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INVITED REVIEW

Water and solute secretion by the choroid plexus

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Abstract The cerebrospinal fluid (CSF) provides mechanical and chemical protection of the brain and spinal cord. This review focusses on the contribution of the choroid plexus epithelium to the water and salt homeostasis of the CSF, i.e. the secretory processes involved in CSF formation. The choroid plexus epithelium is situated in the ventricular system and is believed to be the major site of CSF production. Numerous studies have identified transport processes involved in this secretion, and recently, the underlying molecular background for some of the mechanisms have emerged. The nascent CSF consists mainly of NaCl and $NaHCO_3$, and the production rate is strictly coupled to the rate of Na^+ secretion. In contrast to other secreting epithelia, Na^+ is actively pumped across the luminal surface by the Na^+,K^+-ATPase with possible contributions by other Na^+ transporters, e.g. the luminal Na^+,K^+,$2Cl^-$ cotransporter. The Cl^- and HCO_3^- ions are likely transported by a luminal cAMP activated inward rectified anion conductance, although the responsible proteins have not been identified. Whereas Cl^- most likely enters the cells through anion exchange, the functional as well as the molecular basis for the basolateral Na^+ entry are not yet well-defined. Water molecules follow across the epithelium mainly through the water channel, AQP1, driven by the created ionic gradient. In this article, the implications of the recent findings for the current model of CSF secretion are discussed. Finally, the clinical implications and the prospects of future advances in understanding CSF production are briefly outlined.

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Introduction

The adult human brain is a fragile organ that weighs approximately 1,500 g. The vital structures of this organ contain little connective tissue, and this is only on the outer surface of the brain. The brain normally escapes mechanical damage by being submerged in as little as 150 ml of cerebrospinal fluid, CSF, causing a reduction of its effective weight to less than 50 g [59]. Thus, the CSF cushions the movements of the soft brain tissue relative to the inside of the rigid cranium. The position of the brain in a water-fill cavity may, however, also present a disadvantage, as any mechanical force to the cranium will always be conducted to the brain parenchyma. Water cannot be compressed physically, and therefore the CSF fails to buffer abrupt impacts on the skull efficiently.

A second function of CSF is to provide a constant fluid flow in the ventricular system inside the brain and on the surface of the brain and spinal cord (Fig. 1). The CSF flow rids the central nervous system of waste products and carries a number of secreted mediators to the brain parenchyma (for review, see [32]). The CSF thereby removes excess K^+ ions and transmitters from the brain [51] as well as breakdown products from surface lining epithelial cells, bacteria, viruses and similar debris [26]. In fact, the CSF homeostasis seems to be under strict control so that e.g. the concentration of K^+, [K^+], and the pH value are kept within remarkably tight limits. Raising the plasma [K^+] from 1.6 to 11 mM results in an increase in CSF [K^+] of less than 2 mM [3]. Increases in P_{CO2} change CSF pH

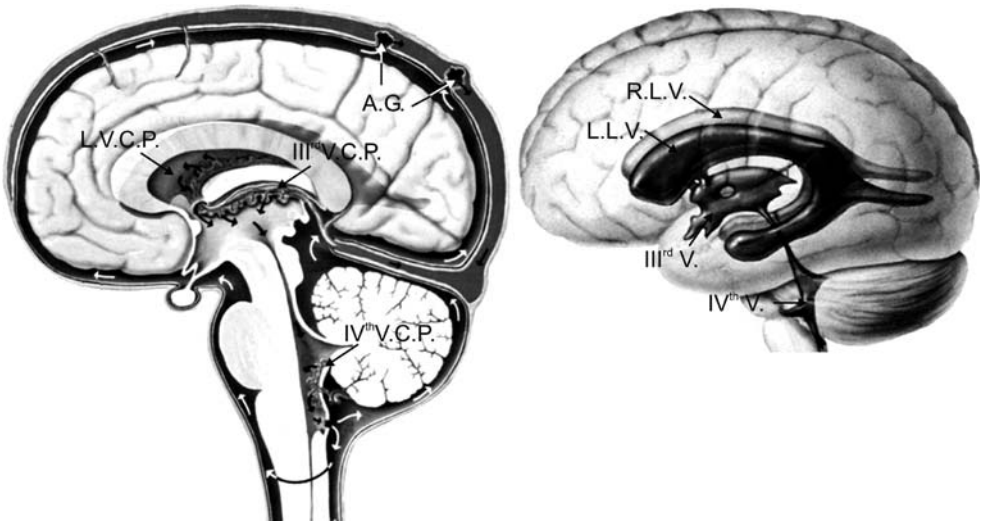

Fig. 1 The brain and the ventricular system. *L.V.C.P.* is the lateral ventricle choroid plexus; *IIIrd V.C.P.* is the third ventricle choroid plexus; *IVth V.C.P.B.* is the fourth ventricle choroid plexus; *A.G.* is arachnoidal granulations. *R.L.V.* is the right lateral ventricle; *L.L.V.* is the left lateral ventricle; *IIIrd V.* and *IVth V.* are the third and fourth ventricles, respectively. Modified from Netter [71]

minimally, as HCO_3^- is secreted by the choroid plexus to compensate for the acidifying effect of CO_2 [4]. This homeostatic capacity is of high physiological and clinical importance, in light that a fall in CSF pH of 0.05 units leads to a tenfold increase in ventilation [35].

It is widely recognised that the major fraction of the CSF is produced by the choroid plexus epithelium. The involvement of the choroid plexus in CSF formation was established by Rougemont and colleagues [89], although this site was already suggested as the site of origin by J. Faivre as early as 1854 [33] and H. Cushing in 1914 [24]. Rougemont and coworkers demonstrated that the content of solutes in the nascent CSF was different than expected from a simple plasma ultrafiltrate. Hence, the secretory function was ascribed to the epithelium of the choroid plexus. Apart from water and solutes, many mediators and nutrients carried by the CSF are actually secreted by the choroid plexus epithelium (for specific review, see [15]). While the main target of these substances is the brain parenchyma, some of the mediators act on the choroid plexus itself, i.e. revealing endocrine, paracrine and even autocrine functions of the epithelium. Another major feature of the CSF is that the balance between its production and reabsorption normally helps keep the intracranial pressure at suitable levels (5–15 mm H_2O), despite fluctuations in blood flow and changes in plasma osmolarity.

The fluid and solutes of the CSF are believed to return to the general circulation via the arachnoidal granulations or villi [110] and dural sinuses. It should be noted that most scientists in the field share this general view on CSF circulation. Recently, evidence has emerged on the connections between cerebrospinal fluid and nasal lymphatic vessels [46]. Furthermore, reports have suggested that CSF is transported through the choroid fissure and recycled through the choroid plexus [62]. Finally, the CSF may to some extent reenter the circulation through the venoles of the brain parenchyma, which represents a large surface area, but has limited permeability to water and solutes. Given the involvement of CSF reabsorption in a number of pathological conditions, surprisingly few studies have described the underlying physiological processes and the possible contribution of specific transport proteins.

The mechanism by which the choroid plexus secrete CSF is much different from other sites of fluid secretion, e.g. the salivary or sweat glands (see below). Decades of studies have revealed the essential physiological transport phenomena and most of the underlying molecular mechanisms. Although several central mechanisms are not fully established, it seems timely to review the current understanding of the transport processes of the choroid plexus. The current understanding of the transepithelial movement of ions and water across the choroid plexus epithelium is based on studies of multiple species as man, monkey, horse, sheep, dog, cat, rabbit, rat, mouse, and non-mammals such as bull frog, mudpuppy, dogfish and shark.

Structure of the choroid plexus

The adult human choroid plexus weighs about 2 g on average [25]. The tissue forms characteristic sheet-like structures in the left and right lateral ventricles, and branched villi-like structures in the the IIIrd and IVth ventricle of the mammalian brain [23].

Both types of structures consist of a single continuous layer of epithelial cells residing on a core of sparse connective tissue, which carries blood vessels of different calibre from small arteries through capillaries to small veins (Fig. 2a). The surface cells are of similar origin as the ependymal cells lining the remaining ventricular surface, and a short transition of the two types of epithelia are seen in the base of the choroid plexus stalk. While the choroid

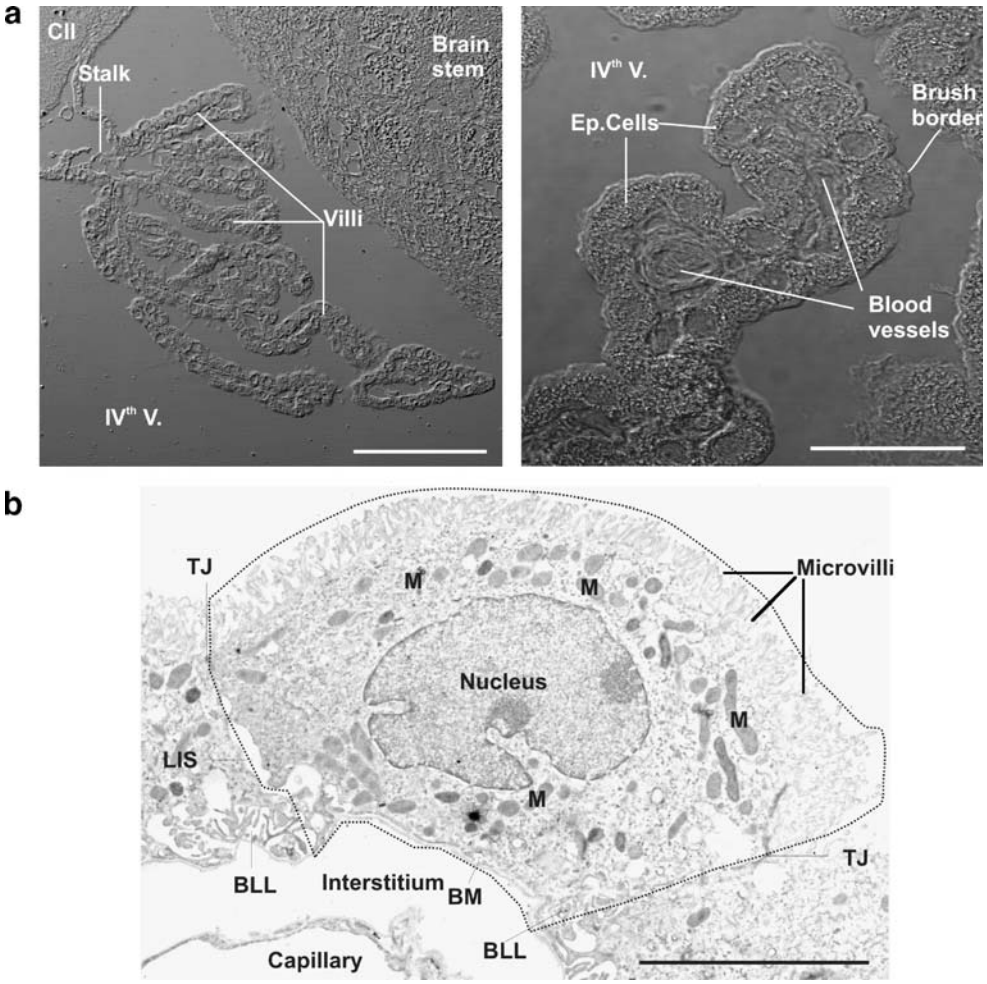

Fig. 2 The structural basis of cerebrospinal fluid metabolism. **a** Differential interference contrast images of the mouse IVth ventricle choroid plexus. The *left panel* is a low magnification micrograph with indications of the cerebellum (*Cll*), the *stalk*, the *brain stem*, *villi* and the lumen of the ventricle (*IVth V.*). The *bar* shows 100 μm. The *right panel* shows a part of a single villus containing blood vessels, single epithelial cells, brush borders, and the lumen of the ventricle (*IVth V.*). The *bar* indicates 40 μm. **b** Electron micrograph of an epithelial cell of rat IVth ventricle choroid plexus. The *broken line* marks the outline of a single cell. The *TJ*s identify tight junctions, the *M*s mark mitochondria, the *BLL*s show basolateral labyrinths, and *BM* is the basement membrane. The *bar* represents 5 μm

plexus epithelium and the ependymal cells form the ventricular lining, the choroid plexus and the arachnoidea on the brain and spinal cord surfaces comprise the blood–CSF barrier.

