Epithelial Pathways in Choroid Plexus Electrolyte Transport

Helle H. Damkier,[1] Peter D. Brown,[2] and Jeppe Praetorius[1]

[1]Department of Anatomy and the Water and Salt Research Center, Aarhus University, Aarhus C, Denmark; and [2]Channels and Transporters Research Group, Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom
jp@ana.au.dk

A stable intraventricular milieu is crucial for maintaining normal neuronal function. The choroid plexus epithelium produces the cerebrospinal fluid and in doing so influences the chemical composition of the interstitial fluid of the brain. Here, we review the molecular pathways involved in transport of the electrolytes Na^+, K^+, Cl^-, and HCO_3^- across the choroid plexus epithelium.

The human brain is a delicate organ that is surrounded by the rigid skull. The effective weight of the brain is reduced from 1,500 to ~50 g by being submerged in the cerebrospinal fluid (CSF). This helps reduce the risk of mechanical injury to the brain parenchyma and cerebral blood vessels (51). The CSF is constantly secreted and thus displaces older fluid, which drains into the systemic circulation via the dural sinuses. This creates a flow of fluid through the ventricular system, which is important in maintaining a stable environment within the brain. The flow of CSF also assists in the removal of waste products from the central nervous system, e.g., excess neurotransmitters (43), debris from the surface lining epithelium, bacteria, and viruses (22).

The balance between CSF production and reabsorption normally helps maintain intracranial pressure at appropriate levels (5–15 mmHg) despite fluctuations in blood flow and changes in plasma osmolarity. In addition, the composition of CSF in the intraventricular compartment is kept remarkably constant over time, despite changes in the blood compartment. For example, an increase in plasma $[K^+]$ from 1.6 to 11 mM results in a <2 mM increase in CSF $[K^+]$ (4). Changes in plasma $[HCO_3^-]$ also have little effect on the corresponding CSF values (34). Furthermore, elevations in blood P_{CO_2} rapidly lead to similar increase in CSF P_{CO_2}; however, this affects CSF pH much less than predicted, taking into account the almost complete lack of protein buffers in CSF (9). This feature remains largely unexplained but is of great physiological and clinical importance since a 0.05-U drop in CSF pH evokes a 10-fold increase in ventilation (29).

The CSF is produced mainly by the cells of the choroid plexus epithelium (FIGURE 1A). In addition to the secretion of water and solutes, the choroid plexuses also transport humoral mediators and nutrients, which are required by the central nervous system, e.g., leptin (14). In this review, however, we will concentrate on discussing the molecular mechanisms involved in transport of the main CSF electrolytes across the epithelium, namely Na^+, Cl^-, HCO_3^-, and K^+.

Choroid Plexus Morphology

The choroid plexuses arise from the ependymal lining of the brain ventricles and protrude into the ventricles. They are quite small structures and in the adult human weigh only ~2 g in total (25). In the lateral ventricles of the mammalian brain, the choroid plexuses form sheet-like structures, whereas branched villus-like structures are found in the third and fourth ventricles (19). Each choroid plexus consists of a continuous monolayer of cuboidal to columnar epithelial cells situated on a core of connective tissue (FIGURE 1, B AND C). The part of the blood-CSF barrier formed by the choroid plexus consists of the fenestrated endothelium of the capillaries with their basal lamina, sparse connective tissue, a basement membrane, and the single layer of epithelial cells. The epithelium is regarded as the major barrier to the movement of substances between the blood and the CSF. The epithelial barrier is defined by the plasma membranes and lateral intercellular spaces with relatively leaky tight junctions. The ventricular surface is characterized by numerous microvilli and a central bundle of long motile cilia, as reviewed previously (86, 75). The basal surface is relatively smooth, but the lateral surfaces appear very irregular with many basal infoldings (44). The blood flow to the choroid plexuses is 3 $ml \cdot g^{-1} \cdot min^{-1}$, which is 10 times higher than the flow to the brain parenchyma and 5 times higher than that to the kidney (15, 30).

Secretion of CSF

Rougemont and colleagues first suggested that CSF is not an ultrafiltrate and that it is secreted by the choroid plexus epithelium, as they demonstrated that the solute contents of nascent CSF differed from those expected for a plasma ultrafiltrate (71). The view is now generally accepted as: 1) the CSF is 5 mOsm hypertonic compared with plasma (23); 2) the $[Na^+]$ and $[HCO_3^-]$ of nascent CSF are higher than expected for an ultrafiltrate (33), whereas $[K^+]$ and $[Cl^-]$ are lower in the CSF (5); 3) a 5-mV lumen positive electrical potential difference exists across

the epithelium (85) (FIGURE 2A). Apart from the plasma and CSF concentrations, it is also important to take the intracellular concentrations into account when the driving forces for ionic movement are discussed in relation to the specific transporters described below. The intracellular ionic composition in the mammalian choroid plexus epithelium has been estimated from the distribution of radioactive tracers after adjustment for extracellular and blood volumes (37). They are (in mM): 45 $[Na^+]_i$, 148 $[K^+]_i$, 12 $[HCO_3^-]_i$, and 65 $[Cl^-]_i$. These numbers, however, must be approximate because the combined concentrations of $[Na^+]_i$ and $[K^+]_i$ are very high compared with those in the CSF and plasma. Such concentrations will result in intracellular hypertonicity and will therefore be almost impossible to maintain in choroid plexus cells, which express high numbers of the aquaporin-1 (AQP1) water channel. Nevertheless, these numbers represent the best estimates available to date.

As will be discussed in detail below, there are fundamental differences between the mechanisms by which the choroid plexus secretes CSF and fluid secretion in other epithelia. Nevertheless, the molecular machinery behind the choroid plexus CSF production is highly efficient, and the rate of fluid transport rate is between 0.2 and 0.4 ml·min^{-1}·g tissue^{-1}, which exceeds the rate of secretion by the exocrine pancreas (80) and fluid reabsorption by the renal proximal tubules (12). Thus the epithelium secretes the equivalent of 10% of the received blood volume, and in total secretes ~0.5 liters/day in the adult human.

