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WATER HOMEOSTASIS IN THE BRAIN: BASIC CONCEPTS

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Abstract—The mammalian CNS is separated from the blood by tight junctions, collectively termed the blood–brain barrier (BBB). This imposes unique features of solvent and water movement into and out of the CNS. The basic equations for water fluxes driven by osmotic gradients are presented. The anatomy of the BBB and the physiology of the transport processes for cerebrospinal fluid production, extracellular fluid production and intercellular water and solute transport are then described. A quantitative analysis of the need for aquaporin-based water movements to accompany the known rates of CSF production is also presented. Finally, the mechanisms and roles of cellular and vasogenic edema in the CNS, especially in relation to aquaporins, are described.

Key words: CSF production, blood brain barrier, blood CSF barrier, choroid plexus, vasogenic edema, cellular edema.

The CNS is as unique in its treatment of water flow from the blood as it is in its other properties. For all other tissues there is convective water flow into the tissue with solute between the endothelial cells of blood vessels, and only plasma proteins are retained in the vascular space. This gives rise to the Starling relationship where the retained protein osmotic pressure offsets the efflux of fluid due to blood hydrostatic pressure, according to the following relations (Rapoport, 1978, 1979, 1997)

$$J_{cap} = L_{cap}[\{P_{plasma} - P_{tissue}\} - \sigma_{protein}\{\Pi_{protein,plasma} - \Pi_{protein,tissue}\}] \quad (1)$$

$P_{plasma} - P_{tissue}$ is the hydrostatic pressure difference between plasma and tissue, $\Pi_{protein,plasma} - \Pi_{protein,tissue}$ is the difference in protein osmotic pressure between plasma and tissue. L_{cap} is capillary hydraulic conductivity. The units are mm (Hg) for the two pressure difference terms and for L the units are cm^3 (mm Hg)$^{-1}$ sec^{-1} g^{-1} to give the flow, J_{cap}, in cm^3 sec^{-1}. This will be per gram of tissue when L is expressed g^{-1} or can be for the whole tissue when the g constant is multiplied by the weight of the whole tissue, such as 1500 for a human 1500 g brain. The critical factor is the osmotic reflection coefficient σ. σ_{salt} equals 0 at peripheral capillaries indicating that plasma fluid entering tissue will contain its normal salt content. $\sigma_{protein}$ has a value of 0.93 at peripheral capillaries which means that about 93% of the plasma protein will be retained in the vascular bed. Protein flux due to bulk fluid flow therefore is in mg sec^{-1} when J_{cap} is in cm^3 sec^{-1} and $C_{protein,plasma}$, the albumin concentration, is in mg cm^{-3};

$$(1 - \sigma_{protein}) \times C_{protein,plasma} \times J_{cap} \quad (2)$$

The retained proteins result in a net osmotic driving force in the venous capillary bed for the return of fluid to the blood and are known as Starling's relationship. The peripheral tissues also have lymphatics to remove additional extravasated fluid and prevent edema.

In the CNS the vascular endothelial cells are linked by tight junctions that form complete zonula occludens. This is formed by a variety of specific proteins (Kniesel and Wolburg, 2000). These junctions effectively prevent the movement of hydrophilic substances, including univalent cations such as Na^+ and K^+. Thus in the brain both σ_{salt} and $\sigma_{protein}$ are effectively one because the zonulae occludens make the blood vessels essentially impermeant to salts and proteins. Thus equation (1) has to be modified to the extent that there is a potential contribution of a salt gradient to fluid movement, as follows;

$$J_{cap} = L_{cap}[\{P_{plasma} - P_{tissue}\} - \sigma_{protein}\{\Pi_{protein,plasma} - \Pi_{protein,plasma}\} - \sigma_{salt}\{\Pi_{salt,plasma} - \Pi_{salt,tissue}\}] \quad (3)$$

Since σ_{salt} is equal to 1, any movement of water into the CNS driven by the blood pressure, which in arteries must always be greater than the intracranial pressure (ICP) to allow adequate blood flow, is immediately opposed by the osmotic pressure gradient set up by the ions retained in the vascular lumen and the dilution of the extravascular ions, as such ions are osmotically the major constituents of all the body fluids. The last term only becomes greater than 0 when theoretically some water is forced into the brain due to the driving force of $P_{plasma} - P_{tissue}$ to dilute $\Pi_{salt,tissue}$ and concentrate $\Pi_{salt,plasma}$. This would immediately set up an opposing salt osmotic driving force to reduce J_{cap} and therefore reduce further movement of water into the CNS (Quigley et al., 2003).

P_{plasma} is in the range 35–20 mm Hg from the arterioles to the capillaries, while P_{tissue}=ICP and is 5 to 10 mm Hg. The hydrostatic pressure gradient driving fluid out of the vasculature into tissue therefore averages 10–30 mm Hg. Since $\Pi_{ECS} = \Pi_{CSF} = 5100$ mm Hg (Rapoport, 1997), small dilutions of cerebral ECS will generate at, say 1% dilution, an opposing osmotic pressure gradient of $\Pi_{salt,plasma} - \Pi_{salt,tissue}$ of 5100–5049=51 mm Hg. 25 mm Hg is the osmotic pressure of the plasma proteins while the CSF and ISF contain a minimal amount of free protein (see Rapoport, 1997 and Table 1), making the maximal net driving force 30−25−51=−46 mm Hg. This

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Abbreviations: ADC, apparent diffusion coefficient; BBB, blood–brain barrier; ECS, extracellular space; ICP, intracranial pressure; MRI, magnetic resonance imaging.

