Chapter 6
Acid/Base Transporters in CSF Secretion and pH Regulation

Dagne Barbuskaite, Helle Damkier, and Jeppe Praetorius

Abstract Early functional studies on the choroid plexus established that the cerebrospinal fluid (CSF) secretion rate was sensitive to inhibitors of acid/base transporters and carbonic anhydrase. In fact, the CO_2/HCO_3^- buffer system is required for effective CSF secretion. Unlike blood, the CSF contains very little protein and yet it responds efficiently to changes in pH utilizing primarily the CO_2/HCO_3^- buffer system. Besides a central role in CSF secretion, recent studies indicate a central role for the choroid plexus epithelium in CSF pH regulation. The luminal plasma membrane expresses a variety of acid and base transport proteins of which one, the NBCe2, was directly shown to regulate CSF pH. This chapter describes the current knowledge on the molecular mechanisms in choroid plexus acid/base transport and the understanding of their possible involvement in both CSF secretion and pH regulation.

6.1 Acid/Base Regulation in Brain Extracellular Fluid Compartments

The brain is composed of two extracellular fluid compartments: the brain extracellular fluid (BECF) surrounding cells in the parenchyma as well as the cerebrospinal fluid (CSF) that fills the ventricles and covers the brain and spinal cord (Hladky and Barrand 2016).

The fluid compartments are separated from the blood by two distinct barriers. The blood-brain barrier (BBB) separates the BECF from the blood, and the blood-cerebrospinal fluid barrier (BCSFB) separates blood from cerebrospinal fluid

D. Barbuskaite
Department of Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
e-mail: dagne@sund.ku.dk

H. Damkier · J. Praetorius (✉)
Department of Biomedicine, Faculty of Health, Aarhus University, Aarhus, Denmark
e-mail: jp@biomed.au.dk

J. Praetorius et al. (eds.), *Role of the Choroid Plexus in Health and Disease*, Physiology in Health and Disease, https://doi.org/10.1007/978-1-0716-0536-3_6

(CSF). These barriers have distinct barrier properties that hinder diffusion of many substances. The BECF and the CSF in the ventricles are only separated by a single layer of ependymal cells which does not constitute a barrier per se. The CSF in the subarachnoid space and the space surrounding the penetrating vessels from the surface of the brain, the Virchow-Robin space, is separated from the BECF by the pia-glial membrane. This membrane consists of a thin layer of connective tissue covering the surface of the brain parenchyma and the glia limitans which is a layer of astrocytic end-feet. This membrane is permeable to diffusion of substances between the CSF and BECF. Both the BBB and the BCSFB are permeable to CO_2 that enters the brain freely and if not buffered will lead to a decrease in brain pH. In order to buffer the pH, bases such as HCO_3^- need to be transported from the blood across the barriers. This requires the presence of transporters in the membranes of the barrier.

Neuronal excitability is greatly affected by pH changes in the brain parenchyma (Baron et al. 1985). Firing of action potentials builds up the acid content in the interstitium. These acids are usually buffered and cleared and do not lead to functional damage (Kazemi et al. 1967). In some circumstances, acid production can even protect brain function. The high neuronal activity during seizures builds up an acidic environment. This activates acid sensing ion channels which leads to inhibition of the neuronal activity, and thus to halting the seizure (Ziemann et al. 2008). In febrile seizures, the fever causes the child to hyperventilate and thereby respiratory alkalosis develops in the immature brain (Schuchmann et al. 2006). The seizure is broken by the central pH decrease that follows the increased neuronal activity.

Proteins play a central role in buffering the effects of sudden changes in pH of the blood plasma and other fluid spaces. In contrast, CSF contains only small amounts of protein but brain pH is nevertheless maintained within a quite narrow range. Fluctuations in plasma pH and pCO_2 are followed over time by similar changes in CSF pH (Lee et al. 1969). While CO_2 crosses all brain barriers rapidly, H^+ and HCO_3^- mainly cross the CPE via membrane transporters. One would expect this to result in sizable fluctuations in brain pH during respiratory acidosis because of the lack of protein buffers. However, in respiratory acidosis the decrease in blood pH surpasses that of CSF pH due to the presence of efficient buffers such as the open CO_2/HCO_3^- buffer system. In support for such buffering, Hasan and collaborators found that inhalation of 5% CO_2 for 4 h lead to a threefold higher increase in CSF HCO_3^- compared to plasma HCO_3^- in dogs (Hasan and Kazemi 1976).

CSF pH is known to directly influence BECF pH in the areas close to the ventricle system (Okada et al. 1993). Also, the CSF in the subarachnoid space enters the brain parenchyma via the blood vessels. The composition of CSF thereby greatly affects the composition of the BECF and thereby the pH regulation of the brain parenchyma.

6.2 Choroid Plexus Acid-Base Transport

The production of CSF amounts to approximately 500 ml CSF daily in humans, of which the major proportion is secreted by the epithelial cells of the choroid plexus (Cserr 1971). The CSF formation by the choroid plexus depends primarily on the

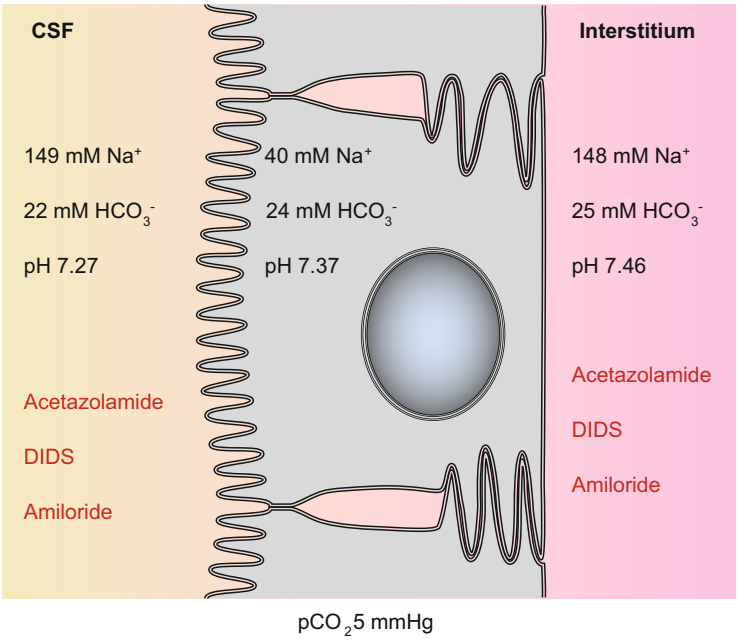

Fig. 6.1 Schematic diagram of normal physiological values and inhibitor actions relevant to choroid plexus epithelial acid-base transport. Acetazolamide and DIDS inhibits CSF secretion both when applied from the CSF side and the interstitial side. Amiloride inhibits CSF secretion when applied from the blood/interstitial side as well as from the luminal side

transepithelial movement of Na^+, Cl^-, HCO_3^- and H_2O (Pollay and Curl 1967; Welch 1963; Davson and Segal 1970; Wright 1972). Apart from the central involvement of typical transport proteins such as e.g. the Na^+, K^+-ATPase (Welch 1963; Davson and Segal 1970; Wright 1972) in CSF production by the choroid plexus, the CO_2/HCO_3^- buffering system and acid-base transporters seem to be of pivotal importance for the process of secretion. Firstly, the carbonic anhydrase inhibitor acetazolamide inhibits CSF secretion significantly (Welch 1963; Tschirgi et al. 1954; Kister 1956; Davson and Luck 1957; Ames et al. 1965; Segal and Burgess 1974; Pollay and Davson 1963; Vogh et al. 1987; Murphy and Johanson 1989a) (Fig. 6.1). Secondly, CSF secretion is sensitive to manipulations of basolateral pH and HCO_3^- concentration (Saito and Wright 1983), and basolateral application of the stilbene-type HCO_3^- transport inhibitor DIDS reduces the secretion rate by choroid plexus epithelial cells (Deng and Johanson 1989). Finally, the NHE inhibitor amiloride inhibits Na^+ import into the choroid plexus from the blood side as well as the CSF secretion (Davson and Segal 1970; Murphy and Johanson 1989a, b; Johanson and Murphy 1990), suggesting a dependence of CSF secretion on basolateral Na^+/H^+ exchange. Nevertheless, injection of amiloride into the ventricles had a similar effect on CSF secretion (Segal and Burgess 1974). In the following, we summarize the current knowledge on choroid plexus carbonic anhydrases and acid-base transport proteins.

