Published in final edited form as:
Neuroscience. 2010 December 29; 171(4): 951–972. doi:10.1016/j.neuroscience.2010.09.037.

Na-coupled bicarbonate transporters of the Slc4 family in the nervous system: function, localization, and relevance to neurologic function

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Abstract

Many cellular processes including neuronal activity are sensitive to changes in intracellular and/or extracellular pH— both of which are regulated by acid-base transporter activity. HCO_3^--dependent transporters are particularly potent regulators of intracellular pH in neurons and astrocytes, and also contribute to the composition of the cerebrospinal fluid (CSF). The molecular physiology of HCO_3^- transporters has advanced considerably over the past ~14 years as investigators have cloned and characterized the function and localization of many Na-Coupled Bicarbonate Transporters of the Slc4 family (NCBTs). In this review, we provide an updated overview of the function and localization of NCBTs in the nervous system. Multiple NCBTs are expressed in neurons and astrocytes in various brain regions, as well as in epithelial cells of the choroid plexus. Characteristics of human patients with *SLC4* gene mutations/deletions and results from recent studies on mice with Slc4 gene disruptions highlight the functional importance of NCBTs in neuronal activity, somatosensory function, and CSF production. Furthermore, energy-deficient states (e.g., hypoxia and ischemia) lead to altered expression and activity of NCBTs. Thus, recent studies expand our understanding of the role of NCBTs in regulating the pH and ionic composition of the nervous system that can modulate neuronal activity.

Keywords

brain; electrogenic; astrocyte; genetic; neuron; pH

The regulation of intracellular pH (pH_i) is critical for proper cell function because many cellular processes are sensitive to changes in pH (Roos and Boron, 1981; Bevensee and Boron, 2007). pH-sensitive processes include fertilization (Johnson and Epel, 1976), cell

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coupling (O'Beirne *et al*., 1987; Roos and Boron, 1981), alterations in cell structure (Parton *et al*., 1991), and the activity of metabolic enzymes such as phosphofructokinase (Trivedi and Danforth, 1966). In the central nervous system (CNS), which is comprised of cells with high surface-to-volume ratios and an extracellular space that is quite tortuous, the movement of acid-base equivalents across plasma membranes (e.g., by passive diffusion and pH_i-regulating transporters) not only changes pH_i, but also has the additional consequence of changing extracellular pH (pH_o) in the opposite direction. Changes in pH_i and/or pH_o have important functional consequences in the nervous system, particularly because the activity of many voltage-sensitive and ligand-gated ion channels associated with neuronal activity are sensitive to changes in pH_i/pH_o.

The regulation and homeostasis of pH_i is controlled primarily by acid-base transporters localized at the plasma membrane. As described in more detail below, acid-base transporters are characterized as HCO_3^--dependent or -independent acid extruders or acid loaders. Na-Coupled Bicarbonate Transporters (NCBTs) in conjunction with Anion Exchangers (AEs) are encoded by *Solute carrier 4* (*Slc4*) genes, and many NCBTs including electrogenic and electroneutral Na/Bicarbonate Cotransporters (NBCs) and Na-Driven Cl-Bicarbonate Exchangers (NDCBEs) are dominant contributors to the pH physiology of cells, especially those in the nervous system. While other *Slc* genes (e.g., *Slc26*) also encode bicarbonate transporters, we restrict our review to the Slc4-encoded Na-coupled ones— primarily from vertebrates. This review in conjunction with recent reviews of Na-independent and/or -dependent Slc4 anion exchangers (Alper, 2009; Romero *et al*., 2009) and Slc26 transporters (Dorwart *et al*., 2008; Romero *et al*., 2009) provides a comprehensive overview of bicarbonate transporters.

Our understanding of the physiologic importance of NCBTs —especially in the nervous system— has expanded tremendously over the past decade following the cloning of the first NCBT cDNA encoding the electrogenic NBC from salamander kidney (Romero *et al*., 1997). Recent data from studies on various NCBT knockout mice have highlighted the importance of cation-coupled bicarbonate transporters in neurologic processes, including neuronal activity and somatosensory function. In the present review on NCBTs, we first provide a general overview of the importance of pH regulation, the energetics of proton movement and acid-base transporter activity, and the molecular physiology of the bicarbonate transporters. Next, we present both historical and contemporary information on the function and localization of NCBTs in the nervous system. Finally, we discuss the latest advances in understanding the role of NCBTs in the functioning nervous system and in energy-deficient neurologic conditions.

1) Overview of pH Regulation and NCBT Activity

A) General importance of pH regulation in the nervous system

Changes in pH can have a powerful influence on brain function, for example by altering neuronal activity and synaptic transmission. Although there are exceptions, an increase in pH_o generally stimulates neuronal firing, whereas a decrease has the opposite effect (Chesler and Kaila, 1992; Balestrino and Somjen, 1988; Ransom, 2000). In fact, an increase in pH can trigger epileptiform activity in both humans and rodents (Cohen and Kassirer, 1982; Aram and Lodge, 1987; Woodbury *et al*., 1984; Jarolimek *et al*., 1989; Lee *et al*., 1996; Marshall and Engberg, 1980; Velísek *et al*., 1994). A decrease in pH has the opposite effect. For example, lowering pH_o from 7.4 to 6.7 reversibly reduced the amplitude, increased the interval, and slowed the propagation of low extracellular Mg^{2+}-induced epileptiform discharges in combined entorhinal cortex-hippocampal slices (Velísek *et al*., 1994). Lowering pH_o to 6.2 inhibited the discharges completely. pH_o-induced increases in neuronal activity are often associated with increased activity of *N*-methyl-D-aspartate (NMDA)

receptors that display pronounced pH sensitivity in the physiologic range (Tang *et al*., 1990; Traynelis and Cull-Candy, 1990) with increased open probability at high pH.

Perhaps it is not surprising that changes in pH can alter neuronal firing in light of the myriad of ligand- and voltage-gated ion channels sensitive to changes in pH_i and/or pH_o (Traynelis, 1998; Tombaugh and Somjen, 1998). Many of these voltage-gated channels generate and propagate action potentials. pH changes presumably alter ion-channel activity by modifying electrostatic interactions between charged amino acids and inducing structural alterations. In isolated rat CA1 hippocampal neurons, a moderate extracellular acidosis (pH 6.4) depresses the peak Na^+ current by ~15%, whereas alkalosis has the opposite effect (Tombaugh and Somjen, 1996). K^+ channels are more sensitive to changes in pH_i than pH_o in the physiologic range. In general, a decrease in pH_i inhibits K channels (Byerly and Moody, 1986).

Compared to Na^+ and K^+ channels, Ca^{2+} channels are typically more sensitive to changes in pH. In neurons, an increase in pH_o reversibly enhances both high voltage-activated Ca^{2+} currents (e.g., from L-, N-, P-, Q-, and R-type Ca^{2+} channels; Tombaugh and Somjen, 1996) and low voltage-activated Ca^{2+} currents (e.g., from the T-type Ca^{2+} channel; Tombaugh and Somjen, 1997). Regarding effects of changes in pH_i, the activity of high voltage-activated Ca^{2+} channels in chick dorsal root ganglion neurons is reversibly enhanced by applying a weak base (which raises pH_i) and depressed by applying a weak acid (which lowers pH_i) (Mironov and Lux, 1991). Similar results have been obtained on rat CA1 neurons (Tombaugh and Somjen, 1997).

Channels in the nervous system that are activated by changes in pH_o can influence neurotransmission. For example, DeVries (2001) has presented evidence that transient acidification at the synaptic cleft can inhibit a presynaptic voltage-gated Ca^{2+} current in retinal cone photoreceptors due to a pH-mediated shift in the voltage dependence of the Ca^{2+} channel. Furthermore, the regulation of pH_o by horizontal cells may modulate or contribute to the feedback inhibition of these Ca^{2+} channels in the cone photoreceptors (Vessey *et al*., 2005). A change in pH is the primary stimulus for activating some channels such as acid-sensing ion channels (ASICs), which are stimulated by a decrease in pH_o (Waldmann *et al*., 1999). The importance of these pH-sensitive channels in neurotransmission is highlighted by studies on ASIC1 knockout mice that have impaired hippocampal long-term potentiation, as well as reduced excitatory postsynaptic potentials and NMDA receptor activation (Wemmie *et al*., 2002). Neurons cultured from the hippocampus of these ASIC1 knockout mice fail to elicit acid (pH 5)-evoked cation currents. The ASIC1-knockout mice also display defects in performance on the Morris water maze test and eye-blink conditioning— findings that implicate these channels in synaptic plasticity, learning, and memory.

pH changes in the brain can also regulate neurotransmission by modulating the release and/or uptake of neurotransmitters and neuromodulators. In studies performed on isolated rat-brain synaptosomes, the depolarization-induced release of total glutamate was 15% less at pH_o 6.0 than 7.4, although the uptake of glutamate was unaltered in the pH_o range 6.0–7.4 (Fedorovich *et al*., 2003). However, such pH effects are dependent on the neurotransmitter/neuromodulator. For instance, in the same study, uptake of the acetylcholine precursor choline displayed a pH_o dependence, with uptake being 80% less at pH_o 6.0 compared to 7.4. In a different study on superfused rat hypothalamic synaptosomes, both an extracellular and an intraterminal acidification stimulated ^3H-dopamine release through a Ca^{2+}-dependent exocytotic process (Cannizzaro *et al*., 2003).

B) Cellular physiology and energetics of acid-base transporters

For a cell with a resting membrane potential of −60 mV, and bathed in a pH-7.3 solution, the electrochemical gradient favors the passive entry of protons (or conjugate weak acid) into the cell, and the passive exit of weak bases such as bicarbonate out of the cell, thereby leading to a decrease in pH_i (towards the equilibrium pH of 6.3). The extent of such passive movement depends on the cell membrane's permeability to protons or acid-base species through nonspecific pathways (see review by DeCoursey, 2003), or ion channels such as GABA-activated Cl^- channels that can conduct HCO_3^- (Kaila and Voipio, 1987; Chen *et al*., 1990, 1992; Kaila *et al*., 1992). Passive H^+ movement can also occur through Hv1-type voltage-gated H^+ channels (Sasaki *et al*., 2006; Ramsey *et al*., 2006) originally described in snail neurons (Thomas and Meech, 1982), although depolarization-induced activation typically drives H^+ out of cells. Finally, the transmembrane electrochemical gradient for H^+ will favor the retention of metabolically produced H^+. Thus, most cells are continually exposed to a chronic acid load that tends to lower pH_i. Probably because so many important cellular processes are sensitive to pH (as described above), cells have evolved a system of acid-base transporters to regulate pH_i above the equilibrium pH.

Acid-base transporters are classified as acid loaders or acid extruders. Acid loaders transport protons into or base equivalents out of cells, whereas acid extruders transport those ions in the opposite direction. Acid-base transporters are further classified as being HCO_3^--independent or -dependent, and for many cells, the HCO_3^--dependent transporters are the more powerful of the two classes in regulating pH_i. NCBTs usually function as acid extruders by transporting HCO_3^- into cells and increasing pH_i, although they can also function as acid loaders. The direction of transport is determined by the transporter stoichiometry, ion gradients, and membrane potential (for an electrogenic transporter). Every NCBT is Na^+-dependent. However, as detailed in the next section, NCBTs represent a diverse family of transporters that are either electroneutral or electrogenic (with more than one $Na:HCO_3^-$ stoichiometry), chloride-independent or chloride-dependent, and stilbene-sensitive or -insensitive.

C) Molecular physiology of NCBTs

We have made significant advances in understanding the importance of NCBTs in the functional nervous system with the cDNA cloning, molecular characterization, and localization of these proteins. Below, we describe the general characteristic features of the different NCBTs and their splice variants. A more extensive review of the molecular nature of NCBTs and their splice variants is presented in Boron *et al*. (2009), as well as Romero *et al*. (2009) who also discuss the contribution of Slc4 transporters to the functioning CNS.

a) NBCe1—The first cDNA encoding a NCBT was identified by Romero *et al*. (1997) who expression cloned the electrogenic NBC (NBCe1) from the proximal tubule of the salamander kidney— a preparation in which the first NBC was functionally identified (Boron and Boulpaep, 1983). Cloned NBCe1 expressed in *Xenopus* oocytes elicits a DIDS-sensitive, Na-dependent increase in pH_i following a CO_2-induced acidification when oocytes are exposed to a CO_2/HCO_3^- solution. The electrogenicity of the transporter is evident by a hyperpolarization when oocytes are exposed to the HCO_3^- solution, and a depolarization when external Na^+ is removed (Romero *et al*., 1997). NBCe1 was subsequently cloned and identified from other preparations (Table I), including zebrafish recently (Sussman *et al*., 2009). Three splice variants of NBCe1 (−A, −B, and −C) have been identified, and these variants differ only at their N and/or C termini. A common theme among the NCBTs is alternative splicing that produces variable N and C termini that can influence transporter function and regulation, and perhaps expression (see review by Boron *et al*., 2009). For example, the N terminus of NBCe1-B and −C (but not −A) contains an autoinhibitory

domain that reduces transporter activity (McAlear *et al*., 2006). NBCe1 variants are also regulated by classic signaling pathways and protein-protein interactions (see Pushkin and Kurtz, 2006; Wu *et al*., 2009).