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Table 1. Typical mammalian CSF and plasma concentrations of ions (meq/l) and glucose and protein (mg/100 ml)

	CSF	Plasma	CSF/plasma
K^+	2.8	4.5	0.6
Na^+	138	138	1.0
Cl^-	119	102	1.2
HCO_3^-	22	24	0.9
Ca^{2+}	2.1	4.8	0.4
Mg^{2+}	2.3	1.7	1.4
PO_4^{3-}	0.5	1.8	0.3
Albumin	7000	20	0.003

cannot be reached, of course because as soon as $(\Pi_{salt,plasma}-\Pi_{salt,tissue})+(\Pi_{oncotic,plasma}-\Pi_{oncotic,tissue})>(P_{plasma}-P_{tissue})$ water is forced back into the blood.

This negative feedback control of water flow into the CNS fails as the blood-brain barrier (BBB) breaks down and the resulting net increase in brain volume leads to edema and a life threatening rise in ICP (see section on ICP). Essentially σ_{salt} goes to 0 and $\sigma_{protein}$ becomes <1. $\sigma_{protein}$ may decrease to the 0.93 value of the capillaries of peripheral tissue (Rapoport, 1997) or less depending on the degree of disruption of the BBB.

The presence of endothelial aquaporins might make the instantaneous water movements faster so there is less of a time lag in the development of the opposing osmotic gradient needed to oppose the hydrostatic pressure difference. However, the density of AQP-4 in both membranes of the endothelial cells is much lower than for the perivascular astrocytic membrane (Kobayashi et al., 2001). Thus one can speculate that the high densities of AQP-4 in the vascular facing astrocytic membrane would seem to have more to do with water movement between the brain extracellular space (ECS) and the astrocytic intracellular space. The advantage is presumably to rapidly, osmotically adapt to the transport of solutes such as glucose from the blood and intracellular K^+ released during the falling phase of the action potential into the astrocyte as part of their CNS functions (Hof et al., 1988; Magistretti et al., 1999; Somjen, 1987).

Blood–CSF barriers

CSF is directly secreted by the choroid plexi into the ventricles and is also derived from the ECF produced principally by the brain capillaries which communicate with the CSF through gaps between the cells forming the ependymal lining of the ventricles (Laterra et al., 1999). There is less precise information known regarding the location of carriers and channels in the capillaries, the principal site of the BBB, than at the choroid plexi because it is more difficult to do experiments with this system compared with the more easily isolated choroid plexi. Fig. 1 shows the asymmetric localization of ion channels, including aquaporins, transporters and the (Na^+-K^+) pump and aquapor-

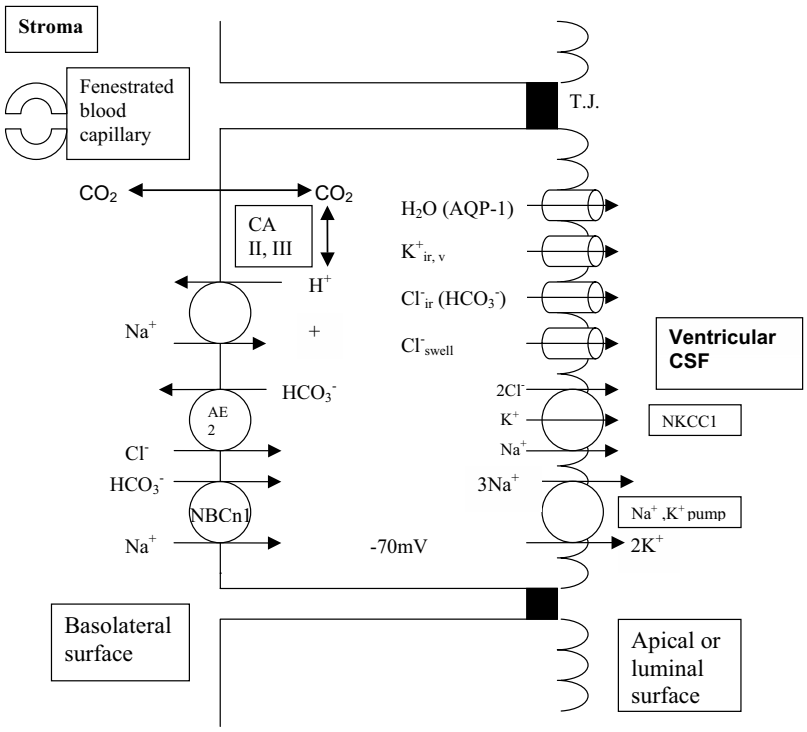

Fig. 1. Model showing known asymmetry of transporters (○) and channels (⛁) on choroid plexus epithelium. CA II and III, NKCC1, AE2, NBCn1, $K_{ir,v}^+$, Cl_{ir}^- Cl^-_{swell} and AQP1 are the forms of the carbonic anhydrase (CA), sodium-potassium-2 chloride co-transporter, anion exchanger, sodium-bicarbonate co-transporter, inward rectifying and voltage sensitive potassium channels, inward rectifying chloride and cell swelling activated chloride channels and aquaporins or water channels, respectively so, found so far in mammalian choroid plexi. a Drawn from data in Johanson, 1989; Laterra et al., 1999; Speake et al., 2001, 2003.