The choroid plexus is supplied with blood by the choroidal arteries that arise from branches of the internal carotid artery. From a rich bed of fenestrated capillaries, the blood drains via choroidal veins into the internal cerebral vein and later to the great vein of Galen [83]. Each gram of the choroid plexus receives 3 ml of blood per minute, which may seem modest at first glance. However, measured relative to the mass of the tissue, the blood flow is ten times higher than that of the brain parenchyma and five times higher than that of the kidney [17, 36]. The choroid plexus is supplied by sympathetic and parasympathetic nerve endings containing several neurotransmitters such as noradrenaline, neuropeptide Y and vasoactive intestinal polypeptide [73], but it is unclear at present to which extent the nerve supply affects CSF formation.

Electron microscopic analyses have revealed that the cuboidal to cylindrical epithelial cells of the choroid plexus display highly differentiated cell surfaces (Fig. 2b). The ventricular or luminal surface is characterised by numerous microvilli and in certain species by a central bundle of long motile cilia (as reviewed earlier [93, 111]). The basal surface appears remarkably smooth, whereas the lateral surface is convoluted, with interdigitations between the basal parts of adjacent cells [52]. This area is often referred to as the basal labyrinth or the basal infoldings. These structures represent a substantial increase of the surface area like the microvilli, which increase the luminal surface by six- to 13-fold depending on species [20].

Both surface area enlargements are thought to be important for the transport capability of the epithelium. The more luminal parts of the lateral intercellular spaces appear almost flat except for adherence zones and tight junctions [79]. The leakiness of the tight junctions to small ions and water may rely on the number of strands in the tight junctions or on the contents of specific proteins such as occludin and the claudins (for specific review, see [88]). Similar to epithelia derived from other germ layers, the choroid plexus cells form focal adhesion contacts with the basement membrane. The cellular ultrastructure as such does not present unusual features, but the cells are relatively rich in mitochondria. These organelles are observed in highest abundance in the luminal domain of the cytosol

[20], and this arrangement is regarded as necessary for supporting the active transport of ions across the luminal membrane that requires high rates of ATP synthesis (i.e. the Na^+,K^+-ATPase).

Thus, the blood–CSF barrier of the choroid plexus consists of the very leaky fenestrated endothelium with basal lamina, sparse connective tissue, a basement membrane and the single layer of epithelial cells. The latter component is regarded as the major obstruction for diffusion of substances from blood to the CSF. This part of the barrier is defined by the plasma membranes and lateral intercellular spaces with the luminal tight junctions, which are electrically leaky to small ions and may permeate water [112].

Overview of the secretion of water and salt by the choroid plexus

The choroid plexus epithelium is responsible for about 70% of the CSF production, as estimated from studies by Welch and others (e.g. [109]). The remainder is thought to cross the blood–brain barrier passing into the CSF through the ependyma or to originate from the arachnoidal surface blood–CSF barrier [82, 91]. The latter process is thought to rely less on specific transport systems and more on passive leakage of water and solutes, despite a quite low permeability of these structures. However, the vast total surface area of these tissues may account for the non-choroidal component of the CSF formation.

The epithelial cell of the choroid plexus ranks as one of the most potent secretory cell types in the organism. The fluid transport rate of about 0.2 to 0.4 ml/g min tissue exceeds estimates from other secretory tissues such as the exocrine pancreas (as referred by [100]) and the water reabsorption by renal proximal tubules (calculated from [12]). Thus, the human choroid plexus produces more than 0.5 l of CSF per day corresponding to a renewal of the entire CSF volume about five times a day (as reviewed previously [23, 111]). The epithelium seems to be capable of secreting an astonishing 10% of the blood volume it receives by comparing the blood flow into the choroid plexus with the rate of secretion.

The CSF cannot be regarded as a simple ultra-filtrate of the interstitial fluid for the following reasons. Firstly, the CSF is slightly hypertonic compared to plasma as described above. Secondly, the [Na^+] and [HCO_3^-] are slightly higher than expected at equilibrium, while [K^+] and [Cl^-] are lower in the CSF [5]. Thirdly, there is a modest positive electrical potential difference across the epithelium, i.e. up to 5 mV lumen positive (for summary, see Fig. 3). It is also noted that there is a 15-mm H_2O pressure difference across the epithelium, and the CSF is about 5 mOsM hypertonic to plasma [27] and contains very little protein.

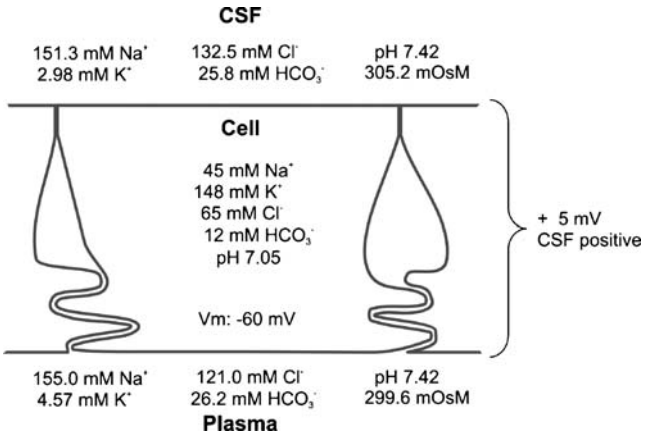

Fig. 3 Estimates of the ionic composition of *CSF*, *plasma* and the intracellular compartment of the choroid plexus. Values from *plasma* and *CSF* are from in vivo observations by Davson & Segal on rabbit [25], while intracellular ionic composition is from in vivo studies on rats by Murphy & Johanson [44]

It should be emphasised that the CSF composition seems to differ somewhat from the choroid plexus surface (nascent CSF) to the ventricular bulk CSF, and between ventricular CSF and CSF of the brain and spinal cord surface [5, 89]. Also, the fluid movement has to be separated into two components. The bulk flow of CSF should be considered a consequence of the continuous secretion of CSF into the ventricles and the absorption of the CSF on the outside of the CNS. In contrast, the movement of fluid and soluble constituents of the CSF by the beating cilia may serve to prevent local buildup of gradients in the glycocalyx or unstirred layer, which may impede secretion.

Mechanisms in the secretion CSF

The following sections are intended to give an overview of the main physiological observations leading to the current understanding of the secretory events in the choroid plexus and to propose a specific role for each of the known molecular processes in the CSF production. The net secretion occurs by the action of several ion and water transport molecules residing in the plasma membranes of the choroid plexus epithelium and most likely to a lesser extent through unspecific passive pathways. It may seem inappropriate to describe each of the processes separately, as these are interdependent in some cases and mutually supplementary in other instances. Hence, one should acknowledge that the overall function of the epithelium reflects a delicate concert of transport processes.

A number of techniques have been successfully applied in the study of choroid plexus physiology over the past decades. However, some confusion arose from the emergence of conflicting results from the rich variety of

approaches and studied species. Also, uncertainty has arisen from the difficulties in some studies to discern the effects of transport inhibitors on the epithelium from those exerted on the local blood supply or on the entire haemodynamic system. Review of the literature also reveals different opinions on the specificity and dosage and target accessibility of transport inhibitors.

Ventriculo-cisternal perfusion was described first in 1950 [90] and is one of the few techniques allowing the acquisition of data on choroid plexus function in vivo [40]. Two inflow cannulae are inserted into the lateral ventricles, and the outflow is collected from the cisterna magna. Then, the ventricular system is perfused with artificial CSF containing radioactive tracer molecules (both transported and non-transported reference molecule); the subsequent analysis of the dilution or concentration of substances allows the determination of net transport to and from the CSF. Thus, the method primarily offers information on the luminal transport.

Johanson and coworkers utilised an alternative method to perturb rat choroid plexus function in vivo [68]. They changed the ionic contents of the plasma or injected a variety of pharmacologically active substances into the blood stream or peritoneum and collected blood plasma, CSF and choroid plexus tissue for flame photometric determination of solutes and traces analysis. The technique allowed determination of the precise ionic composition of all three compartments. Also, the intracellular pH (pH_i) and bicarbonate contents in the choroid plexus cells could be estimated, as well as [Na^+] and [K^+] fluxes into the choroid plexus or CSF. Any haemodynamic changes due to altered renal function were prevented by nephrectomy before the experiment.

It is technically possible to perfuse the lateral ventricle choroid plexus of large mammals as the sheep and horse. The in vitro perfusion method was first described by Csaky and Rigor [22]. Later, Segal and Dean removed the brain and perfused the choroid plexus in situ through the internal carotid artery and outflow collected from the vein of Galen [89]. The technique primarily provides data for the uptake of molecules across the basolateral plasma membrane of the epithelium, as the loss of transported molecule is calculated relative to a non-transported substance. The lateral ventricles could be superfused with artificial CSF for determination of transepithelial movement of the substances.

Studies have also been conducted on the frog choroid plexus, which, in contrast to the mammalian tissue, forms sheets containing only epithelium on one side of the underlying connective tissue. This made the frog choroid plexus ideal for transport studies in Ussing chambers [77, 112]. The method provides robust recordings of unidirectional ion fluxes across the epithelium and determinations of the electrophysiological characteristics of the tissue. The same type of data can be obtained from primary cultures of mammalian choroid plexus epithelia, although one has to take into account the risk that specific transport features are lost in culture.

The current understanding of choroid plexus CSF secretion is described in the following section partly based on the various effects on CSF formation or ion fluxes of inhibitors such as ouabain, bumetanide, furosemide, amiloride, DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid), and acetazolamide. A recent review by Peter Brown's group presents up-to-date systematic information on the molecular identities of the various transporters [10]. Interestingly, the human choroid plexus displays an almost identical epithelial distribution pattern of transporters as the rat and mouse choroid plexuses described below [86]. Thus, the proposed model for CSF formation could be highly relevant for human physiology as well.

Luminal processes

Most transport proteins and enzymatic processes involved in CSF formation are restricted to either of the two plasma membrane domains: the luminal and the basolateral membranes. As described later, a few processes also occur in the cytosol and a single mechanism seems to exist in both plasma membrane domains. Because of the strict coupling between luminal Na^+ translocation and CSF production, it is reasonable to regard this process as the pivotal event in the secretory process.

Ouabain inhibitable Na^+,K^+-ATPase

A fundamental observation was that luminal (ventricular) application of the Na^+,K^+-ATPase inhibitor, ouabain, completely abolished the transepithelial net Na^+ flux and the CSF secretion [28, 109, 117]. This observation was surprising at the time, as the activity of this enzyme was restricted to the basolateral side in almost all other transporting epithelia - both secretory and absorptive. The Na^+, K^+-ATPase of secreting epithelia is usually not directly involved in transcellular Na^+ transport, but creates and sustains the asymmetrical distribution of ions that drives the secretion. In particular, an inward Na^+ gradient is built up, which is necessary for secondary active transport directly involved in the secretion. In those tissues, secreted Na^+ seems to pass to the lumen by paracellular pathways, driven by negative transepithelial potentials resulting from luminal electrogenic anion secretion. In the mammalian choroid plexus epithelium, however, the transepithelial potential difference is 5 mV lumen positive [41], so Na^+ would not readily pass into the CSF paracellularly.