The importance of NBCe1 in physiologic functions is highlighted by results from studies on human patients with NBCe1 gene mutations and data from a more recent NBCe1 knockout mouse. At present, investigators have identified human patients with one of either two frameshift mutations, seven missense mutations, or three nonsense mutations in the *SLC4A4* gene. All these patients exhibit proximal renal tubule acidosis, and most of them also have ocular abnormalities, including glaucoma and/or cataract formation (Igarashi *et al*., 1999; Dinour *et al*., 2004; Horita *et al*., 2005; Demirci *et al*., 2006; Suzuki *et al*., 2010). Although neurological abnormalities are not a consistent finding in all patients, six of them experience migraines (Suzuki et al., 2010), three are mentally retarded (Igarashi *et al*., 1999; Igarashi *et al*., 2001; Horita *et al*., 2005), and two others displayed developmental disorders (Horita *et al*., 2005; Demirci *et al*., 2006). These mutations can lead to loss of NBC-mediated HCO_3^- transport by either decreasing activity of the transporter (Igarashi *et al*., 1999; Dinour *et al*., 2004; Horita *et al*., 2005) or reducing expression at the plasma membrane (Li *et al*., 2005; Horita *et al*., 2005; Toye *et al*., 2006; Suzuki *et al*., 2010).

Gawenis *et al*. (2007) developed an NBCe1 null-mutant mouse by disrupting the *Slc4a4* gene. The NBCe1 null-mutant mice, which died early before weaning, had severe metabolic acidosis, abnormalities in dentition, growth retardation, splenomegaly, hyponatremia, and impaired HCO_3^- secretion in the colon. The mice had very thin and transparent skulls due to defects in bone mineralization— an effect likely due to the metabolic acidosis. Although the null-mutant mice did not exhibit any overt neurological defects, further neurologic studies may reveal alterations in learning and memory and susceptibility to seizures. Of course, the compensatory expression of other acid-base transporters in these knockout mice during development may have masked an important role of NBCe1 in the nervous system.

b) NBCe2—From human heart and testis, Ira Kurtz's group cloned cDNAs encoding two variants of another member of the NBC family —NBCe2 or NBC4— that exhibit sequence similarity to NBCe1 (Pushkin *et al*., 2000a; Pushkin *et al*., 2000b). Subsequently, four additional NBCe2 variants were identified. The six variants (NBCe2-A to −F) differ at their C termini and/or last third of the transmembrane domain region. The A through D variants, but not E and F, are reported in the human genome database. At present, only the C variant of the cloned NBCe2s has been functional characterized and determined to be electrogenic (Virkki *et al*., 2002). Similar to the aforementioned studies on NBCe1, NBCe2-C expressed in oocytes and studied with voltage- and pH-sensitive microelectrodes elicits a DIDS-sensitive, Na-dependent increase in pH_i following a CO_2-induced acidification. The electrogenicity of the transporter is evident by a hyperpolarization when oocytes are exposed to the HCO_3^- solution, a depolarization when external Na^+ is removed, and Na^+- and HCO_3^--induced currents under voltage-clamp conditions. These electrogenic signals are blocked by DIDS. NBCe2 at the RNA and protein levels has been detected in rodent and human tissues (Table I).

c) NBCn1—cDNA encoding the transporter (NBCn1-A) was first cloned from human skeletal muscle (Pushkin *et al*., 1999a), and then three additional variants (NBCn1-B, −C, and − D) were cloned shortly thereafter from rat smooth muscle (Choi *et al*., 2000). When expressed in oocytes, NBCn1-A (originally named NBC3) stimulated Cl^--independent, DIDS-insensitive ^{22}Na uptake (Pushkin *et al*., 1999a). In subsequent pH_i experiments on NBCn1-expressing oocytes, removing external Na^+ in the presence of CO_2/HCO_3^- produced an acidification, while raising external K^+ to depolarize the membrane had no effect on pH_i. These findings are consistent with NBCn1 being electroneutral.

The electroneutrality of NBCn1 was particularly evident from microelectrode studies on oocytes expressing NBCn1-B cloned from rat smooth muscle (Choi *et al*., 2000). NBCn1-B-expressing oocytes displayed a Na^+- and HCO_3^--dependent, yet Cl^-- independent pH_i recovery following a CO_2-induced acid load. This pH_i recovery was only modestly inhibited by DIDS. While the transporter is electroneutral based on the absence of a CO_2/HCO_3^--induced hyperpolarization, a Na^+ conductance is associated with the transporter. NBCn1 is ubiquitously expressed (Table I). In additional to the aforementioned four splice variants, four additional ones have been identified. These eight variants (NBCn1-A to −H) have an identical transmembrane domain region, but different splice cassettes at their cytoplasmic N and/or C termini.

Bok *et al*. (2003) have developed an NBCn1 knockout mouse that is blind and displays defects in hearing. Details of this study are presented in a subsequent section.

d) NBCn2 (NCBE)—cDNA encoding the transporter was first cloned from an insulin-secreting cell line MIN6 cDNA library and called a Na-driven Chloride/Bicarbonate Exchanger (NCBE) (Wang *et al*., 2000). Based on $^{22}Na^+$, $^{36}Cl^-$, and pH_i studies with oocytes or HEK293 cells heterologously expressing the transporter, the authors concluded that the transporter exchanges extracellular Na^+ and HCO_3^- for intracellular Cl^- and H^+. However, the observations reported in the study that removing external Cl^- inhibited both $^{22}Na^+$ uptake and $^{36}Cl^-$ efflux in NCBE-expressing oocytes (which are difficult to Cl^- deplete) are opposite to that predicted for a functional Na-driven Cl-HCO_3 exchanger. Furthermore, according to a study with voltage- and pH-sensitive microelectrodes, the transporter expressed in oocytes is electroneutral and responsible for a DIDS-sensitive, Na^+- and HCO_3^--dependent, but Cl^--independent pH_i recovery following a CO_2-induced acidification (Parker *et al*., 2008a). Using both Cl^--sensitive microelectrodes to measure surface Cl^- transients and $^{36}Cl^-$ to monitor Cl^- efflux, the authors determined that Cl^- flux across the membrane is not coupled to Na^+ and HCO_3^- transport, but rather, is linked to CO_2/HCO_3^--stimulated, but Na^+-independent, Cl^- self exchange (Parker *et al*., 2008a). Interestingly, the group did observe that NBCn2 (NCBE) is capable of NDCBE-like activity in the absence of extracellular Cl^-.

The function of NBCn2 (NCBE) may also be cell-type dependent. Giffard *et al*. (2003) presented evidence that two NBCn2 (NCBE) splice variants (−B and −C) expressed in NIH 3T3 cells display external Na^+, Cl^-, and HCO_3^--dependent pH_i recoveries from acid loads. In a more extensive analysis, Damkier *et al*. (2010) recently reported that NBCn2 (NCBE) functions as a Na-driven Cl-HCO_3 exchanger when expressed in NIH-3T3 fibroblasts. The transporter exhibited a DIDS-sensitive, HCO_3^--dependent pH_i increase elicited by returning external Na^+ following an acid load. In parallel experiments, returning external Na^+ stimulated a DIDS-sensitive, HCO_3^--dependent $^{36}Cl^-$ efflux, but no HCO_3^--dependent $^{36}Cl^-$ influx.

Four splice variants (NBCn2 (NCBE)-A through −D) have been identified with different internal cytoplasmic N terminal splice cassettes and/or different cytoplasmic C termini. NBCn2 (NCBE) displays pronounced expression in brain (Table I), and the −B and −C variants were cloned from rat brain (Giffard *et al*., 2003). Jacobs *et al*. (2008) developed an NBCn2 (NCBE) knockout mouse that displays a high seizure threshold and a reduced brain ventricular volume. Details of the study are described in a subsequent section.

e) NDCBE/NDAE—The first cDNA encoding a Na-Driven Anion Exchanger (NDAE) was identified from *Drosophila*, and the protein expressed in oocytes mediates DIDS-sensitive transport of Na^+, Cl^-, and H^+/HCO_3^- (Romero *et al*., 2000). However, NDAE does not have an absolute requirement for HCO_3^- and exhibits some electrogenicity. Shortly

thereafter, a cDNA encoding a related Na-Driven Cl-Bicarbonate Exchanger (NDCBE) was cloned from human brain (Grichtchenko *et al*., 2001). In functional studies using voltage-, Na^+-, and pH-sensitive microelectrodes, as well as a $^{36}Cl^-$ efflux assay, NDCBE expressed in oocytes induced a DIDS-sensitive, Na^+- and HCO_3^--dependent pH_i recovery from a CO_2-induced acidification. Na^+ and HCO_3^- efflux from oocytes mediated by NDCBE required extracellular Cl^-. Also, CO_2/HCO_3^--induced stimulation of the transporter elicited a DIDS-sensitive and Na^+-dependent increase in $^{36}Cl^-$ efflux, as well as a DIDS-sensitive increase in intracellular [Na^+]. Four variants of NDCBE have been identified that differ at the cytoplasmic N and/or C termini. Similar to NBCn2 (NCBE), NDCBE also exhibits pronounced expression in brain (Table I).

On a related note, the cloned cDNA encoding the anion bicarbonate transporter ABTS-1 from *C. elegans* is ~50% identical to the cDNA encoding NDCBE (Sherman *et al*., 2005). ABTS-1 heterologously expressed in *Xenopus* oocytes exhibits Cl-HCO_3 exchanger activity.

2) Functional Evidence for NCBTs in the Nervous System

A) Neurons

The Na-driven Cl-HCO_3 exchanger was the first pH_i-regulating transporter functionally identified and shown to be the major acid extruding mechanism in classic neuronal preparations including the squid giant axon (Boron and De Weer, 1976a; Boron and De Weer, 1976b; Russell and Boron, 1976; Russell and Boron, 1981; Boron and Russell, 1983) and the snail neuron (Thomas, 1976a; Thomas, 1976b; Thomas, 1977). Work on these two preparations was performed simultaneously, but independently, by Walter Boron and Roger Thomas— two pioneers in the field of pH_i regulation.

In both preparations, the recovery of pH from an acid load required external HCO_3^- (Boron and De Weer, 1976a; Thomas, 1977), external Na^+ (Thomas, 1977; Boron and Russell, 1983), intracellular Cl^- (Russell and Boron, 1976; Thomas, 1977), and intracellular ATP (Russell and Boron, 1976; Boron and Russell, 1983). The transporter was also inhibited by SITS (Russell and Boron, 1976; Boron and Russell, 1983; Thomas, 1977). Based on pH_i measurements, as well as $^{22}Na^+$ and $^{36}Cl^-$ efflux/influx data, the transporter moves 1 Na^+ and 2 HCO_3^- (or equivalent species) in one direction and 1 Cl^- in the opposite direction (Boron and Russell, 1983). The acid extrusion rate was pH_i dependent and stimulated by a decrease in pH_i (Boron and Russell, 1983). In the squid axon, intra-axonal ATP is required for a functional transporter, although not as an energy source (Boron *et al*, 1988) because the ATP effect can be mimicked by non-hydrolysable ATPγS. The transporter's K_M for ATP is 124 μM (Davis *et al*, 2008).

The Na-driven Cl-HCO_3 exchanger also appears to be the major HCO_3^- -dependent acid extruder in mammalian neurons. In CA1 neurons acutely isolated from the hippocampus of neonatal rats, applying CO_2/HCO_3^- elicited a pH_i recovery from the initial CO_2-induced acidification that was Na^+-dependent and DIDS-sensitive (Schwiening and Boron, 1994). Cl^- dependence of the transporter was evident by progressive decreases in the rate and magnitude of HCO_3^--induced pH_i increases when the neurons were exposed several times to the CO_2/HCO_3^- solution in the continued absence of external Cl^- (Fig. 1, *gh vs. de vs. ab*). The transporter appears to slow itself down by transporting Cl^- out of the cell and lowering intracellular Cl^-, which can not be replenished in the absence of external Cl^-. This slowing down could also be explained by a Na/HCO_3 cotransporter that functions like a Na-driven Cl-HCO_3 exchanger in the absence of external Cl^-. In additional studies, results from pH_i experiments implicated the presence of a Na-driven Cl-HCO_3 exchanger in other mammalian neurons (see Bevensee and Boron, 1998).

According to early studies, electroneutral NCBTs are the dominant acid extruding bicarbonate transporters in neurons (see below). However, more recent data support the function and expression of an electrogenic NBC in cultured mammalian neurons. For example, in pH_i experiments on cultured rat hippocampal neurons, removing external Na^+ elicited a pH_i decrease that was partially due to a HCO_3^--dependent, Cl^--independent process sensitive to niflumic acid (Williams *et al*., unpublished data). In whole-cell and perforated patch-clamp experiments on the same preparation, applying a CO_2/HCO_3^- solution elicited a Na^+-dependent outward current, whereas removing external Na^+ in the presence of CO_2/HCO_3^- induced an inward current in a population of the neurons (Majumdar *et al*., unpublished data). Further studies, particularly with NCBT inhibitors, are required to identify and characterize this presumed electrogenic NBC in the hippocampal neurons.

As discussed in the localization section below, using immunohistochemical techniques, Cooper *et al*. (2005) reported the expression of NBCn1 in the synapses and somatodendrites in embryonic rat hippocampal neurons. They proposed that the possible role of NBCn1 is to regulate pH_i at the synapse and therefore modulate neuronal activity.