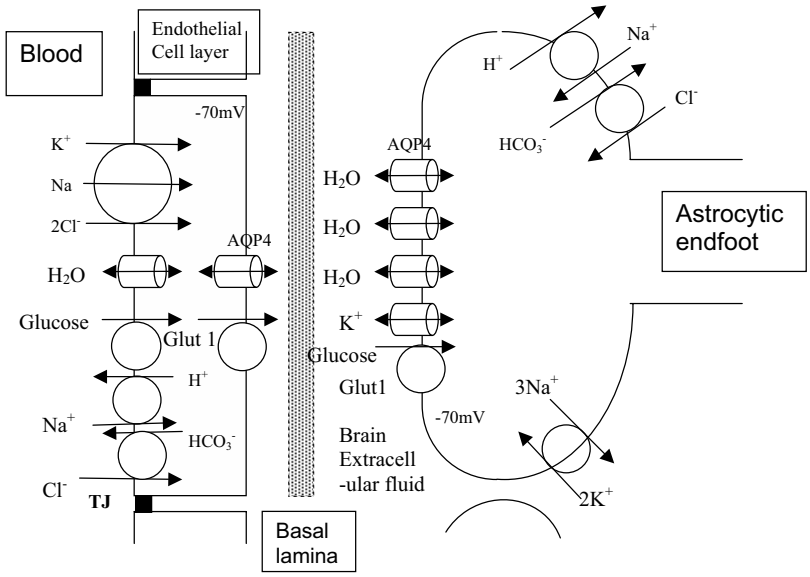

Fig. 2. Model of BBB with likely location of main transporters (○) and channels (▭) as indicated. TJ denotes tight junctions between endothelial cells. Based on several sources (Amiry-Moghaddam et al., 2003; Kimelberg and Ransom, 1986; Kimelberg and Bourke, 1982).

ins at the choroid plexus epithelium, the principal site of the blood–CSF barrier (see figure legend for details). These arrangements are derived from a combination of inhibition of ion transport from blood to brain in intact animals, immunocytochemistry of fixed sections and studies on isolated choroid plexi (Johanson, 1989; Speake et al., 2001). See the chapter by Brown et al. (2004) in this volume for a detailed account of the asymmetric arrangement of channels and transporters and a treatment of current controversies and conflicting data, such as the role of AQP4. The total area of the human vasculature is around 10 m^2, while the choroid plexi have a combined area of 200 cm^2 (Laterra et al., 1999; Stewart, 1997). Thus there is a 500-fold greater area of the vasculature compared with the choroid plexi. Since, as will be discussed in more detail later, the contribution of these two regions in total to the overall CSF secretion appears to be comparable, the rate of water and ion transport per unit area at the choroid plexi should be around 500 times greater than for the vasculature.

BBB

The impermeability of the BBB is at the level of the zonulae occludens (tight junctions) of the endothelial cells but all blood vessels in the CNS are surrounded by astrocytic processes termed foot processes and astrocytes can therefore modulate, by their possession of transporters and intracellular metabolism, the entry and egress of substances across the BBB. The vascular-facing astrocytic membranes are also where AQP-4 is specifically localized at a high density. Since water has to enter from the blood it is, perhaps, not surprising that they are also present, albeit at lower density, in the endothelial cell membranes (Amiry-Moghaddam and Ottersen, 2003).

In terms of the need for AQPs, the major unknown is the intrinsic water permeability of the different CNS membranes without aquaporins. This varies in biological membranes and some, such as the membranes of the epithelial cells of the thin ascending loop of Henle in the kidney, are effectively impermeable to water (Tian et al., 2004). This is thought to be due, at least in part, to the lipid composition of the membranes (Quigley et al., 2003). The AQPs of the perivascular astrocytic membranes are now known to constitute the orthogonal arrays of intramembrane particles disclosed some time ago by freeze fracture electron microscopy (Risau and Wolburg, 1990). These were at one time speculated to be K^+ channels (Kimelberg and Norenberg, 1989), and currently K^+ channels are thought to coexist with the AQP particles in the membranes of rat retinal Müller cells (Nagelhus et al., 1999).

Net transport of water always has to be driven by osmotic forces due to solute movement as there is no primary active transport, ATP-driven, water pump. There are a wide variety of transporters and channels to effect solute transport in the CNS to overcome the lack of free diffusion of the BBB to polar substances. Possible locations for these are shown in Fig. 2. This occurs, 1) to allow adequate entry of important compounds needed by the brain such as glucose, 2) to control brain salt composition and pH, and 3) to get rid of normal waste metabolites. The transporter present in the highest density in endothelial cells is the glucose transporter and the normal rate of glucose transport is approximately 700 nmol/g brain/min (Laterra et al., 1999). The next most actively transported molecule is lactate on the monocarboxylic acid transporter at 60 nmol/g brain/min and then phenyalanine on the large neutral amino acid carrier at 12 nmol/g/min (Laterra et al., 1999). For the 1500 g human brain the normal rate of glucose transport translates to 1.05 mmol glucose/brain/min. Based on a total osmolarity of 290 mOsmol this will add 0.35% molarity or an increase

of 1500×0.0035=5.25 ml water. This should not be significant in terms of the total water load.

The astrocytes contain the *GLUT*-1 glucose transporter while the neurons have the *GLUT*-3 (Dwyer et al., 2002). The possession of a high density of the *GLUT*-3 transporter on neurons has made the astrocytic-lactate hypothesis, where blood glucose first enters the astrocyte exclusively and is then converted to lactate which then constitutes the primary fuel of neurons (Magistretti et al., 1999), difficult to fully accept.

