricle following myocardial infarction (278). This regulation seemed to be dependent on the function of the angiotensin system. Taken together, the increased expression or activity of NBCe1 is likely deleterious to cells. Douglas et al. (94) examined the effect of chronic intermittent hypoxia on NBCe1 protein expression and found downregulation of the transporter. The level of downregulation varies between NBCe1 variants and in different brain regions. Consistent with the downregulation of NBCe1, acute hypoxia inhibits electrogenic Na,HCO_3-cotransport activity in hippocampal astrocytes (25). In addition, recent studies show that NBCe1 activity enhances glutamine efflux via the glutamine

transporter SLC38A3 (335) and fast glycolysis in response to excitatory synaptic transmission (274).

SLC4A4-related diseases in humans—Missense and nonsense mutations in human *SLC4A4* have been identified in patients with proximal renal tubular acidosis (pRTA), visual and hearing defects, and abnormal dentition (90, 91, 133, 137–140, 199, 293, 307, 308, 322). These phenotypes are recapitulated in knockout mice with targeted disruption of the *slc4a4* gene (105,170), or knockin mice with a point mutation (199), although both knockout and knockin mice exhibit additional abnormalities. Studies by many different researchers show that some of these mutations result in loss of NBCe1 activity due to impaired membrane trafficking. For example, by cysteine mutagenesis of these pRTA-associated residues, Zhu et al. (366) found that most of the mutants are not accessible to the methylsulfonate reagents. These pRTA-associated residues are buried in the membrane field and are important for the protein structure. Surface biotinylation or immunoblot experiments by other researchers show comparable impairment of protein expression in membranes (90, 91, 133, 137–140, 199, 293, 307, 308, 322). Some pRTA-associated mutations result in a partial loss of function. For example, G486R and T485S mutants have reduced Na,HCO$_3$-cotransport activity despite normal membrane expression in heterologous expression systems (307). Other mutations result in gain of a new function: A799V gains an unusual HCO$_3^-$-independent conductance that might be associated with hypokalemic paralysis (237).

Signaling molecules affecting NBCe1 activity—Little is known about the effect of signaling molecules on NBCe1 activity. It has been suggested that phosphorylation activated by cAMP or cytosolic [Ca^{2+}] change the stoichiometry of the transporter in heterologous expression systems. In addition, ATP can stimulate NBCe1 activity (129). On the other hand, NBCe1 is negatively affected by protein kinase C. Perry et al. (241) observed that PKC activated by phorbol-12-myristate-13-acetate (PMA) inhibits NBCe1 in *Xenopus* oocytes. Angiotensin II affects NBCe1 endocytosis in a biphasic manner: at 10^{-11} to 10^{-10} M, angiotensin II stimulates NBCe1 currents; but at 10^{-6} M, angiotensin II reduces NBCe1 currents and surface expression (241). This biphasic effect is similar to that in the proximal tubules. Wu et al. (340) reported that NBCe1 is stimulated by phosphatidylinositol 4,5-bisphosphate (PIP$_2$). DIDS-sensitive NBCe1 currents exhibit run-down in oocytes; and by patch-clamp recordings, the authors found that cytosolic PIP$_2$ reduces the rate of rundown and stimulates NBCe1 current. PIP$_2$ is known to bind to a stretch of positively charged amino acids in membrane-associated proteins or signaling molecules near the membrane and thereby regulate their activity (130). It is thus proposed that PIP$_2$ interacts with NBCe1 and stimulates the Na,HCO$_3$-cotransport activity. Hong et al. (132) recently reported that residues 37 to 65 in NBCe1-B are required for the interaction with and regulation by IRBIT as well as PIP2. Phosphorylation of Ser^{65} mediates NBCe1-B regulation by SPAK, while phosphorylation of Thr^{49} is required for regulation by IRBIT and SPAK.

Mechanism of ion transport

(i) Ion dependence: In *Xenopus* oocytes, NBCe1 produces currents in response to Na$^+$ and HCO$_3^-$ (Fig. 6A) (118,210,288). The currents are outwardly directed at the resting

membrane potential and get progressively larger at more positive voltages. The current-voltage relationship exhibits a positive and linear slope with a reversal potential (E_{rev}) ranging between -120 and -140 mV. The NBCe1 current is dependent on Na^+ and the slope of the I/V-curve becomes progressively larger at higher $[Na^+]$. The apparent affinity K_m for Na^+ is 19 to 36 mmol/L for three NBCe1 variants (288). NBCe1 does not produce currents to K^+, $NMDG^+$, or choline, although it appears to generate a very small current to Li^+. The strict dependence on Na^+ is confirmed by pH_i experiments in which pH_i recovery from intracellular acidification requires external Na^+ (17, 210).

The current-voltage relationship for NBCe1 has a positive, almost linear slope and displays a strong dependence on HCO_3^- (118, 210, 288). Determined by peak currents at -60 mV under voltage-clamp conditions, Grichtchenko et al. (118) proposed a kinetic model for a single Michaelis-Menten process (with an apparent K_m for HCO_3^- of 6.5 mmol/L) and a linear component. NBCe1 does not produce current in response to other anions such as Cl^-, Br^-, I^-, or NO_3^- (350). The strict HCO_3^- dependence of NBCe1 is distinct from AEs that can move several monovalent and divalent anions with low affinity in either direction or via anion self exchange (158).

(ii) HCO_3^- versus CO_3^{2-} transport: Since equilibrated solutions containing HCO_3^- will also contain CO_3^{2-}, it has been a challenging question to determine which ion is in fact being transported. To address this question, Boron and his colleagues (114, 184) co-expressed NBCe1 and GPI-linked CA IV in *Xenopus* oocytes and measured surface pH (pH_s) while voltage-clamping oocytes. The idea is that if NBCe1 transports HCO_3^-, CA IV would replenish the lost HCO_3^- at the oocyte surface and then pH_s would decrease. Inhibiting CA IV would consequently blunt the decrease in pH_s. However, if NBCe1 transports CO_3^{2-}, then a large increase in $[H^+]$ would occur because HCO_3^- is deprotonated to produce CO_3^{2-}. CA IV would then consume H^+ by catalyzing the generation of OH^- by conversion of HCO_3^- to $CO_2 + OH^-$ as discussed above. In this case, inhibiting CA IV would enhance the pH_s decrease (Fig. 3). Boron and his colleagues found that stimulating NBCe1 (by positively shifting the holding potential) decreased pH_s, whereas slowing NBCe1 (by negatively shifting the holding potential) increased pH_s. The CA inhibitor acetazolamide augmented all pH_S changes. Thus, NBCe1 appears to transport CO_3^{2-}, not HCO_3^-. These results are comparable with the observation by Grichtchenko and Chesler (115) that the CA inhibitor benzolamide augments a pH_o decrease mediated by NBCe1 in hippocampal glial cultures. It is unclear whether HCO_3^- and CO_3^{2-} can be transported simultaneously (see discussion above). In this case, NBCe1 in the renal proximal tubules would have a stoichiometry of 1 Na^+ to 1 CO_3^{2-} and 1 HCO_3^- (221,292,297).

(iii) Ion translocation: The NBCe1 amino acid sequence has facilitated structure-function studies to determine the molecular mechanism of ion transport and the structural requirements for function. Abduladze et al. (4) performed site-directed mutagenesis of charged and polar residues and identified many mutations affecting transport function. They proposed that charged and polar residues serve as ion binding sites or interact with other charged residues constituting structural components of the transporter. Other researchers focused on amino acid residues in single TMs or cytoplasmic N-and C-terminal domains.

Dinour et al. (91) investigated the function of S427L (located in TM1), which is one of the human mutations exhibiting visual defects and renal tubular acidosis. The mutation was found to cause the protein to lose voltage-sensing capacity or a Na^+ binding site and additionally reduced membrane expression of the protein. The reversal potential for the S427L-mediated current was extremely negative (< -160 mV) indicating no HCO_3^- efflux under normal conditions in native cells. Li et al. (192) report that S427L is incorrectly targeted to the apical membrane instead of the basolateral membrane. Using cysteine mutagenesis of all amino acids in TM1(Gln^{424}–Gly^{448}), Zhu et al. (364) found that Ala^{428}, Ala^{435}, and Thr^{442} affect cotransport function. The access of these mutants to methylsulfonate reagents significantly inhibits pH_i recovery, indicating that these residues are located on the same face of the TM 1 α-helix lining the pore.

Based on sequence comparisons among bicarbonate transporters, Yang et al. (350) identified Asp^{555} as a residue responsible for anion selectivity. Mutation of this residue to a Glu (i.e., D555E) causes NBCe1 to be permissive to other anions. All electroneutral Na,HCO_3-transporters possess a Glu in this position, suggesting that the anion selectivity would be associated with electrogenicity although the mechanism of how the two properties are related is unclear. Yamazaki et al. (346) found that the single nucleotide polymorphism K558R results in reduced transport activity without changing protein trafficking or the apparent K_m for Na^+. McAlear and Bevensee (210) performed cysteine-scanning mutagenesis of amino acid residues in TM8 (Ala^{739}-Thr^{758}) and found Leu^{750} as a residue involved in ion translocation. The L750C mutant mediates HCO_3^- currents that are strongly inhibited by sulfhydryl reagents. Chen and Boron (63) proposed TM6 and TM12 as part of a functional unit.

In addition to the TM domains, the cytoplasmic N-terminal domain is proposed to contribute to ion translocation. Chang et al. (58) demonstrated that the low Na,HCO_3-cotransport activity of the human mutant R298S can be rescued by substituting Glu^{91} with an Arg. The authors propose that an electrostatic interaction or hydrogen bond between Arg^{298} and Glu^{91} forms a permeation tunnel, through which HCO_3^- is translocated. Whether Arg^{298} lines the pore remains unknown.

Taken together, several residues in TMs and the cytoplasmic N-terminus have been identified to be critical for ion translocation (Table 3). Not all of these residues are charged or polar, and they may be lining the pore or serve as structural residues for protein conformation.

Electrogenicity and stoichiometry—To understand the molecular basis of electrogenicity, Choi et al. (71) performed experiments in which a series of chimeric transporters from NBCe1 and NBCn1 were constructed and their electrogenicity tested. They found that the transmembrane domain of NBCe1 is essential for electrogenicity, while the cytoplasmic N- and C-terminal domains and the large third extracellular loop (EL3; located between TM5 and TM6) have negligible effect on transport function despite the fact that these regions are unique to NBCe1. Furthermore, electrogenicity of NBCe1 requires an interaction between the two regions of TM1-5 and TM6-14. The interaction might be essential for Na^+ and HCO_3^- binding or a part of the ion-binding vestibule. The interaction

might not be limited to TMs, but could involve the loops between TMs. Chen et al. (62) identified the loop EL4 between TM7 and TM8 as the region critical for electrogenicity. EL4, which contains 32 amino acids, may coordinate with residues in TM1-5 to produce Na,HCO_3-current.

The issue of Na^+ to HCO_3^- stoichiometry is related to the electrogenicity of NBCe1. In *Xenopus* oocytes, the stoichiometry of NBCe1 was found to be 1 Na^+ to 2 HCO_3^- (129) and unaffected by the ATP concentration (129) and the temperature (95). However, when the $[Ca^{2+}]$ in the oocytes was increased from 100 nmol/L or less to 500 nmol/L the 1 Na^+ to 2 HCO_3^- stoichiometry was shifted to a 1 Na^+ to $3HCO_3^-$ stoichiometry (221). It was suggested by the authors that this was most likely occurring via a Ca^{2+}-dependent protein kinase. In this respect, it may be of interest that an increase of $[Ca^{2+}]$ via calmodulin was shown to inhibit the HCO_3^--dependent uptake of $^{22}Na^+$ into vesicles of basolateral membranes from rabbit proximal tubules (272).