6.2.1 Carbonic Anhydrases

In many biological systems, the hydration of CO_2 to form H_2CO_3 is catalyzed by carbonic anhydrases (Boron and Boulpaep 2012), and H_2CO_3 then dissociates spontaneously to HCO_3^- and H^+. Carbonic anhydrases are among the fastest enzymes studied and are blocked by acetazolamide (Maren 1962). Carbonic anhydrase activity is important for CSF production by the choroid plexus, as acetazolamide reduces secretion in the range of 50–100% depending on the experimental setup (Welch 1963; Davson and Segal 1970; Ames et al. 1965). In the early experiments, carbonic anhydrase activity was mainly considered in relation to the cytosol where HCO_3^- would be produced within the cell by intracellular carbonic anhydrase such as CAII (Fig. 6.2). This led to the hypothesis that HCO_3^- secretion by the CPE depended on intracellular formation from CO_2 and H_2O (Vogh and Maren 1975; Maren 1972). However, this reaction would also produce intracellular H^+, and the choroid plexus cell would therefore require an efficient basolateral H^+

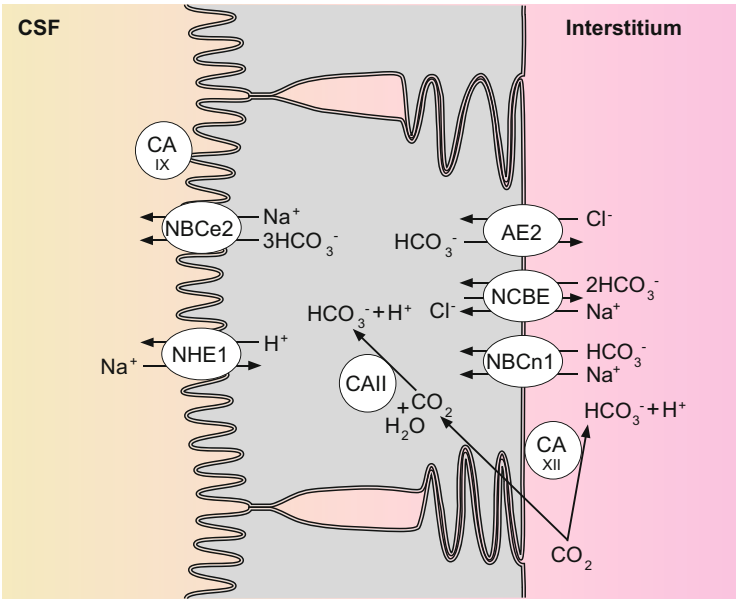

Fig. 6.2 Schematic diagram of choroid plexus epithelial carbonic anhydrases (CAs). Acetazolamide and high concentrations inhibit CAs efficiently, but it is impossible to tell which of the three forms are most relevant for CSF formation and pH regulation. Interestingly, enzyme histochemistry is only detected in the plasma membrane domains (Masuzawa et al. 1981) i.e. corresponding to CAIX and CAXII. The traditional hypothesis predicts intracellular conversion of CO_2 and H_2O to H^+ and HCO_3^- to sustain bicarbonate secretion. The alternative hypothesis involves CAXII in extracellular activity to sustain HCO_3^- import into the epithelial cells and perhaps CAIX for sustaining luminal HCO_3^- extrusion. Thus, like in the duodenal villus cells, there is a notion that HCO_3^- secretion is sustained by transcellular HCO_3^- movement rather than intracellular HCO_3^- generation from CO_2

extrusion mechanism such as a Na^+/H^+ exchanger (NHE) in order to sustain CSF secretion. There is, indeed, an apparent lack of such transport mechanism in the basolateral membrane of choroid plexus epithelial cells, i.e. there is no basolateral membrane expression of NHE's and only minimal acid extrusion capacity in the absence of Na^+ or CO_2/HCO_3^- [see below Bouzinova et al. (2005), Damkier et al. (2009), Christensen et al. (2017)]. The only known basolateral "acid-extruders" are actually two HCO_3^- import proteins (Ncbe and NBCn1, see below).

Thus, the model of intracellular formation of HCO_3^- for secretion and basolateral H^+ extrusion is difficult to sustain despite Maren's pioneering work. One indication for a transcellular route for secreted HCO_3^- came from the clear enzyme histochemical demonstration that choroid plexus epithelial carbonic anhydrase activity was almost entirely restricted to the microvilli and the basal infoldings (Masuzawa et al. 1981). This is consistent with the detection of plasma membrane carbonic anhydrases CAIX and CAXII in the choroid plexus (Christensen et al. 2017; Kallio et al. 2006), where CAXII is localized to the basolateral membrane and CAIX may be enriched in the luminal domain (Fig. 6.2). Both CAIX and CAXII are single-pass transmembrane proteins with extracellular catalytic domains (Tureci et al. 1998; Opavsky et al. 1996). We are not aware of any other normal epithelium expressing both of these CA forms. The close proximity between these enzymes and the plasma membrane HCO_3^- transporters may increase transport rates by supplying sufficient substrate (HCO_3^- or even CO_3^{2-}) for the transport (McMurtrie et al. 2004). The putative direct physical and functional associations of external membrane-bound and/or cytosolic carbonic anhydrases with HCO_3^- transporters such as AE2 and NBCe1 is compelling although still debated (McMurtrie et al. 2004; Boron 2010). Nevertheless, it remains possible that membrane bound CAs with extracellular enzyme activity are the actual targets of acetazolamide in the choroid plexus, and that a reduced HCO_3^- transport rate results in the observed decline in CSF secretion. The primary target for acetazolamide in the choroid plexus needs to be investigated in order to establish to what extent secreted HCO_3^- arise from cytosolic production and from transcellular carrier mediated movement.

6.2.2 *Base Transporters*

As mentioned above, the presence of the CO_2/HCO_3^- buffering system is necessary for efficient CSF secretion thereby indirectly implicating the participation of HCO_3^- transporters in the process of CSF secretion (Haselbach et al. 2001). Indeed, DIDS reduces HCO_3^- and Na^+ transport rates to the luminal side of the epithelium both after basolateral and luminal application (Deng and Johanson 1989; Nattie and Adams 1988; Mayer and Sanders-Bush 1993). Bicarbonate transporters arise from two gene families: the solute carrier (*Slc*) 4 and 26 families (Romero et al. 2013; Cordat and Reithmeier 2014). The *Slc4* derived polypeptides include the classical Cl^-/HCO_3^- exchangers of which Ae2 and, the electrogenic sodium bicarbonate exchanger NBCe2, the electroneutral NBCn1, and Ncbe/NBCn2 expressed in the

choroid plexus (Damkier et al. 2013). Apart from epithelial NBCn1, these transporters are DIDS sensitive and candidate targets mediating the inhibitory effect of stilbene derivatives on CSF secretion.

6.2.2.1 The Anion Exchanger Ae2, Slc4a2

Ae2 mediates electroneutral exchange of extracellular Cl^- for intracellular HCO_3^-, and thus extrudes base from the cell (Alper 2009),is more active at alkaline pH, and is inhibited by DIDS (4). The import of Cl^- into the choroid plexus epithelial cell from the interstitium is necessary for continuous CSF secretion, and most likely involves classical anion exchangers as judged from the sensitivity towards basolateral DIDS application (Deng and Johanson 1989; Frankel and Kazemi 1983) and the requirement for CO_2/HCO_3^- (Hughes et al. 2010). Ae2 is expressed at the basolateral membrane domain of choroid plexus epithelial cells with the highest signal at the basal labyrinth (Lindsey et al. 1990; Alper et al. 1994; Praetorius and Nielsen 2006) (Fig. 6.3). The localization and direction of transport suggests that Ae2 is involved in transcellular Cl^- transport and is in fact the only known basolateral Cl^- entry mechanism for sustaining CSF secretion. Ae2 may also be indirectly involved in CSF pH regulation, as it could facilitate continued H^+ excretion across the luminal membrane as e.g. in CSF pH regulation during alkalosis.

6.2.2.2 The Electroneutral Na^+ and HCO_3^- Transporter Ncbe, NBCn2, Slc4a10

Polypeptides derived from *slc4a10* have all been characterized as DIDS sensitive electroneutral Na^+ driven and HCO_3^- importers (Giffard et al. 2003; Wang et al. 2000; Damkier et al. 2010; Parker et al. 2008). First, the HCO_3^- transport was shown to depend on intracellular Cl^- in mammalian expression systems transfected with *slc4a10* (Giffard et al. 2003; Wang et al. 2000). In one study, the stoichiometry for Na^+ to acid-base equivalents was estimated to 1:2 and a net Cl^- efflux was DIDS-sensitive (Damkier et al. 2010). Thus, the transporter was characterized as a Na^+-dependent Cl^-/HCO_3^- exchanger and named Ncbe. However, convincing results from a study using the human *SLC4A10* gene product expressed in *Xenopus laevis* oocytes did not support the dependence of transport on intracellular Cl^- nor was a net Cl^- extrusion detected by Parker et al. (2008). The protein was therefore renamed NBCn2 as an electroneutral Na^+:HCO_3^- cotransporter. We prefer to apply the transporter names Ncbe in rodents and NBCn2 in humans as long as this controversy is not resolved.