Water which crosses the BBB presumably initially enters the ECS, but because of the close apposition of the astrocytic processes and their high density of AQP4 may rapidly and preferentially end up in the perivascular astrocyte to accompany any of the solute movements shown in Fig. 2. The ECS communicates reasonably freely with ventricular CSF through normal non-barrier spaces between ependymal cells (Cserr, 1971).

Endogenous production of water

The production of water within the CNS due to complete oxidation of glucose by the measured oxygen consumption of 156 μmol/100 g/min for the human brain can be calculated. Thus a 1500 g human brain should produce 0.043 $cm^3.min^{-1}$ of metabolic water from oxygen, or about 12% of the net CSF production (Cserr, 1971; Rapoport, 1978). The solutes accompanying this are presumably various metabolites also produced by cellular respiration. Glucose presumably enters the CNS with its osmotic equivalent of water but is metabolized to trioses and carboxylic acid intermediates in the TCA requiring additional water from the blood or increased endogenous production of water from the oxidation of these substrates.

Formation and resolution of CSF

The choroid plexus forms a classical secretory epithelium between the blood and the CSF of the ventricles. In mammals the choroid plexi are located in all the ventricles; namely the two lateral and the third and fourth ventricles. The compartmental barrier here is not at the level of the blood vessels but between the epithelial cells that form the inner CSF-facing surface of the choroid plexi, while the blood capillaries of the highly vascularized choroid plexi are the normal, fenestrated leaky type typical of systemic capillaries. Since the choroid plexi are of mesodermal origin leaky capillaries in the stroma of the choroid plexi are not one of the exceptions to the rule that almost all capillaries in the CNS form tight junctions between their endothelial cells. Thus the choroid plexi blood vessels allow an adequate amount of blood ultrafiltrate for the secretion of CSF, principally the ions Na^+, Cl^- and HCO_3^- (see Fig. 1).

There is an asymmetric distribution of carriers and channels on the basal and apical membranes of the epithelial cells of the choroids plexi that results in a net transport of Na^+, Cl^- and HCO_3^- with osmotically obligated water (Brown et al., 2004; Wright, 1977; Wu et al., 1998). Differences in the relative proportion of HCO_3^- in the CSF also allows control of CSF pH (Cserr, 1971). AQP-1 is concentrated on the CSF-facing apical surface, allowing rapid transport of the accompanying water transport (Amiry-Moghaddam and Ottersen, 2003). However, it is not clear why there is an asymmetry of AQP-1 as water needs to cross both membranes of the epithelium. Is there a more rapid transport of ions at the apical face but then where is the water coming from? It can hardly be the intracellular water only. Alternatively the apical membrane could be less permeable to water making it more necessary to have water channels located here. There is some question that AQP-4 might be located on the epithelial apical membrane (Brown et al., 2004). As shown in Fig. 1, and Fig. 6 in Brown et al. (2004) (this volume), the arrangement of transporters in the epithelial cells of the choroid plexi clearly corresponds to the basic phenomenon of uptake at the basolateral, blood-facing face and secretion from there to the CSF. The basic composition of the CSF is shown in Table 1 in this chapter, and also Table 1 in Brown et al. (2004).

CSF production is measured by dilution studies of ventricular CSF and is produced at a pressure of around 11 mm Hg (1 mm Hg=1 torr; Rosenberg, 1997). The ICP normally varies from 5 to 10 mm Hg. In man the rate of CSF production is 0.3–0.4 ml/min or 432–576 ml/day. The total volume of CSF is around 130 ml and thus is renewed every 5–7 h. Clearly the fixed volume of the CNS cavity requires that the CSF is removed at the same rate as it is produced. The classic view is that this is the function of the arachnoid villi which are outpouchings of the meninges into the venous sinuses as shown diagrammatically in Fig. 3A. They open at pressures of about 5 mm Hg, and effectively function as one-way valves. Fluid is proposed to pass into the venous sinuses by bulk flow. The fixed volume of the brain consists of:

$$V_{blood} + V_{CSF} + V_{brain}$$

which leads to the problem of an increasing ICP when the volume of any component increases without compensatory decreases in another. The CSF circulates from the ventricles through the foramen of Magendie located in the fourth ventricle, over the surface of the brain and spinal cord within the subarachnoid space (Fig. 3B and Fig. 1 in Brown et al., 2004). The perfusion is rather slow, taking around 2 h to traverse the entire brain surface and 1–1.5 h to reach the lumbar region of the spinal cord or even longer (Greitz and Hannerz, 1996).

An alternative, or complementary, view to the classic view that the arachnoid villi (or pacchionian granulations) provide the only or major outlet for CSF by bulk flow has been based on model and patient studies using dye and radionuclide cisternography measurements, respectively (Greitz and Hannerz, 1996). In this model the villi do not serve as the major site of efflux. Rather the reabsorption of CSF is through the blood vessels to the blood, and that a mixing or pulsatile flow rather than a bulk flow, where injected contrast material should move as a bolus, was implied. Because of the BBB such vascular reabsorption would require transport of the major ion constituents of the CSF on carriers or channels, with simultaneous movement of osmotically obligated