The luminal ouabain-sensitive Na^+ flux of the choroid plexus is consistent with the strong luminal Na^+,K^+-ATPase immunoreactivity, which was found later in all tested species from frog through rodents to humans (e.g. [61, 86, 96] and Fig. 4a). The Na^+,K^+-ATPase extrudes 3 Na^+ in exchange for 2 K^+ under the hydrolysis of 1 ATP molecule. This process creates the electrochemical driving force for a variety of other transport events in most mammalian cells. The mechanism leading to luminal targeting of the Na^+,K^+-ATPase is this tissue has not yet been determined. It has been suggested that the specific molecular variant of the ATPase controls the trafficking event.

The Na^+,K^+-ATPase is composed of two to three subunits. In the choroid plexus, the protein complex consists of α1, β1 and β2 and the cAMP-sensitive accessory protein, phospholemman (FXYD1) [37, 107]. This composition of α and β Na^+,K^+-ATPase subunits is similar to many epithelia, and can therefore not explain the luminal localisation of the Na^+,K^+-ATPase in the choroid plexus. Phospholemman expression has not been reported in other epithelia, so it is unknown whether this subunit plays a role in the luminal targeting. Interestingly, phoshpolemman belongs to a group of ATPase accessory and regulatory proteins of which the gamma subunit (or FXYD2) was discovered first. Sweadner and coworkers suggested that phospholemman could represent a target for the phoshorylation-activated CSF secretion by the choroid plexus [37].

Indeed, phospholemman is activated by PKA [76] and is also shown to conduct an anion current induced by cell swelling [66]. This finding may have some bearing in the discussion below on volume-sensitive anion conductances in the choroid plexus. Recently, Alper and coworkers showed that the cytoskeletal components and linkers fodrin/spectrin, ankyrin, actin, myosin and alpha actinin are all predominantly expressed in the luminal domain of the choroid plexus epithelium [1]. Of these, ankyrin seems of particular interest, as this protein is located basolaterally with Na^+, K^+-ATPase in other epithelia, and as it is shown to actually bind to the Na^+,K^+-ATPase and other ATPases. However, Marrs and colleagues did not find a strict luminal

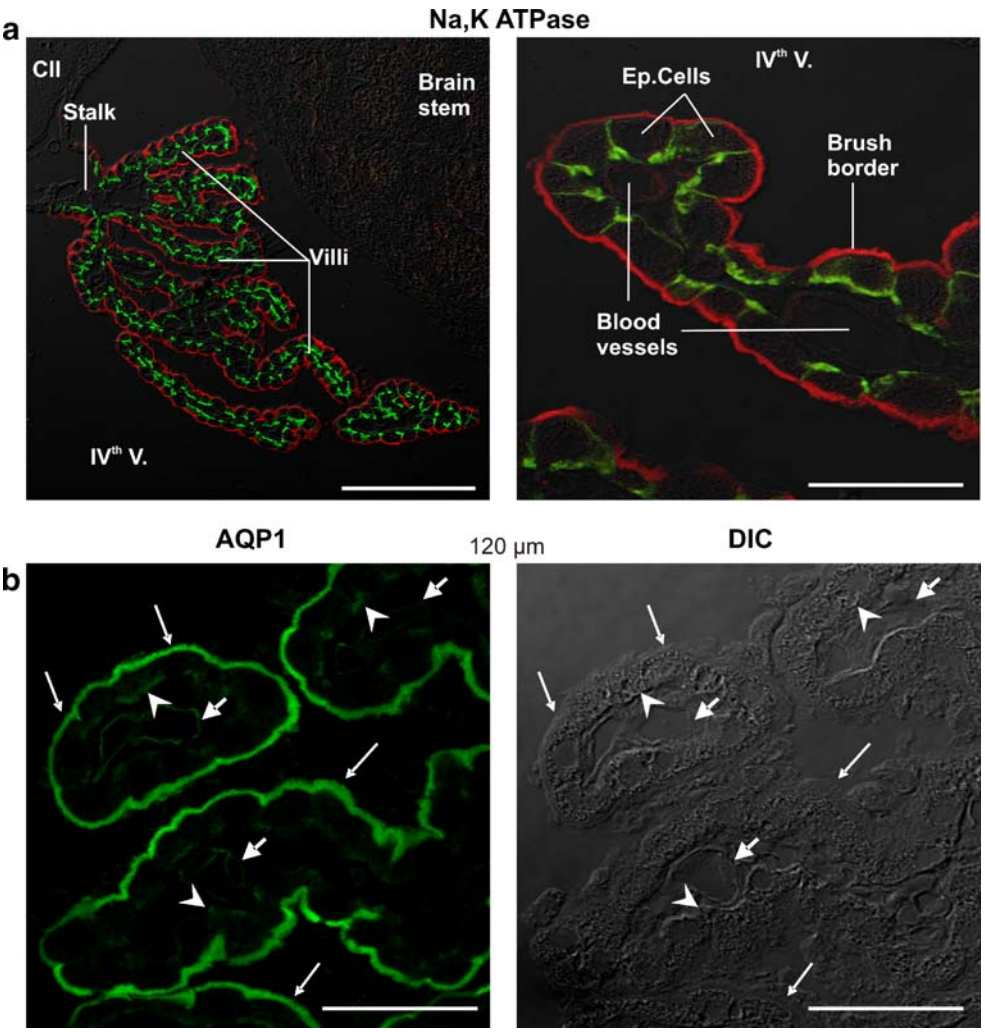

Fig. 4 Immunohistochemical localisation of transporters centrally involved in the production of cerebrospinal fluid. **a** The Na^+, K^+-ATPase. The *left panel* represents a low magnification scanning confocal micrograph of mouse choroid plexus (same as in Fig. 1). *Red* indicates positive anti-Na,K ATPase immunoreactivity and green arises from a basolateral marker (anti-NCBE). The right panel is a high magnification of a villus from a similar choroid plexus using the same markers. The *bars* show 100 μm in the *left panel* and 40 μm in the *right panel*. **b** Aquaporin-1. The *left panel* represents a scanning confocal micrograph of mouse choroid plexus. *Green* indicates positive anti-AQP1 immunoreactivity. The *right panel* is the corresponding differential interference contrast image of the same choroid plexus. *Long arrows* indicate sites of luminal AQP1 staining, *arrow heads* mark basolateral labelling and *short arrows* show the endothelial immunoreactivity. The *bars* indicate 40 μm

localisation of fodrin in their original report, although they found that ankyrin, fodrin formed high-molecular-weight complexes with the Na^+,K^+-ATPase [60]. Thus, it remains to be established how the luminal targeting of the Na^+,K^+-ATPase is controlled. Nevertheless, there is compelling evidence that the Na^+,K^+-ATPase directly pumps Na^+ across the luminal surface in addition to creating the Na^+ and K^+ gradients in the choroid plexus epithelium.

Bumetanide-sensitive Na^+,K^+,2Cl^- cotransport

The Na^+,K^+-ATPase is not the only Na^+ transporter of the luminal plasma membrane. At least one more Na^+-dependent process is described at this site: the bumetanide-sensitive Na^+,K^+,2Cl^- cotransporter, [53, 113]. The molecular identity for this luminal transport in the choroid plexus was shown to be the NKCC1 [81]. In other epithelia this transporter is localised to the basolateral surface and only the related protein, NKCC2, of the renal thick ascending limbs is expressed luminally [114]. In any case, Na^+ and K^+ are cotransported with 2 Cl^- ions into cells, as the collective inward Na^+ and Cl^- gradients by far exceed the outward K^+ gradient, given typical ionic gradients. However, the NKCC1 in the choroid plexus has been reported to transport in both an inward and outward direction.

On one hand, bumetanide inhibited the CSF formation both by in vivo installation in the ventricular system and by luminal administration in vitro [7, 42, 53]. The simplest explanation is that the drug inhibited outward transport of Na^+ and Cl^-, and strongly suggests a role for NKCC1 in CSF secretion. In addition, this mode of cotransport would enrich the CSF with K^+ and hereby feed the luminal Na^+,K^+-ATPase. On the other hand, NKCC1 was suggested to transport in an inward direction as bumetanide (100 μM) induced volume decrease in the choroid plexus in vitro [113]. At this concentration, the drug may have other effects on the cells. In fact, Brown and colleagues found no evidence for inward transport as 10 μM bumetanide did not affect the regulatory volume increase [11].

The driving forces for NKCC1 transport can be estimated from the Nernst equation in a simplified form, as the transporter is electroneutral. Interestingly, the reported intracellular [Na^+], [K^+], and [Cl^-] in rat choroid plexus epithelia are somewhat atypical for epithelia [69]. A remarkably high intracellular [Na^+] (and [Cl^-]) and a relatively low [K^+] concentration in CSF actually point towards a net outward transport by NKCC1 in the choroid plexus. However, one should bear in mind that minor changes in the ionic concentrations would easily change the predicted direction of transport, and it is possible that the direction changes over time to meet the changing requirements of the tissue. Nevertheless, the referred effect of bumentanide on CSF secretion in vivo represents the most compelling evidence to date for the outward transport through NKCC1 during secretion.

The effect of DIDS and other anion conductance inhibitors

DIDS is a relatively unspecific blocker of many Cl^- channels and Cl^-/HCO_3^- transporters. In the choroid plexus, the Cl^- secretion is inhibited by luminal application of DIDS [29]. As in other epithelia, Cl^- and HCO_3^- seem to be secreted mainly by luminal electrogenic processes. Two major anion conductances, both inward rectifiers, have been found in the choroid plexus by patch clamp analysis (for specific review [10]). One is a volume-sensitive anion conductance, while the other is a protein kinase A (PKA)-activated anion conductance with a high permeability to HCO_3^- [55]. The volume-sensitive conductance was sensitive to anion transport inhibitors like glibenclamide and NPPB, while the PKA-sensitive conductance was partially inhibited by DIDS [55] and by H^+ [47].

The relative anion permeability for the PKA-sensitive conductance was: $I^->HCO_3^-=Cl^->Br^-$. This profile is not compatible with a conductance carried by the cystic fibrosis transmembrane conductance regulator (CFTR), which is also PKA-activated. This view was supported by the absence of CFTR mRNA from the choroid plexus, and that the anion conductance of the choroid plexus was unaltered in a CFTR knock-out model [54]. ClC-2 was also rejected as a candidate for the conductance by the use of a knock-out mouse model [98], as was the aforementioned phospholemman (FXYD1) [47]. Thus, the anion channels responsible for the luminal secretion of Cl^- and HCO_3^- are yet to be identified.

An alternative DIDS-sensitive electrogenic anion pathway may be the recently identified Na^+-HCO_3^- cotransporter NBC4 or NBCe2 [87], which was localised to the luminal membrane of mouse and rat choroid plexus epithelium [9]. This transporter is shown to transport the equivalent of 1 Na^+ and at least 2 HCO_3^- across cell membranes by heterologous expression [103]. It is feasible that NBCe2 transport, like NBCe1, can change direction according to variations in the membrane potential.

Inhibition of K^+,Cl^- cotransport and other effects of furosemide

Luminal furosemide-sensitive K^+ and Cl^--dependent transport most likely reflects the functional presence of a K^+,Cl^- cotransporter [115, 116]. The protein mediating the observed transport was later shown to be KCC4 [57]. The luminal KCC was suggested to be important for sustaining CSF secretion by recycling K^+ to the lumen, thereby supporting ongoing Na^+,K^+-ATPase activity, simultaneous

with mediating Cl^- secretion [116]. However, luminal administration of another KCC inhibitor, R(+)-butylindazone, did not have any effect on transport in vitro [53]. It was the notion that KCC was not as important for CSF formation as NKCC1, and that furosemide was more prone to affect CSF formation when administered from the blood side (see below).

Additional luminal processes

K^+ conductances were first shown in choroid plexus by Zeuthen and coworkers in amphibians [19, 116]. These conductances were found on the luminal side of the epithelium. The luminal K^+ conductance in mammalian choroid plexus is inwardly rectifying [56], and the expression of mRNA encoding Kir7.1 [31] was soon supplemented by its localisation to the luminal plasma membrane [70]. Later, Brown and coworkers identified two delayed-rectifying K^+ channels, Kv1.1 and Kv1.3, in the choroid plexus and localised both to the luminal surface [99]. The K^+ channels are thought to set the membrane potential of the epithelial cells and to sustain the Na^+,K^+-ATPase by providing an efflux pathway for K^+ (in addition to NKCC1 and KCC4).