Na$^+$ Pathways in the Choroid Plexus

CSF secretion by the choroid plexus is closely linked to the transepithelial basal to CSF movement of Na$^+$ (3, 24, 68, 84, 86). As mentioned above, the epithelium has relatively leaky tight junctions, and one might predict that Na$^+$ secretion could occur via the paracellular route. This indeed is the case in most secretory epithelia where Na$^+$ secretion is paracellular pathway and is driven by lumen negative potential differences (generated by transcellular Cl$^-$ secretion). In the choroid plexus, however, there is a lumen positive transepithelial voltage (FIGURE 2A), and this exceeds the slight blood to ventricle chemical gradient for Na$^+$. Therefore, the net movement of Na$^+$ from the blood side to the ventricle must be energy dependent and occur by transcellular transport involving both basolateral Na$^+$ loaders and luminal Na$^+$ extruders (FIGURES 2B AND 3A).

Basolateral Na$^+$ Loaders

In vivo and in vitro studies have shown that CSF secretion is inhibited by 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS) applied to the basolateral side and is sensitive to basolateral pH and [HCO$_3$] (26, 54, 72). These findings suggest that Na$^+$-dependent acid/base transporters may be involved in the secretory process. At present, the electroneutral Na$^+$-HCO$_3^-$ cotransporter 2 (NBCn2 or NCBE) is thought to be the main entry route for Na$^+$ at the basolateral membrane. FIGURES 2B AND 3A shows that the NBCn2/NCBE is exclusively expressed in the basolateral membrane of the choroid plexus, where it can transport Na$^+$ and HCO$_3^-$ into the cell in a DIDS-sensitive manner (69). Genetic ablation of NBCn2/NCBE in mice causes an 80% reduction in brain ventricle size, suggesting that the rate of CSF secretion is greatly decreased (35). This hypothesis is supported by a similar reduction in DIDS-sensitive and Na$^+$-dependent HCO$_3^-$ import into the choroid plexus cells (21, 35). Deletion of NBCn2/NCBE not only causes decreased ventricle size, but also leads to
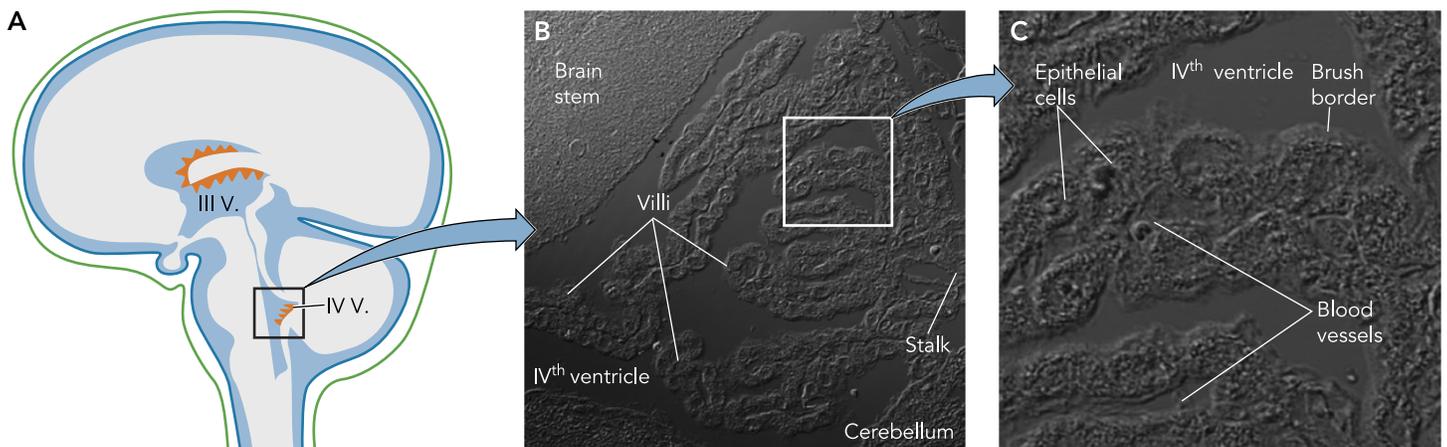

FIGURE 1. Schematic representation and differential interference contrast micrographs
A: schematic representation of the brain and its ventricular system. Choroid plexuses are indicated in orange, cerebrospinal fluid in light blue, brain parenchyma in gray, meninges in dark blue, and skull in green. III V. is the third ventricle, and IV V. is the fourth ventricle. B: differential interference contrast micrograph of the mouse fourth ventricle and choroid plexus at low magnification. C: higher magnification of a single villus of the same choroid plexus.

rearrangements of transporter expression, which presumably promote epithelial cell survival at the expense of maintaining CSF secretion (21). In support of this view, the abundance of the Na^+-K^+-ATPase is greatly decreased in the *slc4a10* knockout mouse (Damkier HH, Praetorius J, unpublished observations).

NBCn1 is another Na^+-HCO_3^- cotransporter that is expressed in the basolateral membrane of rat choroid plexus epithelial cells (69). In the rat choroid plexus, most of the Na^+-dependent HCO_3^- influx was initially ascribed to NBCn1 (10). However, more recent findings indicate that this protein does not play a major role in CSF secretion. First, the transporter is expressed in the luminal membrane of the choroid plexus in some mouse strains (21) and is also detected at both membranes in human choroid plexus (70). Second, the epithelial NBCn1 is not inhibited by DIDS; and third, the NBC activity in isolated choroid plexus cells from NBCn1 knockout mice was indistinguishable from that of wild-type littermates (Damkier HH, Praetorius J, unpublished observations). Thus the role of NBCn1 in the choroid plexus remains uncertain.

Luminal Na^+ Extruders

There are several Na^+ extruding mechanisms in the luminal membrane of the choroid plexus. The Na^+-K^+-ATPase is luminally expressed and directly secretes Na^+ into the CSF (28, 53, 70, 76). This is in marked contrast to the majority of epithelia in which the Na^+-K^+-ATPase is expressed in the basolateral membranes. At the mRNA level, α_1, β_1, and β_2 subunits are expressed in rat choroid plexus (48, 83, 92), and phospholemman (FXYD1) acts as the γ subunit homolog in choroid plexus (31). Phospholemman (FXYD1) is shown to reduce the Na^+ affinity of the Na^+-K^+-ATPase (17), but the change is not great enough to explain the reported high intracellular [Na^+] in the choroid plexus epithelium (39). Thus the Na^+-K^+-ATPase is probably fully activated with respect to the transcellular K^+ and Na^+ distribution.