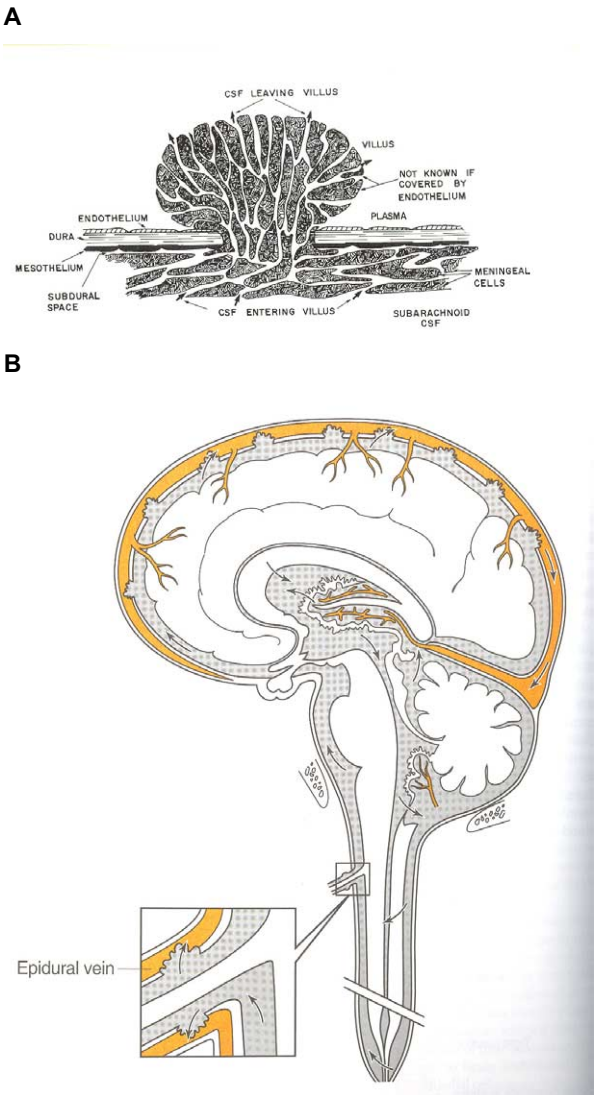

Fig. 3. (A) Diagram of arachnoid villi. (B) Circulation of CSF (arrows) from production sites of choroid plexi (shown in lateral and fourth ventricles) and neuropil and egress from arachnoid villi. From Lewis, 1976.

water. This would also serve to explain the observation that α-syntrophin knockout mice, that do not show the normal perivascular concentration of AQP-4 on astrocytic membranes, have swollen perivascular astrocytic processes (Amiry-Moghaddam et al., 2003).

The functions of the CSF are as a sink for metabolic products and, even more important, to provide buoyancy for the brain (see also Brown et al., 2004). The specific gravity of brain tissue is 1.040 g/ml and CSF is 1.007 g/ml, respectively. The average volume of the brain is 1500 cc^3, and its effective weight is therefore 1500 $cc^3 \times 0.033$ g/cc^3 or 49.5 g, rather than the $1500 \times 1.040 = 1560$ g it would weigh in air.

Aquaporins and ECF production at the cerebral vasculature

Forty percent to 70% of CSF has been estimated to come from the choroid plexi and 30–60% from the interstitial fluid that derives from transport across the blood vessels plus endogenous water production, depending on species (Cserr, 1971). The interstitial fluid contribution is lower in cats and rabbits than nonhuman primates where it is thought to be around 60% (Rosenberg, 1997). If we take the higher figure as indicative of what happens in the human brain and subtract endogenous water production we get 48% production by the vasculature. Total production of CSF by the human brain is 0.30 $cm^3\ min^{-1}$ (Laterra et al., 1999), so the vasculature contributes 0.15 $cm^3\ min^{-1}$. This must be from the movement of ions such as Na^+ and Cl^- through transporters and channels whose likely locations are shown in Fig. 2. Since the upper limit of the permeability coefficient for water in lipid bilayers is 0.005. (Papahadjopoulos and Kimelberg, 1974). So lets say 0.001 cm sec^{-1} for an average biological membrane and apply this to the following equation (Verkman, 2000):

$$J_v = P_f \cdot S \cdot v_w[(c_{i2} - c_{i1}) + \sigma_p(c_{p2} - c_{p1}) + (P_1 - P_2)/RT] \quad (4)$$

Where P_f (cm/sec) is the permeability coefficient, $S(cm^2)$ the membrane surface area, v_w the partial molar volume of water (18 $cm^3\ mol^{-1}$), P the hydrostatic pressure (atm), σ_p the reflection coefficient of the permeant and c_p and c_i the concentration of the permeant and impermeant solutes, respectively; 1 and 2 refer to the two compartments separated by the membrane in question. Now we have noted above that the BBB is effectively impermeant to the ions that represent the bulk of the plasma osmolarity thus all the solute will be treated as c_i.

With a blood vessel surface area of around 10 m^2 or 100,000 cm^2 for the total human brain (Stewart, 1997), and a net CSF production of 0.15 or 2.5×10^{-3} ml/min, equation 4 translates to:

$2.5.10^{-3} cm^3 sec^{-1} = 0.001$ cm $sec^{-1} \times 10^5$ $cm^2 \times 18$ $cm^3\ mol^{-1}(\Delta osm + \Delta P/RT)$.