B) Glia

Boron and Boulpaep (1983) working on perfused proximal tubules from the salamander kidney functionally identified an electrogenic NBC as the first cation-coupled HCO_3^- transporter. The basolateral transporter was responsible for a SITS-sensitive decrease in both pH_i and intracellular Na^+ activity (aNa^+_i), as well as a depolarization of the basolateral membrane upon lowering basolateral pH or HCO_3^-. Removing Cl^- had no effect on these changes in pH_i or basolateral membrane potential. The electrogenicity of the transporter was particularly evident from the following now-recognized hallmark observation: lowering/removing external Na^+ elicited a simultaneous depolarization and decrease in pH_i. This transporter in basolateral membrane vesicles from rabbit kidney cortex has a 1:3 Na^+:HCO_3^- stoichiometry based on a thermodynamic analysis (Soleimani *et al*., 1987).

In the CNS, an electrogenic NBC was first characterized by Deitmer and Schlue working on the giant neuropile glial cells of the leech (Deitmer and Schlue, 1987; Deitmer and Schlue, 1989; Deitmer, 1992). The transporter was responsible for a CO_2/HCO_3^--induced alkalization and hyperpolarization that was DIDS-sensitive and Na^+-dependent (Deitmer and Schlue, 1989). The HCO_3^--induced alkalization was not affected by either removing extracellular Cl^- or depleting the cells of intracellular Cl^-. The HCO_3^- solution also elicited an expected increase in intracellular Na^+. The electrogenic nature of the transporter was also evident from the following simultaneous, HCO_3^--dependent changes elicited by removing external Na^+ (Fig. 2): a pronounced depolarization (top trace), a decrease in pH_i (middle trace), and a faster decrease in aNa^+_i than seen in the nominal absence of HCO_3^- (bottom trace) (Deitmer, 1992). In leech glial cells, the transporter appears to have a stoichiometry of 1:2 Na:HCO_3^- (Deitmer and Schlue, 1989; Deitmer, 1991).

Using similar approaches, the transporter was subsequently identified and/or studied in other glial cell preparations including connective glial cells from leech (Szatkowski and Schlue, 1992), astrocytes from the *Necturus* optic nerve (Astion and Orkand, 1988), as well as mammalian astrocytes cultured from rat forebrain (Boyarsky *et al*., 1993), hippocampus (Bevensee *et al*., 1997a; Bevensee *et al*., 1997b; Pappas and Ransom, 1994), cerebellum (Brune *et al*., 1994) and cortex (Shrode and Putnam, 1994). In at least rat hippocampal astrocytes, the mammalian electrogenic NBC appears to have a 1:2 Na^+:HCO_3^- stoichiometry (Bevensee *et al*., 1997a; Bevensee *et al*., 1997b).

Electrogenic NBC activity has also been functionally characterized in mammalian brain slices. In gliotic hippocampal slices, electrogenic NBC activity contributes to a depolarization-induced alkalization and an extracellular acid shift (Grichtchenko and Chesler, 1994a; Grichtchenko and Chesler, 1994b). Applying Ba^{2+} or raising extracellular K^+ produces a Na^+-dependent intracellular alkalization and an extracellular acidification that are both enhanced in the presence of CO_2/HCO_3^-. In contrast to results from *in vitro* studies on cultured astrocytes, the *in situ* NBC is not sensitive to stilbene derivatives.

Groups have reported evidence for Na-driven Cl-HCO_3 exchanger activity in astrocytes, including those cultured from rat brain (Mellergård *et al*., 1993; Shrode and Putnam, 1994). For example, in rat astrocyte and C6 glioma cell cultures, exposing cells to a CO_2/HCO_3^- solution elicited a pH_i recovery following the initial CO_2-induced acidification. This HCO_3^--induced pH_i recovery was dependent on external Na^+, blocked by DIDS, and inhibited by incubating the cells for 2 h in the absence of external Cl^- (Shrode and Putnam, 1994).

There is evidence for a Na^+- and HCO_3^--dependent, and perhaps Cl^-- independent transporter in cultured oligodendrocytes from embryonic mouse spinal cord (Kettenmann and Schlue, 1988) and adult rat cerebellum (Boussouf *et al*., 1997). However, the electrogenicity/electroneutrality of an oligodendrocyte NBC has yet to be determined. Support for a functional electrogenic NBC in oligodendrocytes comes from immunolocalization data in which an NBCe1-A/B antibody labels dendrites of oligodendrocytes cultured from mouse and rat (Ro and Carson, 2004).

C) Epithelial cells of choroid plexus

As reviewed in more detail by McAlear and Bevensee (2003), according to early studies on pH-regulating transporters in the choroid epithelium, a Na-H exchanger and an anion-exchanger (AE) on the basolateral membrane in conjunction with a possible NBC or HCO_3^--conducting anion channel on the apical membrane contribute to both the ionic composition and pH of the cerebrospinal fluid (CSF). According to more recent functional data, multiple Na-coupled HCO_3^- transporters appear to be involved.

Using the pH-sensitive dye BCECF, Bouzinova *et al*. (2005) measured pH_i of epithelial cells from the rat choroid plexus to characterize HCO_3^- transporters involved in both the pH_i recovery from an acid load and the pH_i increase elicited by applying CO_2/HCO_3^- at resting pH_i in the nominal absence of the physiologic buffer. In both assays, the HCO_3^--induced pH_i increase was Na^+ dependent, but only the pH_i increase elicited by applying CO_2/HCO_3^- at resting pH_i was inhibited by DIDS. Cl^--free media had no effect on the pH_i recoveries. In light of additional immunologic evidence presented that the cells express NBCe2, as well as previous studies demonstrating the expression of NBCn1 and NBCn2 (NCBE) (see below), the authors conclude that rat choroid plexus epithelial cells have both a DIDS-insensitive NCBT (probably NBCn1) and a DIDS-sensitive NCBT (probably either NBCe2 and/or NBCn2 (NCBE)). More recently, Chen *et al*. (2008b) have localized NDCBE to the basolateral membrane of the choroid plexus of fetal (but not adult) rats.

In a recent functional study, Millar and Brown (2008) measured small DIDS-sensitive, Na^+-dependent, HCO_3^--induced outward currents from I–V plots obtained from mouse choroid plexus epithelial cells. From the reversal potentials of these I–V plots, the authors concluded that the NBC —which may be NBCe2 according to the previous discussion— has a 1:3 Na^+:HCO_3^- stoichiometry. With the membrane potential of the choroid plexus epithelial cell estimated to be −35 mV to −60 mV, this NBC would be expected to mediate HCO_3^- secretion into the CSF given the Na^+ and HCO_3^- gradients across the apical membrane.

3) Localization of NCBTs in the Nervous System

A) Electrogenic Na/HCO_3 cotransporter, NBCe1

NBCe1 was the first NCBT localized in the CNS—not surprising because it was the first NCBT cloned and subsequently used to generate molecular probes including PCR primers, cRNA probes, and antibodies. Schmitt *et al*. (2000) and Giffard *et al*. (2000) performed the early NBCe1 localization studies on rat brain using antibodies and polynucleotide probes—nearly all of which did not distinguish among the NBCe1 splice variants. Both groups found wide-spread expression of NBCe1 throughout the brain in a pattern consistent with glial-cell expression. According to double-labeling studies using an antibody to the glial fibrillary acidic protein (GFAP) and an *in situ* hybridization probe to NBCe1, the transporter was evident in astrocytes and Bergmann glia in the cerebellum (Giffard *et al*., 2000). Schmitt *et al*. (2000) performed *in situ* hybridization co-localization studies with probes to the astrocytic glutamate transporter 1 (GLT1) and NBCe1. The authors observed NBCe1 mRNA expression in the astrocytes of the cortex, dentate gyrus, and brainstem. In double-labeling studies using antibodies to GFAP and NBCe1, the authors found expression of NBCe1 in astrocytes in hippocampus and cerebellum. NBCe1 mRNA was also evident in neurons of the piriform and entorrhinal cortex, cerebellum, olfactory bulb, dentate gyrus and striatum. Using an antibody to the microtubule associated protein 2 (MAP2), a neuronal marker, Schmitt *et al*. also found NBCe1 expression in Purkinje neurons of the cerebellum, as well as neurons in the dentate gyrus, pyramidal and molecular cell layers, and stratum oriens of the hippocampus.

NBCe1 has also been identified in human brain. Using human NBCe1 primers that did not discriminate among the NBCe1 splice variants, Damkier *et al*. (2007) found NBCe1 mRNA in human cerebellum, cerebrum, hippocampus, and choroid plexus.

As mentioned earlier, NBCe1 has three splice variants. Recently, Rickmann *et al*. (2007) reported on the expression of NBCe1-A and B in mouse brain using immunohistochemistry and immunoelectron microscopy. The authors found expression of NBCe1-A throughout brain, particularly in neuronal populations in the cerebellum, hippocampus, cerebral cortex, and olfactory bulb. In contrast, they reported the expression of NBCe1-B in astroglia and Bergmann glia. The results from studies by Rickmann *et al*. were different from earlier cloning/localization results in three ways. First, the cDNA encoding the A variant of NBCe1 has not been identified in a homology cloning study of NBCe1 from rodent brain (Bevensee *et al*., 2000). Second, Giffard *et al*. (2000) reported preliminary data that an *in situ* hybridization probe specific to the A variant did not appreciably label cells in rat brain. Finally, the antibody used by Rickmann *et al*. to localize NBCe1-B is also expected to recognize the C variant.

Using mRNA and protein localization techniques, Majumdar *et al*. (2008) examined the presence and expression profiles of the three NBCe1 variants in rat brain. According to *in situ* hybridization data, NBCe1-B and likely −C (rather than −A) are the predominant variants expressed in rat brain, with high levels in the cerebellum and hippocampus. Using immunolabeling techniques and antibodies to neuronal and astrocytic markers, the authors found *i*) diffuse labeling throughout the brain with antibodies to the A/B variant and C variant, and *ii*) evidence that NBCe1-B is localized intracellularly in neurons (e.g., in the hippocampus and cortex), whereas NBCe1-C is expressed at the plasma membrane in glia surrounding neurons and possibly neurons themselves (e.g., in pyramidal and granule layers of the hippocampus, the Purkinje layer of the cerebellum, and the cortex.)

NBCe1 mRNA and protein levels are developmentally regulated. In the aforementioned *in situ* hybridization study on rat brain, Giffard *et al*. (2000) first detected NBC mRNA at

embryonic day-17 (E17) in the spinal cord. In rat forebrain, NBC mRNA was first evident at postnatal day-0 (P0). The authors found that NBC mRNA persisted into adulthood and the level was highest at P15. Using immunoblotting techniques with a nonspecific NBCe1 antibody, Douglas *et al*. (2001) found that NBCe1 expression increases gradually from E16 to P105 in the cerebral cortex, cerebellum, and brain-stem diencephalon, but to a much lesser degree in the cortex.

Sussman *et al*. (2009) recently cloned by homology the cDNA encoding NBCe1 from zebrafish (zNBCe1), which is most similar to mammalian NBCe1-B. By 72 h post fertilization, zNBCe1 transcript was detected in brain, ependymal cells lining the brain ventricles, and the inner nuclear layer of the retina. In the adult zebrafish, zNBCe1 protein was found in the retina, particularly the ganglion cell layer and the photoreceptor layer.

B) Electrogenic Na/HCO_3 cotransporter, NBCe2

NBCe2 mRNA in brain has been localized to rat and mouse choroid plexus epithelial cells (Praetorius *et al*., 2004b). In immunofluorescence studies with an antibody to the N-terminal 22 residues of NBCe2, Bouzinova *et al*. (2005) identified expression in the apical membrane of mouse and rat choroid plexus. Such apical expression was reinforced by results from electron microscopy studies with immunogold labeling of the apical microvillar projections from the mouse choroid plexus. Interesting, in a study by Praetorius and Nielsen (2006), the authors did not detect NBCe2 in human choroid plexus. The specific variant of NBCe2 expressed in rodent choroid plexus is not known.

NBCe2 is also present in human brain. Using RT-PCR and human NBCe2 primers that are expected to amplify all splice variants of NBCe2 cDNA, Damkier *et al*. (2007) found mRNA expression in human cerebellum, cerebrum, hippocampus, and choroid plexus.

C) Electroneutral Na/HCO_3 cotransporter, NBCn1

In a preliminary study, Risso Bradley *et al*. (2001) used immunohistochemistry and found NBCn1 expression in nerve fibers of the hippocampal slice. In a more recent study, Cooper *et al*. (2005) used RT-PCR techniques and found NBCn1-B in hippocampal neurons cultured from embryonic rats and NBCn1-E in neurons cultured from adult rat hippocampal slices. According to single-cell RT-PCR data with primers to NBCn1, glutamic acid decarboxylase 65 (GAD65), and GAD67, the authors found that NBCn1-B was present in both excitatory (GAD-negative) and inhibitory (GAD-positive) embryonic neurons. In immunocytochemistry studies, the authors found expression of NBCn1 at the plasma membrane of both the soma and dendrites of embryonic neurons. According to double-labeling antibody studies, NBCn1 partially colocalized with post synaptic density protein-95 (PSD-95) at excitatory synapses. More recently, the authors have generated new NBCn1 antibodies and identified NBCn1 expression in neurons in many regions of rat brain, including hippocampal pyramidal neurons, dendate gyrus granular neurons, posterior cortical neurons, and cerebellar Purkinje neurons, as well as in the basolateral membrane of the choroid plexus epithelia (Park *et al*., 2010). NBCn1 is expressed at the soma and dendrites of CA3 neurons, and partially colocalizes with PSD-95 in the dendrites.