Although the main Na^+ extruder is the Na^+-K^+-ATPase, other Na^+-coupled transporters may also contribute to the net Na^+ export. For instance, NBCe2 is also located in the luminal membrane (10) (FIGURES 2A AND 3A) and transports 1 Na^+ along with 3 HCO_3^- to the CSF (56). NBCe2 is believed to participate not only in Na^+ secretion but also in the regulation of CSF pH by extruding HCO_3^- (see *HCO_3^- Pathways in the Choroid Plexus* below).

Other Na^+ Transporters

A number of other Na^+ importers have been localized to the ventricular membrane, but their role in CSF secretion remains uncertain. The expression of NHE1 mRNA in the choroid plexus has been demonstrated by a number of groups (21, 41). For many years, this transporter was believed to reside in the basolateral membrane of the choroid plexus, because intravenous application of amiloride was reported to inhibit the rate of CSF secretion (59). Recent work, however, has shown that NHE1 is
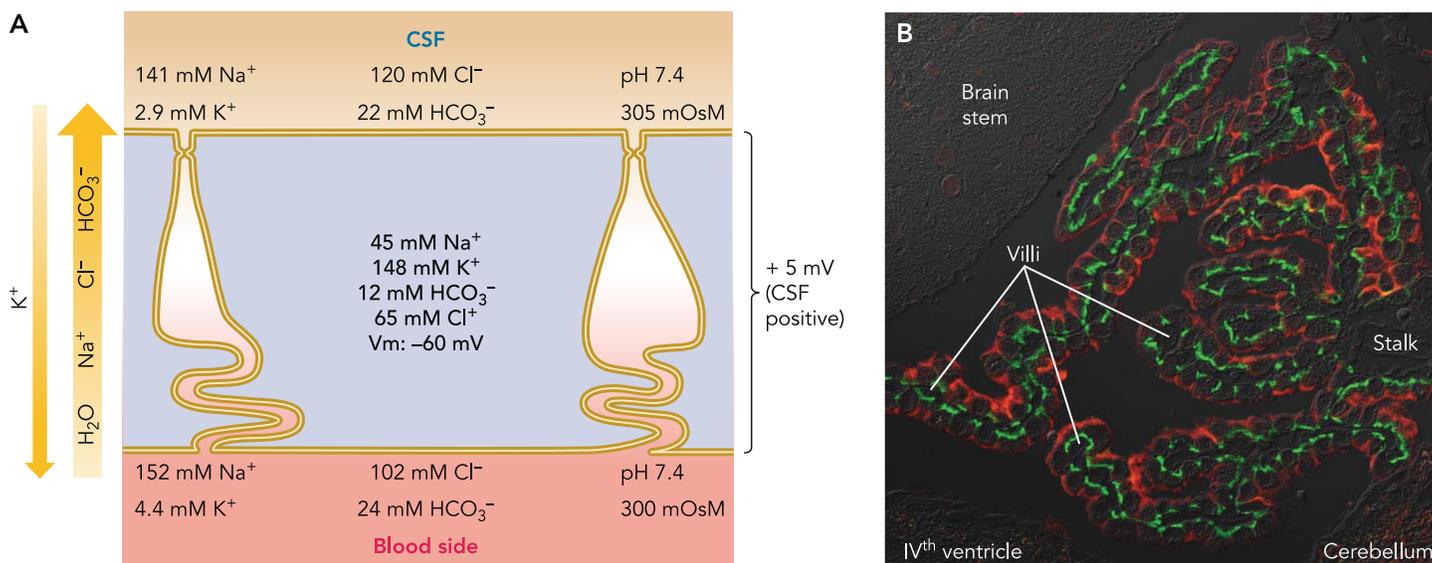
FIGURE 2. Estimates of ionic compositions and immunohistochemical localization of $NaHCO_3$ transporters
A: estimates of the ionic compositions of CSF, plasma, and the intracellular compartment of the choroid plexus. Values from plasma and CSF are from in vivo observations on rabbit (25), whereas intracellular ionic composition is from in vivo studies on rats (60). The luminal hyperosmolarity and the lumen positive transepithelial potential difference are indicated (85). The choroid plexus epithelium mediates net secretion of Na^+, Cl^-, HCO_3^-, and H_2O, whereas there is a net transcellular absorption of K^+. *B*: immunohistochemical localization of $NaHCO_3$ transporters involved in the production of cerebrospinal fluid. A low-magnification scanning confocal micrograph of mouse choroid plexus demonstrates the basolateral localization of NBCn2/NCBE (green) and luminal NBCe2 localization (red).

actually expressed in the luminal membrane of both mouse and human choroid plexus (21). At this location, it extrudes H^+ in exchange for extracellular Na^+. Furthermore, the NHE1 seems to be the only NHE isoform to be functional in the normal choroid plexus, since NHE1 knockout mouse displayed an almost complete the lack of Na^+-dependent pH_i recovery following acidification (21). The role of ventricular NHE1 in choroid plexus epithelium is almost certainly to maintain intracellular pH, and it probably does not participate directly in CSF secretion.

The Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) is also expressed in the ventricular membrane of the choroid plexus cells (45, 67) (FIGURE 3A). In the majority of cells including other secretory epithelia, NKCC1 mediates ion influx. In the choroid plexus, however, the net driving force for transport is close to zero given the low CSF K^+ concentration and high intracellular Na^+ and Cl^- (45). Thus it is debatable as to whether NKCC1 mediates ion influx or efflux, and experimental data in support of the direction of transport are equivocal. For instance, the introduction of bumetanide (an inhibitor of NKCC1) to the brain ventricles decreases CSF secretion rate, suggesting that NKCC1 extrudes Na^+ (8). By contrast, bumetanide has been reported to induce a decrease in choroid plexus cell volume in another study, suggesting that NKCC1 mediates inward transport (87). Most recently, Hughes and colleagues, reported that NKCC1 contributes neither to influx nor to efflux during cell volume regulation in choroid plexus cells (33). This lack of consistency in the data concerning NKCC1 may be due to differences in experimental conditions; alternatively, it may indicate that the activity of NKCC1 in the choroid plexus is carefully regulated. This view is perhaps supported by studies that have demonstrated that the expression of NKCC1 increases in rat choroid plexus after traumatic brain injury (52). This increase appears to be a compensatory response to the increased intracranial pressure, since bumetanide installation attenuated brain edema in the same model. Thus it seems that the role(s) of NKCC1 in the choroid plexus remains to be clarified, but it does not appear to play a major role in CSF secretion.