In the choroid plexus, the Ncbe/NBCn2 transporter is localized on the basolateral membrane (Praetorius and Nielsen 2006; Praetorius et al. 2004a), and is therefore proposed to supply the CPE with both Na^+ and HCO_3^- from the blood side of the epithelium (Fig. 6.4). Consistent with this idea, the Na^+ and CO_2/HCO_3^-dependent base uptake (or acid extrusion) in the choroid plexus epithelium is partly DIDS

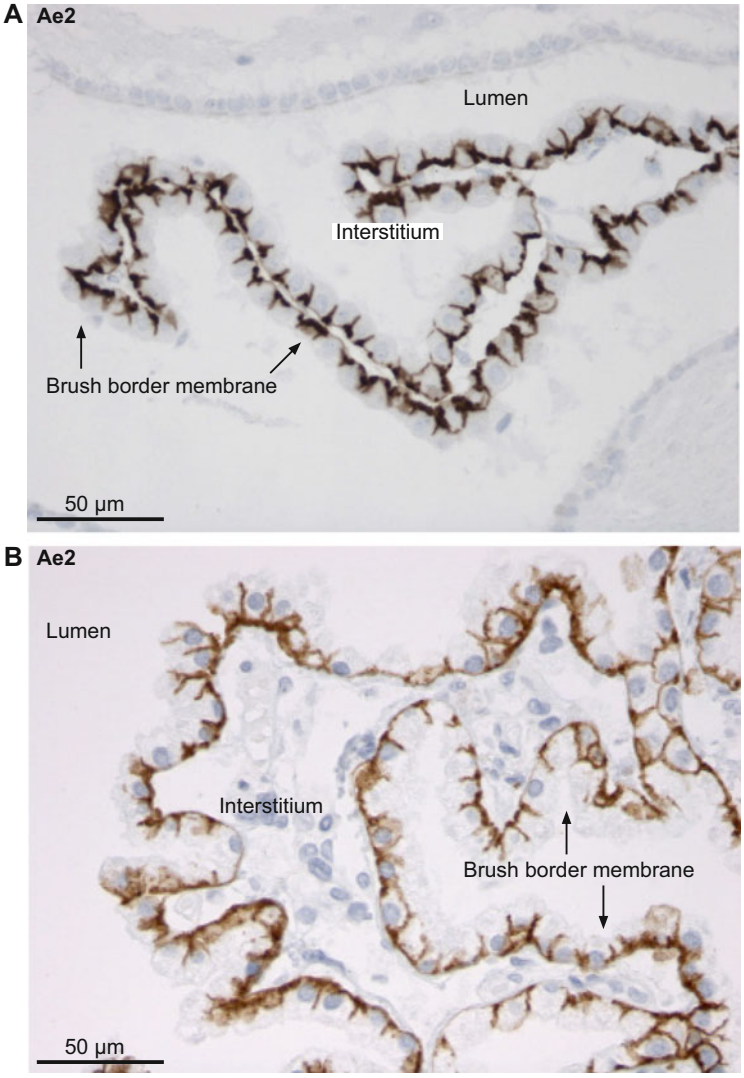

Fig. 6.3 Bright-field micrograph of anti-Ae2 immuno-peroxidase reactivity in the choroid plexus. (**a**) The brown reaction product is confined to the basolateral membrane domains including the basal labyrinth in mouse tissue. (**b**) A similar staining pattern was observed in the human choroid plexus. Nuclei are counterstained with hematoxylin (blue). For methods, see previous publications (Praetorius and Nielsen 2006; Praetorius et al. 2004a)

sensitive, probably reflecting Ncbe activity (Bouzinova et al. 2005). Direct involvement of Ncbe in the pH_i regulation of choroid plexus epithelial cells was demonstrated in studies of Ncbe knockout mice. The choroid plexus from Ncbe knockout mice showed a severely diminished Na^+-dependent acid extrusion compared to normal littermates (Jacobs et al. 2008), and up to 70% of the DIDS-sensitive pH_i

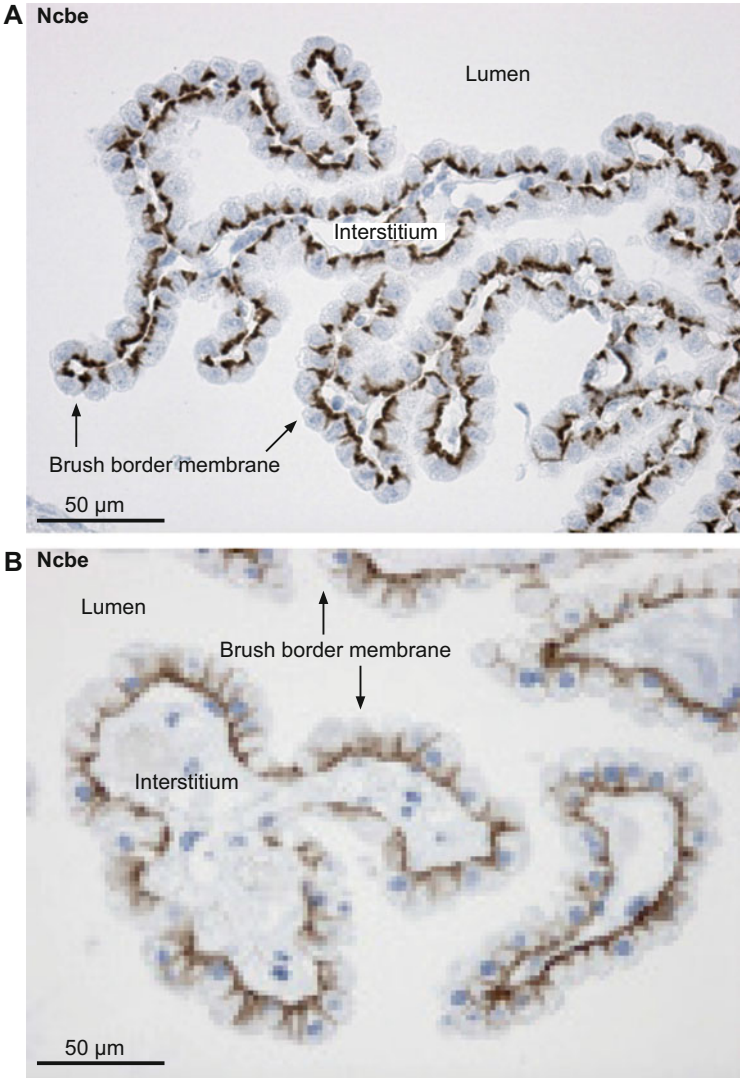

Fig. 6.4 Bright-field micrograph of anti-Ncbe immuno-peroxidase reactivity in the choroid plexus. (**a**) The brown reaction product is confined to the basolateral membrane domains including the basal labyrinth in mouse tissue. (**b**) A similar staining pattern was observed in the human choroid plexus. Nuclei are counterstained with hematoxylin (blue). For methods, see previous publications (Praetorius and Nielsen 2006; Praetorius et al. 2004a)

recovery from acid load depends on this transporter (Damkier et al. 2009). Besides regulating pH_i of the choroid plexus epithelial cells, Ncbe mediated HCO_3^- loading may well facilitate the luminal bicarbonate extrusion. In this way, Ncbe could sustain luminal HCO_3^- secretion and indirectly participate in CSF pH regulation.

As one of a very few Na^+ transporters in the basolateral membrane, the Ncbe/NBCn2 probably constitutes the bottleneck for Na^+ in CSF secretion, although this has not yet been demonstrated directly. Nevertheless, the genetic disruption of Ncbe resulted in a reduction in brain ventricle size to almost the same degree as the Na^+:HCO_3^- cotransport (Jacobs et al. 2008). This may suggest that Ncbe knockout also greatly diminished the rate of CSF secretion. One should take caution suggesting a central role for Ncbe in CSF secretion, although the studies in Ncbe knockout mice are compelling in that regard. Large changes in abundance of other transport proteins of interest may also explain the decreased ventricular volume and the feasibly diminished CSF secretion in Ncbe knockout mice. Ncbe knockout critically affected the expression levels of the Na^+, K^+-ATPase, AQP1, Ae2, and e.g. membrane protein anchoring proteins (Damkier et al. 2009; Christensen et al. 2013; Damkier and Praetorius 2012). The luminal Na^+/H^+ exchanger NHE1 of the normal choroid plexus was demonstrated in the basolateral membrane domain of the epithelial cells from Ncbe knockout mice (Damkier et al. 2009). Thus, studies directly demonstrating the involvement of Ncbe in CSF secretion are highly warranted.

6.2.2.3 The Electrogenic Na^+:HCO_3^- Cotransporter NBCe2, Slc4a5

The electrogenic cotransporter NBCe2 transports 1 Na^+ with 2 or 3 HCO_3^- in or out of cells across the plasma membrane depending on tissue-specific factors (Sassani et al. 2002; Virkki et al. 2002). Typical electrochemical gradients for Na^+ and HCO_3^- favor outward transport in the 1:3 transport mode and inward transport in the 1:2 transport mode. Electrogenic HCO_3^- transport in the luminal membrane of choroid plexus epithelial cells was established in bull frog (Saito and Wright 1984), and the Na^+ dependence of transport indicated an outwardly directed Na^+:HCO_3^- cotransport mechanism was shown in mouse and rat (Banizs et al. 2007; Johanson et al. 1992). These observations could be explained by the expression of NBCe2 in the luminal plasma membrane domain of rat (Bouzinova et al. 2005) and mouse (Christensen et al. 2018) choroid plexus (Fig. 6.5). NBCe2 mRNA was detected in human choroid plexus as well (Praetorius and Nielsen 2006; Damkier et al. 2007), but the protein was not immunolocalized to the epithelium, probably reflecting amino acid sequence differences compared to the rodent immunogenic epitope applied thus far. NBCe2 exports Na^+ and HCO_3^- across the luminal membrane of mouse CPE in an apparent 1:3 stoichiometry (Millar and Brown 2008). Thus, NBCe2 is believed to take part in the Na^+ secretion of the choroid plexus epithelium as well as the regulation of intracellular pH and even extracellular pH.