Also, Gross and colleagues have addressed the question of stoichiometry. In immortalized rat proximal tubule cells grown in a monolayer and placed in Ussing chambers, the apical membrane was permeabilized with amphotericin B. The transport through the basolateral Na,HCO_3-cotransporter was assessed based on the reversal potential and conductance of the Na^+- and HCO_3^--dependent, dinitro-stilbene disulfonate sensitive current and the stoichiometry was found to be 1 Na^+ to 3 HCO_3^- (123). After cloning of the NCBTs, Gross and colleagues expressed the pancreatic variant of NBCe1 in a mouse pancreatic cell line and found the stoichiometry to be 1 Na^+ to 2 HCO_3^- under those conditions (120). These differences in stoichiometry are consistent with the transporter mediating influx of HCO_3^- over the basolateral membrane of pancreatic ducts but efflux of HCO_3^- over the basolateral membrane of the proximal tubule. The same year, Gross and colleagues expressed both the pancreatic and the renal variant of NBCe1 in a mouse proximal tubule cell line as well as in a mouse collecting duct cell line (121). Both NBCe1 variants showed a 1 Na^+ to 3 HCO_3^- stoichiometry in the proximal tubule cell line but a 1 Na^+ to 2 Na^+ stoichiometry in the collecting duct cell line. These findings provide strong further arguments that the same transport molecule can mediate transport with different stoichiometries depending on the cellular environment. To provide insight into which signals in the cells could be important for this shift in stoichiometry, the role of a cAMP-dependent phosphorylation of the transporter was investigated (122). Addition of a membrane permeable variant of cAMP (8-Br-cAMP) resulted in a shift from a 1 Na^+ to 3 HCO_3^- stoichiometry to a 1 Na^+ to 2 HCO_3^- stoichiometry and was associated with phosphorylation of NBCe1. The phosphorylation and shift in stoichiometry was abolished when Ser^{982} in the C-terminus was mutated to an alanine. It was further demonstrated that the catalytic subunit of PKA was able to phosphorylate the protein. These findings are consistent with cAMP-dependent signaling modifying the stoichiometry and could explain how cAMP reduces the reabsorption of HCO_3^- in the proximal tubules through a change in transport stoichiometry.

Pharmacological inhibition—A number of pharmacological agents have been shown to inhibit NBCe1 with reasonable potency (31) but development of more specific compounds applicable under both *in vitro* and *in vivo* experimental conditions would be a substantial improvement.

The stilbene derivatives such as DIDS and SITS are potent inhibitors of Cl,HCO_3-exchangers (12, 51) and also of NBCe1. Studies of erythrocyte AE1 reveal that the DIDS inhibition occurs in two steps (52): a rapid ionic interaction, which can be reversed by scavenging DIDS with albumin, and a slower covalent reaction. In either case, DIDS blocks transport. In AE1, the covalent binding site is Lys[539] in TM5 as determined by site-directed mutagenesis (21) and biochemical analysis (225). At high pH, H_2DIDS also binds to Lys[851]. The DIDS binding motif in AEs was recognized as KLXK ($X = I$ and Y), where the first K binds to DIDS.

Based on the fact that NBCe1 has the similar DIDS motif (KMIK) in TM5, Lu et al. (203) performed mutagenesis of the KMIK motif and found that Lys[559] (first K) produces the most substantial effect on DIDS sensitivity. The inhibitory constant for DIDS increases in the order of Asp > Glu > Gln > Lys at this position, indicating that a charge environment near the site is important for DIDS binding. DIDS is known to block anion channels/transporters by binding to the pore entry of the protein and occluding the pore (317). Thus, Lys[559] is expected to be located near the pore entry. The NBCe1 mutated at Lys[559] has similar HCO_3^- conductance compared to NBCe1 mutated at other residues in the KMIK motif (203). Thus, it is unclear whether Lys[559] serves as the site for HCO_3^- binding. The inhibition constant K_i of NBCe1 for DIDS is 36 μmol/L (196), which is higher than those of the Cl,HCO_3-exchangers AE1-3.

In addition to DIDS and SITS, there are other blockers that have inhibitory effects on NBCe1, and probably other NCBTs. Fluorescent oxonol dyes such as diBA(3)C4 and diBA(5)C4 inhibit NBCe1 (196) and are more potent than DIDS, although DIDS and oxonol dyes partially share the same binding site and compete with each other. Other anion transport blockers, including niflumic acid, benzamil, and tenidap, also inhibit NBCe1 (95,203). The inhibition by a broad spectrum of anion blockers implies that the pore of NBCe1 may contain a relatively large inner vestibule.

The N-cyanosulphonamide compound S0859 has been described as a more specific and reversible inhibitor of Na,HCO_3-cotransport (57, 286, 345). The K_i for S0859 is 1.7 μM in cultured rat ventricular myocytes (57). At 30 μmol/L, which gives full inhibition of Na,HCO_3-cotransport, S0859 does not affect Cl^-/HCO_3^--, Cl^-/OH^--, or Na^+/H^+-exchange activity (57). Thus, its effect is specific to NCBTs, at least in ventricular cardiomyocytes. More recent studies also show that S0859 inhibits NBCn1 activity in the human breast cancer cell line MCF-7 (175) whereas surprisingly S0859 had no effect on Na,HCO_3-cotransport activity in isolated arteries or slices of murine breast tumors (175). NBCn1 is responsible for Na,HCO_3-cotransport activity in both of these tissues and differences in transporter isoform, therefore, does not appear to explain the difference. Instead, the authors suggested that a very strong binding to extracellular proteins (studied for plasma samples) may explain the effect of S0859 in isolated cells but not in tissues (175). Therefore, despite S0859 being a useful generic Na,HCO_3-cotransport inhibitor and a definite improvement compared to the stilbene derivatives, it does not appear to distinguish between different NCBT isoforms and delivery of the compound in tissues and *in vivo* may be an issue.

Also functionally active antibodies have been used to modify NBC activity. An antibody was raised against NBCe1 (155) and shown to inhibit 50% to 66% of the recovery from acidosis in rat cardiomyocytes and HEK cells transfected with NBCe1. Interestingly, the antibody reduced the adverse effects of ischemia on performance of the isolated perfused rat heart in that study (155). Also De Giusti et al. (88) raised antibodies against NBCe1 and reported on two antibodies with concentration dependent activating and inhibitory effects, respectively on Na,HCO$_3$-cotransport activity in isolated cat ventricular myocytes. It would be of substantial interest if this area was further developed.

Autostimulatory versus autoinhibitory domains—In *Xenopus* oocytes, all three variants NBCe1-A, -B, -C move 1 Na$^+$ and at least 2 HCO$_3^-$ into the cells, thus inducing HCO$_3^-$-dependent acid extrusion and electrogenic currents. Nonetheless, the different N- and C-terminal amino acids in the variants contribute to modulating cotransport activity, making them functionally distinct. McAlear and Bevensee (211) found that deleting the N-terminal 41 amino acids uniquely present in NBCe1-A decreases the current, whereas deleting the N-terminal 85 amino acids in NBCe1-B/C increases the current. The authors proposed that the N-terminal region in NBCe1-A serves as an autostimulatory domain, while the N-terminal region in NBCe1-B/C serves as an autoinhibitory domain. The autoinhibitory domain appears to be found in other NCBTs, according to the sequence comparison among the transporters (40). IRBIT binds to the N-terminal 85 (294) amino acids of NBCe1-B, and increases HCO$_3^-$ transport activity (348). Deletion of residues 2 to 16 in the N-terminus abolishes the IRBIT-induced increase in transport activity without disrupting autoinhibition (183). These findings indicate that the structural determinant for autoinhibition is different from that for IRBIT binding.

Interaction with carbonic anhydrases—Some HCO$_3^-$ transporters have been proposed to interact with CAs to form a functionally relevant protein complex (213). Most importantly, AE1 has been proposed to interact with CAII (324) through an acidic cluster DADD in the cytoplasmic C-terminus of AE1, which appears to serve as a consensus CAII binding site. The critical residue is the first Asp (D^{877}) to which the basic amino acids in the N-terminal region of CAII bind by electrostatic interactions involving His and/or Lys residues. The AE1/CAII interaction has been reported to increase the rate of HCO$_3^-$ transport (302). Cl$^-$/HCO$_3^-$ exchange via AE1 in erythrocytes is a rate-limiting step for CO$_2$ delivery from tissues to the lungs (338), and maximizing the HCO$_3^-$ transport rate helps utilize the full CO$_2$ transport capacity of the blood. Other AEs and different CA isoforms are also reported to interact with each other (55).

Similarly, NBCe1 is reported to interact with CAII. NBCe1 contains D^{986}NDD in the C-terminal domain (251), and—based on measurements of membrane conductance and intracellular [Na$^+$]—the interaction has been proposed to help maximize the HCO$_3^-$ gradient local to NBCe1 and enhance the cotransport rate (23, 251). NBCe1 also interacts with CAI and CAIII (285), CA IV (15), and CA IX (226, 309). Mutational studies has suggested that Gly^{767} in the fourth extracellular loop of the transporter is a site important for binding in a GST pull-down assay and for the stimulatory effect of CA IV on HCO$_3^-$ transport (15). Missense mutations in CA IV associated with autosomal dominant rod-cone dystrophy

disrupt NBCe1-mediated HCO_3^- transport in the eye, thus indicating that the interaction is essential for function (351).

Nonetheless, Boron and his colleagues found that neither CAII nor CAII-NBCe1 fusion affect NBCe1 currents or the pH_i recovery rate mediated by NBCe1 in *Xenopus* oocytes (204). They propose a model where CAII in the immediate vicinity of NBCe1 has a minimal effect on local $[HCO_3^-]$ and is thus unlikely to enhance NBCe1 activity (204). Furthermore, a recent report shows that a deletion mutant lacking the putative CAII-binding domain has normal transport activity (342). The role of CA binding to NBCe1 is thus controversial.

Membrane expression—A series of studies by Soleimani and colleagues suggest that the cytoplasmic C-terminal domain of NBCe1 is important for its targeting to the basolateral membrane. Deletion of the residues QQPFLS (position 1010–1015) causes the mutant protein to be misplaced to the apical membrane in the kidney epithelial cell line MDCK (193). In particular, Phe^{1013} and Leu^{1014} are essential. Substitution of Phe^{1013} or Leu^{1014} with Ala causes the transporter to be misplaced to the apical membrane, whereas substitution of Phe^{1013} with a Leu has no effect and substitution of Leu^{1014} with a Phe results in intracellular retention of the transporter (191). Shifting the Phe^{1013}-Leu^{1014} pair by one amino acid position results in either misplacement or intracellular retention (189). These data indicate that the exact orientation and location of the two residues are critical for basolateral expression of NBCe1.

Several studies have been performed to determine structural elements important for intracellular trafficking. Espiritu et al. (97) demonstrated that deletion mutants lacking the cytoplasmic N- or C-terminal domains showed reduced basolateral membrane expression in opossum kidney OK cells. A recent report by Soleimani's group shows that Asp^{405} and Asp^{416} in the N-terminal domain of NBCe1 are critical for protein expression in plasma membranes (190).

Protein structure—Similar to AEs (331, 356), NBCe1 forms a homodimer comprised of two individually functional subunits (109,153). The dimerization is mediated by the N-terminal domain, and also involves an intramolecular interaction between Cys^{630} and Cys^{642} in the EL3, although this stable linkage is unnecessary for function (153). Despite this progress, our knowledge on the NBCe1 protein structure is very limited. The topology is currently based on the hydropathy plot of the amino acid residues and biochemical analyses such as glycosylation, cysteine-scanning mutagenesis, and molecular modeling. It is generally accepted that the N-terminal and C-terminal domains of the SLC4-proteins are intracellular, but it has been debated how many TMs are present in the NCBTs. In particular, the second part of the TM domain (between TM6 and the last TM) has been debated. Zhu et al. (365) addressed this issue by cysteine-scanning mutagenesis of Ala^{800}-Lys^{967} covering the second part of the TM domain, and identified the presence of 6 TMs in the region. The current NBCe1 topology model has 14 TMs. NBCe1 has a large extracellular loop between TM5 and TM6, whereas AEs have a large extracellular loop between TM7 and TM8. Recent work has further shown that in NBCe1 the important TM1 is longer compared to TM1 in AE1 and the TM1 in NBCe1 is predicted to take up a tilted position in the membrane (367).