Boedtkjer *et al*. (2008) used an antibody-independent technique to perform a more systemic analysis of NBCn1 localization in mouse brain. This immunoreactive-independent technique involves generating a transgenic mouse with the LacZ gene under the control of the NBCn1 promoter. A colorometric product of β-galactosidase activity can be used as a marker for NBCn1 transcriptional activity. In brain, β-galactosidase staining was seen in several regions including the cortex, hippocampus, cerebellum, and epithelial cells of the choroid plexus. Staining was evident in all the layers of the hippocampus including the pyramidal cells

layers and the dentate gyrus. Staining was also evident in the dentate nucleus and cortical Purkinje cells of the cerebellum. NBCn1 mRNA has also been detected in the cerebrum and cerebellum of rat, and the choroid plexus of both mouse and rat choroid plexus (Praetorius *et al*., 2004b). Based on immunohistochemistry, the authors reported NBCn1 expression in the basolateral membranes of the epithelial cells of both rat and mouse choroid plexus.

NBCn1 is also present in human brain. Using RT-PCR and human NBCn1 primers that are expected to amplify all splice variants of NBCn1 cDNA, Damkier *et al*. (2007) found mRNA expression in human cerebellum, cerebrum, hippocampus, and choroid plexus.

Chen *et al*. subsequently reported the developmental expression of NBCn1 in cortex, hippocampus, subcortex, and cerebellum of postnatal day 16 (P16), P30, P104, and P118 mice. NBCn1-B expression was highest in cortex and hippocampus of P16 mice (Chen *et al*., 2007). Postnatal changes in NBCn1-B expression were brain region dependent. For example, NBCn1-B expression with age gradually increased in the hippocampus, decreased in the cortex, and remained the same in the subcortex and cerebellum. Changes in NBCn1 expression may be required for proper development of the various brain regions, and may highlight developmental changes in the pH physiology of the regions.

D) Electroneutral Na/HCO_3 cotransporter, NBCn2 (NCBE)

In addition to NBCe2 as described above, NBCn2 (NCBE) is also present in the epithelial cells of the choroid plexus (Praetorius *et al*., 2004b). In RT-PCR studies, Praetorius *et al*. detected NBCn2 (NCBE) mRNA in the cerebrum and cerebellum of rat, and the choroid plexus of both mouse and rat. In immunochemical studies on the choroid plexus, NBCn2 (NCBE)-B protein was evident on the basolateral membrane of the epithelial cells.

Giffard *et al*. (2003) cloned two NBCn2 (NCBE) variants from rat brain: rb1NCBE (NBCn2 (NCBE)-B) and rb2NCBE (NBCn2 (NCBE)-C). Subsequently, they reported developmental and regional mRNA expression patterns of NBCn2 (NCBE) in rat and mouse brain. NBCn2 (NCBE) mRNA in rat brain was evident from Northern blot analysis. According to *in situ* hybridization data, NBCn2 (NCBE)-B mRNA expression was widespread in the brain and spinal cord of rats as early as E19, and such expression persisted until adulthood. At higher magnifications, NBCn2 (NCBE)-B mRNA was detected in the Purkinje cells of the cerebellum and CA3 principal neurons of the hippocampus. According to results from RT-PCR studies, mRNA expression of the C variant was greater than that of the B variant in mouse cultured astrocytes, whereas both variant mRNAs expressed similarly in mouse cultured neurons. The authors report that the C variant compared to B variant mRNA was greater in cortex, striatum, and hippocampus of mouse brain, while the reverse was true for these regions from rat brain. To examine the cellular expression profiles of the two variants in mouse brain, the authors transfected cultured mouse astrocytes with retroviral vectors encoded FLAG-tagged NBCn2 (NCBE) constructs and subsequently performed immunocytochemistry with anti-FLAG antibodies. Although both variants were expressed at the plasma membrane, the B variant was more localized to intracellular vesicles, whereas the C variant was more localized to the actin cytoskeleton.

NBCn2 (NCBE) is also present in human brain. Using RT-PCR and human NBCn2 (NCBE) primers that are expected to recognize all splice variants of NBCn2 (NCBE) cDNA, Damkier *et al*. (2007) found higher NBCn2 (NCBE) mRNA levels in human cerebrum, hippocampus, and choroid plexus than in the cerebellum.

Using immunoblotting techniques and a polyclonal antibody that recognizes the N-terminal 135 amino acids of NBCn2 (NCBE), Chen *et al*. (2008c) found abundant expression of NBCn2 (NCBE) in both rat and mouse hippocampus, cerebellum, cerebral cortex, and

subcortex to a lesser extent. Interestingly, NBCn2 (NCBE) expression was evident in neurons cultured from rat hippocampus or freshly dissociated from mouse hippocampus, but not dissociated astrocytes from mouse. NBCn2 (NCBE) was localized to both the plasma membrane of the soma and the processes of cultured rat hippocampal neurons. In agreement with the aforementioned results by Praetorius *et al*. (2004b), where the authors detected NBCn2 (NCBE) expression in the basolateral membranes of choroid plexus, Chen *et al*. found basolateral expression of NBCn2 (NCBE) in the choroid plexus from E18 and adult rats.

Similar to the results by Chen *et al*. (2008c), Jacobs *et al*. (2008) used a different polyclonal antibody and found NBCn2 (NCBE) protein in lysates from mouse brain, as well as mixed neuron/glia cultures, but not pure glia cultures. In immunohistochemical studies, the authors observed broad NBCn2 (NCBE) expression in cortex, cerebellum, and olfactory bulb. Based on results from colocalization immunohistochemical studies with antibodies to cell-specific proteins, NBCn2 (NCBE) expression was evident in the CA3 layer of the hippocampus, some GAD-positive interneurons in the neocortex, and parvalbumin-positive Purkinje neurons in the cerebellum (but not interneurons in the molecular layer). Based on immunochemical results with three antibodies, all four NBCn2 (NCBE) variants (−A through −D) appear to be expressed in mouse CNS. The A variant appears to be the dominant one throughout mouse brain, whereas the D variant is mainly expressed in the subcortex and medulla (Liu *et al*., 2010).

In a developmental study using *in situ* hybridization techniques, Hübner *et al*. (2004) first detected NBCn2 (NCBE) mRNA as early as E12.5 in mouse brain regions, including cerebellum and cortex. mRNA levels increased from E15.5 to E18.5 in hippocampus, and from E12.5 to E18.5 in cortex. The transcript expression followed a neuronal pattern and was not detected in large fiber tracts. mRNA was found in the neuronal cell layer of the retina, the retinal pigment epithelium, as well as in certain regions of the central auditory pathway including the geniculate nucleus and auditory brain stem nuclei. NBCn2 (NCBE) mRNA was also detected in the choroid plexus as expected from the aforementioned studies.

Chen *et al*. (2007) used immunoblotting to examine and compare expression levels of NBCn2 (NCBE) in the cortex, cerebellum, hippocampus, and subcortex of mouse brain with development from P16 to P118. In general, expression was highest in the cortex and hippocampus at the different age groups, and expression in any given region did not change with age. This developmental expression profile is different than with NBCn1, which displays increased levels with age (Chen *et al*., 2007).

E) Electroneutral Na-driven Cl-HCO_3 exchanger, NDCBE/NDAE

According to in-situ and immunohistochemical studies, NDAE is present in the central and peripheral nervous systems, as well as dorsal and Bolwig's sensory organs in the developing *Drosophila* (Romero, *et al*., 2000; Sciortino *et al*., 2001).

Regarding NDCBE, Risso Bradley *et al*. (2001) in a preliminary immunohistochemistry study reported NDCBE expression in the soma and dendrites of rat cerebellar Purkinje neurons. In a subsequent RNA study on rat brain, Praetorius *et al*. (2004b) used RT-PCR techniques and found NDCBE mRNA in cerebrum and cerebellum, but not in the choroid plexus.

In a more extensive protein study, Chen *et al*. (2008b) recently used immunochemical approaches and an antibody to the N-terminus of human NDCBE to localize protein expression in mouse and rat brain. In immunoblotting studies, the authors assessed NDCBE expression in membrane proteins from the cortex, subcortex, cerebellum, and hippocampus

from mouse brain. NDCBE expression was higher in the cortex, subcortex, and cerebellum compared to the hippocampus. According to results from immunohistochemistry studies, NDCBE is widely distributed in mouse and /or rat brain, with particular expression in cortex, hippocampus, cerebellum, substantia nigra, brainstem, and olfactory bulb. In further studies with cell-specific antibodies, the authors found that NDCBE is expressed more so in neurons than astrocytes in the hippocampus and cerebellum of mouse, and neuronal expression is evident in both the soma and processes. Similar findings were obtained with neurons and astrocytes cultured from rat/mouse hippocampus. NDCBE protein was detected in the basolateral membrane of choroid plexus epithelial cells from E18, but not adult rat brains (Chen *et al*., 2008b).

NDCBE is also expressed in human brain. By RT-PCR, Damkier *et al*. (2007) found NDCBE mRNA expression in human cerebrum, hippocampus, and choroid plexus. In addition, in immunohistochemistry studies using an antibody that is expected to recognize both NDCBE-A and −B, the authors found expression in pyramidal cells of human hippocampus.

3) Role of NCBTs in Nervous System Function

A) General neuronal excitability

a) Neuron-glia interactions—As discussed above, a decrease in pH_o typically inhibits neuronal firing, whereas an increase in pH_o typically stimulates activity. Repetitive neuronal firing or depolarization as seen in spreading depression or epilepsy is often associated with a decrease in pH_i. As mentioned earlier, a predominant acid-extrusion mechanism in astrocytes is an electrogenic NBC. Because an electrogenic NBC when active alters both pH_i and the pH_o by moving HCO_3^- across the plasma membrane of cells, this transporter links neuronal activity with changes in pH. According to a classic model proposed by Chesler (1990) and Ransom (1992), an electrogenic NBC in astrocytes may actually modulate neuronal excitability through such changes in pH. When a neuron fires an action potential, there is an increase in extracellular K^+ (K^+_o) that depolarizes neighboring astrocytes. This depolarization leads to stimulation of an electrogenic NBC activity in astrocytes and transport of Na^+, HCO_3^- and net-negative charge into the cells. The ensuing decrease in pH_o tends to dampen further neuronal activity by inhibiting many pH-sensitive voltage- and ligand-gated channels. This negative-feedback model is predicted to be neuroprotective under pathophysiological conditions associated with repetitive and excessive neuronal firing such as with spreading depression and epilepsy.

The direction of HCO_3^- transport will be influenced by the transporter's stoichiometry, as well as the membrane potential (V_m) of the NCBT-expressing brain cell if the transporter is electrogenic. For an electrogenic NBC transporting net-negative charge and expressed in a typical cell with a negative V_m, the chemical gradient favors NBC-mediated HCO_3^- influx. However, the electrical driving force favors NBC-mediated HCO_3 efflux, which will be enhanced by an NBC that moves more net-negative charges per transport cycle (i.e., has a Na :HCO_3 stoichiometry of 1:3 *vs*. 1:2), and by a more negative membrane potential. For an electrogenic NBC with a 1:2 Na^+:HCO_3^- stoichiometry, the electrochemical gradient would likely favor the transport of HCO_3^- into a neuron or astrocyte with either a typical resting V_m or a depolarized V_m (e.g., during an action potential), thereby decreasing pH_o and inhibiting neuronal activity. However, for an electrogenic NBC with a 1:3 Na^+:HCO_3^- stoichiometry, the electrochemical gradient would likely favor the transport of HCO_3^- out of a neuron or astrocyte at a typical resting V_m, thereby increasing pH_o and promoting neuronal activity.

In addition to regulating pH and modulating neuronal activity, an electrogenic NBC may also contribute to other changes in the extracellular space (ECS) associated with neuronal activity. As mentioned by Østby *et al*. (2009), neuronal stimulation causes a ~30% shrinkage of the ECS in brain. To examine the impact of specific pathways involved in such shrinkage, the authors generated five models that incorporated the following traditional ion/water pathways: both the efflux of K^+ out of and the influx of Na^+ into firing neurons, the influx of Na^+, K^+, and Cl^- via channels into astrocytes, the influx of K^+ and the efflux of Na^+ via the Na-pump into astrocytes, and the osmotic movement of water into/out of astrocytes. In addition, the authors also evaluated the impact of two additional transporter-mediated pathways: *i*) the influx of Na^+ and HCO_3^- via the NBC into astrocytes, and *ii*) the influx of Na^+, K^+, and Cl^- via the Na/K/Cl cotransporter 1 (NKCC1) into astrocytes. Although shrinkage could be reasonably well modeled in the absence of these additional transporter-mediated pathways, their presence provided better, more physiologically relevant results in agreement with the literature. Thus, an astrocytic NBC may act in concert with NKCC1 and additional ion/water channels to mediate neuronal activity-evoked decreases in the volume of the ECS.

b) NBCn2 (NCBE) knockout mouse— elevated seizure threshold—Recent knockout studies have highlighted the functional importance of NCBTs in the nervous system. Jacobs *et al*. (2008) developed an NBCn2 (NCBE) knockout mouse by targeted disruption of exon 12 of *Slc4a10* gene. The knockout mice have a high seizure threshold and exhibit delayed onset of proconvulsive- and hyperthermia-induced seizures. A larger number of knockout *vs*. wild-type mice survive lethal doses of proconvulsants. In measuring pH_i with fluorescence imaging and the pH-sensitive dye BCECF, the authors found that CA3 pyramidal neurons from the knockout mice displayed slower pH_i recoveries from propionate-induced acid loads than neurons from the wild-type mice (Fig. 3A). The slower pH_i recoveries in the knockout mice correlated with propionate-induced decreases in the frequency of 4-aminopyridine (4-AP)-elicited interictal events in the CA3 region (Fig. 3B). Furthermore, in the continued presence of propionate, the frequency of interictal-like events in this region recovered to control levels in slices from the wild-type mice, but not the knockout mice. The lack of frequency recovery correlated with the incomplete pH_i recovery in neurons from the knockout mice. Thus, NBCn2 (NCBE)-mediated pH_i regulation appears to influence excitability of at least the CA3 region.