The mRNAs encoding all three subunits of the epithelial Na^+ channels (ENaC-α, -β, and -γ) have been detected by high cycle number RT-PCR in the rat choroid plexus (7). Subsequent work also identified immunoreactivity for all of three subunits and reported a benzamil-sensitive retention of $^{22}Na^+$ in the cells consistent with the expression of functional ENaC channels (6, 7). The massive inward driving force for Na^+ makes ENaC an obvious candidate as a Na^+ pathway at the basolateral membrane. The membrane localization of the ENaC subunits was not determined in these studies (6, 7). In our immunocytochemical studies, only the nonpore forming γ-subunit has been localized to luminal membrane of rat choroid plexus. Furthermore, we found no evidence for the expression of mRNA of other ENaC subunits by RT-PCR even after gene-specific RT (Praetorius J, unpublished observations). These data are consistent with the absence of ENaC mRNA expression in the brain by Northern blotting (13) and with the fact that there is no electrophysiological evidence for ENaC function in studies of mouse choroid plexus (Millar ID, Brown PD, unpublished observations). The lack of evidence for expression of functional channels raises questions about the potential contribution of ENaC to vectorial Na^+ transport from blood to CSF.

NaBC1 has been localized in the ventricular membrane of choroid plexus in both rodents and humans (20). NaBC1 is a less well characterized member of the *SLC4* family of HCO_3^- transporters. The protein is known to transport Na^+ and borate into cells; however, whether this is the only role of the protein has not yet been established.

Cl^- Pathways in the Choroid Plexus

In many secretory epithelia, secretion is driven by the secondary active, transcellular transport of Cl^- (81). In the choroid plexus, the role of Cl^- may not be so critical to the overall process of secretion because the transport of Na^+ by the Na^+-K^+ ATPase at the apical membrane appears to be the central event. Anion transport must, however, occur to limit the development of a potential difference across the epithelium, and Cl^- must play an important role in this since it is the major anion in the CSF (FIGURE 2A). There is considerable evidence to suggest that anions (Cl^- and HCO_3^-) move via a transcellular route and not the paracellular route across the choroid plexus epithelium: *1*) the CSF concentrations of both Cl^- and HCO_3^- are less than predicted for simple diffusion given the ventricular positive potential difference (FIGURE 2A); *2*) there is no evidence that claudins (the proteins that determine paracellular permeability) are anion selective; *3*) a wide array of anion transporters are expressed in the luminal and basolateral membranes of the choroid plexus epithelium.

Basolateral Cl^- Loaders

As in other epithelia, Cl^- is accumulated by transporters located in the basolateral membrane, and the few measurements of intracellular Cl^- activities support this concept. In bullfrog, choroid plexus measured intracellular Cl^- activity to be as high as 32 mM using ion-sensitive microelectrodes (74), whereas Johanson and Murphy estimated an

even higher concentration of 65 mM in rat choroid plexus epithelial cells on the basis of $^{36}Cl^-$ flux experiments (38). In most other secretory epithelia, Cl^- loading at the basolateral membrane is via the NKCC1 (81). This transporter is, however, expressed in the apical membrane where data to support a role in Cl^- influx are equivocal (see *Other Na$^+$ Transporters* above).

Thus the main mechanism by which Cl^- is thought to accumulate is by the Cl^-/HCO_3^- exchanger (AE2) (FIGURE 3B). This transporter was first immunolocalized exclusively to the basolateral membrane of mammalian choroid plexus by Lindsey et al. (50), an observation that has subsequently been confirmed by others (2, 70, 77). The energetics of such a large accumulation of Cl^- by AE2 are still a matter of conjecture, i.e., Cl^- accumulation will require a large outwardly directed gradient for HCO_3^- efflux. The hypothesis is supported, however, by indirect evidence from patch clamp studies in which the driving force for Cl^- efflux is enhanced in CO_2/HCO_3^--buffered solutions compared with those buffered with Hepes in rat choroid plexus (32). Furthermore, Cl^- accumulation during cell volume regulation in mouse choroid plexus cells has also been shown to be due to the HCO_3^--dependent activity of AE2 (33). One possible explanation for HCO_3^--dependent Cl^- loading is that HCO_3^- is first accumulated across the basolateral membrane through the actions of $NaHCO_3$ loaders, and HCO_3^- is then exchanged for Cl^- by the AE2.

Luminal Cl$^-$ Extruders

In the majority of secretory epithelia, passive movement of Cl^- via ion channels accounts for Cl^- efflux at the apical membrane. Cl^- channels also contribute to Cl^- efflux at the apical membrane of the choroid plexus; however, cation-chloride cotransporters may also contribute to Cl^- extrusion at the apical (and basolateral) membranes.

Electrophysiological studies have identified inward-rectifying anion channels in choroid plexus cells from rat (47), mouse (46), and pig (40). Some of the biophysical properties of these channels are similar to those of ClC-2, e.g., voltage dependence, but others differ, e.g., halide selectivity (47). Any putative link between this channel and ClC-2, however, was disproven when the channels were still found to be functional in choroid plexus from ClC-2 knockout mice (78), and the molecular identity of this channel still remains to be determined. The channel properties, the fact that they make a major contribution to the overall conductance of the cell, and the existence of a large electrochemical gradient for Cl^- efflux led to the conclusion that these channels must make a significant contribution to CSF secretion (47). A second class of Cl^- channels identified in choroid