SLC4A5 (NBCe2)

Physiology of NBCe2—Despite the cloning of NBCe2 in the early 2000s, its physiological importance in tissues is poorly understood. NBCe2 moves 1 Na^+ and at least 2 HCO_3^- across the plasma membrane and thus is electrogenic (Fig. 6B). The transporter operates with a 1 Na^+ to 2 HCO_3^- stoichiometry in *Xenopus* oocytes (326). In the renal proximal tubule cell line mPCT (280) and in intact cells isolated from choroid plexus epithelia (214), however, NBCe2 operates with a 1 Na^+ to 3 HCO_3^- stoichiometry and is expected to mediate HCO_3^- efflux from the cells. NBCe2 is expressed in the apical membrane of choroid plexus epithelia (Fig. 8A) and thus transports Na^+ and HCO_3^- to the cerebrospinal fluid (46). Very interestingly it has recently been shown that the NBCe2 expressed in the apical membrane of the choroid is a novel shorter variant of the known NBCe2, and is under control of an alternative promoter (102). Similarly, NBCe2 in the apical membrane of the renal collecting duct intercalated cells (81) is expected to contribute to HCO_3^- secretion.

Knockout mice with targeted disruption of the *slc4a5* gene have profoundly decreased intracranial volume and pressure (Fig. 8B, 8C, and 8D) due to an abnormal cerebrospinal fluid volume (152). Mitochondria, Na^+ transporting proteins, and cytoskeletal components are misplaced in choroid plexus epithelia from NBCe2 knockout mice, implying that NBCe2 affects the cellular structure of choroid plexus epithelia. These knockout mice have normal livers and kidneys, as well as normal plasma electrolytes. Another line of NBCe2 knockout mice develops arterial hypertension and metabolic acidosis (119). These mice also develop hyporeninemic hypoaldosteronism, elevated fluid intake and urine excretion, and increased glomerular filtration rate. Based on these findings, Groger et al. (119) suggested that lack of renal NBCe2 results in a compensatory increase in HCO_3^- absorption via other Na,HCO_3-transporters such as NBCn1, which could cause an increase in Na^+ uptake due to the difference in transport stoichiometry. While this could contribute to hypertension development, there is currently no strong evidence for luminal expression of NBCn1 in the relevant nephron segments at least in wild type mice.

Genetic polymorphisms—Genome-wide association analyses show significant levels of association between single nucleotide polymorphisms (SNPs) in *SLC4* genes and pathological conditions (Table 4). In particular, several SNPs in *SLC4A5* are associated with hypertension (20). *SLC4A5* is one gene on chromosome 2 that is known to influence blood pressure (134, 163). A significant association of rs10177833 (Celera database: hcv1137534) with blood pressure reactivity, and systolic and diastolic blood pressures was observed in the Utah pedigree samples (136). SNP rs10177833 is also identified as one of the two SNPs (the other is rs7571842) that are associated with salt sensitivity of blood pressure in Caucasian Americans (salt sensitivity is defined as a 7 mmHg increase in mean arterial pressure during a transition between low and high [Na^+] diet) (54). Other studies show that African American women with rs8179526 who consumed high sodium levels have lower systolic blood pressure than controls (311). The results support the idea of *SLC4A5* serving as a hypertension susceptibility gene. In addition, certain SNPs are also associated with atherosclerotic peripheral arterial disease (154). Currently there is no understanding of how a modification in *SLC4A5* influences development of atherosclerosis. Most SNPs in *SLC4A5*

are located in introns or nontranslated regions of the gene, and therefore, the observed associations of *SLC4A5* polymorphisms with the cardiovascular phenotypes are unlikely due to direct changes in transport function. Instead, these SNPs may be in disequilibrium with functionally important SNPs in the coding region or may influence the expression levels of the transporters. The promoter activity of the *SLC4A5* gene is not affected by polymorphisms (305).

SLC4A7 (NBCn1)

Physiology of NBCn1—Among the NCBTs, NBCn1 is the second most studied transporter and transport in an electroneutral manner (Fig. 6C). Molecular and cellular studies have demonstrated the physiological importance of NBCn1 for acid-base regulation in a variety of tissues and expanded our understanding of how acid-base disturbances interfere with normal physiological functions.

(i) Renal acid-base regulation: NBCn1 is found in the renal thick ascending limb epithelium and in intercalated cells of the collecting ducts (167, 248, 327). In the medullary thick ascending limb, where 10% to 15% of filtered HCO_3^- is reabsorbed (112, 228), NBCn1 is localized to the basolateral side of the epithelium. Furthermore, DIDS-insensitive Na^+-dependent HCO_3^- influx has been described in this segment. Considering the direction of transport, the role of NBCn1 in this nephron segment is unlikely associated with HCO_3^- reabsorption but instead is proposed to compensate for intracellular H^+ liberated during transcellular transport of NH_4^+/NH_3 across the thick ascending limb (166, 224). Importantly this function of NBCn1 could—together with NHE4 (44)—assist in transepithelial transport of NH_4^+ in the thick ascending limb. NH_4^+ transport and subsequent dissociation of intracellular NH_4^+ to NH_3 and H^+ are significantly enhanced during metabolic acidosis due to increased ammoniagenesis in the proximal tubules and luminal NH_4^+ delivery to the thick ascending limb. Consistent with this idea, NBCn1 expression and function are significantly upregulated in rats fed with a low-K^+ diet (144) or treated with NH_4^+ in the drinking water (166), both of which increases NH_4^+ delivery to the thick ascending limb. NBCn1 in a medullary thick ascending limb cell line is also upregulated under an acidic incubation and enhances NH_4^+ transport (181). NBCn1 is sensitive to cellular and systemic pH changes, and its expression and activity are affected by acidosis and alkalosis. NBCn1 is downregulated by hypercalcemia-induced chronic metabolic alkalosis (334). Conversely, NBCn1 is upregulated by chronic metabolic acidosis induced by NH_4Cl loading (166). The transporter is also upregulated after long-term lithium treatment that induces hyperchloremic metabolic acidosis (157). The upregulation of NBCn1 under these conditions may account for adaptive changes in HCO_3^- and NH_4^+ absorption in the thick ascending limb during chronic metabolic acidosis (111). Interestingly, acid/base transporters such as NBCn1, NBCe1, and NHE3 are downregulated in rat kidneys following ureter obstruction (332) or calcineurin inhibition (216), both of which cause metabolic acidosis. The downregulation of these transporters might be responsible for the development of metabolic acidosis.

(ii) HCO_3^- secretion in exocrine cells: A variety of secretory epithelial cells express NBCn1, but the physiological contribution of this transporter to HCO_3^- secretion has not been extensively investigated. In most epithelial tissues, NBCn1 is localized to the

basolateral membranes (113, 230, 248, 249, 327). Thus, similar to NBCe1 in many secretory cells, NBCn1 raises intracellular HCO_3^- levels and stimulates apical HCO_3^- secretion mediated by other HCO_3^--releasing proteins. On the other hand, some secretory cells such as pancreatic ducts and salivary glands also express NBCn1 in the apical membranes (113). Park et al. (232) demonstrated that NBCn1 interacts with the chloride channel CFTR in the apical membranes of pancreatic ducts and submandibular glands. At rest, NBCn1 salvages HCO_3^- in the lumen to minimize HCO_3^- secretion, but this salvage mechanism is unnecessary and inhibited upon stimulation of CFTR that regulates the activity of all luminal HCO_3^- secretion. In the duodenum NBCn1 is present in the basolateral membrane (64, 247), where it is shown to play a major role in basal and forskolin-induced secretion of HCO_3^- from the duodenum (64).

(iii) Arterial tone regulation: Both pH_i (Fig. 8) and pH_o (328) affect arterial tone regulation. Many proteins in the vasculature are sensitive to pH, and severe pH changes influence both vasoconstriction and vasodilation in resistance arteries (30). AEs and NHEs have been known to govern pH_i regulation in vascular smooth muscle cells (150, 151), but more recently, it has become clear that HCO_3^--dependent acid extrusion also plays a role for pH_i regulation in vascular smoothmuscle cells (1,2). NBCn1 is found in vascular smooth muscle and endothelial cells determined by RT-PCR analyses, immunohistochemistry, and transgenic reporter mice (33, 34, 80). The transporter is found in intact mouse mesenteric, coronary, and cerebral small arteries. The molecular detection of NBCn1 (33) is consistent with the functional observation of Na^+-dependent HCO_3^- transport in vascular smooth muscles from all of these vascular beds. Furthermore, knocking down NBCn1 in mouse mesenteric small arteries using small interference RNA decreases the steady-state pH_i by ~0.2 pH unit and the net amiloride-insensitive, Na^+-dependent base influx by ~70% (33), reflecting a significant contribution of NBCn1 to the net acid extrusion. Knockout mice with targeted disruption of *slc4a7* show completely abolished Na,HCO_3-cotransport activity and exhibit abnormalities in artery function (Fig. 9) and blood pressure regulation (35). These mice have a lower steady-state pH_i in arterial endothelial cells (Fig. 9A) and smooth muscle cells (Fig. 9C), and also develop reduced nitric oxide-mediated vasorelaxation (Fig. 9B) and smooth muscle Rho-kinase dependent Ca^{2+} sensitivity with consequent reduced contraction to vasoconstrictors like norepinephrine (Fig. 9D). The NBCn1 knockout mice are mildly hypertensive, but resistant to developing hypertension to angiotensin II and nitric oxide synthase inhibition. Together with the recent observation that SNPs in *SLC4A7* are associated with hypertension in humans (96), the knockout mouse study provides very strong arguments for NBCn1's importance in the pathogenesis of hypertension and as a potential target in this condition.

(iv) Development of sensory neurons: Knockout mice with targeted disruption of the *slc4a7* gene slowly develop blindness and auditory impairment due to degeneration of retinal photoreceptors and inner ear hair cells (36,202). In the inner ear, a progressive loss of inner and outer hair cells and spiral ganglia neurons was shown in knockout mice beginning at postnatal day 21. Type II and IV fibrocytes in the spiral ligament were also progressively lost. The pathological mechanism appears to involve a disruption of protein-protein interactions in the macromolecular network in the synaptic terminals of inner ear hair cells

and retinal photoreceptors (259). The *slc4a7* knockout mice have been proposed as a model to study Usher syndrome type IIB, but the clinical and genetic analyses of Usher patients show no mutations in NBCn1 (178).

(iv) Modulation of neuronal activity: Neuronal activity is affected by local pH at synapses and by systemic pH (67). Na^+-driven Cl/HCO_3-exchange has been functionally characterized as a major HCO_3^--dependent acid extrusion pathway in neurons (22, 287). However, Cooper et al. (75) found NBCn1 protein expression in primary cultures of rat hippocampal neurons. NBCn1 in adult neurons lacks the 123-amino acid long cassette II and is localized to somatodendrites and synapses. It is found in both GABAergic and non-GABAergic neurons determined by single-cell RT-PCR analyses. NBCn1 is markedly upregulated following incubation in acidic or Mg^{2+}-free media, probably to compensate intracellular acidification during acid loads or neuronal activity (76). NBCn1 knockdown attenuates excitotoxicity mediated by NMDA receptors in Mg^{2+} depletion, which is consistent with previous data that pharmacological inhibition of Na,H-exchange or Na,HCO_3-transport protects neurons against damage or injury in cerebral ischemia (67). NBCn1 in the brain is upregulated in chronic metabolic acidosis (230), and this upregulation could be the basis for acidic injury of neurons during cerebral ischemia or chronic metabolic acidosis. Chen et al. (59) examined NBCn1 and NBCn2/NCBE expression in mouse brains subjected to chronic continuous hypoxia (11% O_2 for 14 or 28 d) and found a decreased expression of the transporters in many different brain regions. The authors proposed that the decreased expression of NBCn1-B and NBCn2/NCBE reflect decreased energy consumption in the brain.

(v) Other roles: In osteoclasts, NBCn1 is involved in bone resorption (262). Knocking down NBCn1 inhibits bone resorption and increases intracellular acidification in osteoclasts. NBCn1 is also involved in osteoclast survival (45). In addition, NBCn1 is found in matrix vesicles released from osteoblasts during bone development (316). Electroneutral Na,HCO_3-cotransport has been reported in ventricular cardiomyocytes and Purkinje fibers (85, 173). The cotransport affects excitation-contraction coupling and subsequent contractility. The mRNA levels and activities of both NBCn1 and NBCe1 are upregulated following ventricular hypertrophy and are induced by suprarenal abdominal aortic constriction (344). Enhanced NBC activity during hypertrophic development is expected to cause intracellular Na^+ overload, which would be detrimental to myocytes during ischemic reperfusion due to Ca^{2+} overload via Na,Ca-exchangers. Immunohistochemical analysis also shows NBCn1 being localized to the capillaries of the heart ventricles (80).