Basolateral processes

In general, the basolateral Na^+ and water transport processes are not as well elucidated as those of the luminal membrane. The basolateral Na^+ transport mechanisms supply the high luminal extrusion of Na^+ by the Na^+,K^+-ATPase. Nevertheless, it is largely unknown how Na^+ enters the epithelial cells from the basolateral surface. Until now, the only transporters that have been suggested to mediate Na^+ entry into the choroid plexus epithelium are Na^+-dependent acid/base transporters. Na^+/H^+ exchange has to a very large extent been put forward as the most probable mechanism for basolateral Na^+ loading. Recent studies suggest that Na^+/H^+ exchange is less important for CSF formation than basolateral Na^+ and CO_2/HCO_3^--dependent mechanisms. The evidence in favour and against the involvement of Na^+/H^+ exchangers (NHEs) in transepithelial Na^+ movement is outlined below. However, one should avoid neglecting the probability that the Na^+ loading may be ascribed to yet unidentified transport processes as a Na^+-channel or an unselective cation channels, etc.

Amiloride and Na^+/H^+ exchange

As mentioned above, Na^+/H^+ exchange has been widely accepted as the main basolateral Na^+-entry mechanism for several reasons. Firstly, numerous studies have demonstrated an inhibitory effect of the NHE and Na^+-channel blocker, amiloride, on the rate of Na^+ secretion when applied from the blood side of the epithelium [67–69, 94]. This effect was also found when amiloride was applied to both sides of the epithelium [28, 94]. To avoid the renal and haemodynamic effects of the drug, Johanson and coworkers conducted their experiments on functionally nephrectomised rats by clamping the renal arteries. In these studies abdominally installed amiloride inhibited Na^+ flux into the CSF, while the local blood flow was supposedly unchanged.

The estimated local amiloride concentration would have been sufficient to inhibit the most amiloride-sensitive NHE form, NHE1 but was unlikely to inhibit other NHEs effectively. Secondly, the secretion of CSF is dependent on pH-sensitive Na^+ uptake from the serosal side of the epithelium [69]. In line with these observations, Kalaria and colleagues demonstrated the presence of mRNA encoding the Na/H exchanger, NHE1, in the choroid plexus of rat [48]. In the corresponding model of CSF formation, the NHE would be capable of ridding the cell of protons formed by the hydration of CO_2 and subsequent dissociation of carbonic acid to HCO_3^- and H, and in the same process provide the Na^+ necessary to sustain CSF secretion.

Surprisingly, amiloride has little effect on Na^+-dependent pH recovery in the choroid plexus in the presence of CO_2/HCO_3^- in vitro [63]. This suggests that the Na^+/H^+ exchanger plays a minor role in maintaining the pH_i under these conditions. Furthermore, NHE1 immunoreactivity has never been detected in the choroid plexus [1]. The possible existence of an amiloride-insensitive NHE1 splice variant was recently rejected by comparing the full length NHE1 transcript from kidney and choroid plexus (own unpublished data). These findings are in agreement with the notion that NHE1 is often responsible for setting pH_i in epithelia or maintaining cell volume, rather than participating in transepithelial Na^+ movement.

The mRNA encoding NHE3 and NHE8 has also been found in the choroid plexus, but it is not yet determined whether the epithelial cells or the interstitial or vascular components carry the mRNA (own unpublished data). However, none of these displays sufficient amiloride sensitivity to explain the inhibitory effect of amiloride on choroid plexus secretion. Like NHE1, the corresponding NHE3 and NHE8 proteins have not yet been detected in the tissue. Interestingly, the molecular and functional expression of the epithelial Na^+ channel, ENaC, was recently reported for the choroid plexus [6]. The authors found the ENaC at the basolateral surface and suggested it as the amiloride Na^+-loading mechanism. However, the quite modest amiloride sensitivity of CSF formation would not fit well with the high sensitivity of ENaC.

The finding is surprising in light of the ENaC tetramers being found mainly in tight epithelia. Furthermore, another laboratory found no significant amiloride-sensitive conductance in the choroid plexus epithelia (personal communications, Peter D. Brown) and both ENaC mRNA and protein were apparently absent from this tissue in our hand (own unpublished data). Thus, it remains highly important to determine the molecular nature of the basolateral Na^+ loader of the choroid plexus epithelium possibly by a broad range of antibody methods and by studying the CSF formation in gene knock-out models.

DIDS sensitive and insensitive HCO_3^- transport

The basolateral electroneutral Cl^-/HCO_3^- exchanger AE2 was first cloned from the rat choroid plexus [58] and serves as a DIDS-sensitive base extruder to maintain pH_i in many cells. AE2 is also expressed basolaterally in most other epithelia, e.g. the distal renal tubules and collecting ducts [2]. AE2 has been involved in HCO_3^- reabsorption, which may in fact be its net effect in the choroid plexus. Thus, the protein is not likely to participate in the HCO_3^- secretion, but may well support luminal Cl^- secretion by loading this ion into the cells from the basolateral side. This view is supported by the DIDS sensitivity of the transepithelial Cl^- flux and CSF formation when applied from the basolateral side [29]. Other functions of AE2 may include cell-volume regulation working in concert with e.g. the NHE1 or simply protection of the cells against alkalisation.

A few years ago, two $NaHCO_3$ transporters were demonstrated in the basolateral plasma membrane of choroid plexus epithelial cells, which could represent alternative basolateral Na^+ uptake pathways [85]. NCBE is an electroneutral DIDS-sensitive $NaHCO_3$ importer that likely also extrudes Cl^- with a proposed stoichiometry equivalent to the uptake of 1 Na^+, and $2HCO_3^-$ for the extrusion of $1Cl^-$ [106]. NCBE was suggested as a major Na^+-entry route because the secretion of Na^+ in addition to basolateral pH is sensitive to the serosal [HCO_3^-] [39] and to basolateral application of DIDS [63]. The in vitro steady-state pH of CPE was shown to be quite sensitive to DIDS and to depend on extracellular Na^+, suggesting that NCBE or a similar DIDS-sensitive $NaHCO_3$ transporter is responsible for setting pH_i.

In contrast, the second electroneutral basolateral $NaHCO_3$ transporter, NBCn1, is described as relatively DIDS-insensitive protein when expressed in epithelia. For this reason, NBCn1 is not likely to play a major role in the transepithelial movement of Na^+, but might serve to correct intracellular acidosis in the choroid plexus epithelium. In support of this view, Bouzinova et al. showed that a large fraction of the pH recovery from acid load was mediated by DIDS-insensitive $NaHCO_3$ cotransport [9].

A few considerations are necessary to illustrate the current lack of knowledge on the significance of the various Na^+-dependent acid/base transporters for Na^+ uptake. As mentioned above, the pH_i would most likely have to be around or lower than 7.1 for NHE1 to supply the cell with Na^+ to sustain CSF production. However, the reported values for pH_i in the choroid plexus epithelium differ substantially with the observer. The in vitro data on pH_i in rat CP by fluorescence imaging (BCECF) indicates a resting pH_i of 7.38 in the presence of CO_2/HCO_3^- in our laboratory [9]. This is quite a bit higher than the values reported in vivo where pH_i was 7.05 as assessed by the distribution of radioactively labelled weak acid, 5, 5-dimethyl-2,4-oxazolidinedione (DMO) [44, 69].

On the other hand, our data are relatively close to the pH_i value of 7.3 obtained with the benzoate method in primary cultures [63]. It is always reasonable to regard results obtained in vivo, as more physiologically relevant than those from isolated tissue. However, the accuracy of the DMO method relies on various factors, and independent determinations of cellular pH in vivo are necessary to confirm the low pH_i. Nevertheless, a steady-state pH_i around 7.1 indicates that the outward chemical H^+ and inward HCO_3^- gradients would be about twofold. These numbers are relevant when considering electroneutral processes and equally favour Na^+/H^+ exchange and $NaHCO_3$ transport.

If the proposed stoichiometry for NCBE is correct, the activity of this particular transporter would be more influenced by low pH_i than other transporters. For example, AE2 transport would be halved at pH_i 7.05 compared to pH_i 7.4; the pH gradients acting on NHE1 and NBCn1 would be doubled, while NCBE would go from net zero to three times inward gradient (for 45 mM intracellular Na^+) or even to ten times net inward gradient (for 15 mM intracellular Na^+), if everything else was equal.

In addition to the pH_i, one has to take into account the distribution of Na^+, Cl^- and HCO_3^- inside and outside the cells to estimate the forces working on these electroneutral transporters. Reports on intracellular ionic concentrations have been provided by Johanson and colleagues (e.g. [44]). The values were calculated from distribution of radioactive tracers and carefully adjusted for extracellular volume and blood constituents in the tissue. The mean values from lateral ventricles and fourth ventricles were roughly $[Na^+]_i$=45 mM, $[K^+]_i$=148 mM, $[HCO_3^-]_i$=12 mM and $[Cl^-]_i$=65 mM. The high $[Na^+]_i$ is somewhat striking in the presence of a very potent and active Na^+ extrusion mechanism, i.e. the Na^+,K^+-ATPase.

Also, the sum of cations predicts a somewhat hyperosmolar intracellular milieu, which would be impossible given the high abundance of water channels in the luminal membrane and a CSF of around 305 mOsM. The computational error on $[K^+]_i$ is expected to be less than for $[Na^+]_i$,

as tissue K^+ is mainly found intracellularly and most Na^+ is extracellular. Thus, parts of the extracellular Na^+ space may be inaccessible to the extracellular space marker (radioactively labelled raffinose). Furthermore, it would take an immensely powerful basolateral Na^+ loading mechanism to build up cellular Na^+ despite constitutive open cation channel with very high conductance or similar. Thus, one could speculate that the true $[Na^+]_i$—as in other epithelia—may lie closer to 15 mM in choroid plexus epithelial cells. This way the sum of cations inside the cells would reach the expected 150 mM.

Clearly, further studies on the polarised mammalian choroid plexus are needed to uncover the role of each basolateral Na^+-dependent acid extruder and base loaders. Again, the use of transporter-deficient mice may play a central role and one should consider confirming the reported intracellular ionic composition.

KCl cotransport and furosemide effects

As mentioned above there is a net absorption of K^+ from the CSF to the plasma, and it is therefore of some interest to define how this cation leaves the cell basolaterally. Systemic administration of furosemide was shown to inhibit CSF formation [64, 105]. Although some of the effect might have been caused by vasoconstriction, it is noteworthy that the K^+,Cl^- cotransporter, KCC3, has been identified in the choroid plexus of mice [78]. This mechanism to date represents the only known K^+ efflux pathway from cells to blood. However, the CSF secretion itself is not changed by furosemide from the blood side, when renal effects of the drug are prevented [105]. It was found that furosemide has seven times higher affinity for carbonic anhydrase than bumetanide, and this affinity is indeed relevant with the applied dosage. Thus, it is feasible that the effect of furosemide on CSF formation in vivo and in the perfused tissue is mediated partly by haemodynamic changes and partly by the inhibition of carbonic anhydrases in the choroid plexus epithelium.

Unpolarised processes

Inhibition of carbonic anhydrases by acetazolamide

Inhibition of the carbonic anhydrases by acetazolamide reduced CSF secretion by about 50% [104]. This was interpreted as an inhibition of the cytosolic carbonic anhydrase in the choroid plexus. Consequently, it was concluded that most, if not all secreted HCO_3^-, was formed inside the epithelial cells. The H^+ formed in the reaction would leave the cell through a basolateral NHE. However, recent studies imply that HCO_3^- transporters of the SLC4a family (as AE2, and electrogenic NBC) are functionally and physically coupled to both intracellular CAII and external plasma membrane bound forms of the carbonic anhydrase [101, 102]. The current notion is that the high transport rate through bicarbonate transporters may depend on the local formation of HCO_3^- or perhaps CO_3^{2-}.