plexus belongs to the volume-regulated anion channel (VRAC) family of channels observed in most types of cell (46, 47). The molecular identities of VRAC channels are unknown in all cells. The fact that the channels make only a minor contribution to the Cl^- conductance at normal cell volumes suggest that these channels may only be peripherally involved in the process of CSF secretion.
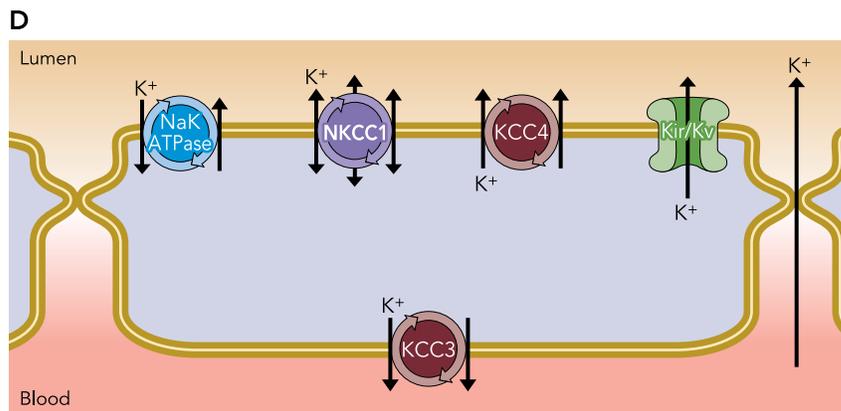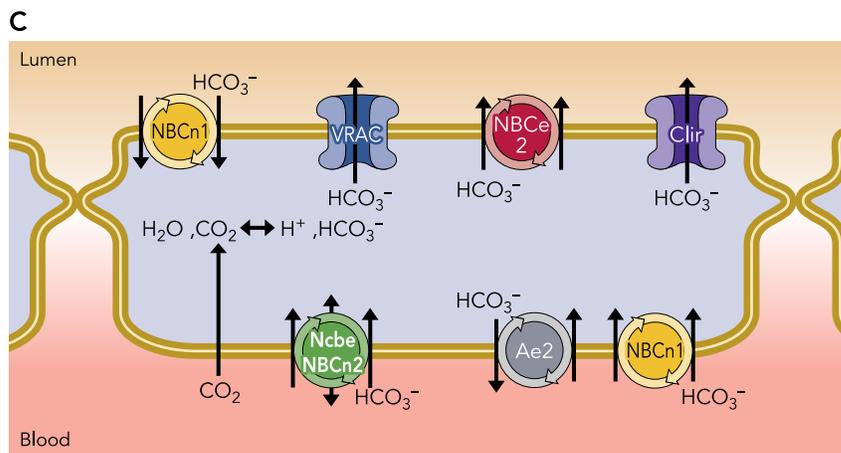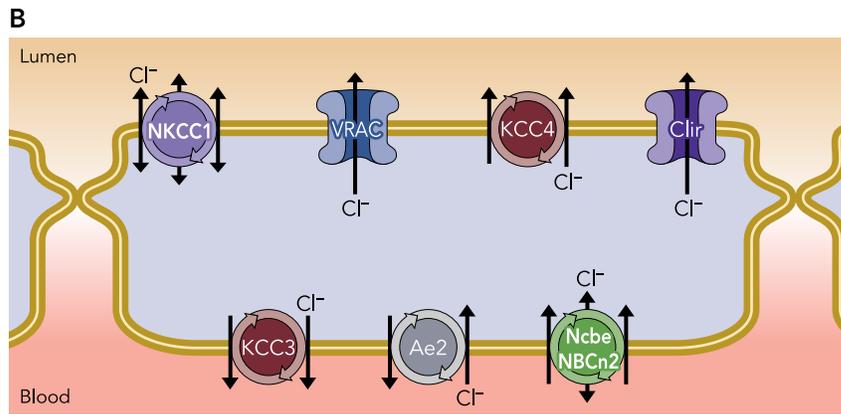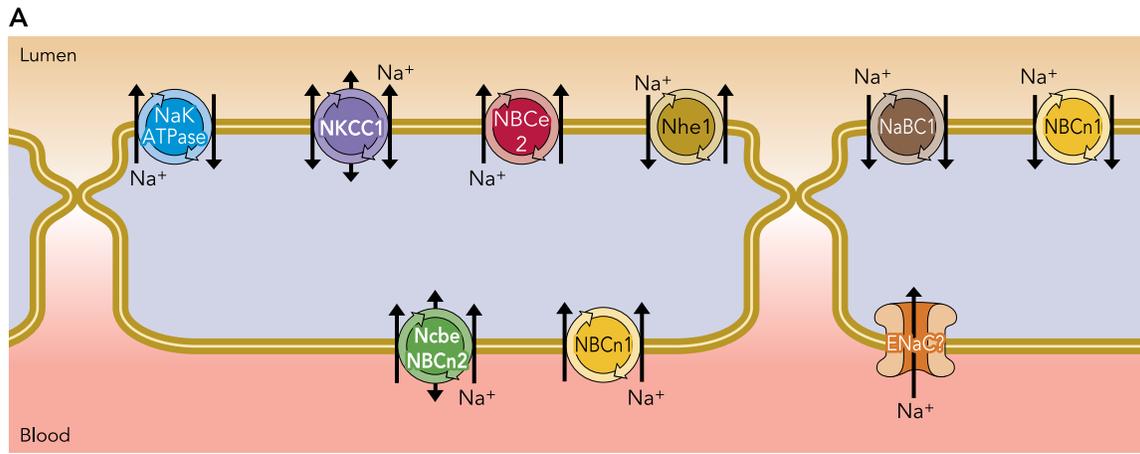
The choroid plexus expresses several cation-chloride cotransporters, including KCC3a, KCC4, and NKCC1 (FIGURE 3B). Of these, KCC3a and KCC4 will almost certainly contribute to Cl^- efflux from the choroid plexus cells at the basolateral and apical membranes, respectively. The exact contributions of these transporters to Cl^- efflux have yet to be evaluated by methods such as siRNA or using tissues from knockout mice. At the apical membrane, KCC4 may make a more significant contribution to both K^+ and Cl^- efflux. The precise contribution will, however, depend on the relative activity of the K^+ and anion channels in this membrane. However, it is likely that KCC3a makes only a minor contribution to overall Cl^- transport, since only a small amount of K^+ is transported across the basolateral membrane (see *K$^+$ Pathways in Choroid Plexus* below). Finally, given the high intracellular concentrations of Cl^- and Na^+ in choroid plexus cells, a possible minor contribution of NKCC1 to Cl^- efflux under certain conditions cannot completely be disregarded.