Association with cancer development—The challenges to pH homeostasis in tumors are different from those in normal tissues. The pH_i of tumor cells is near neutral or slightly alkaline, while the extracellular pH is very acidic usually between pH 6.5 and 6.8 (110). This reversed pH gradient is largely due to abnormal metabolism and affects tumor growth and progression (104). Tumor cells can thrive in this acidic pH environment and expand into the area of the dying normal tissues. NHE1 is one of the proteins responsible for extruding acids from tumor cells (212), but recent studies suggest that NBCn1 also plays a role (176, 238). Boedtkjer et al. (32) demonstrated that NBCn1 expression in membranes of human

primary breast carcinomas and metastases is 20% to 30% higher than that in matched normal breast tissue (10). Functionally, cellular acid extrusion in the human primary breast carcinomas is mainly governed by Na,HCO_3-cotransport (Fig. 10D) that is modestly sensitive to DIDS, a hallmark for NBCn1. NBCn1 is upregulated in tumors obtained after subcutaneous injection of breast cancer cells to mice and is one of the novel target proteins for tyrosine kinase phosphorylation in these tumors (66). Furthermore, expression of NBCn1, but not NHE1, is upregulated by the N-terminally truncated tyrosine kinase ErbB2/ HER-2 (i.e., ΔNErbB2 or p95HER-2) (176), a constitutively active kinase involved in increased metastasis in breast cancers (72) and a protein conveying poor prognosis. This upregulation induced by ΔNErbB2 is comparable with an increased rate of pH_i recovery after acid load. However, whereas NHE1 knockdown or inhibition causes ΔNErbB2-expressing cells to undergo programmed cell death and enhance cancer cell motility, NBCn1 knockdown or pharmacological inhibition shows no effects on these parameters (176,177). Thus, NBCn1 and NHE1 appear to have different effects on cancer progression although they share similar acid extrusion functions (31).

Genetic polymorphisms—Genome-wide association studies have identified the SNP rs4973768 in *SLC4A7* as one of the new breast cancer susceptibility loci (7, 127, 201, 215, 306). The SNP rs4973768 is tightly linked to increased breast cancer risk for BRCA2 carriers (18,223) and is particularly associated with the Luminal A subtype (127). This SNP is located in the 3′ untranslated region of the *SLC4A7* gene, implying that the observed association with breast cancer phenotypes does not involve altered function of the transporter, but instead could be due to a change in NBCn1 mRNA stability.

SLC4A7 gene polymorphisms are also linked to other pathological or behavioral states. The marker rs3278 is identified as one of the addiction phenotypic marker loci (321). The minor allele frequency of rs3278 is prevalent in drug abusers who are strongly dependent on at least one illegal substance of abuse (142). The marker rs3278 is located in intron 7 of the gene. Another SNP rs13082711 is associated with hypertension (96), reflecting the complex blood pressure phenotype in *slc4a7* knockout mice (35). In addition, the *SLC4A7* gene locus shows a strong linkage to the levels of environmental toxic elements in erythrocytes (336, 337).

Channel-like activity—NBCn1 has intrinsic channel-like activity (68, 75). This channel-like activity is mediated by Na^+ and raises intracellular Na^+ levels in HEK 293 cells and *Xenopus* oocytes. It is not coupled to Na,HCO_3-cotransport and occurs in the absence of HCO_3^-. Chimeric transporters with NBCe1 and NBCn1 show that the region of TM6–14 of NBCn1 is responsible for channel-like activity (71). Replacing the TM6-14 region of NBCn1 with the corresponding region of NBCe1 abolishes steady-state Na^+ conductance, whereas the opposite replacement retains the conductance. The channel-like activity is not just an oddity of NBCn1 because other HCO_3^- transporters exhibit similar activities. Erythrocyte AE1 has an anion conductance that progressively increases as the membranes are hyperpolarized (101). Trout AE1 is known to produce a robust Cl^- current and amino acids responsible for the currents have been examined (98). Other HCO_3^- transporters such as SLC26A7 and SLC26A9 have the capability to produce anion currents in addition to

being exchangers (92, 156). The channel-like activity affects membrane potential and ion movement across the membrane at least in heterologous expression systems. Whether a similar effect occurs in the cells *in vivo* is unclear.

Interaction with other proteins—NBCn1 contains the PDZ binding motif in the C-terminal. NBCn1 can interact with many different PDZ proteins in cell/tissue-specific manners. In the kidney collecting ducts (254, 256), NBCn1 interacts with the 56 kDa subunit of H-ATPase. The interaction may help coordinate the two acid-base proteins NBCn1 and H-ATPase in the same side of the membrane. In the pancreatic ducts and salivary glands, NBCn1 interacts with ezrin-binding protein 50 (EBP50) (232), which is a binding protein of CFTR. NBCn1/EBP50 interaction allows NBCn1 to cluster with CFTR so that NBCn1 and CFTR are coordinately regulated by ATP. In the ear, NBCn1 interacts with harmonin to form a protein complex (259). In the nervous system, NBCn1 interacts with the postsynaptic density protein PSD-95 at synapses (230). Interestingly, the interaction does not affect Na,HCO$_3$-cotransport activity, but stimulates channel-like activity (182).

NBCn1 has also been shown to interact physically and functionally with calcineurin Aβ (84). This interaction involves a calcineurin binding motif PTVVIH in cassette II in NBCn1. Calcineurin is required for Ca^{2+}-induced activation of NBCn1 and inhibition of calcineurin augments intracellular acidification during rat mesenteric artery contraction (84). Since cassette II is predominantly expressed in smooth, cardiac and skeletal muscle tissues (74) this observation is an important step towards understanding the functional implication of different splice variants and a mechanism of tissue specific regulation of NBCn1. The role of this regulation for vascular function is still not clear.

NBCn1 expressed as GST-fusion protein has also been suggested to interact with CAII (200). The interaction purportedly involves the consensus CAII binding sites D^{1135}D^{1136} in the C-terminal domain of NBCn1. The dominant-negative CAII mutant V143Y was furthermore found to reduce the rate of pH$_i$ recovery mediated by NBCn1 in HEK 293 cells, indicating that the interaction is required for optimal NBCn1 function. It should be noted, however, that binding of CAII to SLC4-termini (AE1, NBCe1, and NDCBE) was subsequently questioned by another group (244) who suggested that the original signs of binding could be explained by the ability of GST alone to bind CAII.

PKA reduces NBCn1 transport activity by a mechanism that neither involves CAII/NBCn1 interaction nor phosphorylation of the C-terminus.

SLC4A8 (NDCBE)

Paradoxically, the first Na$^+$-coupled HCO$_3^-$ transport to be described (i.e., Na$^+$-dependent Cl,HCO$_3$-exchange) has probably been the least studied in the postcloning period. When expressing the Slc4a9 gene product an electroneutral, sodium-dependent transport is apparent (Fig. 6E). Antibodies against NDCBE have been developed though and used to describe the distribution of NDCBE in the brain of humans (81), rats (49, 81, 179), and mice (61, 296). In all species immunohistochemistry suggested the presence of NDCBE in pyramidal neurons in hippocampus (Fig. 11A) and in Purkinje cells in cerebellum. In both mice and rats, where a more extensive study of the brain distribution of NDCBE was made,

the immunostaining showed a widespread distribution in neurons of the cerebral cortex, in several places in the cerebellum as well as in substantia nigra and in medulla. A detailed analysis revealed that NDCBE is predominantly expressed in presynaptic glutamatergic terminals (49, 296). These morphological results were strongly supported by data from Slc4a8 knockout mice (296). Cultured hippocampal neurons from these mice had a low steady-state pH_i and reduced acid extrusion, thus strongly supporting that NDCBE is an important acid extruder in hippocampal neurons. Functionally, it was shown that spontaneous glutamate release from the CA1 pyramidal layer of *slc4a8* knockout mice was reduced compared to the release from the same site in wild-type mice, and more importantly this reduction could be rescued by increasing pH_i. These data provide very strong arguments for NDCBE serving an important role in regulating pH_i in glutamatergic neurons with consequences for glutamate release. This conclusion was corroborated by the finding that the knockout mice have an increased seizure threshold (296).

The antibody developed by Chen et al. (61) was also used to determine the effect of chronic hypoxia on the expression of NDCBE in the brain (60). In this study, neonatal (postnatal age of 2 days) and adult mice were exposed to normobaric hypoxia (11%) for 14 or 28 days. Based on Western blot analyses, it was found that NDCBE expression was reduced in the brain of adult mice. In the neonatal mice, the pattern was more complex because reduced expression was only seen in the hippocampus and subcortical areas. Also, the effect of metabolic acidosis on NDCBE expression was assessed (179). As expected, metabolic acidosis was associated with upregulation of NDCBE. Interestingly, the upregulation was not general but confined to specific areas in the brain, and the NDCBE downregulation was even seen in some areas such as the olfactory bulb.

In addition, a novel role of NDCBE for sodium reabsorption in mouse cortical collecting ducts has been suggested (187). In intercalated cells in the cortical collecting ducts of Na^+-deprived mice a Na^+-dependent regulation of intracellular pH occurs, which disappears in NDCBE knockout mice (Fig. 11B). In this segment an amiloride-insensitive and thiazide-sensitive sodium absorption is also present. This Na^+ absorption is not explained by transport via the thiazide-sensitive Na,Cl-cotransporter, which is normally present in the distal tubules, but not present in this segment. This conclusion was further strengthened by the observation that the amiloride-insensitive and thiazide-sensitive sodium absorption was also present in knockout mice for the Na,Cl-cotransporter (187). Based on a series of experiments, it was suggested that the uptake was mediated by the combined activity of the Na^+-independent Cl,HCO_3-exchanger (pendrin or slc26a4) and NDCBE. Part of the evidence for this mechanism was the observation that in NDCBE knockout mice the thiazide-sensitive Na^+ absorption is lost in the cortical collecting ducts. It now remains to be seen whether NDCBE is important during the development of salt-dependent hypertension.

SLC4A10 (NBCn2/NCBE)

Following the cloning and characterization of the *SLC4A10* gene product as an electroneutral Na^+-coupled (Fig. 6D) Cl,HCO_3-exchanger NCBE, an important concern over the substrate dependence of the transporter has arisen (Fig. 12). Parker et al. (236) expressed *SLC4A10* in oocytes and used electrodes to monitor surface [Cl^-] and pH_i. They furthermore

measured the transmembrane flux of $^{36}Cl^-$ and provided convincing evidence that the *SLC4A10* gene product does not provide net transport of Cl^- under physiological conditions, although net transport of Cl^- was found when extracellular Cl^- was removed. Therefore, they suggested that the *SLC4A10* gene product mediates electroneutral Na,HCO_3-cotransport in parallel to Cl^- self-exchange under physiological conditions and should be given the name NBCn2. This name reflects that it is the second electroneutral Na,HCO_3-cotransporter. However, Damkier et al. (79) provided equally convincing evidence that net transport of Cl^- was associated with activity of the *SLC4A10* gene product when expressed in a mammalian fibroblast cell line. In this case, $^{36}Cl^-$ efflux was also examined, while pH_i and $[Na^+]_i$ were measured with fluorescent dyes. The authors found that, although expression of *SLC4A10* induced a Na^+- and HCO_3^--dependent unidirectional efflux of $^{36}Cl^-$, no matching increase in unidirectional ^{36}Cl influx was seen. Apart from the very different experimental conditions, it is presently difficult to explain the different findings. An interesting possibility is that one of the HCO_3^- sites (i.e., one of the 2 HCO_3^- sites in the Na^+-driven Cl,HCO_3-exchanger of Fig. 12) prefers Cl^- under some conditions. If this is the case, the transporter would behave as an NBCn2 with Cl^- self-exchange when Cl^- is the preferred substrate, and behave as an NCBE when HCO_3^- is the preferred substrate.