It was recently shown that CAXII and perhaps CAIX are also expressed in the choroid plexus [38, 49]. Interestingly, CAXII was found in association with the basolateral plasma membrane where more bicarbonate transporters are located. It is, however, not established whether the acetazolamide effect is entirely or partly due to inhibition of extracellular carbonic anhydrases. In that case, the secreted HCO_3^- may arise from the plasma instead of intracellular CO_2 and H_2O metabolism. Hence, it is of great importance to settle this issue probably using membrane impermeant CA inhibitors.

Water transport in the choroid plexus

Water transport across the epithelium is driven by the modest luminal hyperosmolarity. The small osmotic gradient and the high rate of water transport resemble that of the renal proximal tubules. In both epithelia, constitutive water permeability is desirable to meet the transport requirements. Thus, it is not surprising that the choroid plexus epithelium—like the proximal tubules—expresses a specialised protein, the water channel AQP1, allowing the majority of the water molecules to permeate the epithelium by a transcellular pathway. Nielsen and colleagues showed that AQP1 is expressed in high abundance in regions corresponding to the luminal plasma membrane of the rat choroid plexus [72].

The AQP1 protein is also detected at the basolateral membrane and in the endothelial cells, although in lower abundance as exemplified in Fig. 4b. The epithelial localisation is especially interesting considering the apparent lack of other water-transporting aquaporins in a tissue with such high secretory rate [84]. Thus, AQP1 potentially provides the basolateral membrane with a substantially higher water permeability than the lipid bilayer would offer. The only other two AQP transcripts found in the choroid plexus, AQP4 and AQP11, were not expressed at detectable levels in plasma membranes ([97], and own observations). Although water may pass paracellularly from the blood side to the CSF, it is feasible that the transcellular water transport mainly occurs through basolateral and luminal AQP1.

In fact, the significance of AQP1 in choroid plexus water permeability and transepithelial water flux was recently studied in AQP1 gene knock-out mice. The authors reported an 80% decrease in water permeability in the AQP1 knock-out mice that was accompanied by a decrease in secretory rate of only 25%. The potential discrepancy suggests that compensatory changes in ionic gradients may have devel-

oped, as proposed for the renal tubules of AQP1 knock-out mice [74, 75, 92].

Other processes studied by patch clamp and mRNA analysis

A number of transcripts for additional transporters have been identified without confirmation of protein expression, knowledge of exact site of expression or determination of functional significance. For example, the KCC1 mRNA expression was determined by two groups [50, 57]. The same is the case for the anion exchangers of the SLC26a gene family SLC26a7, SLC26a10 and SLC26a11, which are in rat and human choroid plexus (unpublished observations). Future investigations will hopefully bring clarity to the localisation and significance of these and other additional transporters. Meanwhile, the transport proteins of known localisation can be represented as shown in Fig. 5.

Working models for CSF secretion by the choroid plexus

It is tempting to suggest a single elaborate model for the processes leading to the net secretion of NaCl and $NaHCO_3$ and net reabsorption of KCl. However, there would be several shortcomings in such a model, as more potentially important details are not yet fully elucidated. Instead, central aspects of the basolateral and luminal processes are discussed in a few partial models of secretion. The

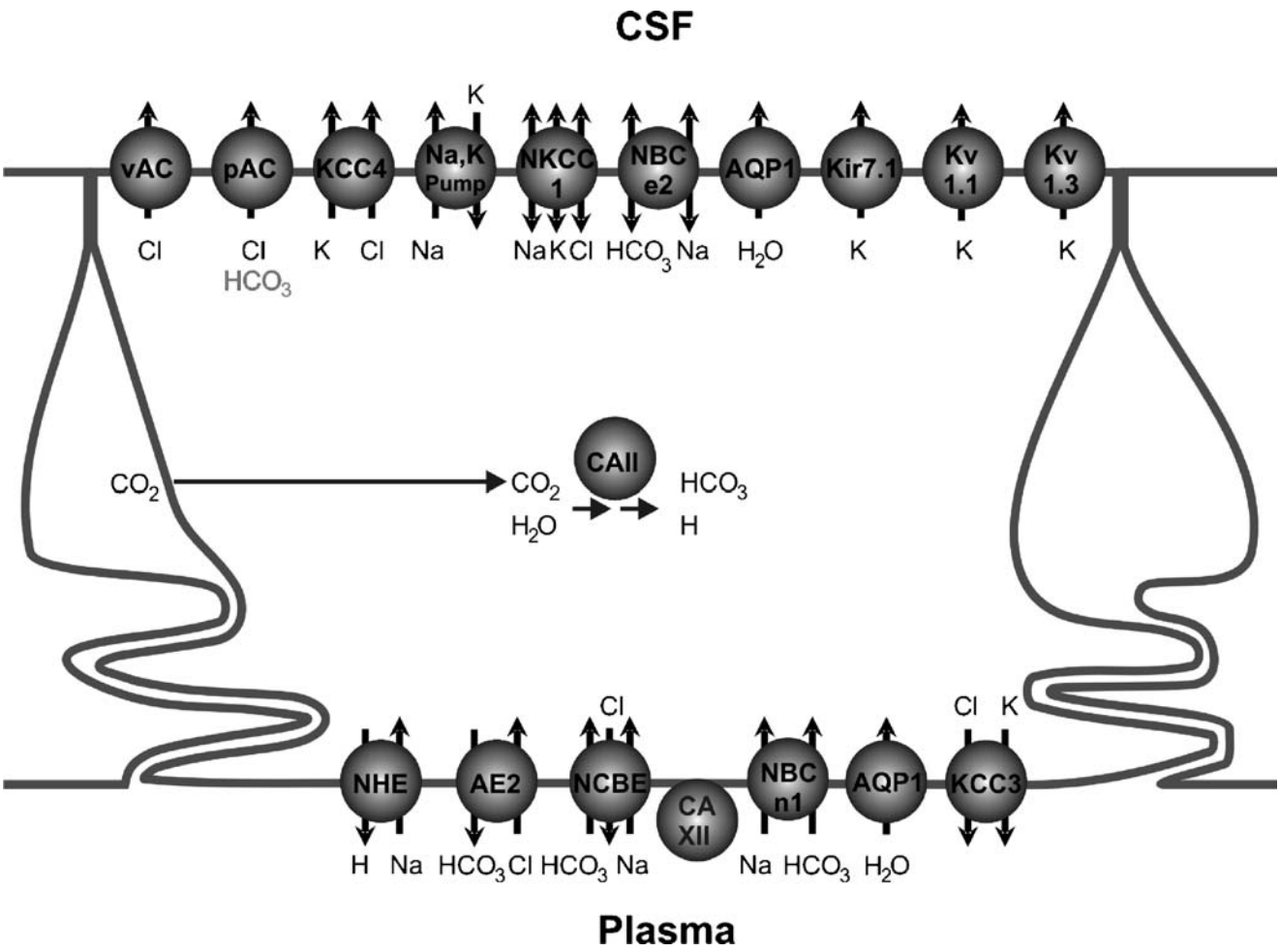

Fig. 5 Schematic representation of the ion and water transporters in the choroid plexus epithelium. Aquaporin1, *AQP1*, is situated in both the luminal and basolateral membranes, and the carbonic anhydrase (*CA*) *II* and *XII* are found in the cytosolic and basolateral compartments, respectively. The 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 conductance of unknown identity (a volume-sensitive anion conductance, *vAC*, and a *PKA*-activated anion conductance, *pAC*). The electrogenic Na^+,HCO_3^- cotransporter *NBCe2* or *NBC4* is also found at the luminal membrane. Three HCO_3^- transporters are localised 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

models I suggest should not be regarded as mutually exclusive and indicate that the interplay between the many transporters is more complex than originally thought. In fact, combinations of the models may be necessary to explain all the effects of transport inhibitors described above. For simplicity, the main task is to obtain a net gain of roughly 14–15 Na^+, per 11–12 Cl^- and 3 HCO_3^- in the CSF originating from the plasma. As indicated above, one could regard the luminal Na^+ extrusion as the primary event in CSF formation, although the hydration of CO_2 or the basolateral Na^+ entry could just as well be chosen as a starting point when modelling secretion.

At the luminal membrane, it is clear the Na^+,K^+-ATPase is a crucial extruder of Na^+, while it is uncertain whether NKCC1 is normally involved in secretion or reabsorption. Therefore, two models are considered for the luminal aspect of secretion. In one model, the Na^+,K^+-ATPase secretes virtually all Na^+ molecules in nascent CSF above the choroid plexus (Fig. 6a). Thus, 15 Na^+ would be extruded, and 10 K^+ are taken up by the Na^+,K^+-ATPase, leaving no room for contributions by NKCC1. As the net transepithelial K^+ absorption is very modest compared to the Na^+ secretion, roughly all 10 K^+ should re-enter the CSF. This could be accomplished by the KCC4, which would also rid the cells of ten of the 12 Cl^- molecules needed for net NaCl secretion. The remaining 2 Cl^- may leave the cells through the luminal Cl^- channels.

Alternatively, the combined action of K^+ and Cl^- channels may substitute for KCC4 by extruding KCl. Actually, some K^+ channel conductance is necessary to retain a membrane potential suitable for sustaining secretion. In this model, HCO_3^- would be excreted by the HCO_3^- permeable anion conductance pathway.

In another model, the Na^+,K^+-ATPase contributes less to the extrusion of Na^+, e.g. three times turnover of the Na^+, K^+-ATPase would yield the exit of 9 Na^+ and entry of 6 K^+ (Fig. 6b). In parallel, four times turnover of the Na^+, K^+,2Cl^- cotransporter would provide the exit of 4 Na^+, 4 K^+ and 8 Cl^-. Using these numbers, there is still a need for extrusion of additional 1 Na^+, 3 Cl^-, 3 HCO_3^- and the K^+ secreted is not sufficient to meet the Na^+,K^+-ATPase's requirement for extracellular K^+. Again, the luminal KCC4 or combined action of Cl^- and K^+ channels may sustain secretion by exporting 2 K^+ and 2 Cl^-. The turnover of the luminal NBCe2 may mediate the translocation of the remainder of the Na^+ and all the secreted HCO_3^-, given a stoichiometry of 1:3 for outwards transport (1× turnover yielding 1 Na^+ and 3 HCO_3^-).