Inhalation of 11% CO_2 causes a significant increase in the Na^+ content in rodent CSF (Nattie 1980). This effect may represent indirect evidence for NBCe2 function in the choroid plexus. More recently, the physiological significance of NBCe2 has been studied more directly in three different NBCe2 knockout mouse strains. The first study reported that NBCe2 deficiency resulted in decreased ventricular volume and intracranial pressure, changes in CSF electrolyte composition, and resistance to seizure induction (Kao et al. 2011). Taken together, the study suggested NBCe2 to

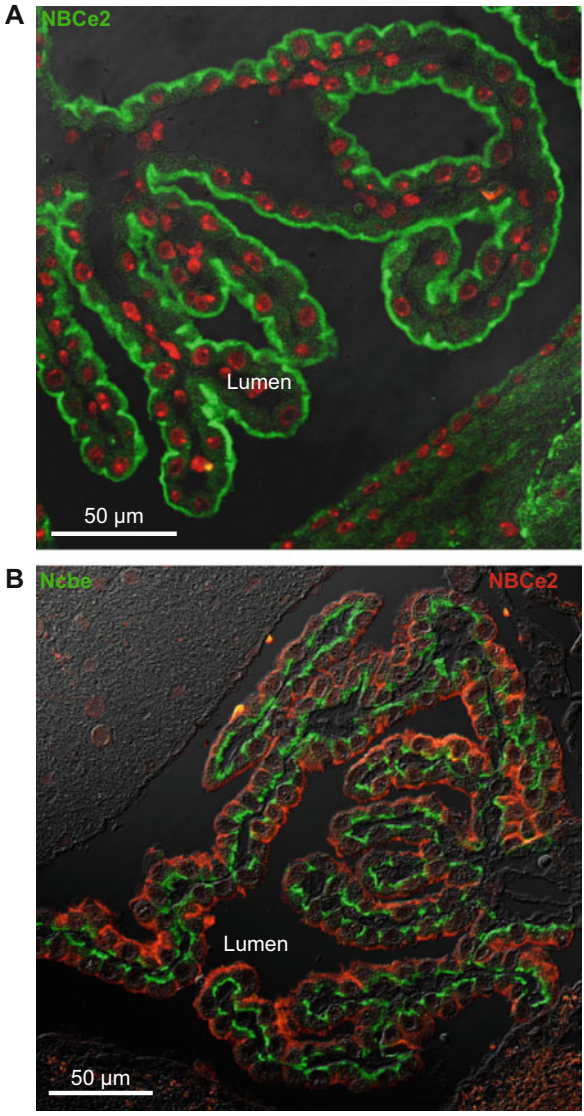

Fig. 6.5 Confocal micrograph of anti-NBCe2 immuno-fluorescence reactivity in the choroid plexus. (**a**) The green immunoreaction is confined to the luminal membrane domain in mouse tissue. (**b**) A similar staining pattern was observed in the rat choroid plexus. Nuclei are counterstained with Topro3 (red). For methods, see previous publications (Bouzinova et al. 2005; Christensen et al. 2018)

be involved in both the regulation of CSF [HCO_3^-] and in CSF secretion. However, the interpretation of the findings was compromised by the changes in expression profiles of other transporter proteins in choroid plexus. For example, the changes in expression and localization of Ncbe and Na,K-ATPase units in this gene trap NBCe2 knockout would most likely contribute considerably to the observed phenotype. The second study focused on the renal consequences of NBCe2 knockout in the kidney. It was, however, noted that the ventricle volume did not seem to be greatly affected by the gene disruption (Groger et al. 2012) and therefore this study seems to

contradict a central role for NBCe2 in CSF secretion. In the latest study of NBCe2 ko mice, caution was again taken to validate the model by describing the expression profiles of various transporters in the choroid plexus epithelium (Christensen et al. 2018). This model did not reveal any changes in abundance or subcellular distribution of other transport proteins or gross changes in ventricle dimensions in the knockouts. However, NBCe2 was directly involved in base extrusion after intracellular alkalization ex vivo, and critically implicated in CSF pH recovery during hypercapnia-induced acidosis in vivo (Christensen et al. 2018). Thus, this study provided direct mechanistic evidence of the implication of the choroid plexus epithelium in CSF pH regulation, and established NBCe2 as the central mediators of this physiological process.

6.2.2.4 The Electroneutral Na^+:HCO_3^- Cotransporter NBCn1, Slc4a7

NBCn1 is an electroneutral sodium bicarbonate cotransporter that mediates Na^+ and HCO_3^- uptake into cells (Romero et al. 2013). The epithelial NBCn1 form is relatively DIDS insensitive, and in the rat choroid plexus the Na-dependent HCO_3^- influx was partly ascribed to this transporter (Bouzinova et al. 2005). Experiments in Ncbe and NBCn1 knockout mice indicate that NBCn1 may contribute less to pH recovery from acidosis in this species (Jacobs et al. 2008; Damkier, unpublished observation). The polarized plasma membrane distribution of NBCn1 in the choroid plexus varies among species and even among stains (Fig. 6.6). NBCn1 was localized to basolateral membrane domain in rats and humans (Praetorius and Nielsen 2006; Praetorius et al. 2004a), but can also be detected in the luminal membrane domain within the same human choroid plexus (Praetorius and Nielsen 2006). In the mouse, the luminal or basolateral NBCn1 expression seems to depend on the strain (Damkier et al. 2009; Praetorius et al. 2004a; Kao et al. 2011).

It is unlikely that NBCn1 takes part significantly in the vectorial HCO_3^- transport across the choroid plexus epithelium during correction of CSF pH, as it is not always expressed in the same membrane domain. As a HCO_3^- loader, NBCn1 could be involved in regulating pH within the choroid plexus epithelium, which may require separate acid-base transporters than those involved in the secretory process. It is unknown whether choroid plexus NBCn1 abundance and function is regulated in acid-base disturbances as elsewhere (Praetorius et al. 2004b). In neurons, NBCn1 is upregulated during metabolic acidosis (Park et al. 2010), thus this transporter could possibly have a similar role in the choroid plexus. Further studies are warranted to elucidate the roles of NBCn1 in this tissue.

6.2.2.5 The *slc4a11* Gene Product

The transport function of *SLC4A11* gene products is much debated, and consensus has not yet been reached regarding a unifying name for the transporter. The controversy probably results from the application of varying experimental

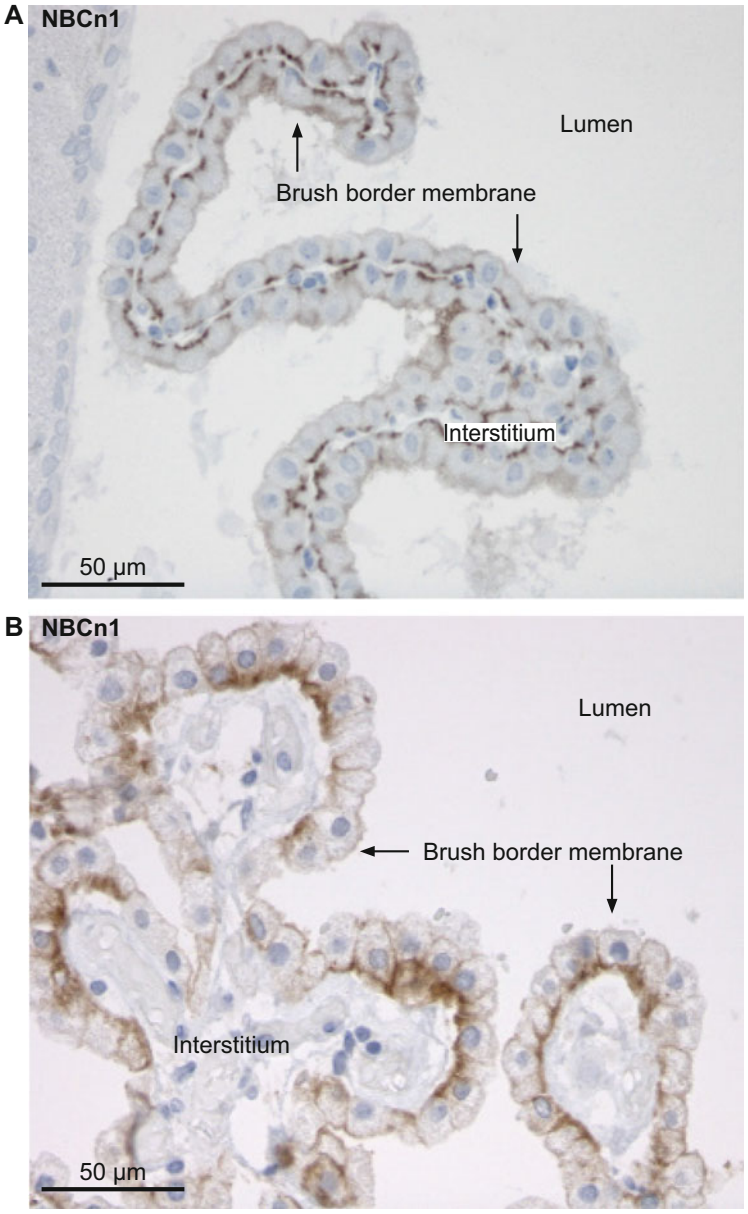

Fig. 6.6 Bright-field micrograph of anti-NBCn1 immuno-peroxidase reactivity in the choroid plexus. (**a**) The brown reaction product is confined to the basolateral membrane domains including the basal labyrinth in mouse tissue. (**b**) A similar staining pattern was observed in the human choroid plexus. Nuclei are counterstained with hematoxylin (blue). For methods, see previous publications (Praetorius and Nielsen 2006; Praetorius et al. 2004a)

techniques and species differences among genes and expression systems in these studies. In bovine corneal endothelial cells, the Na^+-coupled OH^- transport depended on the expression of *SLC4A11* (Jalimarada et al. 2013). The human *SLC4A11* forms seem to transport H^+ in both Na^+-dependent and -independent modes. In the presence of ammonia, *SLC4A11* generated inward currents that were comparable in magnitude (Kao et al. 2016). In another study, however, human *SLC4A11* seems to mediate electroneutral NH_3 transport in a Na^+ independent manner (Loganathan et al. 2016). By contrast, mouse *slc4a11* mediates a OH^- (or H^+) selective conductance without cotransport characteristics (Myers et al. 2016). Both the rat and the human choroid plexus epithelia express *SLC4A11* derived proteins in the luminal membrane domains (58), but *slc4a11* was not detected in the mouse choroid plexus (Damkier et al. 2018). Thus, the significance of NaBC1 in relation to choroid plexus function is unknown.