Interestingly, in contrast to results from the knockout mice studies by Jacobs *et al*. (2008) described above, disruption of the *SLC4A10* gene in a human patient is associated with partial complex epilepsy, in addition to loss of cognitive function, and moderate mental retardation (Gurnett *et al*., 2008). However, this disruption resulted from a chromosomal translocation with multiple unidentified genes being affected. Therefore the role of NBCn2 (NCBE) in epilepsy and seizure disorders has yet to be fully elucidated.

c) Gerbil seizure model— elevated NBCe1 expression—The expression of NCBTs in the nervous system may also be regulated by neuronal activity. Kang *et al*. (2002) compared NBCe1 expression in the hippocampus of gerbils that were separated into seizure-resistant and seizure-sensitive groups depending on the degree of motor arrest induced by stroking the back of the neck. In seizure-sensitive animals, NBCe1 expression by immunohistochemistry increased in the CA1–3 regions of the hippocampus at the 30-min time point following seizure induction. At the 3-h time point, expression increased further in the CA2–3 regions and also increased in the dentate gyrus (granule cells). At the 6-h time point, NBCe1 expression in all hippocampal regions decreased to pre-seizure levels.

The functional impact of a change in the activity/expression of an NCBT such as NBCn2 (NCBE) or NBCe1 on seizure activity is anticipated to be complex because of changes in

pH_i, pH_o, the concentrations of transported ions, and intracellular/extracellular volumes. Further studies are required to elucidate the mechanisms by which altered function/expression of NCBTs influence neuronal activity.

B) Somatosensory function

a) NBCn1 knockout mouse— visual impairments—NBCn1, which is expressed in both the eye and ear, is necessary for somatosensory function (Bok *et al*., 2003; Lopez *et al*., 2005). Mice with a disruption of the *Slc4a7* gene that encodes for NBCn1 (*Slc4a7*$^{-/-}$) are blind and have hearing defects (Bok *et al*., 2003). We will initially focus on the eye, and then discuss the ear in the next paragraph. Based on results from confocal immunofluorescence studies on wild-type mice (*Slc4a7*$^{+/+}$), NBCn1 is expressed in the outer plexiform layer of retina, and more specifically in photoreceptor synaptic terminals. In knockout compared to wild-type mice, there was a progressive deterioration of the photoreceptor region of the retina beginning at two months of age and leading to complete loss at 11 months. At four months of age, the morphology of the fundi region of knockout mice had markedly deteriorated as demonstrated by attenuation of blood vessels and diffuse granularity. The authors propose that eye defects in the NBCn1-knockout mice may result from interrupted NBC-mediated buffering of H^+ that would lower pH_i and inhibit Ca^{2+} efflux via the Ca^{2+}-pump and/or Ca^{2+} influx via pH-sensitive L-type voltage-gated Ca^{2+} channels at the photoreceptor synaptic terminal.

b) NBCn1 knockout mouse— auditory impairments—In the same study introduced above, Bok *et al*. (2003) also found hearing defects in the NBCn1 knockout mice. Based on results from cochlear immunohistochemical studies on wild-type mice, NBCn1 is expressed in fibrocytes of the spiral ligament. The authors found that one-month old knockout mice displayed degenerating inner and outer hair cells of cochlea (which progressed with age), and abnormal morphology of the stria vascularis and spiral ligament. The Reissner's membrane was collapsed— a finding consistent with a role for NBCn1 in endolymph formation. In knockout mice (−/−) compared to wild-type (+/+) mice at three months of age, auditory brainstem responses (ABR) elicited by acoustic clicks to the ear were blunted (Fig. 4A); the mean ABR amplitude from the combined stimulus intensities was ~70% less at three months (Fig. 4B). Thus, NBCn1 plays an important role in the development and function of the auditory system.

According to Bok *et al*. (2003), the NBCn1 knockout mouse serves as a model for Usher syndrome, which is a human disorder associated with similar hearing and vision disabilities. *SLC4A7* appears responsible for type 2B of Usher syndrome (Pushkin *et al*., 1999b), which has been mapped in a consanguineous Tunisian family with vision and hearing defects (Hmani-Aifa *et al*., 2002; Hmani *et al*., 1999).

More recently, Lopez *et al*. (2005) performed a detailed characterization of the time course of cochlear hair cell degeneration and loss from postnatal P2 to P90 NBCn1 knockout mice. Based on results from light and transmission electron microscopy studies, the authors observed degeneration of the hair cells as early as P21. The degeneration progressed with age until complete degeneration at P90. There was atrophy in the organ of Corti beginning at P21. At P90, both the organ of Corti and the myelin that surrounded spiral ganglia neurons were degenerated. In addition, there was formation of vacuoles in the cytoplasm of the spiral ganglia neurons. Apoptotic cells were detected in the cochlea as early as P8.

C) Cerebrospinal Fluid Secretion

a) NBCn2 (NCBE) knockout mouse—In the same study described above on NBCn2 (NCBE) knockout mice, Jacobs *et al*. (2008) examined the impact of NBCn2 (NCBE) loss

on choroid plexus function and ventricle volume. NBCn2 (NCBE) expression was evident on the basolateral membrane of the choroid plexus epithelium from wild-type mice (Fig. 5A, green), but not knockout mice (Fig. 5B). Using magnetic resonance imaging, the authors observed a ~75% reduction in brain ventricular volume in knockout *vs*. wild-type mice (Figs. 5C–E). The decreased volume was not the result of edema or increased intracranial pressure, but was instead due to enlarged lateral intercellular spaces and reduced apical microvilli in the choroid plexus according to data from histological and electron microscopy studies (Fig. 5). To examine NBCn2 (NCBE)-mediated CSF production, the authors performed fluorescence imaging experiments with the pH-sensitive dye BCECF to characterize HCO_3^--dependent pH_i regulation in the epithelial cells of choroid plexus from the knockout mice. Na^+- and HCO_3^--dependent pH_i recoveries from NH_4^+ prepulse-induced acid loads were reduced in the knockout vs. wild-type mice. The authors propose that NBCn2 (NCBE) promotes CSF secretion by contributing to net vectorial movement of Na^+ and HCO_3^- across the polarized choroid plexus epithelium. NBCn2 (NCBE) transports Na^+ and HCO_3^- from blood across the basolateral membrane of the choroid plexus epithelium. Another NBC and the Na^+ pump then transport the intracellular Na^+ and/or HCO_3- across the apical membrane into the CSF. H_2O follows the solute movement through aquaporin-1 H_2O channels. Decreased HCO_3^--dependent CSF production is likely responsible for the reduced brain ventricular volume in the NBCn2 (NCBE) knockout mice.

On a related note, Sussman *et al*. (2009) observed that morpholino knockdown of NBCe1 in zebrafish leads to hydrocephalus, as well as ocular abnormalities such as retinal distention and small eyes. As proposed by the authors, NBCe1 may be required for fluid absorption from CSF to the brain parenchyma, or proper ion homeostasis of cells in the ventricular lining.

There is evidence that NCBT knockout mice may express compensatory acid-base transporters to regulate pH_i. For example, compensatory pH_i-regulating mechanisms appear in the choroid plexus epithelial cells of the NBCn2 (NCBE) knockout mice. According to results from immunofluorescence studies, Damkier *et al*. (2009) found that the Na-H exchanger 1 (NHE1), which is usually localized to the apical membrane in the choroid plexus, is expressed in the basolateral membrane in the choroid plexus epithelial cells from the knockout mice. In functional studies on the choroid plexus epithelium from knockout mice, there was an increase in the activity of an apical EIPA-insensitive NHE responsible for Na^+-dependent pH_i recoveries from NH_4^+-induced acid loads. The apical NHE was sensitive to EIPA in the wild-type mice. According to the authors, NBCn2 (NCBE) knockout increases the expression of an EIPA-sensitive NHE (NHE1) from the apical to basolateral membrane, and also increases the activity of an EIPA-insensitive NHE on the apical membrane.

D) Genetic links to human neurologic disorders

As described above, many of the human patients with frameshift, missense, or nonsense mutations in the *SLC4A4* gene have ocular abnormalities, including glaucoma and/or cataract formation (Igarashi *et al*., 1999; Demirci *et al*., 2006; Dinour *et al*., 2004). In addition, three of them are mentally retarded and another two display developmental disabilities. Findings from additional genetic studies have yielded associations between neurologic disorders and other more recently identified NCBT genes. For example, in a genetic linkage study, Kok *et al*. (2003) identified two families in which a form of hereditary sensory neuropathy type I (HSN I) with gastroesophageal reflux (GER) and cough is linked to chromosome 3p22-p24. Although, 28 genes are mapped to this interval, one of two positional candidate genes identified based on expression in peripheral nerve and spinal cord is *SLC4A7* (encodes NBCn1).

NBCn1 also appears associated with a propensity to substance addictions. In examining allelic variations in the *SLC4A7* gene, Ishiguro *et al*. (2007) found a strong association between single nucleotide polymorphism (SNP) markers of the gene and substance abusers. The authors proposed that allelic variations of *SLC4A7* may increase addiction vulnerability by influencing drug and/or neurotransmitter pharmacodynamics and pH-dependent transport across cell membranes.

Finally, NCBTs have been linked to autism. In performing a high-resolution genomic microarray analysis on 264 families with or without autistic individuals, Sebat *et al*. (2007) identified a pair of monozygotic autistic twins with deleted exon 1 of *SLC4A10* (encoding NBCn2 (NCBE)), as well as a patient with Asperger syndrome linked to a genomic deletion that included the *SLC4A11* gene (encoding a unique Na-dependent borate transporter in the *SLC4* family).

4) Role of NCBTs in Energy-deficient Neurologic Conditions

A) Ischemia

Jung *et al*. (2007) examined the expression profiles of four sodium transporters, including NBCe1, in the ischemic penumbra in a rat model of focal cerebral ischemia induced by occlusion of the left middle cerebral artery. By immunoblotting procedures, the authors reported increased NBCe1 expression in ischemic penumbra tissues 3 and 6 h post surgery in the ischemic model vs. sham-operated controls. Although the reason for this ischemia-induced increase in NBCe1 expression is not known, the accompanying increase in electrogenic NBC activity may protect against associated decreases in pH_i. Furthermore, ischemia-induced increases in extracellular K^+ (Leis *et al*., 2005) may be involved. According to the model of Jung *et al*., an ischemia-induced increase in extracellular K^+ will depolarize brain cells (e.g., astrocytes) and stimulate electrogenic NBC activity. The increased cellular influx of HCO_3^- will buffer ischemia-induced decreases in pH_i. On the other hand, the accompanying Na^+ influx is expected to promote cellular edema.

One of the hallmarks of ischemia is both intracellular and extracellular acidification. Furthermore, McKee *et al*. (2005) have demonstrated a decrease in Mg^{2+} concentration in both the CSF and serum early on in ischemic stroke. Cooper *et al*. (2009) recently examined the expression of NBCn1-B and its contribution to the cytotoxicity of rat hippocampal neurons under ischemic-like conditions with low pH_o and the absence of extracellular Mg^{2+}. Using immunoblot procedures on cultured rat hippocampal neurons, the authors reported increased expression of NBCn1 in cultured neurons under the ischemic-like conditions. Regarding the effects of low pH_o, the authors found an increase in NBCn1-B protein expression by immunofluorescence in the cell body, plasma membrane, and dendrites of neurons cultured at pH_o 6.5, but not 7.4 for 1–3 h. According to results from subsequent cytotoxicity studies, glutamate cytotoxicity was decreased in neurons from NBCn1 siRNA knockdown animals after incubation in Mg^{2+}-free media for 2 and 6 h, but not when the cells were incubated at a low pH_o of 6.3 for 6 h. The authors speculated that 0 Mg^{2+} stimulates NMDA receptor activity, which in turn induces cytotoxicity. The cytotoxicity appears to be promoted by NBCn1-mediated acid extrusion. More recently, the group has reported that chronic metabolic acidosis in mice increases NBCn1 expression in CA3 hippocampal, posterior cortical, and cerebellar granular neurons (Park *et al*., 2010).

Hypoxia

Hypoxia influences both the function and expression of NCBTs. In pH_i experiments with BCECF on astrocytes cultured from the rat hippocampus, Bevensee and Boron (2008) observed that acute hypoxia (3% O_2) stimulated total acid extrusion during pH_i recoveries from NH_4^+-induced acid loads in the nominal absence of CO_2/HCO_3^-, but actually inhibited

SITS-sensitive acid extrusion during pH_i recoveries in the presence of CO_2/HCO_3^-. The data are consistent with acute hypoxia stimulating the activity of the Na-H exchanger activity, but inhibiting the activity of the SITS-sensitive electrogenic Na/HCO_3^- cotransporter in the astrocytes. Hypoxia also stimulated an acid-loading mechanism responsible for the pH_i recovery from an alkali load in the astrocytes. Thus, acute hypoxia stimulates a HCO_3^--independent acid extruder and loader, but inhibits a HCO_3^--dependent acid extruder in the astrocytes.