HCO$_3^-$ Pathways in the Choroid Plexus

There is a considerable amount of data to indicate that HCO_3^- plays an important role in CSF secretion. For instance, CSF secretion in rats is significantly inhibited by carbonic anhydrase inhibitors (82), and secretion by amphibian choroid plexus is greatly reduced in the absence of HCO_3^- (72). The Na^+ dependence and the DIDS sensitivity of the CSF secretion as well as the many HCO_3^- transporters from the *slc4a* gene family expressed in the choroid plexus epithelium argue for transcellular secretory HCO_3^- transport (FIGURE 3C). To our knowledge, paracellular HCO_3^- secretion has not been investigated systematically.

Basolateral HCO$_3^-$ Loaders

At the basolateral membrane, the protein NBCn2/NCBE is most likely to be involved in the uptake of HCO_3^-. As mentioned in *Na$^+$ Pathways*, this protein transports Na^+ and HCO_3^- into the choroid plexus (35). NBCn2/NCBE knockout mice show a 70% decrease in Na^+-dependent HCO_3^- uptake capacity of isolated cells, indicating that this transporter is the major HCO_3^- importer (21, 35). The other possible HCO_3^- uptake pathway is the NBCn1. The localization of this protein, however,

A

Lumen

Na$^+$ · Na$^+$ · Na$^+$ · Na$^+$ · Na$^+$ · Na$^+$

NaK ATPase · NKCC1 · NBCe2 · Nhe1 · NaBC1 · NBCn1

Na$^+$ · Na$^+$

Ncbe NBCn2 · NBCn1 · ENaC?

Na$^+$ · Na$^+$ · Na$^+$

Blood

B

Lumen

Cl$^-$

NKCC1 · VRAC · KCC4 · Clir

Cl$^-$ · Cl$^-$ · Cl$^-$

Cl$^-$

Cl$^-$

KCC3 · Ae2 · Ncbe NBCn2

Cl$^-$

Blood

C

Lumen

HCO$_3^-$

NBCn1 · VRAC · NBCe2 · Clir

HCO$_3^-$ · HCO$_3^-$ · HCO$_3^-$

H$_2$O ,CO$_2$ ⟷ H$^+$,HCO$_3^-$

HCO$_3^-$

Ncbe NBCn2 · Ae2 · NBCn1

CO$_2$ · HCO$_3^-$ · HCO$_3^-$

Blood

D

Lumen

K$^+$ · K$^+$ · K$^+$

NaK ATPase · NKCC1 · KCC4 · Kir/Kv

K$^+$ · K$^+$ · K$^+$

K$^+$

KCC3

Blood

displays a variation in membrane localization among the studied mammalian species and even between different strains of mice (10, 21, 69, 70). This indicates that the protein is most likely to participate in regulation of the intracellular pH and not in vectorial HCO_3^- movement.

Luminal HCO_3^- Extruders

CSF pH is tightly regulated, and this is vital in regulating respiratory center function (9) and in maintaining normal neuronal excitability because there is no diffusion barrier between the interstitial fluid in the CNS and CSF. Saito and Wright proposed that cAMP-regulated, HCO_3^--permeable ion channels may be the main efflux pathway in the apical membrane of amphibian choroid plexus (73). This idea was supported by work on the mammalian choroid plexus, which indicated that the Clir channels have an unusually high HCO_3^- permeability [P_{HCO3}:P_{Cl} = 1.5; (47)]. The role of these channels to HCO_3^- efflux has, however, recently been challenged by the discovery of NBCe2 in the apical membrane (10). The NBCe2 in the choroid plexus transports 1 Na^+ along with 3 HCO_3^- equivalents (56). This means that the transport mediates HCO_3^- efflux into the CSF, given the ion gradients that exist across the ventricular membrane. Furthermore, the transporter generates a small HCO_3^--dependent electrical current (due to the fact that it transports unequal numbers of anions and cations), and this fact was not taken into account in the calculation of P_{HCO3} via the Clir channels. Thus the P_{HCO3} is probably much smaller than originally estimated so that the channels will contribute much less to HCO_3^- efflux than previously anticipated. The relative roles of NBCe2 and Clir in HCO_3^- transport, and hence in the regulation of CSF pH, remains to be elucidated. However, studies demonstrated that exposure of rodents to 10% CO_2 causes a significant increase in CSF Na^+ but not a fall in K^+ (3). This would be consistent with the participation of a Na^+-dependent acid/base transporter in the compensation for the CSF P_{CO_2} change.

Other HCO_3^- Transporters

As mentioned above, AE2 is also expressed in the basolateral membrane of the choroid plexus epithelial cells (1, 50, 70). The transporter, however, is not involved in the net HCO_3^- secretion across the epithelium but possibly functions as a Cl^- loader involved in secretion and/or a base extruder protecting cells against alkalization as described above.

K$^+$ Pathways in the Choroid Plexus

The concentration of K^+ in the CSF is regulated, i.e., the CSF concentration remains relatively constant as plasma K^+ is varied experimentally (4, 34). There is normally a movement of K^+ from the ventricle to the blood as new CSF is produced. However, there is no transcellular route for K^+ secretion; in fact, the choroid plexus epithelium mediates a net transcellular absorption of K^+. Thus secretion of K^+ must take place via the paracelluar route, i.e., via junctional complexes between the cells (FIGURE 3D). The chemical gradient for K^+ favors paracellular secretion, but the lumen-positive transepithelial voltage limits the final CSF concentration to 3.6 mM, given the plasma concentration of 4.4 mM (FIGURE 2A). Thus the final CSF K^+ concentration of 2.9 mM must be maintained by very efficient transcellular absorption of K^+. In the following sections, we first consider the ventricular K^+ importers and then basolateral K^+ extruders.