6.2.2.6 Anion Conductances

As mentioned above, HCO_3^- is secreted across the luminal membrane by electrogenic processes and can be stimulated by cAMP. This transport could be mediated by NBCe2 alone or in concert with HCO_3^- conductive anion channels. Indeed, protein kinase A stimulates inward-rectifying anion conductances in mammalian choroid plexus epithelial cells (Kibble et al. 1996, 1997). In the amphibian choroid plexus, a HCO_3^- conductive and cAMP-regulated ion channel was proposed as the major HCO_3^- efflux mechanism of the luminal membrane (Saito and Wright 1983, 1984). It seems that the inward-rectifying anion channels in the mammalian choroid plexus possess such high HCO_3^- permeability (Kibble et al. 1996). The molecular identities of the anion channels have not been fully established yet, but CFTR and Clc-2 have been ruled out as mediators of the inward-rectifying anion channels (Kibble et al. 1996, 1997; Speake et al. 2002). Recent mass spectrometry analysis of mouse choroid plexus epithelial cells indicated the expression of voltage-dependent anion channels (Vdac1–3) (Damkier et al. 2018).

6.2.2.7 *Slc26*-Derived HCO_3^- Transporters

It is possible that membrane proteins belonging to the *slc26* gene family could serve as HCO_3^- transporters in the choroid plexus epithelium. The mRNA for *slc26a2* (Kant et al. 2018), *slc26a4* (Saunders et al. 2015), *slc26a7*, *slc26a10*, and *slc26a11* (own unpublished observations) have all been detected at the mRNA level in the choroid plexus, but the corresponding proteins have not been detected in isolated epithelial cells by mass spectrometry (Damkier et al. 2018). Thus, it remains to be established to what extent these transporters contribute to base transport in the choroid plexus epithelium.

6.2.3 Acid Transporters

6.2.3.1 The Na^+/H^+ Exchangers, NHE1, slc9a1 and NHE6, slc9a6

The Na^+/H^+ exchanger NHE1 is almost ubiquitously expressed and mediates the electroneutral exchange of Na^+ and H^+, driven by the inward Na^+ gradient. It is inhibited by amiloride derivatives, such as EIPA (233). Na^+/H^+ exchange has been suggested as basolateral entry pathway for Na^+ and therefore centrally involved in CSF secretion, as amiloride applied from the blood side of the CPE inhibits Na^+ accumulation and CSF secretion (Davson and Segal 1970; Murphy and Johanson 1989a, b). This type of transporter would at the same time provide a basolateral H^+ extrusion mechanism to support luminal HCO_3^- secretion. NHE1 mRNA was demonstrated by RT-PCR analysis (Kalaria et al. 1998) and EIPA-sensitive Na/H exchange was detected in isolated epithelial cells (Bouzinova et al. 2005; Damkier et al. 2009). Thus, for some time NHE1 was thought as the primary Na^+ loader in the choroid plexus epithelium.

Some evidence seems to contradict a central role of basolateral NHE activity in choroid plexus function. Firstly, amiloride applied form the luminal side was as efficient as basolateral amiloride in inhibiting CSF secretion (Segal and Burgess 1974). Secondly, amiloride failed to inhibit the Na^+-dependent pH recovery in the choroid plexus cultures in the presence of the CO_2/HCO_3^- buffer system (Mayer and Sanders-Bush 1993). Finally, NHE1 was immunolocalized to the luminal membrane domain of the choroid plexus epithelium in both mice and humans (Damkier et al. 2009; Praetorius and Nielsen 2006; Kao et al. 2011) (Fig. 6.7). NHE1 expression was required for detection of any Na^+-dependent pH_i recovery from acid load in choroid plexus, as judged from experiments on NHE1 knockout mice (Damkier et al. 2009). At first glance, this is indicative of NHE1 being the only NHE in the choroid plexus. However, the abundance of NHE1 is low in the choroid plexus as judged from the immunoreactivity in the referred studies and from mass spectrometry (Damkier et al. 2018). Another Na^+/H^+ exchanger detected in the CPE is NHE6. Although this transporter is usually found in the endosomes, it has been immunolocalized partly in the luminal membrane domain in mouse CPE (Damkier et al. 2018) (Fig. 6.8a). No mRNA for NHE2–5 and NHE7–9 have been detected in the CPE in the same study. NHEs are usually considered as regulators of intracellular pH_i, but since NHE1 and NHE6 transport H^+ out of the cell into the CSF, they could partake in CSF pH regulation. Future studies of the respective roles of NHE1 and NHE6 in intracellular pH and/or CSF pH regulation are warranted.

6.2.3.2 H^+-ATPases

Transmembrane ATPases catalyze the "uphill" movement of H^+ across membranes utilizing the energy generated by the hydrolysis of ATP into ADP and free phosphate. The vacuolar H^+-ATPase (V-ATPase) lowers the pH of vesicles in the endo-

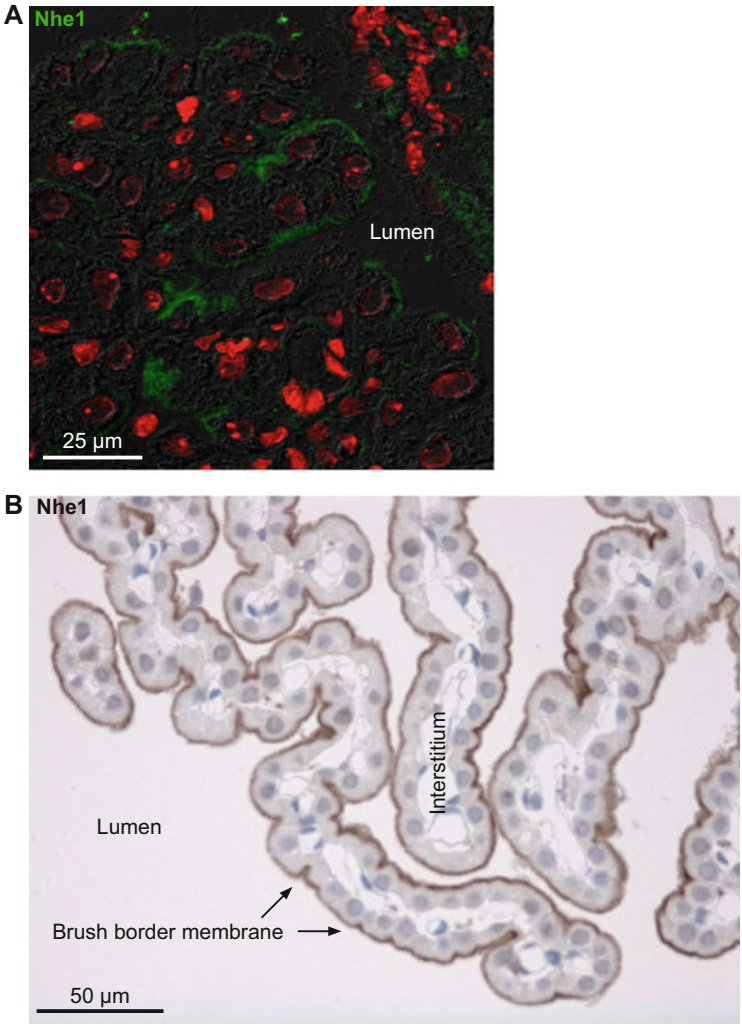

Fig. 6.7 Immunolocalization of Nhe1 in the choroid plexus epithelium. (**a**) Confocal micrograph of luminal membrane domain anti-Nhe1 immuno-fluorescence reactivity (Orlowski AB, green) in the mouse choroid plexus. Nuclei are counterstained with Topro3 (red). (**b**) Bright-field micrograph of anti-NBCn1 immuno-peroxidase reactivity in the rat choroid plexus produced a similar staining pattern with a separate antibody (Nöel AB, brown). Nuclei are counterstained with hematoxylin (blue). For methods, see previous publications (Damkier et al. 2009)

lysosomal system, but can also be plasma membrane acid extruders in epithelia. The B2 isoform of the V-ATPase is usually restricted to the lysosomes and endosomes (Puopolo et al. 1992), yet a small population of the polypeptide was immunolocalized to the luminal membrane domain in the mouse choroid plexus epithelium (Christensen et al. 2017) (Fig. 6.8b). However, the contribution of