Notwithstanding the ambiguity concerning the substrate dependence, an understanding of the physiological role of NBCn2/NCBE is beginning to emerge. The transporter is predominantly found in the brain with the strongest expression in the cortex, hippocampus and cerebellum (59, 107, 143, 198). The expression appears in neurons rather than glia. With the development of antibodies distinguishing some subtypes, it was possible to demonstrate that four different subtypes have differential distribution profiles in the brain (198). Subtype A is the dominant form in the brain, while subtype D is mainly expressed in the medulla and subcortical regions. There is, however, currently little information on the specific physiological importance of NBCn2/NCBE in the brain. Chen et al. (59) report that chronic hypoxia leads to downregulation of NBCn2/NCBE (and also NBCn1) in several brain regions. This downregulation might be part of a general downregulation of transporters seen in the brain during hypoxia to reduce energy consumption. The relevance of the reduced expression of these transporters for the pathology associated with hypoxia was not studied.

A better defined role for NBCn2/NBCE has been obtained in the choroid plexus (Fig. 13A and 13B). The presence of NBCn2/NCBE in the basolateral membranes of choroid plexus is reported in several papers (46, 107, 249). With the development of *slc4a10* knockout mice, the role of NBCn2/NCBE in producing cerebrospinal fluid became apparent (143). The ventricular volume of the knockout mice was substantially reduced (Fig. 13C, 13D, and 13E), consistent with NBCn2/NCBE playing an important role in production of CSF. This is consistent with an old observation demonstrating that the CSF production is dependent on HCO_3^- (277) and may be of clinically relevant as it has been suggested that CSF production may be important in, for example, Alzheimer's disease (339). Although basal pH_i in neurons from the *slc4a10* knockout mice was not different from that of wild type mice, the knockout mice exhibit increased seizure threshold. No other major CNS phenotype was detected (143). The increased seizure threshold in the knockout mice is consistent with the observation that knockout of AE3 lowers the threshold for seizures (128).

These findings in mice are, however, contrasted with the observation that in a patient with epilepsy, mental retardation, and cognitive impairment, a disruption of the *SLC4A10* gene was found as the sole genetic abnormality (126, 161). Interestingly, partial deletion of *SLC4A10* has also been associated to autism (289). Association between gene variations in *SLC4A10* and open-angle glaucoma (198) (which is dependent on CSF production) and major depression (284) were investigated, but no convincing association was found. Interestingly, in the choroid plexus of *slc4a10* knockout mice, NHE1 is abnormally expressed in the basolateral membranes, whereas in the wild type mice, this transporter is located to the apical membrane (83). The expression of the water channel AQP1 and the Na,K-pump in the choroid plexus were also reduced in NHE1 knockout mice (82). The importance of this is currently unknown.

Conclusion

With the molecular characterization of the five NCBTs described in this article, the field of pH regulation has come to a new level. Virtually, every cell in the body has one or more of these transporters. By transporting the base HCO_3^- into or out of cells, these transporters play a central role in regulation of pH_i. With the expression in many epithelial cells, they also contribute substantially to the whole body acid-base homeostasis. For example, HCO_3^- reabsorption mediated by NBCe1 in the proximal tubules is crucial for normal acid-base homeostasis in the body. Despite the substantial advance in our knowledge regarding the molecular, cellular, and physiological implications of the transporters, much more experimental evidence is required to fully understand the physiological and pathophysiological importance of the transporters. It is unclear how the transporters are precisely regulated, both at the expression and activity levels. Also, since only very limited crystal structure information is available, the knowledge on structure-function relationships is primitive. We also need specific drugs to alter transport activity and help characterize the physiological importance and therapeutic potential of the transporters. Some examples of mutations leading to severe conditions have been described and there are likely many more conditions to be described, where these transporters are dysfunctional. Also, even though disturbed bicarbonate transport clearly is associated with conditions like cancer, cardiovascular disease, and metabolic disturbances, the precise mechanistic role and therapeutic potential of the NCBTs remains to be investigated. In conclusion, we expect very novel and exciting results coming from the studies of bicarbonate transporters in the near future.