The $(\Delta osm + \Delta P/RT)$ equals $2.5.10^{-3}/1.8 \times 10^3$ or 1.40×10^{-6} mol/cm^3. If ΔP, the hydrostatic pressure equals 0 then the measured production of CSF from the parenchymal vasculature can be achieved with an osmotic gradient of 1.4 mosmol kg^{-3}. In the absence of an osmotic gradient this volume of CSF would be produced by an average 26.6 torr hydrostatic gradient. The role of aquaporins would be to increase P_f, i.e. decrease the resistance to water flow, allowing this same rate of water transport at a lower osmotic gradient. Since the total osmolarity is around 300 the maximum volume fluctuations would be 0.5%, to be rapidly resolved by the CSF reabsorption processes at work in the CNS. At the choroid plexi we have a 500-fold greater production of CSF because it is a 500-fold smaller area (vide supra). In the absence of increased P_f this would require an osmotic gradient of 500×1.4 mM. Thus a large decrease in the resistance to water flow by aquaporins to raise P_f to 0.1–1.0 cm sec^{-1} might seem necessary. This is provided by AQP-1, but so far the presence of this water channel is established only in the apical epithelial membrane. In any case, the location of the highest density of aquaporins in the CNS, namely not at the BBB but in the perivascular astrocytic membrane abluminal to the endothelial solute barrier, seems the place least likely to need it for water movement from blood to brain. Thus

for astrocytes the perivascular AQPs may be mainly for intra-CNS water transport (see following section).

Since it seems unlikely that water transport is normally rate-limiting for CSF production at the vasculature in the absence of AQP4, it may explain why knockouts of AQP4 had no effect on the normal phenotype (Van Os et al., 2000). However, such knockouts did reduce astrocytic cellular edema in ischemia suggesting aquaporin-dependent water fluxes are involved in situations where there is a need for faster channel-mediated water transport (Manley et al., 2000). In all these cases, however, the caveat is that we do not know the unmodified water permeability of the brain vasculature. It may be relatively impermeant, somewhere between the impermeability of some membranes in the kidney tubules and cell membranes whose water permeability is at least as great as generalized lipid bilayers. Not knowing this makes it difficult to quantitatively predict the effects of aquaporins. More surprisingly knockout of AQP 1 had no effect on choroidal CSF production (Van Os et al., 2000). The role of the AQPs in CNS water transport remains enigmatic.

Intra-brain water transport between neural cells

The transfer of water between the ECS and the various neural cells is the pathway for water transport within the brain. There is presumably continuous transport of water in neurons, astrocytes and other cells accompanying such things as glutamate and potassium transport, although *in situ* such water movements cannot be directly measured. This is usually uncovered by measurements of cell swelling which can be done in isolated systems where the fast rate of water influx is too fast for compensatory efflux movements, or the efflux can be artificially inhibited. Water influx cannot be effectively isolated *in situ* or *in vivo*. Magnetic resonance imaging (MRI) measurements of total water content (T1 or 2 diffusion weighted) or diffusion-weighted imaging, from which an apparent diffusion coefficients (ADC) for water can be obtained and which measure the steady state at any time instant, are the best one can do (Fatouros and Marmarou, 1999). These are also only average brain measurements and compartmental and cell specificity cannot yet be achieved.

AQP-4 is located specifically to astrocytes in the brain and, as noted above, it is predominantly perivascular. Therefore movements of ions and metabolic substrates other than movement from blood to astrocytes may not require rapid water transport. The whole question of a permissive need for AQP channels is puzzling in view of the known relatively high permeability of pure lipid bilayers as discussed above for the fluxes required for net flux of water to form CSF, whose net rate of formation has been measured. However, as also emphasized above we do not know the water permeability of the unmodified membranes and this could be significantly lower than the water permeability of generalized lipid bilayers because of membrane composition modifications. It seems possible that an asymmetry of aquaporins on opposite faces of a water transporting cell is necessary to achieve a net water flux because it is difficult to reduce the high water permeability of biological membranes. This is one way of achieving a net flux of water by producing an additional cellular unidirectional net flux on the base of rapid movements of water in membranes.

There have been a number of studies showing AQPs in astrocytes *in situ* and in culture (Amiry-Moghaddam and Ottersen, 2003; Badaut et al., 2002). This may have something to do with the choroid plexi occupying a smaller proportion by total weight and secretory area than the vasculature in larger brains. Solenov et al. (2004) have recently shown that the P_f was reduced 7.1-fold in astrocytes cultured from AQP-4 deficient mice from a value approximately 0.05 cm sec^{-1} in wild type mice, as measured from hypotonic media-induced swelling. Note from the calculations in the section on “Aquaporins and ECF production at the cerebral vasculature” that a P_f value 50-fold less than 0.05 seemed sufficient to account for extrachoroidal plexus CSF production. We do not know what solute, and therefore water fluxes, astrocytes experience *in situ*, only that in pathological states steady state swelling of astrocytes is frequently observed by electron microscopy (Kimelberg, 2000). There have not been many studies on water transport in neurons and none as yet that have focused on AQP involvement. There is an experimental suggestion that water permeability in neurons may be unusually low based on the absence of volume changes in response to exposure to hypotonic medium in freshly isolated hippocampal pyramidal neurons (Aitken et al., 1998).

Cellular and vasogenic edema

Swelling of brain tissue is a common feature accompanying several pathological states (Kimelberg, 1995; Kimelberg and Ransom, 1986; Mongin and Kimelberg, 2004). Early workers in the field in the first two decades of this century distinguished two forms of brain swelling following injury, stroke or seizures. As described by Long (1982): “One was considered to be extracellular accumulation of water and was called edema. The diagnostic feature was that the cut brain ‘wept fluid.’ Edema was differentiated from brain swelling, which was thought to be intracellular and characterized by the dryness of the cut brain. Brain edema was thought to be traumatic in origin while brain swelling was usually toxic or metabolic. Throughout this period of time the origins of the two forms of brain swelling were completely unknown.”