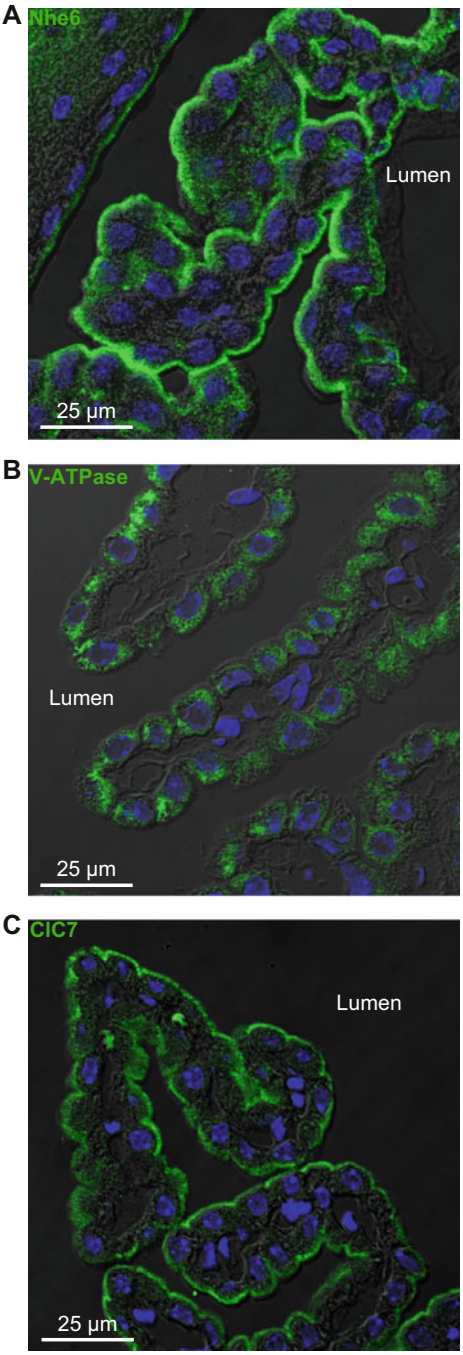

Fig. 6.8 Immunolocalization of additional acid transport proteins in the mouse choroid plexus. Varying degrees of intracellular and luminal membrane domain immunoreactivity (green) is shown for (**a**) Nhe6, (**b**) V-ATPase, and (**c**) ClC-7. Nuclei are counterstained with Topro3 (red). For methods, see Christensen et al. (Christensen et al. 2017*;* Damkier et al. 2018)

luminal V-ATPase to acid-extrusion in the choroid plexus of mice seems to be insignificant compared to NHE activity (Christensen et al. 2017). H^+/K^+-ATPases comprise a separate group of cellular acid extruders, that might contribute to choroid plexus acid-base transport. In rabbits, application of omeprazole, an inhibitor of gastric H^+/K^+-ATPase from the luminal side reduced CSF secretion rate (Lindvall-Axelsson et al. 1992). Thus far, only the non-gastric isoform of H^+/K^+-ATPase has been detected in the rabbit and mouse choroid plexus (Damkier et al. 2018; Pestov et al. 1998). In the unstimulated mouse choroid plexus the Na^+-independent acid extrusion is negligible (Christensen et al. 2017), leaving little room for H^+/K^+-ATPase to have a significant role in this tissue.

6.2.3.3 Acid Transporters Related to Cl^- Channel

Several polypeptides belonging to the ClC group of Cl^- channels have been characterized as Cl^-/H^+ exchangers. Among these, ClC3, ClC4, ClC5 and ClC7 were detected by mass spectroscopy in isolated epithelial cells from the mouse choroid plexus (Damkier et al. 2018). A fraction of the ClC7 immunoreactivity was detected in the luminal CPE membrane domain in the same study, where the expected vesicular ClC7 was also described (Fig. 6.8c). Cl^-/H^+ exchange activity was demonstrated in isolated mouse CPE clusters, as removal and reintroduction of extracellular Cl^- in the absence of Na^+ and HCO_3^- caused the relevant changes in pH_i. Whether ClC7 is the main Cl^-/H^+ exchanger, or other ClC isoforms also contribute to pH regulation in the CPE in vivo requires further investigations. It is peculiar that, like osteoclasts, choroid plexus epithelial cells seems to express a fraction of endo-lysosomal acid transport proteins in the luminal membrane (Fig. 6.9).

6.3 Choroid Plexus Acid-Base Transporters and Cerebrospinal Fluid Secretion

Some evidence supports the central involvement of choroid plexus acid-base transporters in the secretion of CSF. Firstly, the concentrations of Na^+ and HCO_3^- in CSF are higher than expected from an ultrafiltrate (Hughes et al. 2010). Secondly, the CSF secretion and the choroid plexus transport rates are highly affected by acetazolamide, amiloride, and DIDS (Welch 1963; Davson and Segal 1970; Tschirgi et al. 1954; Davson and Luck 1957; Ames et al. 1965; Murphy and Johanson 1989a; Johanson and Murphy 1990; Johanson et al. 1992; McCarthy and Reed 1974; Vogh and Godman 1985). Thirdly, the ventricle volume is affected by genetic disruption of Ncbe and in one report of NBCe2 (Jacobs et al. 2008; Kao et al. 2011). AE2 and Ncbe are most likely mediating the major fraction of Na^+, Cl^- and HCO_3^- import to the epithelial cells from the blood side that is necessary for secretion across the

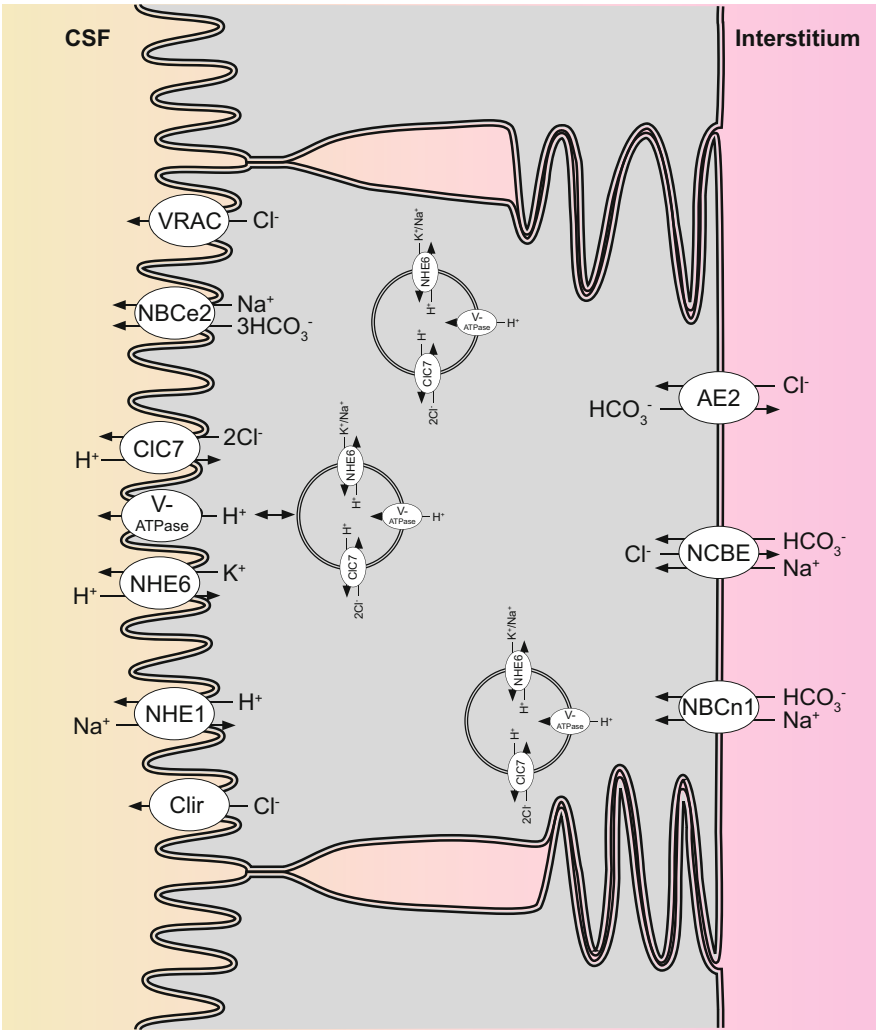

Fig. 6.9 Schematic diagram of the hypothesized exchange of endo-lysosomal transport proteins with the luminal plasma membrane, as a model for the observation of partial luminal expression of Nhe6, the V-ATPase, and ClC-7

luminal membrane. These proteins are expressed in high abundance in the basal labyrinth of the choroid plexus across species and very few alternative absorptive pathways exist in the basolateral membrane. Thus, there are indications for a dependence of fluid secretion by the CPE on acid-base transporters.

6.4 Choroid Plexus and Cerebrospinal Fluid pH Regulation

The acute CSF pH greatly depends on the breathing rate, yet over time CSF pH is maintained within narrow limits (Lee et al. 1969). Upon changes in respiration frequency or tidal volume, pCO_2 in the blood changes accordingly. Since CO_2 can easily cross the blood-brain and blood-CSF barriers, increased pCO_2 in the blood results in increased pCO_2 in the CSF and brain ECF, and thus low pH (Johnson et al. 1983). Changes in metabolic acid-base balance are not reflected in CSF pH, as HCO_3^- and H^+ have to be actively transported over these barriers. As mentioned previously, contrary to the blood plasma, CSF contains very little protein and phosphate buffers, thus should be subjected to a great level of pH variation during respiratory acidosis. However, several studies have demonstrated that increased CSF pCO_2 is compensated by an increase in CSF HCO_3^-, independently of blood acid-base balance. The robust expression of a HCO_3^- export protein NBCe2 in the luminal plasma membrane of the CPE spurred the idea that this tissue could serve a CSF pH regulatory function (Bouzinova et al. 2005) (Fig. 6.5).