Luminal K^+ Loaders

The main mechanisms responsible for K^+ import are quite well described. Alongside the importance of the Na^+-K^+-ATPase in Na^+ secretion, it also transports significant amounts of K^+ ions from the CSF into the epithelial cells (11, 90, 91). The Na^+-K^+-ATPase, however, is probably operating at maximum rate with normal K^+ concentrations because of a quite high affinity for K^+ (18). By contrast, NKCC1 has a higher Km for the ion (65) and may potentially enhance K^+ absorption at high CSF [K^+]. Normally, NKCC1 cotransports Na^+ and K^+ with 2 Cl^- ions into cells, since the combined inward Na^+ and Cl^- gradients by far exceed the outward K^+ gradient, given typical ionic gradients. As described above, the driving forces for Na^+, K^+, and Cl^- transport in the choroid plexus, however, are probably very close to equilibrium. Thus even a small increase in CSF [K^+]

FIGURE 3. **Transporters in the choroid plexus**
A: Na^+ transporters in the choroid plexus. NBCn2/NCBE is an important Na^+ loader in the basolateral membrane, whereas the Na^+-K^+-ATPase is the main luminal Na^+ extruder. NBCe2 is another luminal Na^+ extruder. NKCC1, NHE1, NBCn1, and NaBC1 may not make a direct contribution to the vectorial transport of Na^+. *B*: Cl^- transporters in the choroid plexus. AE2 is the main basolateral Cl^- loader. In the luminal membrane, Clir and KCC4 are important luminal Cl^- extruders. NKCC1, KCC3, and VRAC are unlikely to have a major role in the vectorial transport of Cl^- from blood to CSF. *C*: HCO_3^- transporters in the choroid plexus. Among the HCO_3^- loaders, only NBCn2/NCBE is consistently found in the basolateral membrane. Luminal HCO_3^- extrusion may be mediated by NBCe2 or via the anion channels Clir and VRAC. The roles of NBCn1 and AE2 are unlikely to be the vectorial HCO_3^- transport from blood side to lumen. *D*: K^+ transporters in the choroid plexus. The K^+ transport from blood to CSF occurs via K^+ leak via the paracellular pathway. However, there is a net transcellular flux of K^+. The Na^+-K^+-ATPase is the main K^+ loader in the luminal membrane. The majority of the K^+ pumped into the cell at the luminal membrane is recycled across this membrane via the Kir, KCC4, and Kv channels. KCC3 is the only K^+ extruder in the basolateral membrane. The role of the luminal NKCC1 is uncertain, but it may facilitate K^+ uptake if CSF K^+ concentrations are high.

may sufficiently alter the driving force to favor ion influx. This idea is supported by the ventricular localization of NKCC1 in the choroid plexus (67) and the observation that an increase in extracellular K^+ from 3 to 25 mM causes a 40% increase in choroid plexus cell volume (87). The fact that this volume increase is inhibited by bumetanide suggests the involvement of NKCC1 in ion influx rather than increased Na^+-K^+-ATPase activity or decreased efflux via K^+ channels. Thus NKCC1 may contribute vitally to the regulation of $[K^+]$ in the CSF, although it seems not to be a main K^+ import pathway under normal conditions. Most of the imported K^+ is actually recycled into the CSF by the ventricular KCC and K^+ channels as described below.

Luminal K^+ Extruders and K^+ Recycling

Most of the imported K^+ leaves the cell, back into the CSF across the luminal membrane, thus avoiding excessive accumulation of K^+ in the cells and large changes in cell volume. In amphibians, it is suggested that at least 90% of K^+ efflux occurs via ion channels in the luminal membrane, and

luminal K^+ conductances were originally shown in the choroid plexus by Zeuthen and coworkers (16, 89, 91). In mammals, two K^+ conductances have been identified in rat choroid plexus: a time-independent, inward-rectifying conductance (Kir), and a time-dependent, outward-rectifying conductance (Kv) using whole cell patch-clamp methods (49). The inward-rectifying conductance is carried by Kir7.1 channels (27) in the apical membrane of the choroid plexus (61). The Kv conductance is thought to be mainly carried by Kv1.1 and Kv1.3 channels, which are also expressed in the apical membrane (79). The luminal K^+ channels also make a major contribution to the generation of the intracellular negative membrane potential of the choroid plexus epithelial cells.

The luminal KCC may also be involved in sustaining CSF secretion by the recycling of K^+ to the ventricle lumen and thereby simultaneously supporting the Na^+-K^+-ATPase and mediating Cl^- secretion at the same time (91). Luminal K^+,Cl^- cotransport was evidenced as a furosemide-sensitive K^+- and Cl^--dependent transport in the choroid
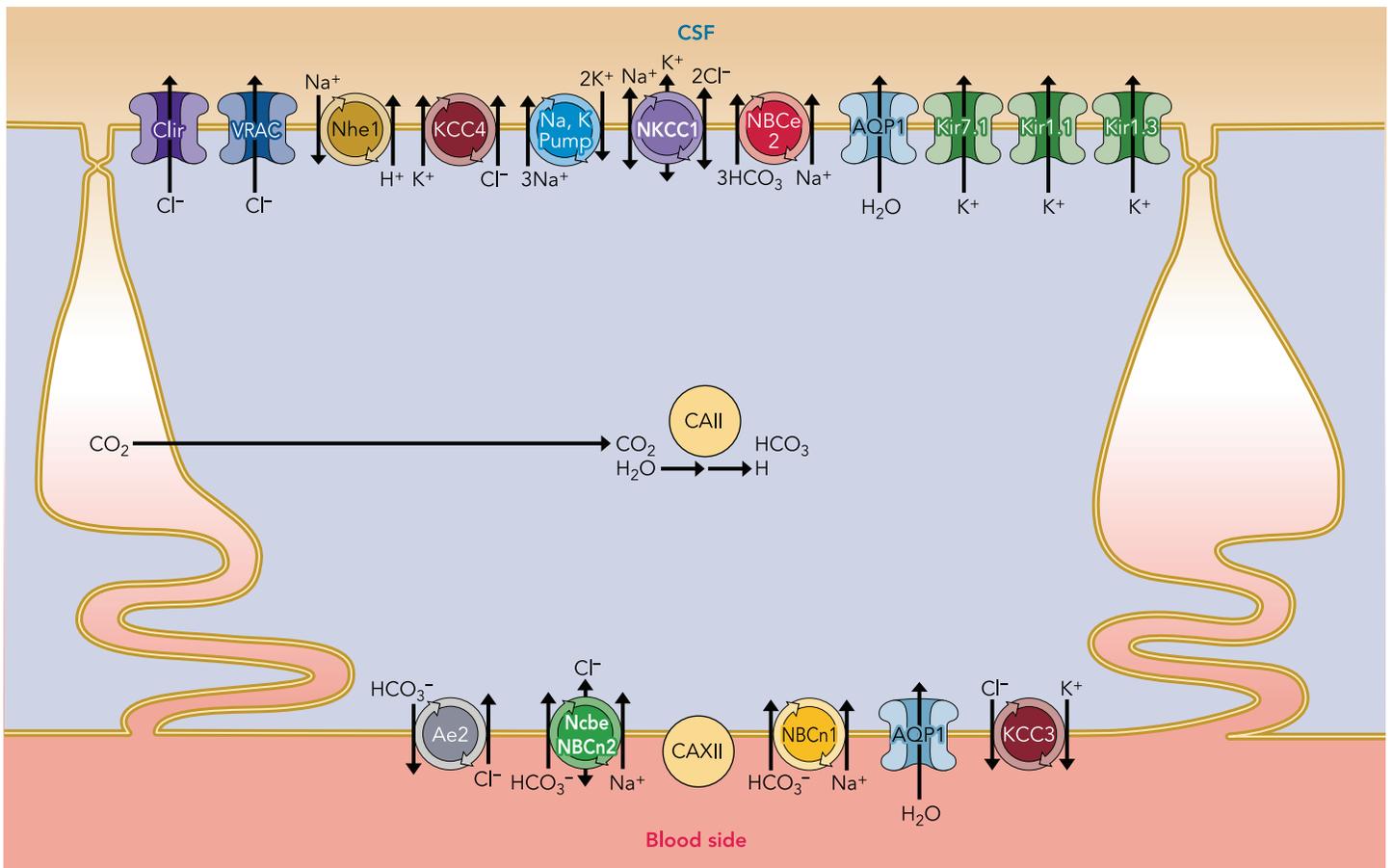