Acknowledgments

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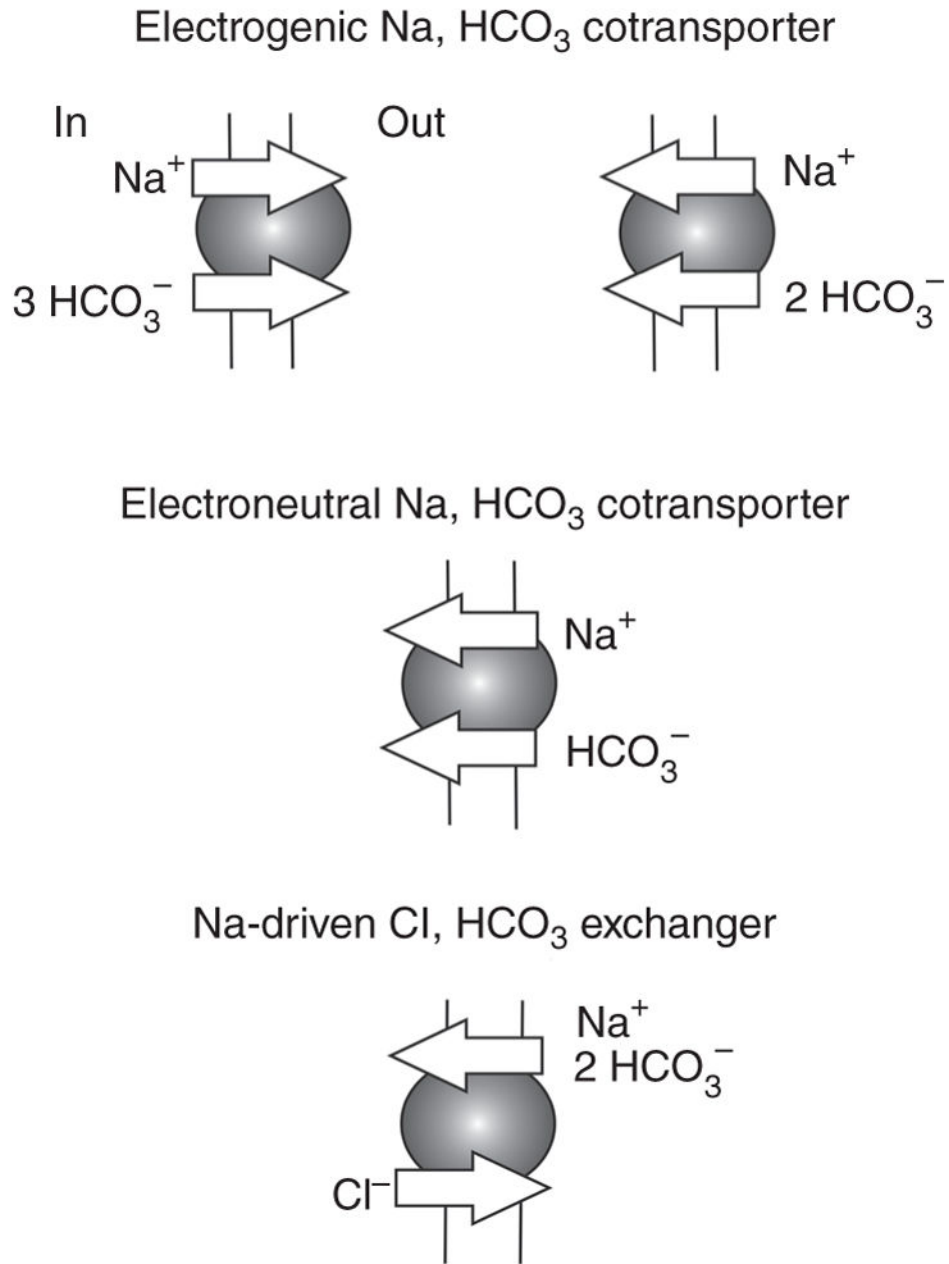
Figure 1.
Na^+-coupled bicarbonate transporters NCBTs. Electrogenic Na,HCO$_3$ cotransporters mediate HCO_3^- efflux or HCO_3^- influx in a tissue-specific manner. Electroneutral Na,HCO$_3$ cotransporters mediate net HCO_3^- influx. Na^+-driven Cl,HCO$_3$ exchangers electroneutrally mediate Cl^- influx in exchange for Cl^- efflux.
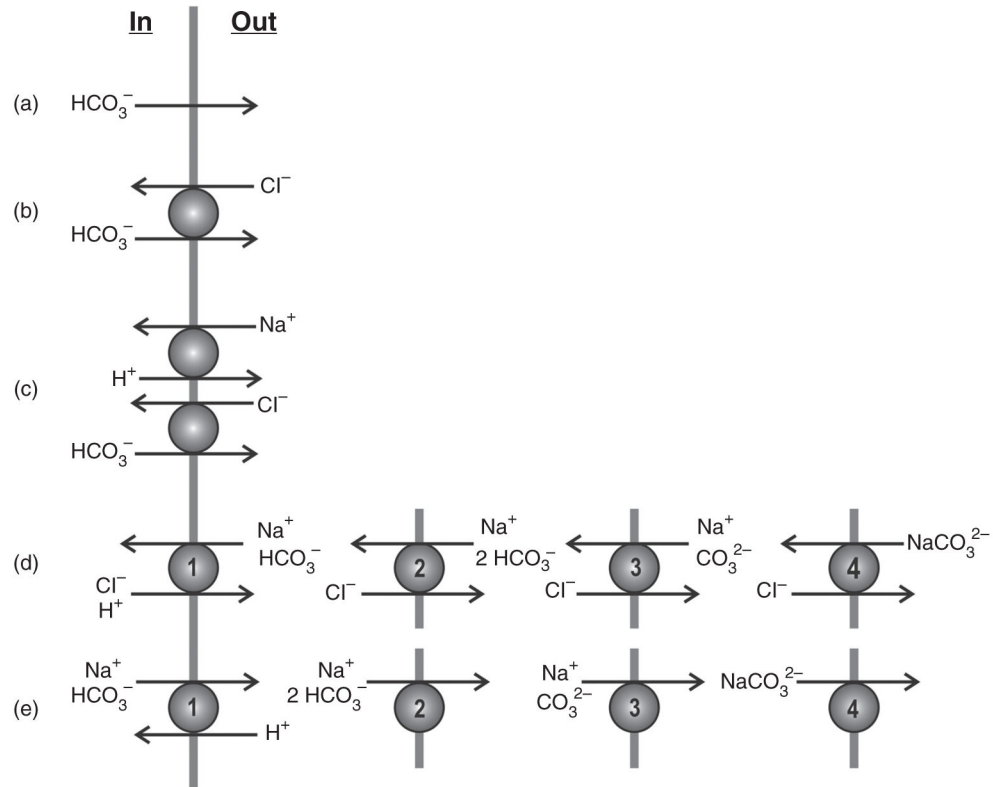
Figure 2.
Five different modes for effective HCO_3^- extrusion over the basolateral membrane of the proximal tubule [adapted, with permission, from (39)]. The authors ruled out the first four modes because the transport was known to be Na^+ dependent and electrogenic. Although this leaves option 5 (e) as the correct transport mode, it is still not decided which of the four different transport modes of (e), that is, which ion species, are actually transported by the transporter. This is not only the case for the Na^+-dependent transport of HCO_3^- or a related species over the basolateral membrane of the proximal tubule, but indeed for all Na^+-dependent transporters discussed in this article.
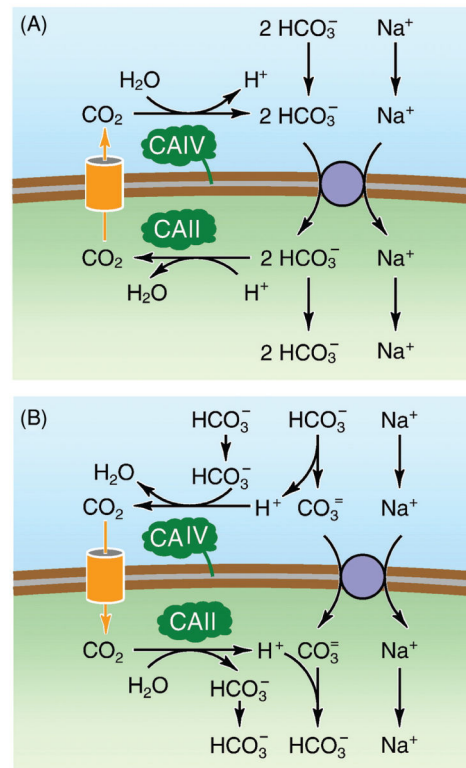
Figure 3.
Schematic diagram showing the rationale for determination of whether HCO_3^- or CO_3^{2-} is the transported ion. It has been suggested (38, 292) that it is possible to distinguish between HCO_3^- and CO_3^{2-} transport by measuring pH in the extracellular space near the membrane following a sudden change in transport activity, before and after inhibition of the carbonic anhydrase. If CO_3^- is the transported species an exaggerated decrease in pH would develop when the carbonic anhydrase is inhibited, while if HCO_3^- is the transported species a blunted decrease in pH would be the result. From (38).
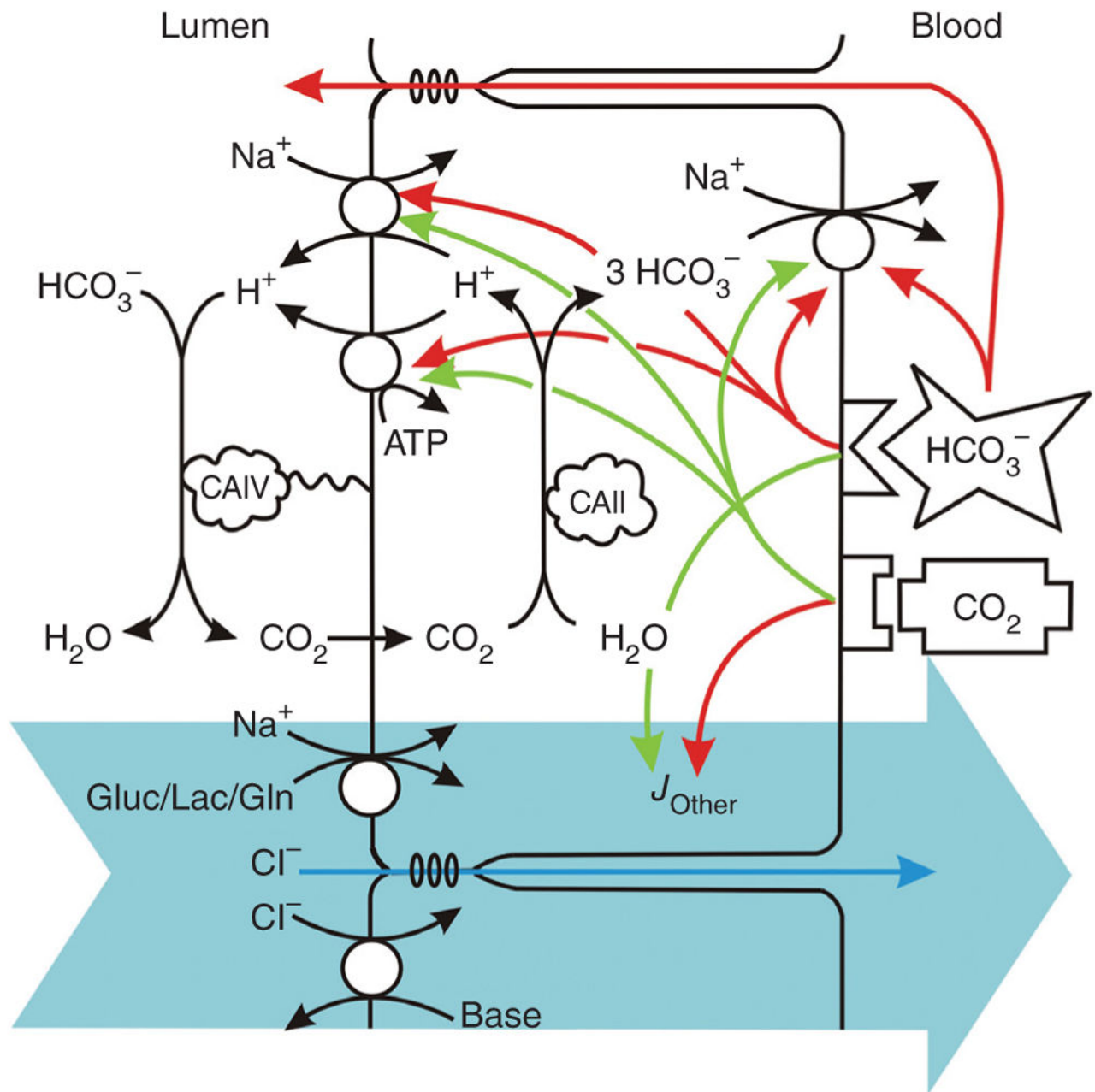
Figure 4.
Model of reabsorption of HCO_3^- in the proximal tubules. The figure underlines the importance of basolateral HCO_3^- and CO_2 for the regulation of HCO_3^- reabsorption suggested by Zhou et al. (363) and recently documented by Fukuda et al. (102) and others. From (363).
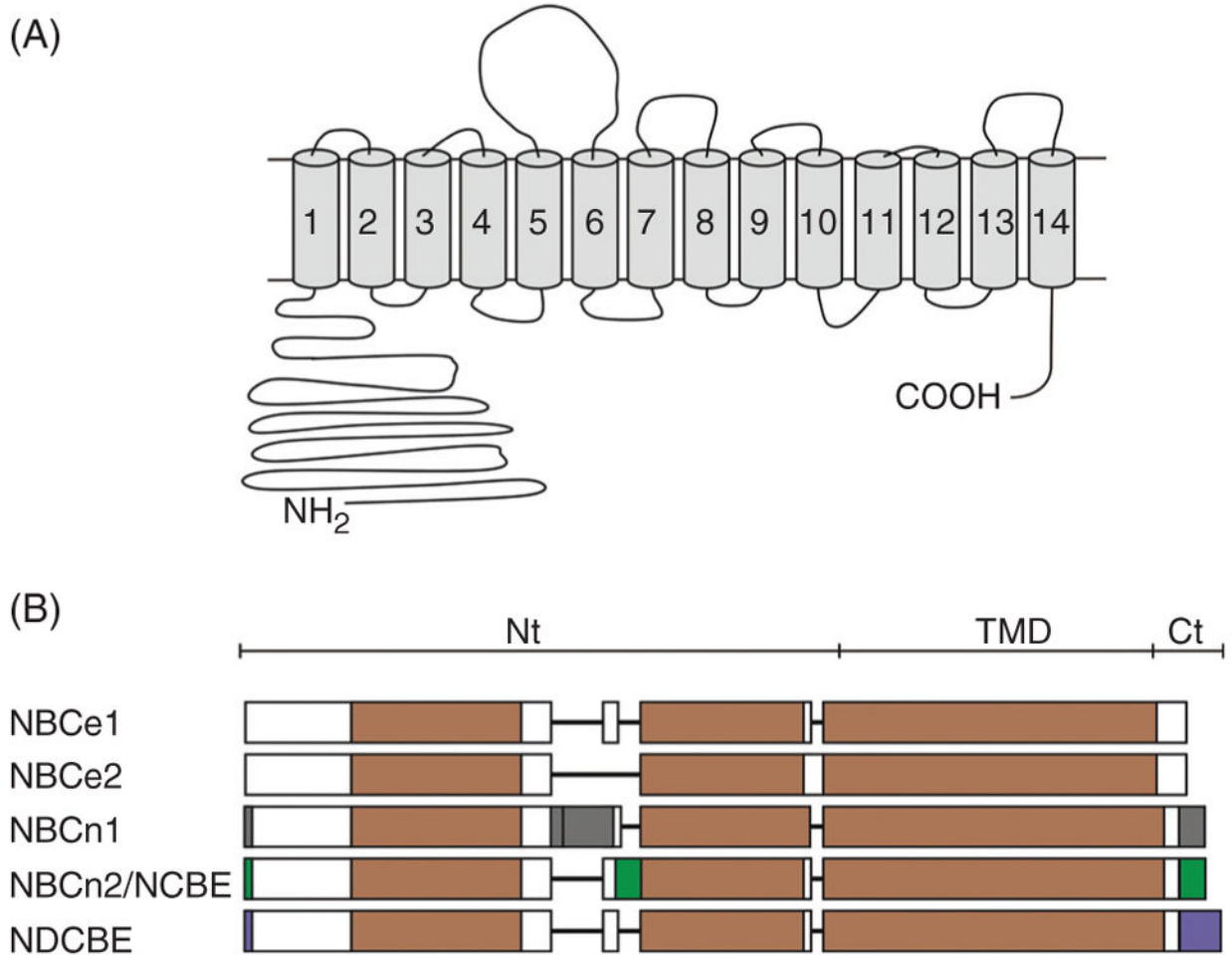
Figure 5.
Structure of NCBT proteins. (A) A generic structure of NCBTs is predicted to have an extended N-terminus, a transmembrane domain containing 14 transmembrane segments, and a relatively short C-terminal domain. The extracellular loop between segments 5 and 6 contains two N-glycosylation sites. (B) Alignment of protein sequence comprising human NCBTs. The alignment was performed using the UniProt (www.uniprot.org) with each canonical protein sequence for NBCe1 (Uniprot ID: Q9Y6R1), NBCe2 (Q9BY07), NBCn1 (Q9Y6M7), NBCn2 (Q2Y0W8), and NDCBE (Q6U841). Sequences highly conserved among NCBTs are shown in brown bars, while sequences moderately conserved are in open bars. Sequences with negligible homology are shown as a horizontal line. Internal splice cassettes are in different colors.
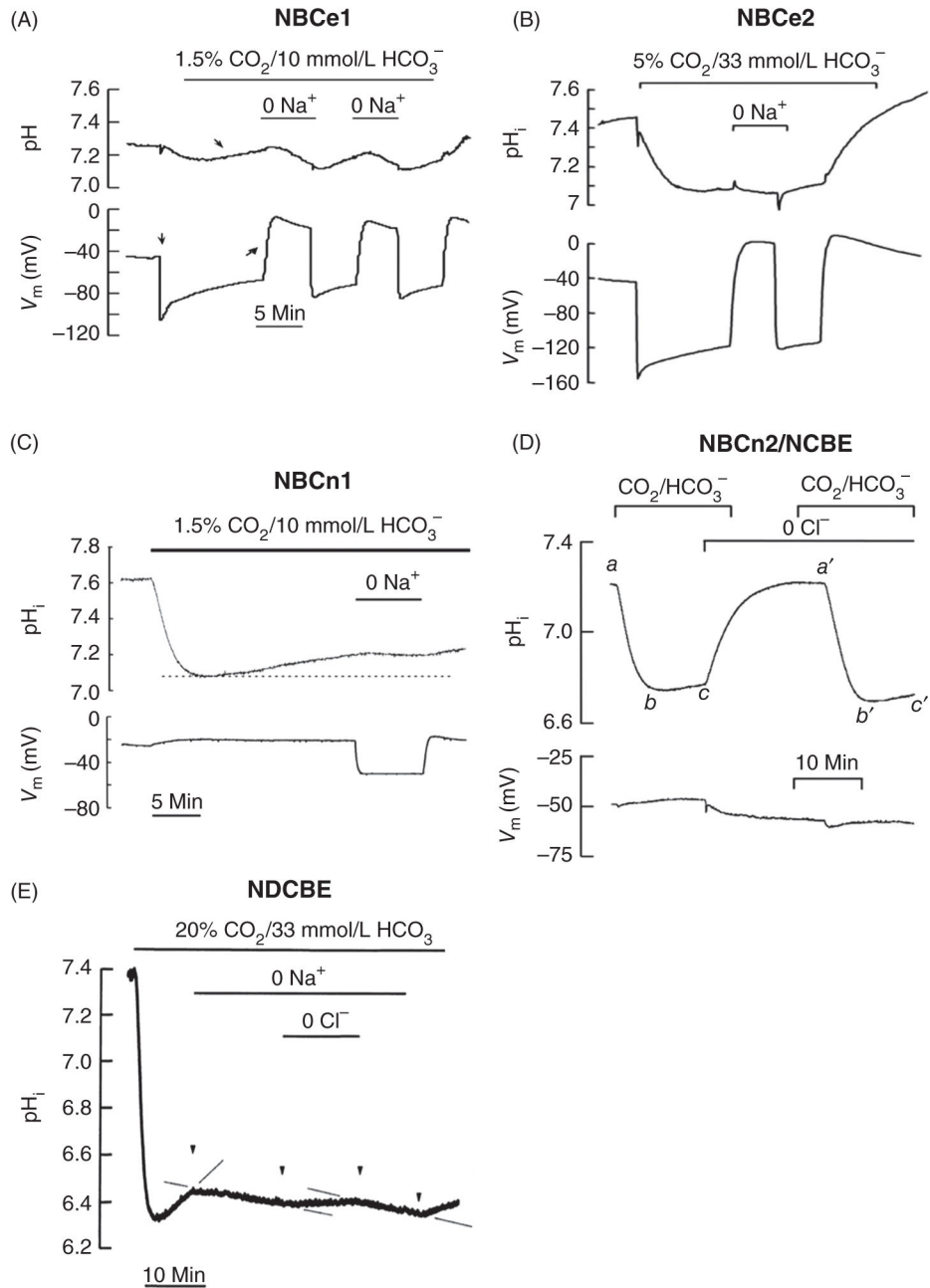
Figure 6.
Functional characterization of NBCs expressed in *Xenopus* oocytes demonstrating Na$^+$-dependent pH$_i$ recovery from a CO$_2$/HCO3$^-$-induced acidification. The electrogenic transporters NBCe1 (A) [adapted, with permission, from 70] and NBCe2 (B) [adapted, with permission, from 326] produce a large hyperpolarization due to net negative charge movement into oocytes. Activation of the electroneutral transporters NBCn1 (C) [adapted, with permission, from 75], NBCn2/NCBE (D) [adapted, with permission, from 236], and NDCBE (E) [adapted, with permission, from 117] is not associated with hyperpolarization.
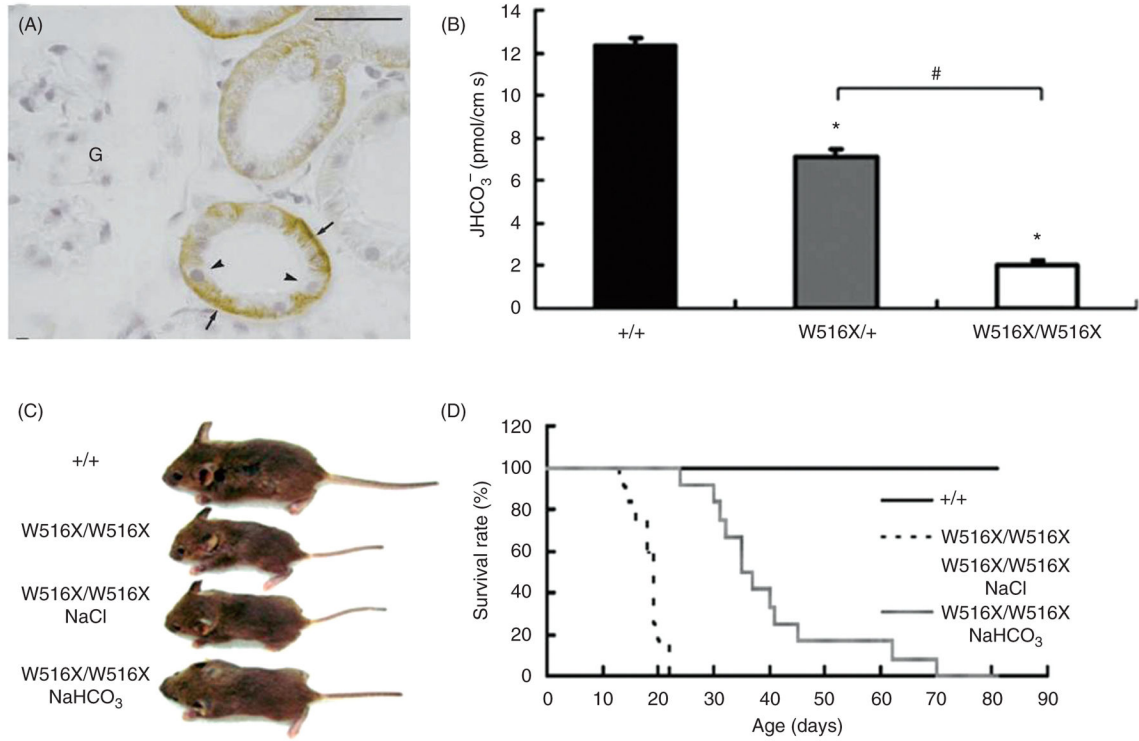
Figure 7.
NBCe1 (A) NBCe1 is highly expressed in the basolateral membranes of the cortical collecting ducts (arrows) but not in glomeruli (G) (209). [(B)–(D)] Functional knockout of NBCe1 in mice (199). (B) Rates of HCO_3^- absorption from isolated renal proximal tubules. Mice with functional knockout of NBCe1 (W516/W516×) had severely reduced reabsorption of HCO_3^-, while heterozygous mice had a mildly reduced reabsorption. (C) Mice with functional knockout of NBCe1 were growth retarded compared to wild-type mice (+/+). NaCl in the drinking water had no effect on the growth retardation while $NaHCO_3$-treated mice had attenuated growth retardation. (D) Mice with functional knockout of NBCe1 had severely reduced survival rates with a sharp increase in mortality starting around 17 days, but $NaHCO_3$ treatment of these mice prolonged the survival time up to 81 days of age.