Klatzo (1967) sought to clarify ambiguities regarding brain edema and its neuropathology. First he eliminated the Anglo-Saxon term swelling (from Old English *swellan* or German *schwellen*) and used brain edema (from Greek *oidema*) as the generic term defining it as “an abnormal accumulation of fluid associated with volumetric enlargement of the brain.” This did not, of course, by itself clarify anything. However, he then defined two species of brain edema based on mechanism which, he emphasized, usually co-existed. One was vasogenic edema in which there is injury to the vessel wall leading to “escape of water and plasma constituents” into the surrounding brain tissue. The other he termed cytotoxic edema in which “a noxious factor

Table 2. Characteristics of the brain edemas

Vasogenic	Cellular or cytotoxic
Increased permeability of the BBB leading to net gain of fluid. Also cell swelling is seen, mainly of astrocytes, in gray and white matter.	Intracellular swelling without increased permeability of the BBB. Mainly seen as swelling of astrocytes but can involve swelling of myelin lamellae and dendritic swelling.

directly affects the stranded elements of the parenchyma producing intracellular swelling; vascular permeability remaining relatively undisturbed." These two forms had the persuasive feature that they corresponded to the two conditions observed by earlier workers as quoted by Long (1982) (vide supra). The characteristics of these two forms of edema are summarized in Table 2 and are shown diagrammatically in Fig. 4.

Cellular edema

Brain edema in response to injuries such as stroke and trauma is now well-recognized to initially involve astroglial swelling, or cytotoxic edema, occurring in both gray and white matter (Kimelberg, 1995). Such swelling may be deleterious for a number of reasons that will be discussed later. The mechanisms of the cellular swelling are less clear, and several possibilities are shown in Fig. 4. Originally, cellular edema was termed cytotoxic edema by Klatzo (1967) because it could be produced by cell toxins, such as triethyltin. More recently, cellular swelling has been found to occur in a number of nontoxin-induced pathological states. For example, astrocytic swelling has been described as a prominent and early feature associated with cerebral contusion in head-injured patients as compared with its lower incidence in control patients operated on for epilepsy or glial tumors (Popp et al., 1996).

In its most limited form, cellular swelling could simply consist of a shift of osmoles from extracellular to intracellular compartments, followed by osmotically obligated water (Kimelberg, 2000). The ECS would then be proportionately decreased in volume as the intracellular space expands. Microscopically, cellular edema is seen to consist principally of swelling of the astrocytic soma and processes, plus some swelling of the neuronal dendrites. A corresponding reduction of the ECS is observed, as measured by an increase in electrical impedance and increased concentrations of extracellular membrane-impermeable molecules (Sykova et al., 1994; Van Harreveld, 1966). Swelling of the neuronal cell soma and axons, and

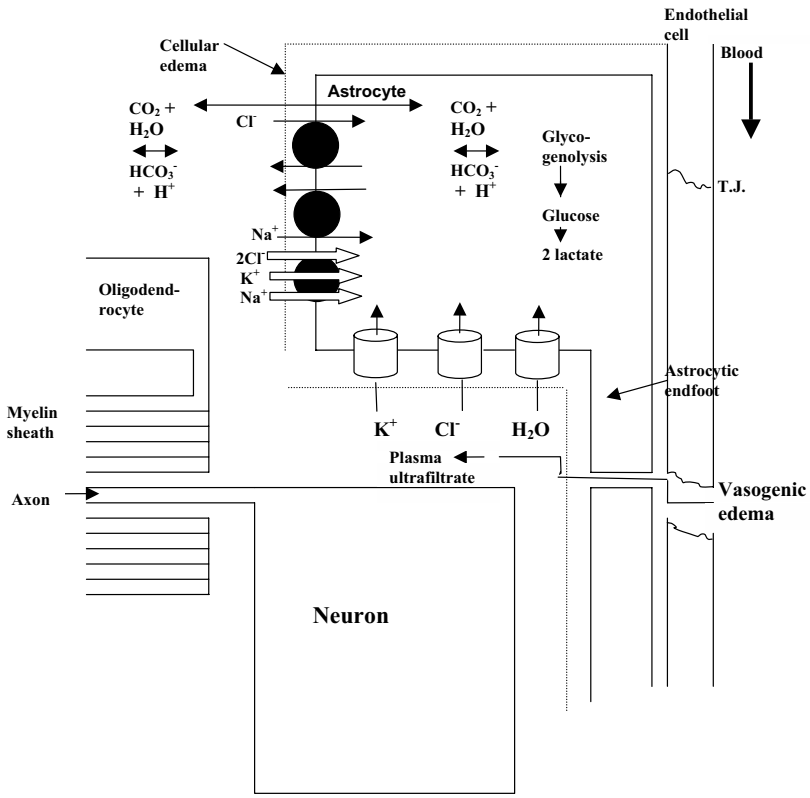

Fig. 4. Diagram of BBB region showing vasogenic edema as an increased permeability across the endothelial cells by due to increased leakage of the tight junctions. Cellular or cytotoxic edema is shown as an increase of the astrocytic cell compartment with diminution of the ECS, which can, however, be maintained at its normal extent or even enlarged if, as can be the case, there is co-existent vasogenic edema. Possible transport mechanisms are shown. They are discussed in the text; ● transporters; ⌭; channels; T.J., tight junctions (modified from Kimelberg, 1995, with permission).

oligodendroglia is generally not seen. It is of interest that in pathological state such as ischemia, electron microscopic studies show that astrocytes appear swollen and neurons shrunken within 30 min or so of initiation of the ischemia (Kimelberg, 1995; Popp et al., 1996; Van Harreveld, 1966). There is also around a 50% decrease in the ECS as measured by impedance methods at the same time (Van Harreveld, 1966). Physiologically, it has been suggested that the water loss from the shrunken neurons is taken into the astrocytes plus some of the ECS water as this is also shrunken. Using a setting of MRI termed diffusion weighting imaging an apparent diffusion coefficient (ADC) for the major nuclei measured, which are water protons, can be obtained. Within minutes of the initiation of experimental ischemia a sharp decline in ADC is measured (Hoehn-Berlage et al., 1995). This has been interpreted as due to the movement of water molecules from the less hindered ECS to the more hindered environment of the intracellular space. While this is consistent with the observed astrocytic swelling the morphological studies cannot achieve the time resolution of the ADC measurements and other possibilities such as increased viscosity of intracellular or extracellular fluid could be responsible (Mongin and Kimelberg, 2004).