This would also give a net electrogenic CSF secretion, as the positive charges moved out by the Na^+,K^+-ATPase would not be balanced by the negative charges extruded by NBCe2. This may in turn increase the drive for the remaining Cl^- transport through luminal Cl^- conductance pathways. Interestingly, these models seem to imply a role

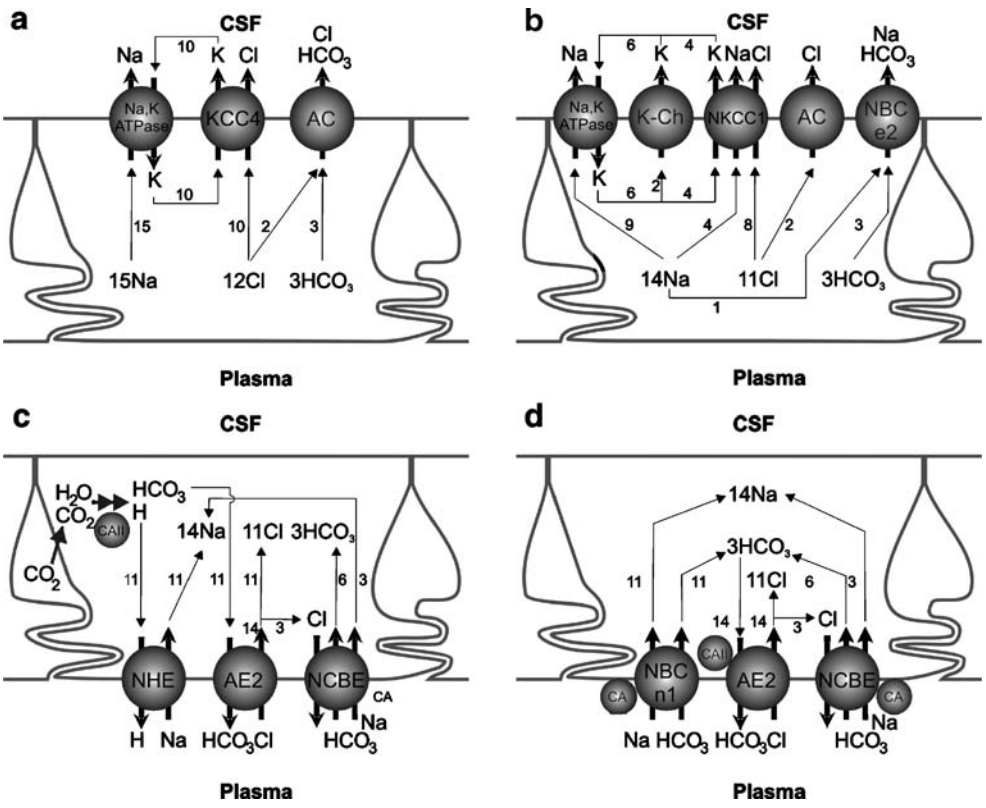

Fig. 6 Models for the translocation of Na^+, Cl^-, and HCO_3^- from plasma to the *CSF*. The main task is to transport the ions across the epithelium in the proportion of roughly 14 Na^+, 11 Cl^-, to 3 HCO_3^-. **a** and **b** show two representations of the putative of luminal processes in *CSF* formation. **c** and **d** are two suggestions for the interplay between basolateral processes supporting *CSF* production. Abbreviations are as in Fig. 5, except for *K-Ch*, which represents any of the three *K* channels of the choroid plexus, and *AC* is one or both of the anion conductances. The models are not mutually exclusive (see text for details)

for K^+ in recycling across the Na^+,K^+-ATPase-bearing membrane, as is suggested for Na^+ in other epithelia. Of course, the actual transport mechanism could be any combination of the two models.

The basolateral transport processes all seem to be electroneutral, and the task is, again, to translocate Na^+, Cl^- and HCO_3^- across the plasma membrane with virtually the same proportion of the major ions as in the nascent CSF, i.e. to take up 14 Na^+, with 11 Cl^- and 3 HCO_3^-. In one model, the first step would be mediated by the combined action of the NHE and AE2 to yield net NaCl transport (Fig. 6c). The 11 times turnover for each of the two transporters would yield a net import of 11 Na^+ and 11 Cl^- into the cell. This process of net NaCl loading would be entirely dependent on the rapid intracellular formation of H^+ and HCO_3^- catalysed by intracellular carbonic anhydrase. In addition to the NaCl import, $NaHCO_3$ would enter the cell by the combined action of NCBE and AE2, which would mediate net $NaHCO_3$ uptake. Three times turnover for each transporter would provide the cell with the remaining 3 Na^+ and 3 HCO_3^- needed to sustain $NaHCO_3$ secretion.

In the other model, the NBCn1 rather than NHE acts in concert with the AE2 to yield net NaCl transport (11× turnover for 11 NaCl into cell). Again, 11 times turnover for each of the two transporters would yield a net import of 11 Na^+ and 11 Cl^- into the cell. As opposed to the first basolateral model, the NaCl uptake may be largely independent on intracellular carbonic anhydrase, as HCO_3^- is recycled across the basolateral plasma membrane. Interestingly, both AE2 and NBCn1 are found in highest abundance in the basolateral membrane infoldings, which may allow rapid HCO_3^- recycling. However, it remains an open question whether efficient bicarbonate transport requires fast production HCO_3^-/CO_3^{2-} in close proximity to the transporter.

The difference between the two models is that intracellular CO_2 is actually only consumed in the model with AE2/NHE synergy, while the AE2/NBCn1 model would have no net effect on intracellular CO_2. Nevertheless, three times turnover of NCBE and AE2 would provide the cell with the remaining 3 Na^+ and 3 HCO_3^- as for the first model.

It is also noted that the two combinations of transporters AE2/NHE and AE2/NBCn1 may have slightly different effects on pH_i, although the combinations seem equivalent at first glance. The combination AE2/NBCn1 is pH_i neutral as 1 HCO_3^- is shuttled in and 1 HCO_3^- extruded from the cells with each 1 Na^+ and 1 Cl^- imported. In contrast, one should bear in mind that the NHE and AE2 removes 1 H^+ and 1 HCO_3^- for each imported NaCl. This is actually not pH neutral to the cytosol, as H^+ or H_3O^+ is the strongest acid known and HCO_3^- is weaker as base. Thus, the equimolar removal of H^+ and HCO_3^- from the cytosol would mimic cytosolic CO_2 removal and increase pH_i, unless regarded as tightly coupled with intracellular carbonic anhydrase activity. Nevertheless, both models of basolateral transport may explain both the acetazolamide and the DIDS sensitivity of the CSF production, while only the first would be consistent with inhibition by amiloride.

As an alternative to these two models, the unconfirmed presence of ENaC subunits may contribute to basolateral cellular Na^+ loading. Again, the actual transport mechanism could be any combination of the two models of basolateral transport. However, the basolateral KCC3 or paracellular K^+ leak could mediate the K^+ reabsorption observed in the choroid plexus.

The water formed by ATP production from glycolysis and oxidative phosphorylation—to fuel the Na^+,K^+-ATPase with ATP—almost equals the amount consumed by hydration of CO_2 to produce HCO_3^- for the Cl^- uptake. This amount of H_2O is extremely modest compared to that accompanying the transepithelial movement of ions. The transepithelial H_2O would actually be more than two orders of magnitude larger than the number of transported Na^+ ions to maintain the modest osmolarity gradients during secretion. Thus, the cellular water metabolism is of minimal importance for the transepithelial water flux. It is feasible that intracellular osmolarity is much closer to that of the nascent CSF than the interstitial osmolarity, as AQP1 is much more abundant at the luminal surface than the basolateral membrane. Thus, a larger gradient for water is needed basolaterally than luminally to permit sufficient movement of H_2O.

The presented models do not take into account the luminal K^+ channels in setting the membrane potential, which should be more negative than −40 mV in order for e.g. NBCe2 to contribute to HCO_3^- secretion. Neither does the model consider the contribution of paracellular H_2O movement, Cl^- secretion and K^+ absorption that may take place.

Regulation of CSF production

The receptors for several mediators have been located in the choroid plexus, although it is presently unknown how many of these actually affect the transport processes leading to secretion of water and solutes into the CSF. Many of the mediators may have roles in haemodynamics, metabolism of nutrients and other compounds secreted into CSF. Among the most interesting discoveries are the detection of vasopressin-1 receptors [80] and receptors for atrial natriuretic factor, angiotensin II, endothelin-1, serotonin, bradykinin and insulin (for specific review [15]). It is noteworthy that although many of the mediators are found in blood; the transport pathways of the choroid plexus seem relatively resistant to changes in plasma levels of these

compounds. It seems that the mediators have to be formed locally; some even appear in the CSF, to act on choroid plexus function. CSF vasopressin levels are up to tenfold higher than in plasma.

In fact, the choroid plexus itself produces or at least releases a number of mediators as reviewed previously [15]. Peptides synthesised in CP with putative interest to the secretory function may include adrenomodulin, endothelin-1 and vasopressin. For example, vasopressin mRNA was demonstrated in the choroid plexus [13], and immunoreactivity for the corresponding peptide was found mainly in the luminal part of the epithelial cells [18].

Vasopressin and angiotensin II have been shown to decrease CSF formation by epithelial effects and not solely through changes in blood supply. Faraci and colleagues demonstrated that vasopressin in the systemic circulation decreased blood flow and CSF formation through action on V1 receptors [34]. It was not possible to discern a specific epithelial effect of basolateral vasopressin from the type of experiment. However, Johanson and coworkers showed a dependence on ventricular V1 receptors of the decreased luminal Cl^- efflux when vasopressin was delivered via artificial CSF [45]. A transient increase in $[Ca^{2+}]_i$ in the choroid plexus epithelium was observed upon vasopressin administration [8], whereas vasopressin was without effect on choroid plexus cAMP [21]. This is in line with the presence of V1 rather than V2 receptors in the choroid plexus. It is still unknown how the increase in $[Ca^{2+}]_i$ brings about the inhibition of Cl^- efflux, as none of the present anion conductances is known to be Ca^{2+} sensitive.

Ventricular angiotensin II was shown to reduce CSF formation through AT1 receptors [17]. Interestingly, angiotensin II seems to act on CSF formation through the release of vasopressin from the choroid plexus to the ventricles [16]. This probably involved Ca^{2+} signalling [8], as cAMP/PKA induction by angiotensin II in choroid plexus was only reported for isoproterenol, prostaglandin, serotonin and histamine [21]. However, one preliminary report indicates that angiotensin II may also act on vasopressin release through cAMP/PKA [14]. Is should be interesting to validate the latter finding, as the transepithelial Cl^- flux from plasma to CSF, and therefore most likely also the CSF formation increases when cAMP signalling is induced [30]. In light of the many putative agents acting on the choroid plexus, numerous investigations are necessary to further our understanding of the hormonal and nervous control of CSF production.

Diseases with altered cerebrospinal fluid secretion

Several medical conditions are associated with changes in choroid plexus function and/or morphology. These conditions have recently been reviewed in great detail by others [32, 43, 95, 108]. The most striking conditions are aging, Alzheimer's disease, stroke, brain edema and hydrocephalus. It is evident that although the choroid plexus may only have a direct influence on the pathogenesis of a few conditions, the functional adaptation of the tissue would be of utmost importance in all of the disorders. Recent analyses suggest adaptive regulation of specific transport proteins such as NKCC1 and AQP1 in Alzheimer's disease and systemic hyponatremia [43, 65]. Hopefully, future studies will pursue similar aspects of adaptational changes in choroid plexus function to severe strain. This would enable researchers to determine the extent to which this tissue is capable of meeting requirements of altered CSF formation and ultimately reduce the impact on the CNS.

Conclusions and perspectives

Opposite the intentions, this review poses more questions than answers to central aspects of the choroid plexus function in water and solute secretion. However, it is the hope that it still may inspire researchers in and outside the field to undertake further investigations to clarify the many unresolved issues. The choroid plexus is a minuscule yet truly a fascinating tissue. It comprises less than 0.003% of the total body mass and is nevertheless proven essential for brain function and thereby for life in mammals. Thus, the choroid plexus is both a biologically and clinically important tissue.

It is concluded that more information on transport processes and the responsible proteins is required to incorporate the present detailed information into a fully coherent model of the entire secretory processes. In this author's opinion the imperative questions are: How is the Na^+,K^+-ATPase targetted to the luminal plasma membrane? Is the net effect of NKCC1 ion secretion or reabsorption, or does the direction, shift to meet varying demands? What is the molecular identity of the two luminal anion conductances, and does NBCe2 contribute to the luminal HCO_3^- secretion? What Na^+-dependent acid/base transporter mediates the Na^+ uptake from the serosal side of the epithelium? Is ENaC in fact expressed in relevant numbers in the choroid plexus epithelium or are yet undiscovered Na^+ transporters present? Is intracellular or extracellular carbonic anhydrase necessary for CSF secretion?