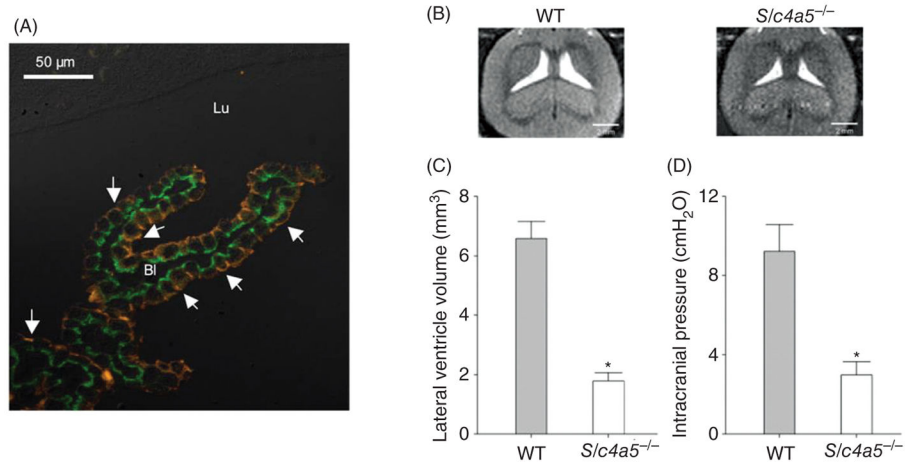
Figure 8.
(A) Double-labeling immunofluorescence microscopic analysis of NBCe2 (red) and NCBE/NBCn2 (green) localization in rat choroid plexus (46). The fluorescence image was overlaid a differential interference contrast image and shows apical localization of NBCe2 (arrows) and basolateral localization of NCBE/NBCn2. Panels B and C show ventricular volume and (D) intracranial pressure in wild-type (WT) and NBCe2 knockout (Slc4a5$^{-/-}$) mice (152). Panel B shows MRI imaging (horizontal plane) of the lateral ventricles in WT and *Slc4a5*$^{-/-}$ mice. The ventricular volume and intracranial pressure are significantly reduced in *Slc4a5*$^{-/-}$ mice.
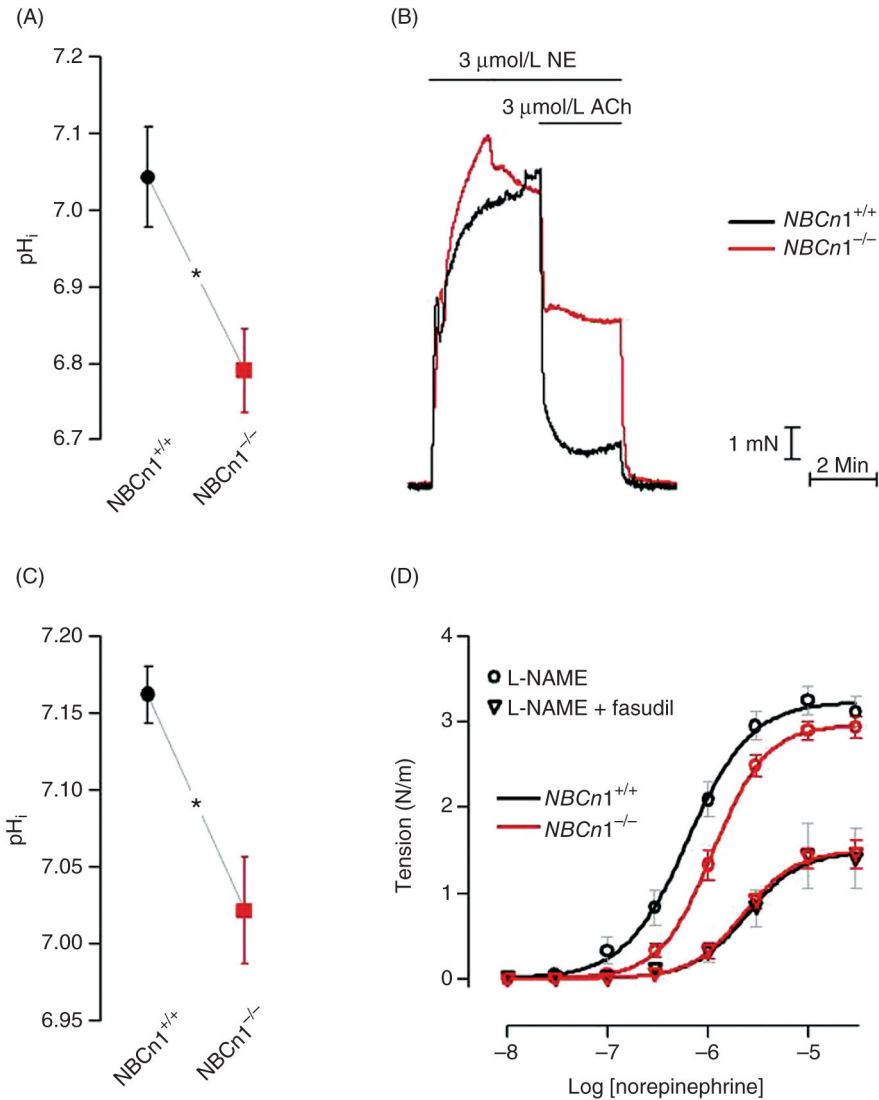
Figure 9.
Effect of intracellular pH on vascular tone (35). In the NBCn1 (Slc4a7) knockout mouse (red) vascular endothelial cell (A) and smooth muscle cell (C) pH is reduced (vertical bars show SEM, $n = 5$ and 6, respectively). This is associated with a reduced endothelial cell mediated smooth muscle cell relaxation to acetylcholine (ACh) of norepinephrine (NE) activated mesenteric small arteries (B) and reduced tension development to NE in the presence of 100 µmol/L nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) E), (vertical bars show SEM, $n = 10$). In the presence of 10 µmol/L Rho-kinase inhibitor fasudil, the tension development to NE is similar in arteries from wild type and knockout mice (D) ($n = 5$), consistent with the Rho-kinase being pH sensitive allowing for an effect of intracellular pH on smooth muscle cell tone.
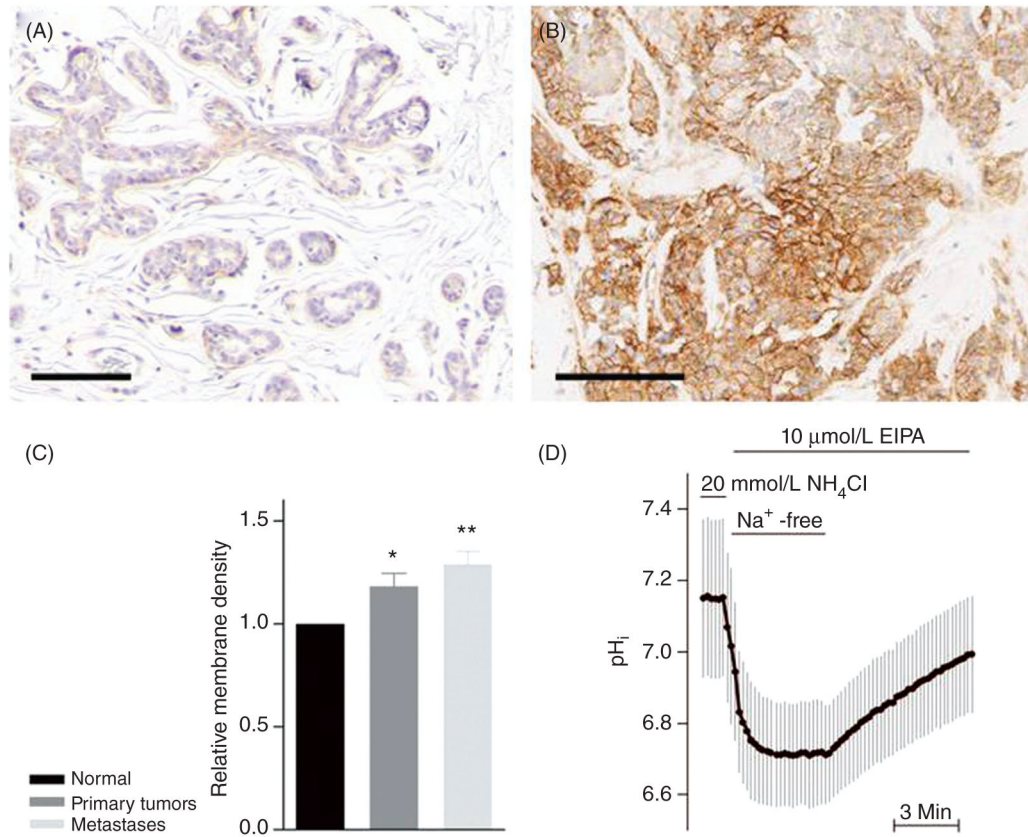
Figure 10.
NBCn1 expression in cancer cells. Micrographs of normal human breast (A) and breast cancer (B) immunostained (brown) for NBCn1. (C) Average (with SEM, $n = 5$) membrane density of NBCn1 immunostaining in normal breast, primary breast carcinomas, and metastases as indicated. (D) Na^+-dependent ethylisropylamilroide (EIPA) insensitive recovery of intracellular pH in a biopsy of human breast cancer following washout of NH_4Cl (vertical bars show SEM, $n = 5$). The recovery of intracellular pH was Na^+ and HCO_3^- dependent and thus demonstrates a NCBT transport activity in breast cancer. The authors also showed that the NCBT activity was modestly DIDS-sensitive ($34\pm9\%$), consistent with NBCn1 being responsible. Scale bars = 20 μm. Adapted, with permission, from (32).
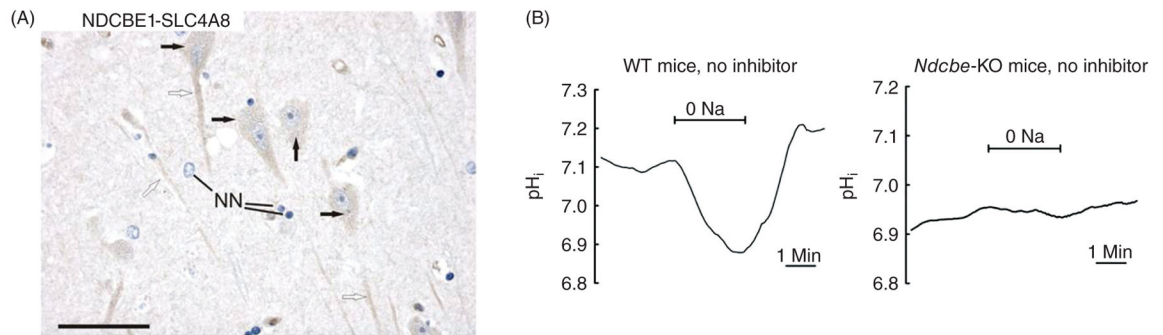
Figure 11.
Localization of NDCBE to the hippocampus of the human brain (adapted, with permission, from 81). (B) Effects of knockout of NDCBE (Slc4a8) on Na^+-dependent pH_i changes in intercalated cells in mice cortical collecting ducts (adapted, with permission, from 187). Traces are the average of pH_i changes recorded when luminal Na^+ was removed and readded, in the presence of extracellular HCO_3^-. The data provides functional evidence for the presence of NDCBE in the apical membrane, which is important for reabsorption of Na^+ by these cells.
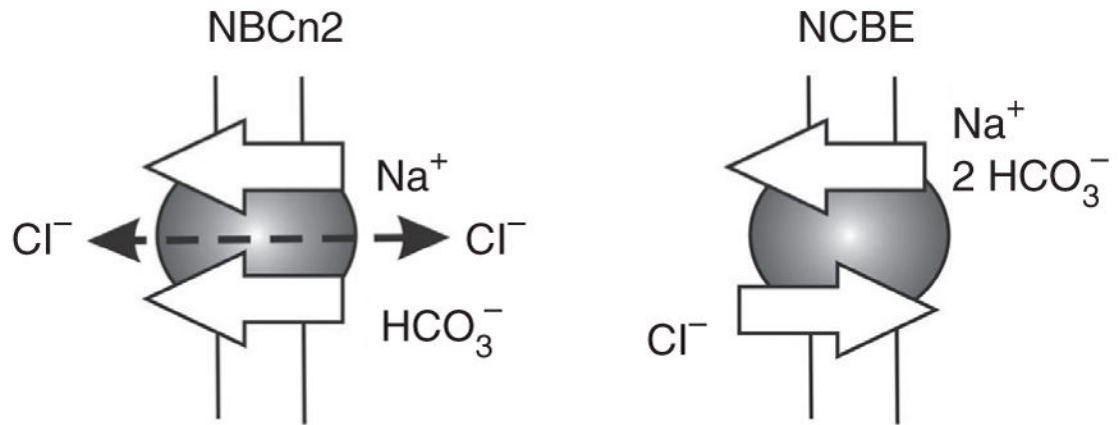
Figure 12.
NBCn2 versus NCBE. In Xenopus oocytes and HEK 293 cells, SLC4A10 encoding NBCn2/ NCBE produces Cl^- flux. This flux is either due to Cl^- self-exchange uncoupled to Na/ HCO_3 transport (236) or Na,HCO_3-dependent Cl^- transport (79).
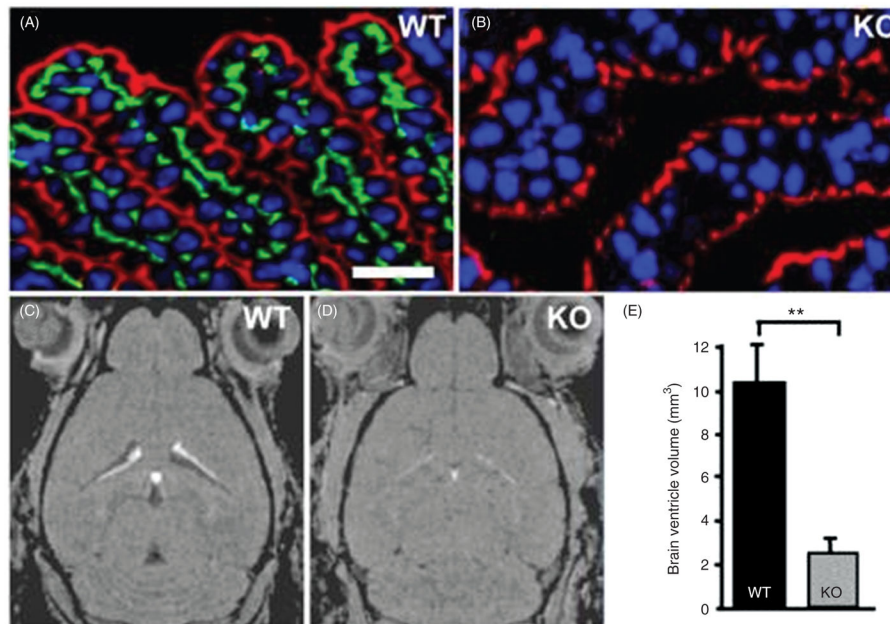
Figure 13.
NBCn2/NCBE (Slc4a10) is present in the basolateral membrane of the choroid plexus epithelial cells and Slc4a10 knockout mice have reduced decreased volume of the brain ventricles (adapted, with permission, from 143). (A) Slc4a10 (green) localizes to the basolateral, but not the apical, membrane of the choroid plexus epithelium. The apical membrane is stained for the Na^+/K^+-ATPase (red) and the blue stain is nuclei (scale bar 30 μm). (B) In Slc4a10 knockout mice no NBCn2/NCBE staining was seen. [(C) and (D)] MRI scans of mice brain show the brain ventricles. (E) The volume of the brain ventricles from knockout mice is reduced to ≈25% of the volume in wild-type mice.