NBCe2 transports Na^+ and HCO_3^- out of the CPE cells, most likely with a 1:3 stoichiometry (Millar and Brown 2008), and with its membrane localization it constitutes an ideal candidate for the HCO_3^- secretion into the CSF. As discussed above, two sources of HCO_3^- for the secretion has been proposed. The classic view is an origin from the hydration of intracellular CO_2. The high levels of carbonic anhydrase II in the CPE would efficiently convert CO_2 to HCO_3^- to supply the luminal transporter with HCO_3^- (Johanson et al. 1992). The problem with this explanation is the apparent lack of NHE proteins in the basolateral membrane. The alternative view is transcellular passage of HCO_3^-, where e.g. Ncbe mediates the import of HCO_3^- across the basolateral membrane (Praetorius et al. 2004c).

CSF pH seems to respond more efficiently to respiratory acidosis than to alkalosis, indicating that the acid extrusion by the CPE is less effective than the alkaline export (Kazemi et al. 1967). Nevertheless, the CPE cells express several proteins that could be involved in acid extrusion to the CSF. In the luminal membrane, Na^+/H^+ exchangers NHE1 (and NHE6) export H^+ in exchange for Na^+ (Damkier et al. 2009) and thereby candidate as mechanisms to lower CSF pH. NHE1 is involved in the regulation of intracellular pH in the CPE cells (Damkier et al. 2009), but its putative role in CSF pH regulation has not been investigated. Recently, we demonstrated that a fraction of the vacuolar H^+-ATPase (V-ATPase) is expressed in the luminal membrane of CPE cells (Christensen et al. 2017). Although this pump is involved in extracellular acidification elsewhere, our study rejects the V-ATPase as a significant contributor to CSF pH regulation (Christensen et al. 2017). The $2Cl^-/H^+$ antiporter ClC-7 was also immunolocalized to the luminal membrane of CPE cells in the same study. However, the highly electrogenic nature of the transport by this protein makes ClC7 highly unlikely as an efficient acid extruder. It is unknown whether AE2 plays a role on CSF pH regulation, but the basolateral expression and the export HCO_3^- in exchange for Cl^- (Alper et al. 1994) could be required sustain luminal H^+ secretion.

References

Alper SL (2009) Molecular physiology and genetics of Na+-independent SLC4 anion exchangers. J Exp Biol 212(Pt 11):1672–1683

Alper SL et al (1994) The fodrin-ankyrin cytoskeleton of choroid plexus preferentially colocalizes with apical Na+K(+)-ATPase rather than with basolateral anion exchanger AE2. J Clin Invest 93 (4):1430–1438

Ames A III, Higashi K, Nesbett FB (1965) Effects of Pco2 acetazolamide and ouabain on volume and composition of choroid-plexus fluid. J Physiol 181(3):516–524

Banizs B et al (2007) Altered pH(i) regulation and Na(+)/HCO3(-) transporter activity in choroid plexus of cilia-defective Tg737(orpk) mutant mouse. Am J Physiol Cell Physiol 292(4):C1409–C1416

Baron R et al (1985) Cell-mediated extracellular acidification and bone resorption: evidence for a low pH in resorbing lacunae and localization of a 100-kD lysosomal membrane protein at the osteoclast ruffled border. J Cell Biol 101(6):2210–2222

Boron WF (2010) Evaluating the role of carbonic anhydrases in the transport of HCO3--related species. Biochim Biophys Acta 1804(2):410–421

Boron WF, Boulpaep EL (2012) Medical physiology. Saunders, Philadelphia, PA

Bouzinova EV et al (2005) Na+-dependent HCO_3^- uptake into the rat choroid plexus epithelium is partially DIDS sensitive. Am J Physiol Cell Physiol 289(6):C1448–C1456

Christensen IB et al (2013) Polarization of membrane associated proteins in the choroid plexus epithelium from normal and slc4a10 knockout mice. Front Physiol 4:344

Christensen HL et al (2017) The V-ATPase is expressed in the choroid plexus and mediates cAMP-induced intracellular pH alterations. Physiol Rep 5(1):e13072

Christensen HL et al (2018) The choroid plexus sodium-bicarbonate cotransporter NBCe2 regulates mouse cerebrospinal fluid pH. J Physiol 596(19):4709–4728

Cordat E, Reithmeier RA (2014) Structure, function, and trafficking of SLC4 and SLC26 anion transporters. Curr Top Membr 73:1–67

Cserr HF (1971) Physiology of the choroid plexus. Physiol Rev 51(2):273–311

Damkier HH, Praetorius J (2012) Genetic ablation of Slc4a10 alters the expression pattern of transporters involved in solute movement in the mouse choroid plexus. Am J Physiol Cell Physiol 302(10):C1452–C1459

Damkier HH, Nielsen S, Praetorius J (2007) Molecular expression of SLC4-derived Na+-dependent anion transporters in selected human tissues. Am J Physiol Regul Integr Comp Physiol 293(5): R2136–R2146

Damkier HH et al (2009) Nhe1 is a luminal Na+/H+ exchanger in mouse choroid plexus and is targeted to the basolateral membrane in Ncbe/Nbcn2-null mice. Am J Physiol Cell Physiol 296 (6):C1291–C1300

Damkier HH, Aalkjaer C, Praetorius J (2010) Na+−dependent HCO_3^- import by the slc4a10 gene product involves Cl^- export. J Biol Chem 285(35):26998–27007

Damkier HH, Brown PD, Praetorius J (2013) Cerebrospinal fluid secretion by the choroid plexus. Physiol Rev 93(4):1847–1892

Damkier HH et al (2018) The murine choroid plexus epithelium expresses the 2Cl(-)/H(+) exchanger ClC-7 and Na(+)/H(+) exchanger NHE6 in the luminal membrane domain. Am J Physiol Cell Physiol 314(4):C439–C448

Davson H, Luck CP (1957) The effect of acetazoleamide on the chemical composition of the aqueous humour and cerebrospinal fluid of some mammalian species and on the rate of turnover of 24Na in these fluids. J Physiol 137(2):279–293

Davson H, Segal MB (1970) The effects of some inhibitors and accelerators of sodium transport on the turnover of 22Na in the cerebrospinal fluid and the brain. J Physiol 209(1):131–153

Deng QS, Johanson CE (1989) Stilbenes inhibit exchange of chloride between blood, choroid plexus and the cerebrospinal fluid. Brain Res 510:183–187

Frankel H, Kazemi H (1983) Regulation of CSF composition--blocking chloride-bicarbonate exchange. J Appl Physiol 55(1 Pt 1):177–182

Giffard RG et al (2003) Two variants of the rat brain sodium-driven chloride bicarbonate exchanger (NCBE): developmental expression and addition of a PDZ motif. Eur J Neurosci 18 (11):2935–2945

Groger N et al (2012) Targeted mutation of SLC4A5 induces arterial hypertension and renal metabolic acidosis. Hum Mol Genet 21(5):1025–1036

Hasan FM, Kazemi H (1976) Dual contribution theory of regulation of CSF HCO3 in respiratory acidosis. J Appl Physiol 40(4):559–567

Haselbach M et al (2001) Porcine choroid plexus epithelial cells in culture: regulation of barrier properties and transport processes. Microsc Res Tech 52(1):137–152

Hladky SB, Barrand MA (2016) Fluid and ion transfer across the blood-brain and blood-cerebrospinal fluid barriers; a comparative account of mechanisms and roles. Fluids Barriers CNS 13(1):19

Hughes AL, Pakhomova A, Brown PD (2010) Regulatory volume increase in epithelial cells isolated from the mouse fourth ventricle choroid plexus involves Na^+-H^+ exchange but not Na^+-K^+-$2Cl^-$ cotransport. Brain Res 1323:1–10

Jacobs S et al (2008) Mice with targeted Slc4a10 gene disruption have small brain ventricles and show reduced neuronal excitability. Proc Natl Acad Sci U S A 105(1):311–316

Jalimarada SS et al (2013) Ion transport function of SLC4A11 in corneal endothelium. Invest Ophthalmol Vis Sci 54(6):4330–4340

Johanson CE, Murphy VA (1990) Acetazolamide and insulin alter choroid plexus epithelial cell [Na +], pH, and volume. Am J Phys 258(6 Pt 2):F1538–F1546

Johanson CE, Parandoosh Z, Dyas ML (1992) Maturational differences in acetazolamide-altered pH and HCO3 of choroid plexus, cerebrospinal fluid, and brain. Am J Phys 262(5 Pt 2):R909–R914

Johnson DC, Hoop B, Kazemi H (1983) Movement of CO2 and HCO-3 from blood to brain in dogs. J Appl Physiol Respir Environ Exerc Physiol 54(4):989–996

Kalaria RN et al (1998) Identification and expression of the Na^+/H^+ exchanger in mammalian cerebrovascular and choroidal tissues: characterisation by amiloride-sensitive [^{3}H]MIA binding and RT-PCR analysis. Brain Res Mol Brain Res 58:178–187