FIGURE 4. Schematic representation of the ion and water transporters in the choroid plexus epithelium
Aquaporin1 (AQP1) is situated in both luminal and basolateral membranes, and the carbonic anhydrase (CA) II and XII are found in the cytosolic and basolateral compartments, respectively. The Na^+-K^+-ATPase and the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1) are strictly luminal plasma membrane transport proteins along with the KCC4, three K^+ channels (Kir7.1, Kv1.1, and Kv1.3), and two types of anion conductances of unknown molecular identity (VRAC and Clir). The electrogenic Na^+-HCO_3^- cotransporter NBCe2 or NBC4 is also found at the luminal membrane. Three HCO_3^- transporters are localized to the plasma membrane domain along with a Na^+/H^+ exchanger: the base extruder AE2 and the two Na-base loaders, NBCn1 and NCBE. KCC3 is the only known candidate for mediating basolateral K extrusion.

plexus (88, 91). A recent immunocytochemical study has shown that the KCC4 protein is expressed in the apical membrane of mouse choroid plexus (42). Although luminal K^+ efflux may take place via the K^+,Cl^- cotransporter, there is no functional evidence for a contribution from this transporter in vivo.

Basolateral K^+ Extruders

As mentioned above, the choroid plexus mediates net transcellular absorption of K^+ from the CSF to the plasma. At present, a K^+,Cl^- cotransporter (KCC) represents the only known K^+ efflux pathway from choroid plexus cells to the blood side. There are two isoforms of KCC3: KCC3a and the truncated form KCC3b (57). Expression of KCC3a, but not KCC3b, was determined in mouse choroid plexus by Northern analysis (66). KCC3 protein was localized to the basolateral membrane of rat choroid plexus using an antibody, which did not discriminate between KCC3a and KCC3b (66).

Water Transport by the Choroid Plexus

H_2O is transported across the choroid plexus epithelium from the blood side to the slightly hyperosmolar CSF. The small osmotic gradient and the high rate of H_2O transport resemble that of the renal proximal tubules, and both epithelia express the highly H_2O-permeable channel AQP1 (FIGURE 4). In the choroid plexus, AQP1 is abundantly expressed in the luminal plasma membrane and much less in the basolateral membrane (62, 70). The significance of AQP1 in choroid plexus water permeability and transepithelial H_2O flux was studied in AQP1 knockout mice (63, 64). The H_2O permeability was decreased by 80% in AQP1 knockout mice, whereas the secretory rate was only reduced by 35%. The only other two AQP transcripts found in the choroid plexus are AQP4 and AQP11, for which the cellular localization is uncertain (Ref. 77; Damkier HH, Brown PD, Praetorius J, unpublished observations). The main problem with the transcellular route is that it requires that the intracellular osmolarity always be kept between the luminal and the basolateral levels. There are no data at present to sustain that requirement, and, in fact, all data point to a relative intracellular hyperosmolarity (37). Even H_2O transport by cotransporters as suggested by Zeuthen (88) would present a problem in that no basolateral cotransporters transport inward. Thus the H_2O transport by the choroid plexus is far from understood, and significant amounts of H_2O may also pass paracellularly from the blood side to the CSF.

Future Directions

Many more investigations on transport processes and the involved proteins are necessary to refine the present model of the CSF secretory processes. The extended use of genetically modified animal models may help in defining the most important transporters. A functional interplay between carbonic anhydrases and bicarbonate transporters in the choroid plexus is expected, since application of acetazolamide is an effective blocker of, e.g., Na^+ uptake by the choroid plexus and CSF secretion (3, 37, 55, 58). It is yet to be determined whether extracellular or intracellular carbanhydrases are responsible for the observed effects on secretion and cell pH changes. It remains to be established whether NBCn2/NCBE is the main Na^+ entry pathway from the blood side, what the role of NKCC1 is, and to what extend NBCe2 and NHE1 contribute to regulation of CSF pH. More information is also required on the expression of claudins to understand the molecular basis of the paracellular transport of H_2O and K^+. The molecular sorting and intracellular trafficking of membrane proteins in the choroid plexus cells is of great cell biological interest: How is the Na^+-K^+-ATPase, NHE1, and NKCC1 directed to the luminal plasma membrane, while the basolateral sorting motif of other proteins functions normally? The perspectives in precise knowledge on the molecular machinery in CSF production include optimized clinical management of diseases involving altered CSF turnover or intracranial pressure.

Conclusions

The choroid plexus transports Na^+, Cl^-, and HCO_3^- from blood to CSF and in doing so creates an osmotic gradient that drives the movement of water into the ventricle. A large and unique array of membrane proteins is thought to contribute to CSF secretion (for overview, see FIGURE 4). The precise roles of many ion transporters expressed by the choroid plexus cells, however, remains to be determined. ∎

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