Table 1

Human Na$^+$-Coupled HCO$_3^-$ Transporters

Protein	Gene	Amino acids[*]	Variants	UniProt ID
NBCe1	*SLC4A4*	1079	5	Q9Y6R1
NBCe2	*SLC4A5*	1137	8	Q9BY07
NBCn1	*SLC4A7*	1214	5	Q9Y6M7
NDCBE	*SLC4A8*	1093	7	Q2Y0W8
NBCn2/ NCBE	*SLC4A10*	1118	2	Q6U841

[*] Amino acids in canonical proteins assigned by the UniProt.

Table 2

Slc4 Knockout and Knockin Mice

Gene	Site	Method[*]	Major phenotypes	Reference
Slc4a4	Exon 9	H	Growth retardation and death before weaning Metabolic acidosis Abnormal dentition and intestinal obstruction Low plasma Na^+, hyperaldosteronism, splenomegaly No depolarization-induced alkalinization in neurons	(105, 170, 310)
	W516X (exon 11)	H	Growth retardation and death before weaning Proximal renal tubular acidosis Ocular abnormalities Anemia, volume depletion, prerenal azotemia	(199)
Slc4a5	Exon 15	R	Reduced intracerebral volume and pressure Remodeling choroid plexus epithelia	(152)
	Exon 7	H	Hypertension Metabolic acidosis Hyporeninemic hypoadosteronism	(119)
Slc4a7	Exon 5	H	Blindness and auditory impairment	(36, 202)
	Exon 1	R	Inhibition of NO-mediated vasorelaxation Resistant to hypertension development	(35)
Slc4a8	Exon 12	H	No thiazide-sensitive Na,Cl transport in collecting ducts Reduced spontaneous glutamate release in CA1 pyramidal layer Increased seizure threshold	(187, 296)
Slc4a10	Exon 12	H	Reduced ventricular volume and neuronal excitability Altered expression of other transporters in choroid plexus	(82, 143)

[*]
H: homologous recombination, R: retroviral integration.

Table 3

Amino Acid Residues in NBCe1 Identified as Sites for Ion Transport

Domain	Residue	Potential role	Reference
Nt	Arg^{298}, Glu^{91}	HCO_3^- permeation tunnel	(58)
TM1	Ser^{427}	Na^+ binding or voltage sensor	(91)
	Ala^{428}, Ala^{435}, Thr^{442}	Ion translocation in the pore	(364)
TM5	Asp^{555}	Anion selectivity	(350)
	Lys^{558}	Pore entry or DIDS-binding site	(346)
	Lys^{559}	DIDS-binding site	(203)
EL4	not specified	Electrogenicity	(62)
TM6	not specified	Functional unit	(63)
TM8	L^{750}	Ion translocation in the pore	(210)
TM12	not specified	Functional unit	(63)

Table 4

SLC4 Genetic Polymorphisms Associated with Diseases

Gene	Chrom	Total SNPs	Syn	Nonsyn	Disease-associated SNP	Disease	Reference
SLC4A4	4q21	7163	56	57	Rs72650362 A/G (exon)	reduced activity*	(346)
SLC4A5	2p13	9750	68	93	rs10177833 A/C (intron)	hypertension	(136)
SLC4A7	3p22	2357	51	73	rs7571842 A/G (intron)	hypertension	(54)
SLC4A8	12q13	2312	46	47	rs4973768 C/T (3′ UTR)	breast cancer	(7, 65, 127, 195, 201, 306)
SLC4A10	2q24	6234	46	43	rs3278 A/G (intron)	drug addiction	(142, 188, 321)
					rs13082711 C/T (5′ untream)	hypertension	(96)

*
Association with disease is not known.