In addition to the above questions, it is highly relevant to uncover the entire signalling pathways for the various agonists and antagonists of CSF formation. For example, many of the luminal transporters are shown in other systems to be subjected to phosphorylation by PKA, so it would be important to find out whether acute cAMP mediated regulation of some or all of these proteins hold

a bearing for CSF production. One perspective would be optimisation for the clinical management of diseases involving altered CSF turnover or intracranial pressure. In the long term, however, further investigations into choroid plexus function are pertinent in the search for unique pharmacological targets for either the reduction or stimulation of CSF formation and optimised clinical procedures. These targets may be found among any "choroid plexus specific" isoforms of the transporters, enzymes, receptors, and transcription factors, or perhaps in the mechanisms underlying the unique membrane targeting of proteins in this tissue.

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References

1. Alper SL, Stuart-Tilley A, Simmons CF, Brown D, Drenckhahn D (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 93:1430–1438
2. Alper SL, Stuart-Tilley AK, Biemesderfer D, Shmukler BE, Brown D (1997) Immunolocalization of AE2 anion exchanger in rat kidney. Am J Physiol 273:F601–F614
3. Ames A III, Higashi K, Nesbett FB (1965) Relation of potassium concentration in choroid plexus fluid to that in plasma. J Physiol 181:506–515
4. Ames A III, Higashi K, Nesbett FB (1965) Effects of Pco2 acetazolamide and ouabain on volume and composition of choroid-plexus fluid. J Physiol 181:516–524
5. Ames A III, Sakanoue M, Endo S (1964) Na, K, Ca, Mg, and Cl concentrations in choroid plexus fluid and cisternal fluid compared with plasma ultrafiltrate. J Neurophysiol 27:672–681
6. Amin MS, Wang HW, Reza E, Whitman SC, Tuana BS, Leenen FH (2005) Distribution of epithelial sodium channels and mineralocorticoid receptors in cardiovascular regulatory centers in rat brain. Am J Physiol Regul Integr Comp Physiol 289: R1787–R1797
7. Bairamian D, Johanson CE, Parmelee JT, Epstein MH (1991) Potassium cotransport with sodium and chloride in the choroid plexus. J Neurochem 56:1623–1629
8. Battle T, Preisser L, Marteau V, Meduri G, Lambert M, Nitschke R, Brown PD, Corman B (2000) Vasopressin V1a receptor signaling in a rat choroid plexus cell line. Biochem Biophys Res Commun 275:322–327
9. Bouzinova EV, Praetorius J, Virkki LV, Nielsen S, Boron WF, Aalkjaer C (2005) Na^+-dependent HCO_3^- uptake into the rat choroid plexus epithelium is partially DIDS sensitive. Am J Physiol Cell Physiol 289:C1448–C1456
10. Brown PD, Davies SL, Speake T, Millar ID (2004) Molecular mechanisms of cerebrospinal fluid production. Neuroscience 129:957–970
11. Brown PD, Pakhomova A, Millar ID (2006) NKCC1 does not contribute to volume regulation in epithelial cells isolated from mouse choroid plexus. Proc Physiol Soc 2:PC15 (Abstract)
12. Burg MB, Orloff J (1968) Control of fluid absorption in the renal proximal tubule. J Clin Invest 47:2016–2024
13. Chodobski A, Loh YP, Corsetti S, Szmydynger-Chodobska J, Johanson CE, Lim YP, Monfils PR (1997) The presence of arginine vasopressin and its mRNA in rat choroid plexus epithelium. Brain Res Mol Brain Res 48:67–72
14. Chodobski A, Summy-Long J, Lin MWS, Wojcik BE, Bui V, Kil E, Johanson CE (2000) Regulation of choroidal vasopressin secretion by the cAMP/PKA signalling patheway and the possible autocrine actions. Soc Neurosci Abstr 26:337 (Abstract)
15. Chodobski A, Szmydynger-Chodobska J (2001) Choroid plexus: target for polypeptides and site of their synthesis. Microsc Res Tech 52:65–82
16. Chodobski A, Szmydynger-Chodobska J, Johanson CE (1998) Vasopressin mediates the inhibitory effect of central angiotensin II on cerebrospinal fluid formation. Eur J Pharmacol 347:205–209
17. Chodobski A, Szmydynger-Chodobska J, Vannorsdall MD, Epstein MH, Johanson CE (1994) AT1 receptor subtype mediates the inhibitory effect of central angiotensin II on cerebrospinal fluid formation in the rat. Regul Pept 53: 123–129
18. Chodobski A, Wojcik BE, Loh YP, Dodd KA, Szmydynger-Chodobska J, Johanson CE, Demers DM, Chun ZG, Limthong NP (1998) Vasopressin gene expression in rat choroid plexus. Adv Exp Med Biol 449:59–65
19. Christensen O, Zeuthen T (1987) Maxi K^+ channels in leaky epithelia are regulated by intracellular Ca^{2+}, pH and membrane potential. Pflugers Arch 408:249–259
20. Cornford EM, Varesi JB, Hyman S, Daiman RT, Raleigh MJ (1997) Mitochondrial content of choroid plexus epithelium. Exp Brain Res 399–405
21. Crook RB, Farber MB, Prusiner SB (1984) Hormones and neurotransmitters control cyclic AMP metabolism in choroid plexus epithelial cells. J Neurochem 42:340–350
22. Csaky TZ, Rigor BM (1967) The choroid plexus as a glucose barrier. In: Lajtha A, Ford DH (eds) Progress in brain research. Elsevier, Amsterdam, pp 147–158
23. Cserr HF (1971) Physiology of the choroid plexus. Physiol Rev 51:273–311
24. Cushing H (1914) Studies on the cerebrospinal fluid. J Med Res 26:1–19
25. Davson H, Segal MB (1996) Physiology of the CSF and blood–brain barriers. CRC Press, Boca Raton
26. Davson H, Kleeman CR, Levin E (1962) Quantitative studies of the passage of different substances out of the cerebrospinal fluid. J Physiol 161:126–142
27. Davson H, Purvis C (1954) Cryoscopic apparatus suitable for studies on aqueous humour and cerebro-spinal fluid. J Physiol 124:12P–13P
28. Davson H, Segal MB (1970) The effects of some inhibitors and accelerators of sodium transport on the turnover of ^{22}Na in the cerebrospinal fluid and the brain. J Physiol 209:131–153
29. Deng QS, Johanson CE (1989) Stilbenes inhibit exchange of chloride between blood, choroid plexus and cerebrospinal fluid. Brain Res 501:183–187
30. Deng QS, Johanson CE (1992) Cyclic AMP alteration of chloride transport into the choroid plexus–cerebrospinal fluid system. Neurosci Lett 143:146–150
31. Doring F, Derst C, Wischmeyer E, Karschin C, Schneggenburger R, Daut J, Karschin A (1998) The epithelial inward rectifier channel Kir7.1 displays unusual K^+ permeation properties. J Neurosci 18:8625–8636
32. Emerich DF, Skinner SJ, Borlongan CV, Vasconcellos AV, Thanos CG (2005) The choroid plexus in the rise, fall and repair of the brain. Bioessays 27:262–274
33. Faivre J (1854) Structure du conarium et des plexus choroïde chez l'hommes et des animaux. Gaz Med Paris 9:555–556

34. Faraci FM, Mayhan WG, Heistad DD (1990) Effect of vasopressin on production of cerebrospinal fluid: possible role of vasopressin (V1)-receptors. Am J Physiol 258: R94–R98
35. Fencl V, Vale JR, Broch JA (1969) Respiration and cerebral blood flow in metabolic acidosis and alkalosis in humans. J Appl Physiol 27:67–76
36. Fenton RA, Flynn A, Shodeinde A, Smith CP, Schnermann J, Knepper MA (2005) Renal phenotype of UT-A urea transporter knockout mice. J Am Soc Nephrol 16:1583–1592
37. Feschenko MS, Donnet C, Wetzel RK, Asinovski NK, Jones LR, Sweadner KJ (2003) Phospholemman, a single-span membrane protein, is an accessory protein of Na,K-ATPase in cerebellum and choroid plexus. J Neurosci 23:2161–2169
38. Halmi P, Parkkila S, Honkaniemi J (2006) Expression of carbonic anhydrases II, IV, VII, VIII and XII in rat brain after kainic acid induced status epilepticus. Neurochem Int 48:24–30
39. Haselbach M, Wegener J, Decker S, Engelbertz C, Galla HJ (2001) Porcine choroid plexus epithelial cells in culture: regulation of barrier properties and transport processes. Microsc Res Tech 52:137–152
40. Heisey SR, Held D, Pappenheimer JR (1962) Bulk flow and diffusion in the cerebrospinal fluid system of the goat. Am J Physiol 203:775–781
41. Held D, Fencl V, Pappenheimer JR (1964) Electrical potential of cerebrospinal fluid. J Neurophysiol 27:942–959
42. Javaheri S, Wagner KR (1993) Bumetanide decreases canine cerebrospinal fluid production. In vivo evidence for NaCl cotransport in the central nervous system. J Clin Invest 92:2257–2261
43. Johanson C, McMillan P, Tavares R, Spangenberger A, Duncan J, Silverberg G, Stopa E (2004) Homeostatic capabilities of the choroid plexus epithelium in Alzheimer's disease. Cerebrospinal Fluid Res 1:1–16
44. Johanson CE, Murphy VA (1990) Acetazolamide and insulin alter choroid plexus epithelial cell [Na^+], pH, and volume. Am J Physiol 258:F1538–F1546
45. Johanson CE, Preston JE, Chodobski A, Stopa EG, Szmydynger-Chodobska J, McMillan PN (1999) AVP V1 receptor-mediated decrease in Cl^- efflux and increase in dark cell number in choroid plexus epithelium. Am J Physiol 276:C82–C90
46. Johnston M, Zakharov A, Papaiconomou C, Salmasi G, Armstrong D (2004) Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species. Cerebrospinal Fluid Research 1:2 DOI 10.1186/1743-8454-1-2
47. Kajita H, Brown PD (1997) Inhibition of the inward-rectifying Cl^- channel in rat choroid plexus by a decrease in extracellular pH. J Physiol 498 (Pt 3):703–707
48. Kalaria RN, Premkumar DR, Lin CW, Kroon SN, Bae JY, Sayre LM, LaManna JC (1998) Identification and expression of the Na^+/H^+ exchanger in mammalian cerebrovascular and choroidal tissues: characterization by amiloride-sensitive [^{3}H] MIA binding and RT-PCR analysis. Brain Res Mol Brain Res 58:178–187
49. Kallio H, Pastorekova S, Pastorek J, Waheed A, Sly WS, Mannisto S, Heikinheimo M, Parkkila S (2006) Expression of carbonic anhydrases IX and XII during mouse embryonic development. BMC Dev Biol 6:22 (Epub ahead of print)
50. Kanaka C, Ohno K, Okabe A, Kuriyama K, Itoh T, Fukuda A, Sato K (2001) The differential expression patterns of messenger RNAs encoding K–Cl cotransporters (KCC1,2) and Na–K–2Cl cotransporter (NKCC1) in the rat nervous system. Neuroscience 104:933–946
51. Kazemi H, Johnson DC (1986) Regulation of cerebrospinal fluid acid–base balance. Physiol Rev 66:953–1037
52. Keep RF, Jones HC (1990) A morphometric study on the development of the lateral ventricle choroid plexus, choroid plexus capillaries and ventricular ependyma in the rat. Brain Res Dev Brain Res 56:47–53
53. Keep RF, Xiang J, Betz AL (1994) Potassium cotransport at the rat choroid plexus. Am J Physiol 267:C1616–C1622
54. Kibble JD, Garner C, Colledge WH, Brown S, Kajita H, Evans M, Brown PD (1997) Whole cell Cl^- conductances in mouse choroid plexus epithelial cells do not require CFTR expression. Am J Physiol 272:C1899–C1907
55. Kibble JD, Trezise AE, Brown PD (1996) Properties of the cAMP-activated C1—current in choroid plexus epithelial cells isolated from the rat. J Physiol 496 (Pt 1):69–80
56. Kotera T, Brown PD (1994) Evidence for two types of potassium current in rat choroid plexus epithelial cells. Pflugers Arch 427:317–324
57. Li H, Tornberg J, Kaila K, Airaksinen MS, Rivera C (2002) Patterns of cation-chloride cotransporter expression during embryonic rodent CNS development. Eur J Neurosci 16:2358–2370
58. Lindsey AE, Schneider K, Simmons DM, Baron R, Lee BS, Kopito RR (1990) Functional expression and subcellular localization of an anion exchanger cloned from choroid plexus. Proc Natl Acad Sci USA 87:5278–5282
59. Livingston RB (1949) Cerebrospinal fluid. In: Fulton JF (ed) A textbook of physiology. Saunders, Philadelphia, pp 916–980
60. Marrs JA, Napolitano EW, Murphy-Erdosh C, Mays RW, Reichardt LF, Nelson WJ (1993) Distinguishing roles of the membrane-cytoskeleton and cadherin mediated cell–cell adhesion in generating different Na+,K(+)-ATPase distributions in polarized epithelia. J Cell Biol 123:149–164
61. Masuzawa T, Ohta T, Kawamura M, Nakahara N, Sato F (1984) Immunohistochemical localization of Na^+, K^+-ATPase in the choroid plexus. Brain Res 302:357–362
62. Maurizi CP (2003) The puzzle of where cerebrospinal fluid is absorbed: new pieces. Med Hypotheses 60:102–103
63. Mayer SE, Sanders-Bush E (1993) Sodium-dependent antiporters in choroid plexus epithelial cultures from rabbit. J Neurochem 60:1308–1316
64. Melby JM, Miner LC, Reed DJ (1982) Effect of acetazolamide and furosemide on the production and composition of cerebrospinal fluid from the cat choroid plexus. Can J Physiol Pharm 60:405–409
65. Moon Y, Hong SJ, Shin D, Jung Y (2006) Increased aquaporin-1 expression in choroid plexus epithelium after systemic hyponatremia. Neurosci Lett 395:1–6
66. Morales-Mulia M, Pasantes-Morales H, Moran J (2000) Volume sensitive efflux of taurine in HEK293 cells overexpressing phospholemman. Biochim Biophys Acta 1496:252–260
67. Murphy VA, Johanson CE (1989) Alteration of sodium transport by the choroid plexus with amiloride. Biochim Biophys Acta 979:187–192
68. Murphy VA, Johanson CE (1989) Acidosis, acetazolamide, and amiloride: effects on ^{22}Na transfer across the blood-brain and blood–CSF barriers. J Neurochem 52:1058–1063
69. Murphy VA, Johanson CE (1990) Na(+)-H+ exchange in choroid plexus and CSF in acute metabolic acidosis or alkalosis. Am J Physiol 258:F1528–F1537
70. Nakamura N, Suzuki Y, Sakuta H, Ookata K, Kawahara K, Hirose S (1999) Inwardly rectifying K^+ channel Kir7.1 is highly expressed in thyroid follicular cells, intestinal epithelial cells and choroid plexus epithelial cells: implication for a functional coupling with Na^+,K^+-ATPase. Biochem J 342 (Pt 2):329–336
71. Netter FM (2003) Atlas der Anatomie des Menschen. 3rd ed. Thieme, Stuttgart