Mechanisms of cellular edema

There have been many suggestions for the underlying transport processes driving the water transport (see Fig. 4). The role for aquaporins, if there is a role, seems likely to be permissive for the osmotically obligated water transport. As diagrammed in Fig. 4 one mechanism of cellular swelling involves the uptake of KCl when extracellular [K^+] increases following traumatic brain injury (Mongin and Kimelberg, 2004). Another mechanism that has been proposed is the transport of HCO_3^- and H^+ out of the cell in exchange for Cl^- and Na^+ respectively, on the appropriate exchangers. The HCO_3^- and H^+ can turn over many times by cycling as follows; membrane-permeable CO_2 diffuses into the cell, where it is hydrated to HCO_3^- and H^+; it is then exchanged for extracellular Na^+ and Cl^- and is dehydrated back to CO_2 in the ECS (Kimelberg, 1979). Cell swelling in astrocytes can also result from ischemia because of the breakdown of macromolecular glycogen, primarily localized in astrocytes, to a number of moles of metabolic products. Fatty acids and free radicals can also cause swelling secondary to breakdown of the selective permeability of the cell membranes, resulting in the influx of Na^+ and Cl^-. Cellular swelling or shrinkage can also occur when systemic hypo- and hypernatremia are encountered after traumatic brain injury.

Cellular swelling can produce numerous deleterious secondary effects in the cell, including (1) membrane depolarization secondary to loss of intracellular K^+ leading to influx of Ca^{2+} through voltage-gated channels; (2) activation of mechanosensitive channels, leading to direct influx of Ca^{2+}; and (3) release and decreased uptake of excitatory amino acids, as seen in astrocytes (Kimelberg, 2000).

Vasogenic edema

Net swelling of the brain must always involve a gain of water. Its physiological basis can be found in equations 1–3. It can readily be measured as a rise in ICP after a certain point is reached when the capability of the brain compartment to compensate for limited increases in the volume of the brain (i.e. its compliance) is exceeded (Popp et al., 1996), or by measuring total brain water using MRI (Fatouros and Marmarou, 1999).

In vasogenic edema increased osmoles and water enter the brain due to an, as yet, ill-defined breakdown of the BBB. The BBB is primarily due to intercellular, occluding tight junctions between the endothelial cells lining the blood vessels and capillaries of the nervous systems of more highly developed animals and it means that a modified version of Starling's principle applies in the brain, as discussed at the beginning of this chapter. Excess water and solute are efficiently kept out since any water entering the brain does so without any solute and purely under hydrostatic pressure. The opposing osmotic force immediately developed by the retained ions and plasma proteins in the blood then sets up an immediate opposing force driving the water back into the blood (Rapoport, 1978; Cserr and Patlak, 1991), as discussed previously. With the breakdown of the BBB this control is lost, the barrier is breached, and a filtrate of blood, including plasma proteins, is driven by the blood pressure into the brain. The blood cells are presumably retained by the residual permeability of the BBB, except in injury sufficient to lead to hemorrhage. Most of this ultrafiltrate fluid accumulates in the white matter due to the greater compliance of this region, i.e. white matter will more easily accept the increased volume at a given pressure. This is likely due to the greater ease of separation of the parallel oriented white matter tracts (Klatzo, 1967; Rapoport, 1979). Astrocytic swelling also commonly occurs concomitantly in vasogenic edema and can also involve uptake of the extravasated plasma proteins (Trachtenberg, 1982). Both cytotoxic and vasogenic edema are seen after triethyl tin intoxication in the anterior commissure of the rat, which thus specifically illustrates the coexistence of cytototoxic and vasogenic edemas (Otani et al., 1986).

The frequency of vasogenic edema in stroke and closed head injury and the degree to which it contributes to an impaired outcome is by no means clear. Certainly the existence of an increased ICP is often seen after head injury and is clearly life-threatening (Popp et al., 1996). As has been suggested, "cytotoxic edema" with its potential for widespread metabolic dysfunction may be an equally serious problem (Kimelberg, 1995). The two phenomena are both termed *edema* and involve unbalanced water movements, but in one case it is cellular, primarily involving astrocytes, and the other due to increased permeability or breakdown of the BBB. Thus while water movements and its steady state content underlies both phenomena their mechanisms and effects are quite different. It seems that AQP-mediated edema could only be involved in cellular edema. Since the consequences of this type of

edema in CNS pathologies are less clear than vasogenic edema the role of AQPs in CNS pathologies is correspondingly less clear. Therefore on this basis alone, studies of the physiological roles of AQPs in the brain are important and will lead to a better understanding of water movements accompanying solute movements involved in water homeostasis in the brain which, when the homeostasis breaks down in brain trauma and ischemia, is life-threatening.

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