Kallio H et al (2006) Expression of carbonic anhydrases IX and XII during mouse embryonic development. BMC Dev Biol 6:22

Kant S et al (2018) Choroid plexus genes for CSF production and brain homeostasis are altered in Alzheimer's disease. Fluids Barriers CNS 15(1):34

Kao L et al (2011) Severe neurologic impairment in mice with targeted disruption of the electrogenic sodium bicarbonate cotransporter NBCe2 (Slc4a5 gene). J Biol Chem 286 (37):32563–32574

Kao L et al (2016) Multifunctional ion transport properties of human SLC4A11: comparison of the SLC4A11-B and SLC4A11-C variants. Am J Physiol Cell Physiol 311(5):C820–C830

Kazemi H, Shannon DC, Carvallo-Gil E (1967) Brain CO2 buffering capacity in respiratory acidosis and alkalosis. J Appl Physiol 22(2):241–246

Kibble JD, Tresize AO, Brown PD (1996) Properties of the cAMP-activated Cl^- conductance in choroid plexus epithelial cells isolated from the rat. J Physiol 496:69–80

Kibble JD et al (1997) Whole-cell Cl^- conductances in mouse choroid plexus epithelial cells do not require CFTR expression. Am J Phys 272:C1899–C1907

Kister SJ (1956) Carbonic anhydrase inhibition. VI. The effect of acetazolamide on cerebrospinal fluid flow. J Pharmacol Exp Ther 117(4):402–405

Lee JE et al (1969) Buffering capacity of cerebrospinal fluid in acute respiratory acidosis in dogs. Am J Phys 217(4):1035–1038

Lindsey AE et al (1990) Functional expression and subcellular localization of an anion exchanger cloned from choroid plexus. Proc Natl Acad Sci U S A 87(14):5278–5282

Lindvall-Axelsson M et al (1992) Inhibition of cerebrospinal fluid formation by omeprazole. Exp Neurol 115(3):394–399
Loganathan SK et al (2016) Functional assessment of SLC4A11, an integral membrane protein mutated in corneal dystrophies. Am J Physiol Cell Physiol 311(5):C735–C748
Maren TH (1962) The binding of inhibitors to carbonic anhydrase in vivo: drugs as markers for enzyme. Biochem Pharmacol 9:39–48
Maren TH (1972) Bicarbonate formation in cerebrospinal fluid: role in sodium transport and pH regulation. Am J Phys 222(4):885–899
Masuzawa T et al (1981) Ultrastructural localization of carbonic anhydrase activity in the rat choroid plexus epithelial cell. Histochemistry 73(2):201–209
Mayer SE, Sanders-Bush E (1993) Sodium-dependent antiporters in choroid plexus epithelial cultures from rabbit. J Neurochem 60:1308–1316
McCarthy KD, Reed DJ (1974) The effect of acetazolamide and furosemide on cerebrospinal fluid production and choroid plexus carbonic anhydrase activity. J Pharmacol Exp Ther 189 (1):194–201
McMurtrie HL et al (2004) The bicarbonate transport metabolon. J Enzyme Inhib Med Chem 19 (3):231–236
Millar ID, Brown PD (2008) NBCe2 exhibits a 3 HCO_3^-:1 Na^+ stoichiometry in mouse choroid plexus epithelial cells. Biochem Biophys Res Commun 373:550–554
Murphy VA, Johanson CE (1989a) Acidosis, acetazolamide, and amiloride: effects on 22Na transfer across the blood-brain and blood-CSF barriers. J Neurochem 52(4):1058–1063
Murphy VA, Johanson CE (1989b) Alteration of sodium transport by the choroid plexus with amiloride. Biochim Biophys Acta 979(2):187–192
Myers EJ et al (2016) Mouse Slc4a11 expressed in Xenopus oocytes is an ideally selective H+/OH- conductance pathway that is stimulated by rises in intracellular and extracellular pH. Am J Physiol Cell Physiol 311(6):C945–C959
Nattie EE (1980) Brain and cerebrospinal fluid ionic composition and ventilation in acute hypercapnia. Respir Physiol 40(3):309–322
Nattie EE, Adams JM (1988) DIDS decreases CSF HCO3- and increases breathing in response to CO2 in awake rabbits. J Appl Physiol (1985) 64(1):397–403
Okada Y et al (1993) Depth profiles of pH and PO2 in the isolated brain stem-spinal cord of the neonatal rat. Respir Physiol 93(3):315–326
Opavsky R et al (1996) Human MN/CA9 gene, a novel member of the carbonic anhydrase family: structure and exon to protein domain relationships. Genomics 33(3):480–487
Park HJ et al (2010) Neuronal expression of sodium/bicarbonate cotransporter NBCn1 (SLC4A7) and its response to chronic metabolic acidosis. Am J Physiol Cell Physiol 298(5):C1018–C1028
Parker MD et al (2008) Characterization of human SLC4A10 as an electroneutral Na/HCO3 cotransporter (NBCn2) with Cl- self-exchange activity. J Biol Chem 283(19):12777–12788
Pestov NB et al (1998) Ouabain-sensitive H,K-ATPase: tissue-specific expression of the mammalian genes encoding the catalytic alpha subunit. FEBS Lett 440(3):320–324
Pollay M, Curl F (1967) Secretion of cerebrospinal fluid by the ventricular ependyma of the rabbit. Am J Phys 213(4):1031–1038
Pollay M, Davson H (1963) The passage of certain substances out of the cerebrosphinal fluid. Brain 86:137–150
Praetorius J, Nielsen S (2006) Distribution of sodium transporters and aquaporin-1 in the human choroid plexus. Am J Physiol Cell Physiol 291(1):C59–C67
Praetorius J, Nejsum LN, Nielsen S (2004a) A SCL4A10 gene product maps selectively to the basolateral plasma membrane of choroid plexus epithelial cells. Am J Physiol Cell Physiol 286 (3):C601–C610
Praetorius J et al (2004b) NBCn1 is a basolateral Na+-HCO3- cotransporter in rat kidney inner medullary collecting ducts. Am J Physiol Renal Physiol 286(5):F903–F912
Praetorius J, Nejsum LN, Nielsen S (2004c) A SLC4A10 gene product maps selectively to the basolateral membrane of choroid plexus epithelial cells. Am J Phys 286:C601–C610

Puopolo K et al (1992) Differential expression of the "B" subunit of the vacuolar H(+)-ATPase in bovine tissues. J Biol Chem 267(6):3696–3706
Romero MF et al (2013) The SLC4 family of bicarbonate (HCO(3)(-)) transporters. Mol Asp Med 34(2–3):159–182
Saito Y, Wright EM (1983) Bicarbonate transport across the frog choroid plexus and its control by cyclic nucleotides. J Physiol 336:635–648
Saito Y, Wright E (1984) Regulation of bicarbonate transport across the brush border membrane of the bull-frog choroid plexus. J Physiol 350:327–342
Sassani P et al (2002) Functional characterization of NBC4: a new electrogenic sodium-bicarbonate cotransporter. Am J Physiol Cell Physiol 282(2):C408–C416
Saunders NR et al (2015) Influx mechanisms in the embryonic and adult rat choroid plexus: a transcriptome study. Front Neurosci 9:123
Schuchmann S et al (2006) Experimental febrile seizures are precipitated by a hyperthermia-induced respiratory alkalosis. Nat Med 12(7):817–823
Segal MB, Burgess AM (1974) A combined physiological and morphological study of the secretory process in the rabbit choroid plexus. J Cell Sci 14(2):339–350
Speake T et al (2002) Inward-rectifying anion channels are expressed in the epithelial cells of choroid plexus isolated from ClC-2 'knock-out' mice. J Physiol 539:385–390
Tschirgi RD, Frost RW, Taylor JL (1954) Inhibition of cerebrospinal fluid formation by a carbonic anhydrase inhibitor, 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (diamox). Proc Soc Exp Biol Med 87(2):373–376
Tureci O et al (1998) Human carbonic anhydrase XII: cDNA cloning, expression, and chromosomal localization of a carbonic anhydrase gene that is overexpressed in some renal cell cancers. Proc Natl Acad Sci U S A 95(13):7608–7613
Virkki LV et al (2002) Functional characterization of human NBC4 as an electrogenic Na+-HCO cotransporter (NBCe2). Am J Physiol Cell Physiol 282(6):C1278–C1289
Vogh BP, Godman DR (1985) Timolol plus acetazolamide: effect on formation of cerebrospinal fluid in cats and rats. Can J Physiol Pharmacol 63(4):340–343
Vogh BP, Maren TH (1975) Sodium, chloride, and bicarbonate movement from plasma to cerebrospinal fluid in cats. Am J Phys 228(3):673–683
Vogh BP, Godman DR, Maren TH (1987) Effect of $AlCl_3$ and other acids on cerebrospinal fluid production: a correction. J Pharmacol Exp Ther 243(1):35–39
Wang CZ et al (2000) The Na+−driven cl-/HCO3- exchanger. Cloning, tissue distribution, and functional characterization. J Biol Chem 275(45):35486–35490
Welch K (1963) Secretion of cerebrospinal fluid by choroid plexus of the rabbit. Am J Phys 205:617–624
Wright EM (1972) Mechanisms of ion transport across the choroid plexus. J Physiol 226 (2):545–571
Ziemann AE et al (2008) Seizure termination by acidosis depends on ASIC1a. Nat Neurosci 11 (7):816–822