To examine hypoxia-induced changes in the expression of NDCBE in mouse brain, Chen *et al*. (2008a) performed immunoblotting of membrane proteins from different regions of the brain from adult and neonatal mice subjected to chronic continuous hypoxia (11% O_2) for 14 or 28 d. At both the 14- and 28-d time points, chronic hypoxia decreased NDCBE-B expression by 20–50% in cortex, subcortex, hippocampus, and cerebellum of adults. In the neonates, the NDCBE-B protein expression was decreased by ~25% in both the hippocampus after 14 d and the subcortex after 28 d hypoxic treatments. The authors proposed that the general reduced effect of chronic hypoxia on NDCBE expression in neonates vs. adults might be due to a smaller compensated respiratory alkalosis and/or reduced hypoxia sensitivity of the neonates.

Chen *et al*. (2007) performed similar studies on NBCn1-B and NBCn2 (NCBE). At both the 14- and 28-d time points, chronic hypoxia decreased the expression of NBCn1-B and NBCn2 (NCBE) by 15–50% in cortex, subcortex, hippocampus, and cerebellum of both neonates and adults. The authors proposed that the hypoxia-induced decrease in protein expression of NBCn1-B and NBCn2 (NCBE) may result in decreased energy consumption in the brain by reducing either protein synthesis, the requirement for Na^+-pump mediated Na^+ extrusion, and/or neuronal activity caused by associated changes in pH_i/pH_o.

Unlike Chen *et al*. (2007) who examined the effect of chronic continuous hypoxia on expression of NCBTs in mouse brain, Douglas *et al*. (2003) examined the effect of chronic intermittent hypoxia on the expression of NBCe1 in different regions of the postnatal mouse CNS. The authors performed immunoblotting on membrane fractions from cerebellum, cortex, hippocampus, and brainstem-diencephalon using three different NBCe1 antibodies. One antibody recognized all three NBCe1 variants, one recognized the C terminus of the NBCe1-A and −B variants, and the final one recognized the different C terminus of the C variant. Using the antibody that recognized all three variants, Douglas *et al*. observed a 33% decrease in NBCe1 expression in the cerebellum of hypoxia-exposed mouse compared to controls. Using the antibody to the C-terminus of NBCe1-A/B, the authors reported a decrease in the expression of NBCe1-A/B in the hippocampus of hypoxic mice. However, with the antibody to the C-terminus of the C variant, NBCe1-C did not appear to show any appreciable change in expression in brain regions from hypoxic and normoxic mice. Therefore, the effects of chronic intermittent hypoxia on NBCe1 expression are both transporter variant and brain-region dependent.

5) Conclusions and Future Directions

We have provided a detailed overview of the function and localization of NCBTs in the nervous system and their relevance to neurologic function. Although the primary role of NCBTs in the nervous system is likely to regulate pH_i/pH_o tightly, some NCBTs such as NBCe2 also contribute to the production and ionic composition of CSF, thereby controlling brain ventricular volume. The following three noteworthy observations have arisen from molecular studies of NCBTs in the nervous system. First, individual NCBTs or associated variants are expressed in different regions and cell types in the brain. Differential expression of NCBTs may contribute to heterologous pH_i regulation of brain cells, and results from

studies designed to characterize the functional properties of specific NCBTs in both endogenous cells and heterologous expression systems will provide further insight into such heterologous pH_i regulation.

The second noteworthy observation is that more than one NCBT can be expressed in a single type of brain cell. Such apparent redundancy reinforces the hypothesis that cells make use of multiple NCBTs with different regulatory properties to modulate pH_i/pH_o under various conditions and stimuli. For instance, many NCBTs such as NBCn1, NBCe1-C, and NBCn2 (NCBE)-C and −D contain PDZ-binding motifs that are likely targets for regulatory proteins containing PDZ domains. NBCn1 at least partially colocalizes with post synaptic density protein-95 (PSD-95) in neurons (Cooper *et al*., 2005; Park *et al*., 2010). In addition, the IP_3-receptor binding protein released with IP_3 (IRBIT) binds to the N terminus of NBCe1-B (but not −A) to increase transporter activity (Shirakabe *et al*., 2006). Further studies are required to identify neurologically relevant protein partners and classic signaling pathways that may regulate specific NCBTs in the brain under both physiologic and pathophysiologic conditions.

Finally, results from animal models of disease and NCBT knockout mice have provided considerable insight into the importance of NCBTs in somatosensory function and CSF production, as well as during pathologic states including epilepsy and ischemia/hypoxia. However, future studies are required to determine if altered expression of NCBTs in disease states is a causative effect or adaptive response. Furthermore, generating and characterizing additional mice with more specific NCBT variant knockdowns will be required to understand the importance of individual NCBT variants, as well as to expand our knowledge of the complexity of the bicarbonate transporter family in the brain.

LIST OF ABBREVIATIONS

AEs	anion exchangers
ASICs	acid-sensing ion channels
BT	bicarbonate transporter
CNS	central nervous system
CSF	cerebrospinal fluid
GABA	γ-aminobutyric acid
GAD	glutamic acid decarboxylase
GFAP	glial fibrillary acidic protein
$a\mathbf{Na^+_i}$	intracellular Na^+ activity
MAP2	microtubule associated protein 2
NCBT	Na-coupled bicarbonate cotransporter
NBC	Na/bicarbonate cotransporter
NBCe1 (or 2)	electrogenic Na/bicarbonate cotransporter 1 (or 2)
NBCn1 (or 2)	electroneutral Na/bicarbonate cotransporter 1 (or 2)
NDAE	Na-driven anion exchanger
NDCBE	Na-driven Cl-bicarbonate exchanger
NCBE	Na-driven Cl-bicarbonate exchanger

NHE	sodium hydrogen exchanger
PDZ	post synaptic density-Drosophila disc large tumor suppressor-zonula occludens-1
pH_i	intracellular pH
pH_o	extracellular pH
PIP_2	phosphatidylinositol 4,5-bisphosphate
PSD-95	post synaptic density protein-95
Slc4	solute carrier 4

Acknowledgments

We are very grateful to Dr. Mark D. Parker (Department of Physiology and Biophysics, Case Western Reserve University) who critiqued our manuscript and provided many valuable comments and suggestions. This work was supported by NIH/NINDS NS046653 (MOB).

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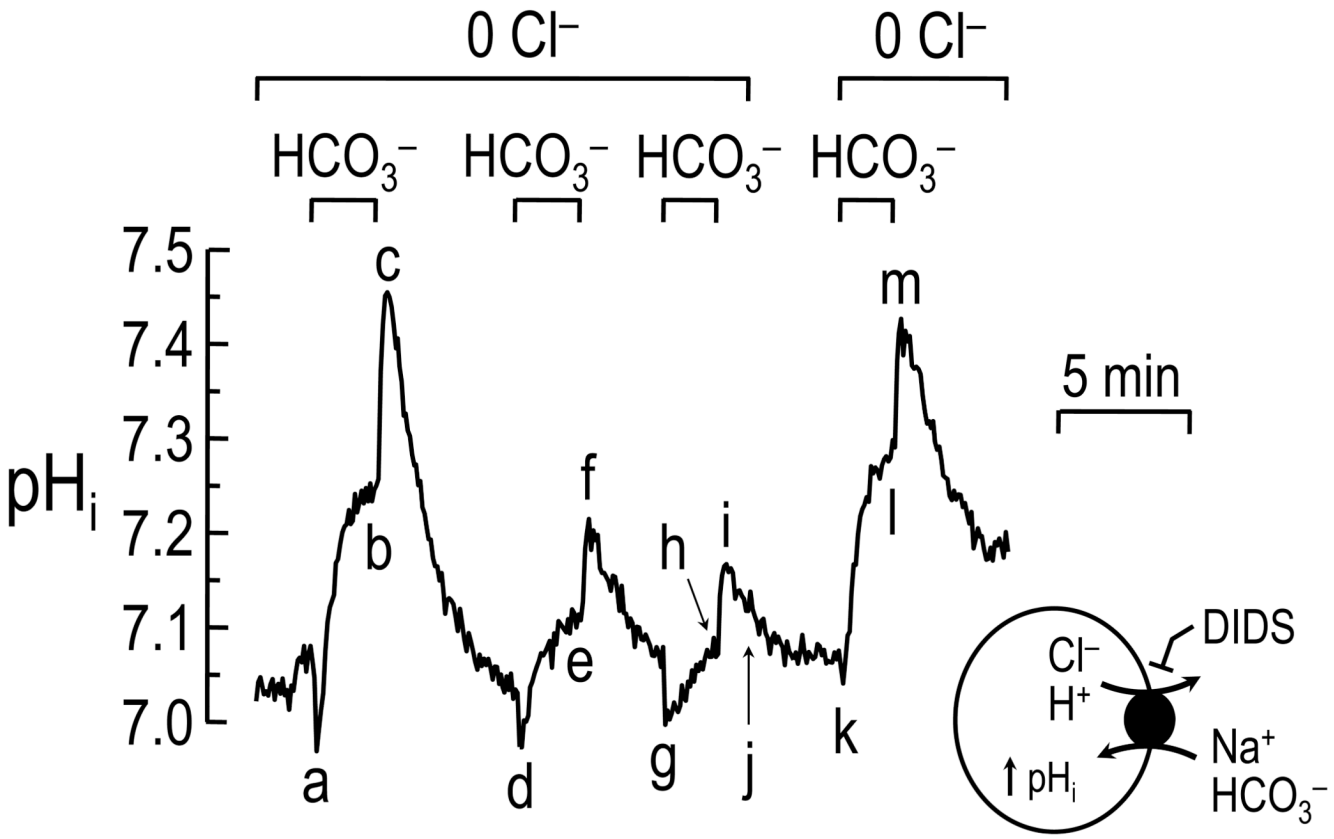

Fig. 1.

Intracellular Cl^- dependence of the Na-coupled HCO_3^- transporter in a single CA1 neuron acutely isolated from the hippocampus of a neonatal rat. pH_i was measured using the pH-sensitive dye BCECF. At the beginning of the experiment, the neuron was bathed in a Cl^--free, HEPES-buffered solution. Switching to a Cl^--free solution containing CO_2/HCO_3^- elicited an initial decrease in pH_i (point *a*) due to CO_2 influx, followed by a rapid increase in pH_i (*ab*) due to a DIDS-sensitive, Na^+- and HCO_3^--dependent transporter. Returning to the HEPES-buffered solution caused a large increase in pH_i (*bc*) due to CO_2 efflux, followed by a slower decrease in pH_i due to the activity of acid-loading mechanisms. Exposing the neuron to the Cl^--free, HCO_3^- solution two additional times (*def* and *ghi*) elicited progressively slower and less pronounced pH_i recoveries from the initial CO_2-induced acidifications (*de* and *gh*), presumably due to a progressive decrease in intracellular Cl^-. Returning the neuron to a Cl^--containing solution briefly (*jk*) presumably refills intracellular Cl^-, thereby allowing the return of the robust HCO_3^--induced pH_i increase (*kl*), even in the absence of external Cl^-. Modified from Schwiening and Boron (1994) with kind permission from John Wiley & Sons Ltd.