72. Nielsen S, Smith BL, Christensen EI, Agre P (1993) Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. Proc Natl Acad Sci USA 90:7275–7279
73. Nilsson C, Ekman R, Lindvall-Axelsson M, Owman C (1990) Distribution of peptidergic nerves in the choroid plexus, focusing on coexistence of neuropeptide Y, vasoactive intestinal polypeptide and peptide histidine isoleucine. Regul Pept 27:11–26
74. Oshio K, Song Y, Verkman AS, Manley GT (2003) Aquaporin-1 deletion reduces osmotic water permeability and cerebrospinal fluid production. Acta Neurochir Suppl 86:525–528
75. Oshio K, Watanabe H, Song Y, Verkman AS, Manley GT (2005) Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel Aquaporin-1. FASEB J 19:76–78
76. Palmer CJ, Scott BT, Jones LR (1991) Purification and complete sequence determination of the major plasma membrane substrate for cAMP-dependent protein kinase and protein kinase C in myocardium. J Biol Chem 266:11126–11130
77. Patlak CS, Adamson RH, Oppelt WW, Rall DP (1966) Potential difference of the ventricular fluid in vivo and in vitro in the dogfish. Life Sci 5:2011–2015
78. Pearson MM, Lu J, Mount DB, Delpire E (2001) Localization of the K(+)-Cl(−) cotransporter, KCC3, in the central and peripheral nervous systems: expression in the choroid plexus, large neurons and white matter tracts. Neuroscience 103:481–491
79. Peters A, Palay SL, Webster HDF (1976) The fine structure of the nervous system. Saunders, Philadelphia
80. Phillips PA, Abrahams JM, Kelly J, Paxinos G, Grzonka Z, Mendelsohn FA, Johnston CI (1988) Localization of vasopressin binding sites in rat brain by in vitro autoradiography using a radioiodinated V1 receptor antagonist. Neuroscience 27:749–761
81. Plotkin MD, Kaplan MR, Peterson LN, Gullans SR, Hebert SC, Delpire E (1997) Expression of the Na(+)-K(+)-2Cl- cotransporter BSC2 in the nervous system. Am J Physiol 272:C173–C183
82. Pollay M, Curl F (1967) Secretion of cerebrospinal fluid by the ventricular ependyma of the rabbit. Am J Physiol 213:1031–1038
83. Pollay M, Stevens E, Estrada S, Kaplan R (1972) Extracorporal perfusion of choroid plexus. J Appl Physiol 32:612–617
84. Praetorius J, Bouzinova EV, Boron WF, Aalkjaer C, Nielsen S (2005) Expression of bicarbonate transporters and aquaporins in the choroid plexus. EB/IUPS 922.2 (Abstract)
85. Praetorius J, Nejsum LN, Nielsen S (2004) A SCL4A10 gene product maps selectively to the basolateral plasma membrane of choroid plexus epithelial cells. Am J Physiol Cell Physiol 286: C601–C610
86. Praetorius J, Nielsen S (2006) Distribution of sodium transporters and aquaporin-1 in the human choroid plexus. Am J Physiol Cell Physiol 291:C59–C67
87. Pushkin A, Abuladze N, Newman D, Lee I, Xu G, Kurtz I (2000) Cloning, characterization and chromosomal assignment of NBC4, a new member of the sodium bicarbonate cotransporter family. Biochim Biophys Acta 1493:215–218
88. Redzic ZB, Segal MB (2004) The structure of the choroid plexus and the physiology of the choroid plexus epithelium. Adv Drug Deliv Rev 56:1695–1716
89. de Rougemont J, Ames A III, Nesbett FB, Hofmann HF (1960) Fluid formed by choroid plexus; a technique for its collection and a comparison of its electrolyte composition with serum and cisternal fluids. J Neurophysiol 23:485–495
90. Royer P (1950) Perfusion of the cerebral spaces. Biol Med (Paris) 39:237–269
91. Sato O, Bering EA (1967) Extra-ventricular formation of cerebrospinal fluid. No To Shinkei 19:883–885
92. Schnermann J, Chou CL, Ma T, Traynor T, Knepper MA, Verkman AS (1998) Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. Proc Natl Acad Sci USA 95:9660–9664
93. Segal MB (2001) Transport of nutrients across the choroid plexus. Microsc Res Tech 52:38–48
94. Segal MB, Burgess AM (1974) A combined physiological and morphological study of the secretory process in the rabbit choroid plexus. J Cell Sci 14:339–350
95. Serot JM, Bene MC, Faure GC (2003) Choroid plexus, aging of the brain, and Alzheimer's disease. Front Biosci 8:s515–s521
96. Siegel GJ, Holm C, Schreiber JH, Desmond T, Ernst SA (1984) Purification of mouse brain (Na++K+)-ATPase catalytic unit, characterization of antiserum, and immunocytochemical localization in cerebellum, choroid plexus, and kidney. J Histochem Cytochem 32:1309–1318
97. Speake T, Freeman LJ, Brown PD (2003) Expression of aquaporin 1 and aquaporin 4 water channels in rat choroid plexus. Biochim Biophys Acta 1609:80–86
98. Speake T, Kajita H, Smith CP, Brown PD (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
99. Speake T, Kibble JD, Brown PD (2004) Kv1.1 and Kv1.3 channels contribute to the delayed-rectifying K^+ conductance in rat choroid plexus epithelial cells. Am J Physiol Cell Physiol 286:C611–C620
100. Speake T, Whitwell C, Kajita H, Majid A, Brown PD (2001) Mechanisms of CSF secretion by the choroid plexus. Microsc Res Tech 52:49–59
101. Sterling D, Alvarez BV, Casey JR (2002) The extracellular component of a transport metabolon. Extracellular loop 4 of the human AE1 Cl^-/ HCO_3^- exchanger binds carbonic anhydrase IV. J Biol Chem 277:25239–25246
102. Sterling D, Reithmeier RA, Casey JR (2001) A transport metabolon. Functional interaction of carbonic anhydrase II and chloride/bicarbonate exchangers. J Biol Chem 276:47886–47894
103. Virkki LV, Wilson DA, Vaughan-Jones RD, Boron WF (2002) Functional characterization of human NBC4 as an electrogenic Na^+-HCO cotransporter (NBCe2). Am J Physiol Cell Physiol 282:C1278–C1289
104. Vogh BP, Godman DR, Maren TH (1987) Effect of AlCl3 and other acids on cerebrospinal fluid production: a correction. J Pharmacol Exp Ther 243:35–39
105. Vogh BP, Langham MR Jr. (1981) The effect of furosemide and bumetanide on cerebrospinal fluid formation. Brain Res 221:171–183
106. Wang CZ, Yano H, Nagashima K, Seino S (2000) The Na^+-driven Cl^-/HCO_3^- exchanger. Cloning, tissue distribution, and functional characterization. J Biol Chem 275:35486–35490
107. Watts AG, Sanchez-Watts G, Emanuel JR, Levenson R (1991) Cell-specific expression of mRNAs encoding Na+,K(+)-ATPase alpha- and beta-subunit isoforms within the rat central nervous system. Proc Natl Acad Sci USA 88:7425–7429
108. Weaver CE, McMillan PN, Duncan JA, Stopa EG, Johanson CE (2003) Hydrocephalus disorders: their biophysical and neuroendocrine impact on the choroid plexus epithelium. Advances in Molecular and Cell Biology 31:269–293
109. Welch K (1963) Secretion of cerebrospinal fluid by choroid plexus of the rabbit. Am J Physiol 205:617–624
110. Welch K, Pollay M (1961) Perfusion of particles through arachnoid villi of the monkey. Am J Physiol 201:651–654
111. Wright EM (1978) Transport processes in the formation of the cerebrospinal fluid. Rev Physiol Biochem Pharmacol 83:3–34

112. Wright EM (1972) Mechanisms of ion transport across the choroid plexus. J Physiol 226:545–571
113. Wu Q, Delpire E, Hebert SC, Strange K (1998) Functional demonstration of Na^+-K^+-$2Cl^-$ cotransporter activity in isolated, polarized choroid plexus cells. Am J Physiol 275:C1565–C1572
114. Yang T, Huang YG, Singh I, Schnermann J, Briggs JP (1996) Localization of bumetanide- and thiazide-sensitive Na-K-Cl cotransporters along the rat nephron. Am J Physiol 271:F931–F939
115. Zeuthen T (1994) Cotransport of K^+, Cl^- and H_2O by membrane proteins from choroid plexus epithelium of Necturus maculosus. J Physiol 478 (Pt 2):203–219
116. Zeuthen T, Wright EM (1981) Epithelial potassium transport: tracer and electrophysiological studies in choroid plexus. J Membr Biol 60:105–128
117. Zeuthen T, Wright EM (1978) An electrogenic NA^+/K^+ pump in the choroid plexus. Biochim Biophys Acta 511: 517–522