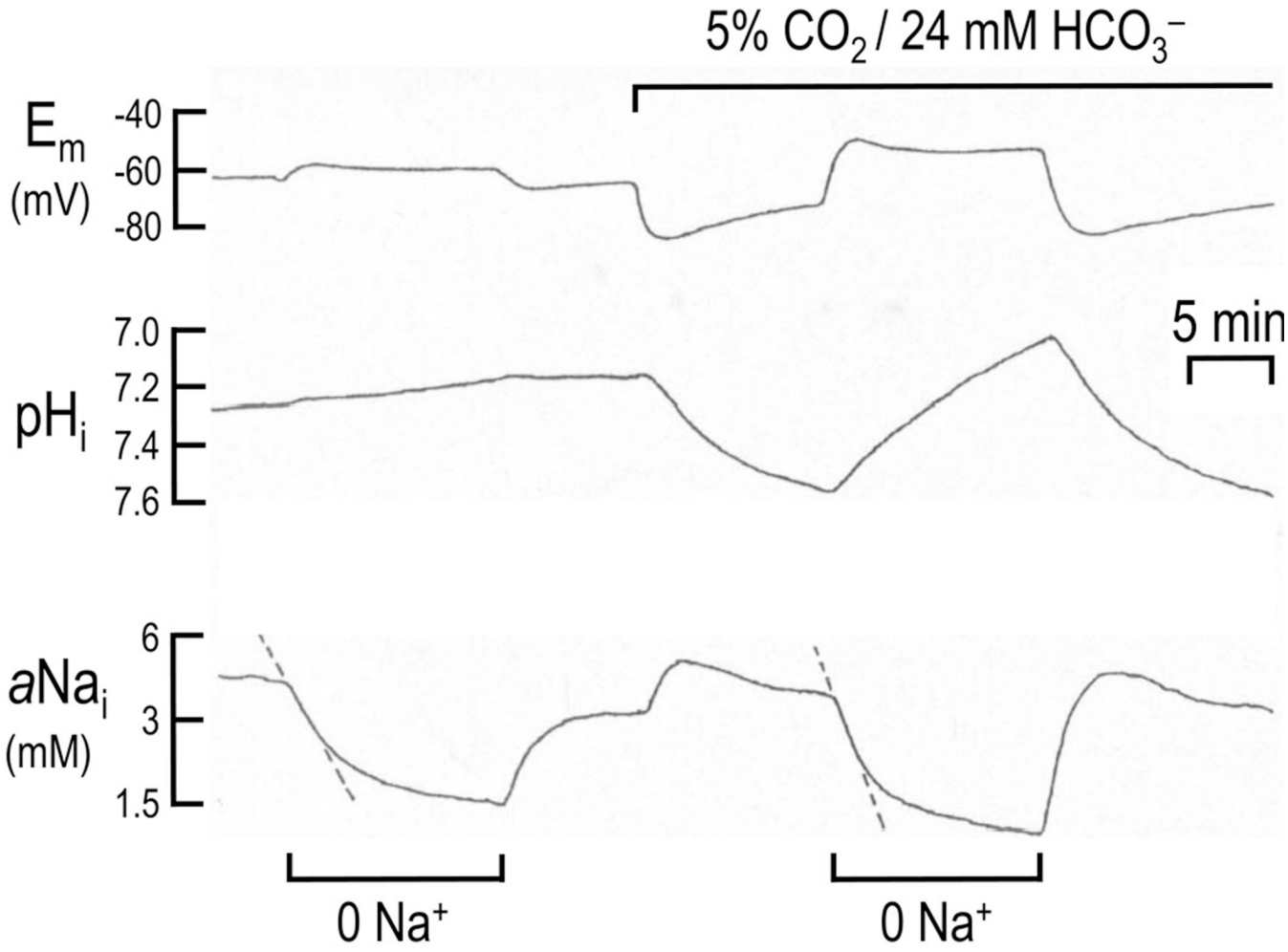

Fig. 2.

Electrogenic Na/Bicarbonate Cotransporter (NBC) activity in a giant neuropile glial cell from leech. Membrane potential (E_m), pH_i, and aNa_i were measured using ion-sensitive and voltage electrodes. For the glial cell bathed in a HEPES-buffered solution, removing external Na^+ had little effect on E_m and pH_i, and elicited a decrease in aNa_i. However, after the glial cell was switched to a CO_2/HCO_3^- solution, removing external Na^+ caused a depolarization, a decrease in pH_i, and a faster decrease in aNa_i than observed in HEPES. These three responses are consistent with an electrogenic NBC moving Na^+, HCO_3^-, and net-negative charge out of the cell when exposed to the Na^+-free solution. Modified from Deitmer (1992), Fig. 3, © Springer-Verlag, 1992 with kind permission of Springer Science +Business Media.

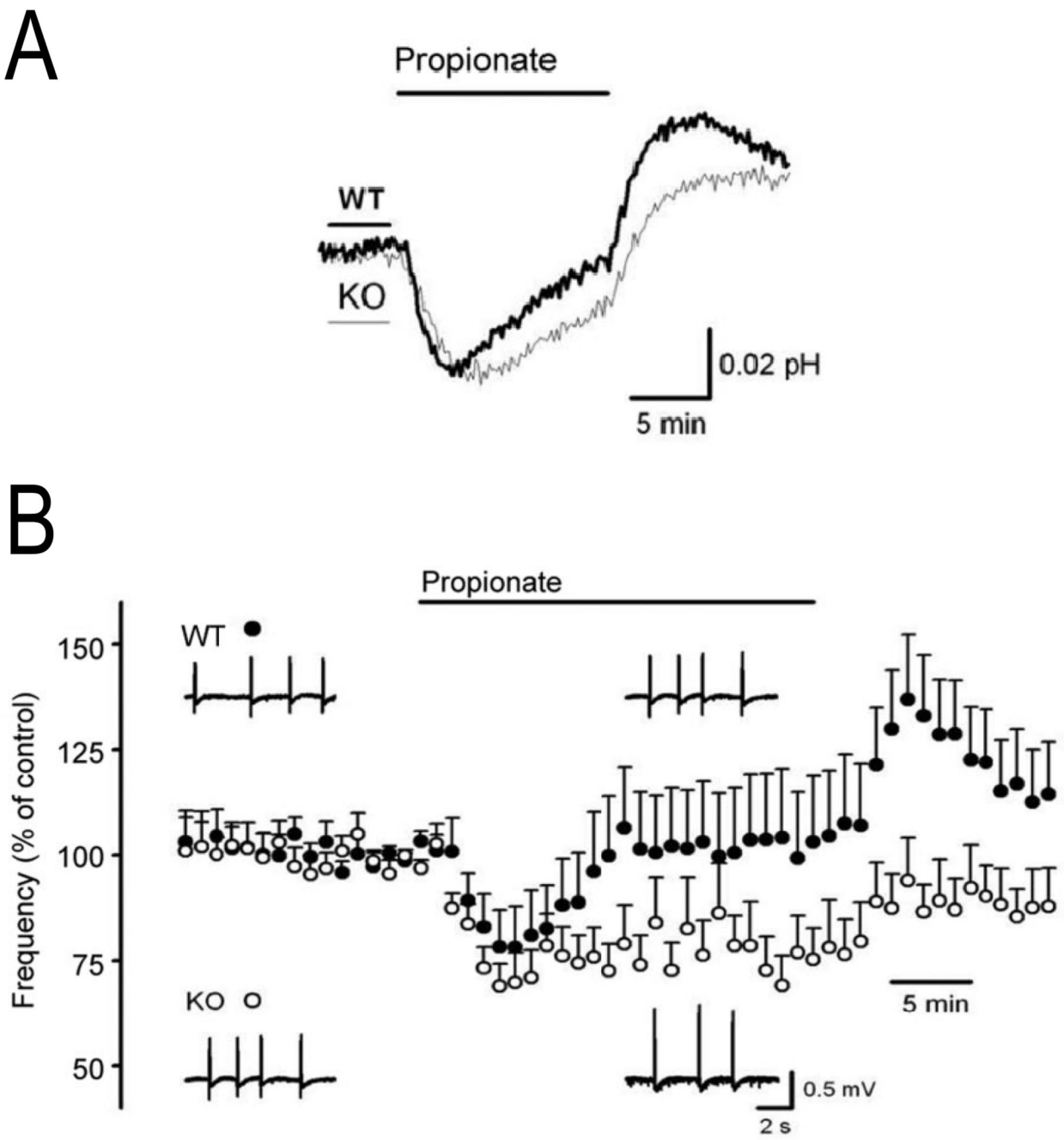

Fig. 3.

Reduced pH_i regulation and neuronal excitability in the CA3 stratum pyramidale layer of hippocampal slices from NBCn2 (NCBE) Slc4a10 knockout mice. (A) pH_i in the CA3 layer of slices from wild-type (WT) and NBCn2 (NCBE) knockout (KO) mice was measured by fluorescence imaging with the pH-sensitive dye BCECF. Exposing slices to a bath solution containing 20 mM propionate elicited an initial decrease in mean pH_i due to the influx of propionic acid, followed by an increase in pH_i due to acid extruders such as NBCn2 (NCBE). Both the rate and magnitude of the mean pH_i increase was less in slices from KO mice (grey trace, n=12) *vs*. WT mice (black trace, n=10). Removing bath propionate elicited a pH_i increase due to the efflux of propionic acid, followed by a pH_i decrease due to acid

loading mechanisms. (B) 4-aminopyridine (4-AP)-elicited interictal-like events in the CA3 layer of slices from WT and NBCn2 (NCBE) KO mice were measured using field potential electrodes. The slower propionate-induced pH_i recoveries in the KO slices shown in panel A correlated with a propionate-induced decrease in the relative mean (+SEM) frequency of interictal events in the KO slices (open circles, n=27) compared to the WT slices (filled circles, n=20). The frequency of the events recovered to control levels in WT slices, but not KO slices during the propionate exposure. Modified from Jacobs *et al*. (2008). © 2007 by The National Academy of Sciences of the USA.

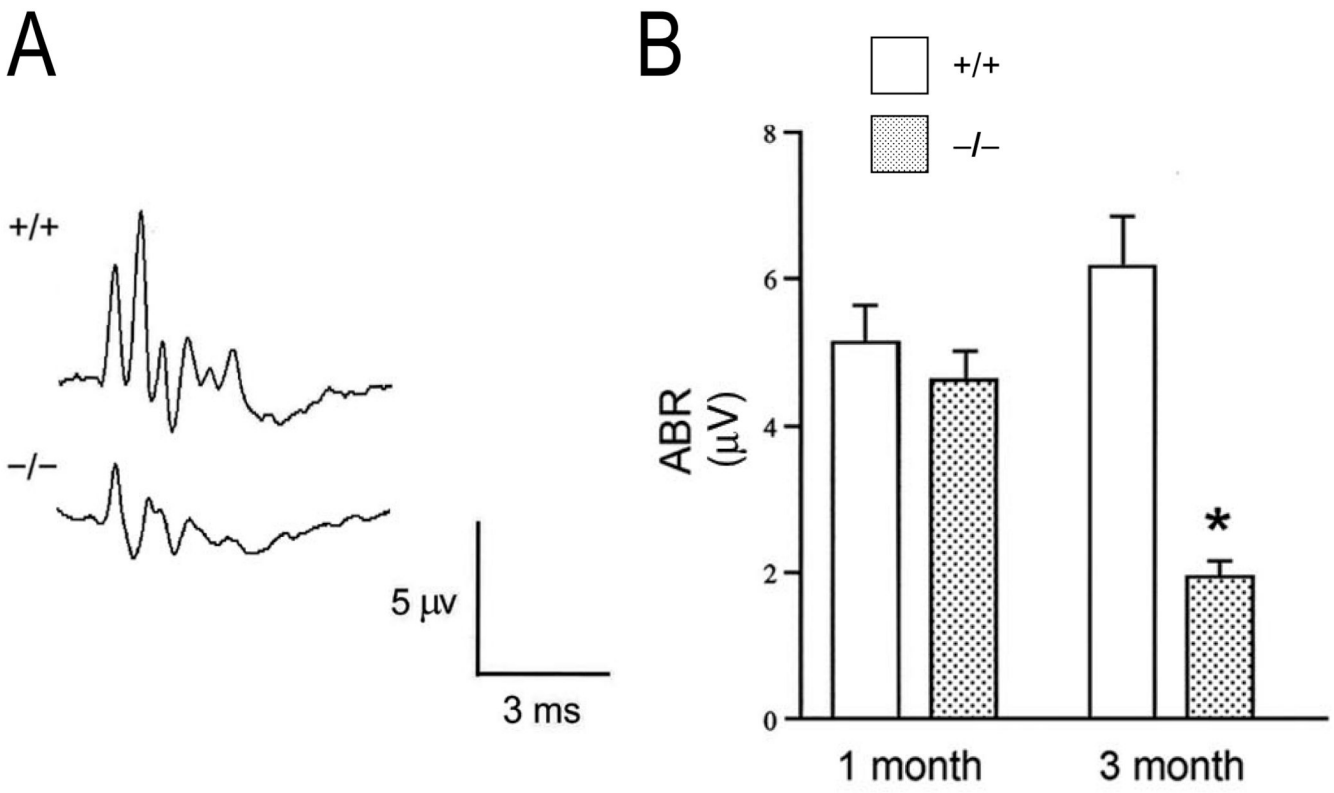

Fig. 4.

Auditory impairment in NBCn1 (Slc4a7) knockout mice. (A) Auditory function was assessed by examining auditory brain responses (ABRs) in response to various click stimuli. Click stimuli of 60 dB sound-pressure level elicited an ABR waveform that was blunted in three-month-old *Slc4a7*$^{-/-}$ *vs. Slc4a7*$^{+/+}$ mice. (B) Summary data from panel-A type responses with means (+SEMs) computed from ABR waves I, II, and III, and from all stimulus intensities for *Slc4a7*$^{+/+}$ (open bars) and *Slc4a7*$^{-/}$ mice (closed bars). n = 5 for each bar. *P < 0.04. Modified from Bok *et al*. (2003). Reprinted by permission from Macmillan Publishers Ltd: Nature Genetics (Bok *et al*., (2003)), © Nature Publishing Group (2003).

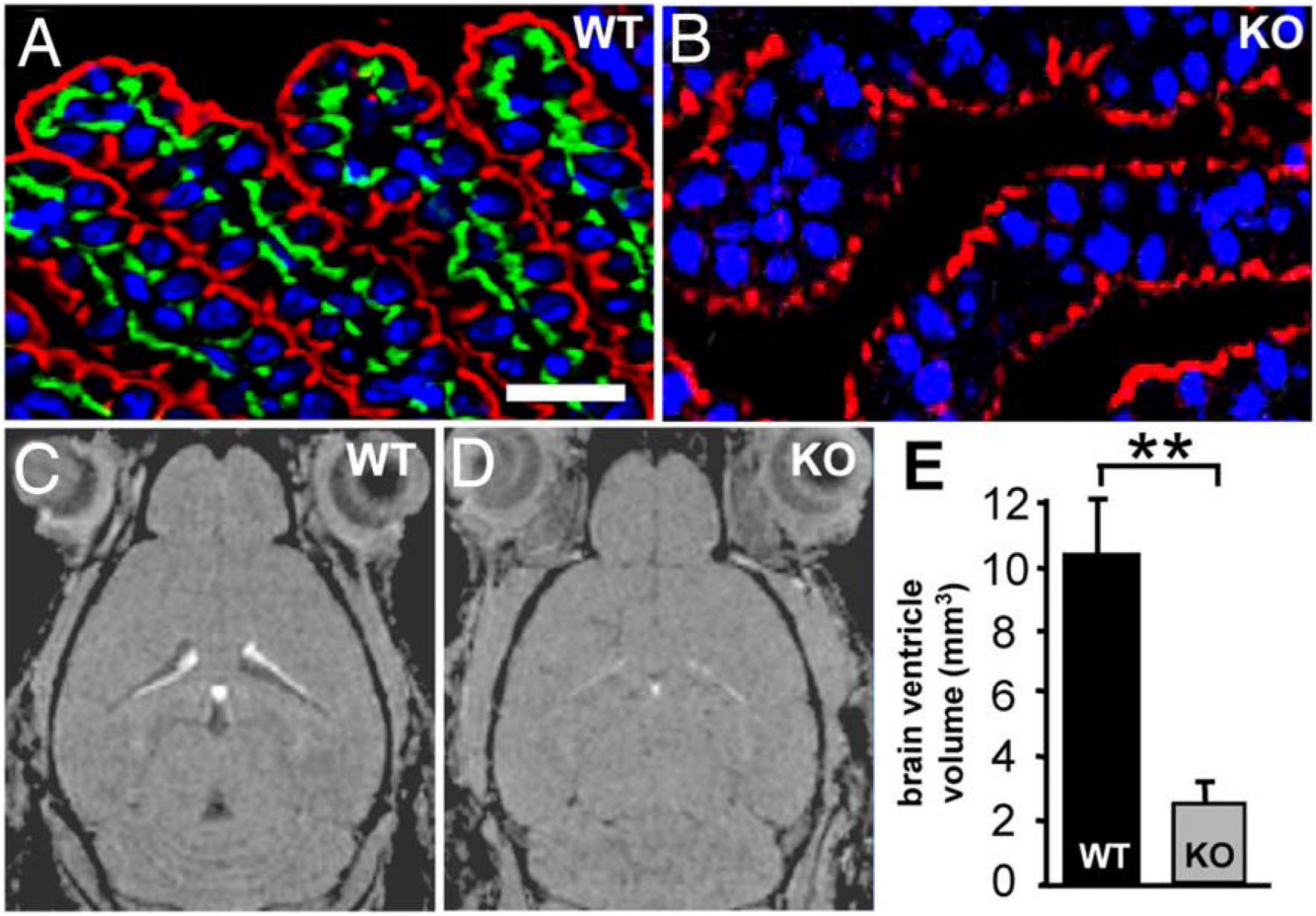

Fig. 5.

Reduced brain ventricular volume in NBCn2 (NCBE) Slc4a10 knockout mice. (A, B) According to results from immunohistochemical studies, NBCn2 (NCBE) is expressed on the basolateral membrane of the choroid plexus epithelium from wild-type but not knockout mice (green), but not on the apical membrane labeled with an antibody to the Na-pump (red). (C–E) Based on magnetic resonance imaging, the mean (+SEM) brain ventricular volume is ~75% less in knockout mice (n=4) *vs*. wild-type mice (n=3). ***P* < 0.04. Modified from Jacobs *et al*. (2008). © 2007 by The National Academy of Sciences of the USA.

Table I

Molecular characterization and localization of Na-Coupled Bicarbonate Transporters (NCBTs) in tissues

NCBT	Variants	Alternate names	SLC	Reported tissue distribution[1]	References
NBCe1	A–C	aNBC[2], akNBC[3], hhNBC[4], hpNBC[5], kNBC[6], kNBC-1, NBC1, pNBC[7], rb1NBC[8], rb2NBC[9], rkNBC[10], zNBCe1[11]	*SLC4A4*	Zebrafish (cloning, B variant)[12], zebrafish tissues (mRNA), zebrafish eye, gill (protein)	Sussman *et al*., 2009
				Ambystoma kidney (cloning, A variant), *Ambystoma* tissues (mRNA)	Romero *et al*.,1997
				gerbil brain (protein)	Kang *et al*., 2002
				murine duodenum (mRNA, protein), murine pancreas (mRNA)	Praetorius *et al*., 2001
				murine colon, other tissues (mRNA)	Bachmann *et al*., 2003
				mouse brain (protein)	Douglas *et al*., 2003
				mouse kidney (protein)	Pushkin *et al*., 2004
				mouse brain (protein)	Rickmann *et al*., 2007
				mouse kidney, small intestine & colon (mRNA)	Gawenis *et al*., 2007
				rat kidney (cloning, A variant), rat tissues (mRNA)	Romero *et al*.,1998
				rat kidney (cloning, A variant), rat tissues (mRNA)	Burnham *et al*.,1998
				rat epididymis (mRNA, protein)	Jensen *et al*., 1999
				rat, *Ambystoma*, & rabbit kidney (protein)	Schmitt *et al*., 1999
				rat salivary gland (protein)	Roussa *et al*., 1999
				rat pancreas (cloning, B variant), rat pancreas (protein)	Thévenod *et al*.,1999
				rat brain (cloning, B & C variants), rat tissues (protein)	Bevensee *et al*., 2000
				rat brain (cloning, B variant), kidney, brain, & tissues (mRNA)	Giffard *et al*., 2000
				rat brain (mRNA, protein)	Schmitt *et al*., 2000
				rat & *Ambystoma* kidney (protein)	Maunsbach *et al*., 2000
				rat eye & conjunctiva (protein)	Bok *et al*., 2001
				rat brain (protein)	Douglas *et al*., 2001
				rat kidney (protein)	Kwon *et al*., 2002
				rat kidney (mRNA)	Xu *et al*., 2003
				rat kidney & pancreas (mRNA,protein),human pancreas (protein)	Satoh *et al*., 2003
				rat kidney (mRNA)	Abuladze *et al*., 2004
				rat pancreas (mRNA)	Abuladze *et al*., 2004

NCBT	Variants	Alternate names	SLC	Reported tissue distribution[1]	References
				rat kidney (mRNA)	Praetorius *et al*., 2004b
				rat brain (protein)	Jung *et al*., 2007
				rat heart (mRNA)	Yamamoto *et al*., 2007
				rat brain (mRNA, protein)	Majumdar *et al*., 2008
				rat pancreas (mRNA, protein)	Soyfoo *et al*., 2009
				bovine cornea (mRNA, protein)	Sun *et al*., 2000
				human kidney (cloning, A variant), human kidney & pancreas (mRNA)	Burnham *et al*.,1997
				human kidney (mRNA)	Abuladze *et al*.,1998
				human pancreas (cloning, B variant), human tissues & mouse pancreas (mRNA)	Abuladze *et al*., 1998
				human heart (cloning, B variant), human pancreas (mRNA)	Choi *et al*., 1999
				human cornea (mRNA)	Usui *et al*., 1999
				human pancreas (mRNA, protein)	Marino *et al*., 1999
				human cornea (mRNA, protein)	Sun and Bonanno, 2003
				human tissues (mRNA), human duodenum (protein)	Damkier *et al*., 2007
NBCe2	A–F	NBC4	SLC4A5	mouse & rat choroid plexus (mRNA)	Praetorius *et al*., 2004b
				mouse & rat choroid plexus (protein)	Bouzinova *et al*., 2005
				mouse & rat choroid plexus (protein)	Damkier *et al*., 2009
				rat liver (cloning, E & F variants), rat tissues (mRNA),	Xu *et al*., 2003
				rat liver (cloning, C variant), rat testis (mRNA), rat liver & kidney (mRNA, protein)	Abuladze *et al*., 2004
				rat heart (mRNA)	Yamamoto *et al*., 2007
				human testis (cloning, A variant), human tissues (mRNA)	Pushkin *et al*., 2000b
				human heart (cloning, B variant)	Pushkin *et al*., 2000a,b
				human testis (cloning, C variant), human tissues (mRNA)	Sassani *et al*., 2002
				human tissues (cloning, C variant)	Virkki *et al*., 2002
				human tissues (mRNA), human kidney (protein)	Damkier *et al*., 2007
NBCn1	A–H	NBC3	*SLC4A7*	mouse ear & eye (protein)	Bok *et al*., 2003
				mouse ear (protein)	Lopez *et al*., 2005
				mouse brain (protein)	Chen *et al*., 2007
				mouse tissues (mRNA, protein)	Boedtkjer *et al*., 2008
				mouse choroid plexus (protein)	Damkier *et al*., 2009
				rat smooth muscle (cloning, B–D variants), rat tissues & aorta (mRNA)	Choi *et al*., 2000

NCBT	Variants	Alternate names	SLC	Reported tissue distribution[1]	References
				rat kidney (protein)	Vorum *et al*., 2000
				rat aorta & murine duodenum (mRNA), murine duodenum (protein)	Praetorius *et al*., 2001
				rat brain (protein)	Risso-Bradley *et al*., 2001
				rat kidney (protein)	Kwon *et* al., 2002
				rat salivary glands, kidney (mRNA)	Gresz *et al*., 2002
				rat kidney (mRNA, protein)	Odgaard *et al*., 2004
				rat kidney (mRNA, protein)	Praetorius *et al*., 2004a
				rat brain (mRNA), rat, mouse choroid plexus (mRNA, protein)	Praetorius *et al*., 2004b
				rat brain (cloning, E variant), rat brain (mRNA, protein)	Cooper *et al*., 2005
				rat tissues (mRNA, protein)	Damkier *et al*., 2006
				rat heart (mRNA)	Yamamoto *et al*., 2007
				rat brain (protein)	Cooper *et al*., 2009
				human skeletal muscle (cloning, A variant), human skeletal muscle & heart (mRNA)	Pushkin *et al*.,1999a
				human salivary glands & kidney (mRNA), rat & human salivary glands & kidney (protein)	Gresz *et al*., 2002
				human choroid plexus (protein)	Praetorius & Nielsen, 2006
				human tissues (mRNA), human kidney & duodenum (protein)	Damkier *et al*., 2007
NBCn2	A–D	NCBE, rb1NCBE[13], rb2NCBE[14]	*SLC4A10*	insulin-secreting cell line (cloning, A variant), the cell line, mouse pancreas, & rat tissues (mRNA)	Wang et al., 2000
				mouse brain, other tissues (mRNA)	Hübner *et al*., 2004
				mouse brain (mRNA, protein)	Jacobs *et al*., 2008
				mouse brain (protein)	Damkier*et al*., 2009
				mouse brain (protein)	Liu *et al*., 2010
				rat brain (cloning, B & C variants), mouse brain (mRNA & protein), rat tissues (mRNA)	Giffard *et al*., 2003
				rat brain (mRNA), rat & mouse choroid plexus (mRNA, protein)	Praetorius *et al*., 2004b
				rat & mouse brain (protein)	Chen *et al*., 2007
				rat & mouse brain (protein)	Chen *et al*., 2008c
				human choroid plexus (protein)	Praetorius & Nielsen, 2006
				human brain & choroid plexus, other tissues (mRNA)	Damkier *et al*., 2007
				human brain (cloning, A & B variants), human brain (mRNA, A & B variants)	Parker *et al*., 2008a
NDAE		NDAE1		*Drosophila* (cloning), *Drosophila* CNS & other tissues (mRNA)	Romero *et al*., 2000
				Drosophila nervous system, sensory organs, & other tissues (protein)	Sciortino *et al*., 2001
NDCBE	A–D, D'	kNBC-3, NDCBE1	*SLC4A8*	squid giant fiber lobe (cloning, A variant), squid tissues (mRNA)	Virkki *et al*., 2003

NCBT	Variants	Alternate names	SLC	Reported tissue distribution[1]	References
				mouse kidney (cloning, A variant), mouse tissues (mRNA)	Wang *et al*., 2001
				rat brain (protein)	Risso-Bradley *et al*., 2001
				rat brain (mRNA)	Praetorius *et al*., 2004b
				rat & mouse brain (protein)	Chen *et al*., 2008a,b
				human brain (cloning, B variant), human brain & tissues (mRNA)	Grichtchenko *et al*., 2001
				human brain, choroid plexus, & other tissues (mRNA), human brain (protein)	Damkier *et al*., 2007
				human brain (cloning, A, C, D, D' variants), human brain (mRNA, A variant), human tissues (mRNA, C, D, & D')	Parker *et al*., 2008b

[1] In many studies, the molecular probes used (e.g., primers in PCR, oligonucleotides with in-situ hybridization, and antibodies with immunochemical techniques) recognized or are expected to recognize multiple variants (often unavoidably).

[2] *Ambystoma* NBC (aNBC)

[3] *Ambystoma* kidney NBC (akNBC)

[4] human heart NBC (hhNBC)

[5] human pancreas NBC (hpNBC)

[6] kidney NBC (kNBC)

[7] pancreas NBC (pNBC)

[8] rat brain 1 NBC (rb1NBC)

[9] rat brain 2 NBC (rb2NBC)

[10] rat kidney NBC (rkNBC)

[11] zebrafish NBCe1 (zNBCe1)

[12] The amino sequence is 82% identical to NBCe1-B

[13] rat brain 1 NCBE (rb1NCBE)

[14] rat brain 2 NCBE (rb2NCBE)