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REVIEW

WATER TRANSPORT BETWEEN CNS COMPARTMENTS: CONTRIBUTIONS OF AQUAPORINS AND COTRANSPORTERS

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Abstract—Large water fluxes continuously take place between the different compartments of the brain as well as between the brain parenchyma and the blood or cerebrospinal fluid. This water flux is tightly regulated but may be disturbed under pathological conditions that lead to brain edema formation or hydrocephalus. The molecular pathways by which water molecules cross the cell membranes of the brain are not well-understood, although the discovery of aquaporin 4 (AQP4) in the brain improved our understanding of some of these transport processes, particularly under pathological conditions. In the present review we introduce another family of transport proteins as water transporters, namely the cotransporters and the glucose uniport GLUT1. In direct contrast to the aquaporins, these proteins have an inherent ability to transport water against an osmotic gradient. Some of them may also function as water pores in analogy to the aquaporins. The putative role of cotransport proteins and uniports for the water flux into the glial cells, through the choroid plexus and across the endothelial cells of the blood–brain-barrier will be discussed and compared to the contribution of the aquaporins.

Key words: cotransporters, cell swelling, aquaporins, water transport, glial cells, choroid plexus.

Contents

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Abbreviations: AQP4, aquaporin 4; CSF, cerebrospinal fluid; EAAT, glutamate transporter; GAT, GABA transporter; KCC, K^+/Cl^- cotransporter; MCT, monocarboxylate transporter; NKCC, $Na^+/K^+/2Cl^-$ cotransporter.

Brain water is continuously shifted between different compartments and across the blood–brain and CFS-brain interface. Disturbances in this well-regulated water homeostasis may have deleterious effects on brain function and may be fatal in cases where water accumulates in the brain following pathologies such as ischemia, haemorrhage, or brain trauma. It is not well-understood via which molecular pathways the water enters and exits the brain, although the glial aquaporin 4 (AQP4) is clearly an important factor in brain edema formation (Zador et al., 2009). In this review, we describe the water transport properties of a different class of membrane transport proteins and discuss their putative role in the brain water homeostasis. It is now generally accepted that cotransporters and uniports can transport water (Agre et al., 2004; King et al., 2004), although the underlying molecular mechanism is debated.

The fundamental physiological issue we want to address is the following: Transport of ions or substrate between two compartments will require an associated water transport in the order of around 165 water molecules per particle in order to achieve isotonicity. In an entirely osmotic model, water transport would take place through aquaporins or via the lipid phase of the membrane. This would require the build-up of an osmotic gradient that may become quite high and lead to unwarranted secondary movements of water. Such an osmotic mechanism is problematic, particularly in a closed system such as the brain where osmotic pressures have to be strictly controlled. We have previously presented extensive data that demonstrate that cotransporters and uniports cotransport significant amounts of water coupled to the substrate by a mechanism within the protein. We will discuss how this cotransport of water mitigates or even removes the requirement for a build-up of osmotic gradients within the brain and its cells.

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WATER TRANSPORT BY COTRANSPORT PROTEINS

Cotransporters are membrane-spanning transport proteins that couple ion and substrate transport. In mammalian cells, Na^+ is usually employed as the principal cotransported ion, due to its large inwardly-directed electro-chemical gradient. Through this coupled transport, the Na^+ can drive the uptake of a substrate against gradients as large as several thousand-fold in case of the glutamate transporters (Danbolt, 2001). We propose that this cotransport may carry a fixed amount of water molecules along with each transported solute, in a manner that will allow for water to be transported against an osmotic gradient. This feature is not found in the aquaporins but is nonetheless physiologically important as well as necessary in certain tissues where the osmotic gradients would favour water transport in the wrong direction, as in the small intestine after a meal or in the retinal pigment epithelium (Pappenheimer, 1998; Hamann et al., 2000, 2003; Zeuthen et al., 1996).

Various cotransport proteins have been shown to possess this ability for transport against an osmotic gradient or "uphill water transport". Examples are the K^+/Cl^- cotransporter, KCC (Zeuthen, 1991a,b, 1994); the $Na^+/K^+/2Cl^-$ cotransporter, NKCC1 (Hamann et al., 2005); the glucose cotransporter, SGLT1 (Loo et al., 1996; Zeuthen et al., 1997, 2001, 2002, 2006; Meinild et al., 1998; Zeuthen and Zeuthen, 2007); the glial glutamate transporter, EAAT1 (MacAulay et al., 2001); the GABA transporter, GAT-1 (MacAulay et al., 2002b); the monocarboxylate transporter 1, MCT-1 (Hamann et al., 2000, 2003; Zeuthen et al., 1996); the dicarboxylate transporter 1, NaDC-1 (Meinild et al., 2000), for recent reviews see (Loo et al., 2002; MacAulay et al., in press; Zeuthen and MacAulay, 2002). The phenomenon has also been observed for the glucose uniports GLUT1 and GLUT2 (Zeuthen et al., 2007; Zeuthen et al., unpublished observations). The amount of cotransported water is significant, ranging from 35 to 500 water molecules (Table 1) and the water transport proceeds along with the substrate in a manner that is independent of the osmotic gradient. This cotransport of water has been studied in various preparations, such as native tissue, cultured mammalian cells, and by heterologous expression in *Xenopus laevis* oocytes. Various techniques have been employed: ion-selective micro-electrodes, fluorescence, and sensitive optical methods for volume measurements. It seems evident, therefore, that secondary active water transport is not due to an artefact arising from the expression system and/or the experimental technique. Although the ability for water transport in cotransport proteins and uniports is generally accepted (Agre et al., 2004; King et al., 2004), the underlying molecular mechanism is debated. Any conventional unstirred layer effect can been ruled out, mainly because the diffusion coefficient in the cytoplasm is too high to result in a significant build-up of osmolarity in the vicinity of the transport protein (Charron et al., 2006; Zeuthen et al., 2002, 2006, 2007; Zeuthen and Zeuthen, 2007; Naftalin, 2008; Zifarelli and Pusch, 2009). The coupling between water and substrate must therefore take place by a mechanism within the protein itself (Zeuthen, 1994; Zeuthen and Stein, 1994; Naftalin, 2008).

Secondary active cotransport of water has several characteristics. First, it is initiated instantaneously after the cotransport begins without the lag that would be observed if the water transport was based on the osmotic build-up of solutes in the cytoplasm (Zeuthen et al., 2006). This point is illustrated by data from the GABA transporter expressed in *Xenopus laevis* oocytes in Fig. 1. Panel A shows that the strict proportionality between the accumulated current associated with the GABA transport and the instant influx of water determined from the swelling of the oocyte. Second, the observed water accumulation is not simply due to an intracellular build-up of osmotic particles but requires a functional turnover of the protein. The GABA transporter, in addition to the GABA translocation, possesses an Li^+-leak current mode which in the absence of Na^+ and GABA and in the presence of Li^+ leads to a large influx of Li^+ into the voltage-clamped GAT-1-expressing oocyte (MacAulay et al., 2002b; Mager et al., 1996). Importantly, this current, being equal or larger than the GABA-coupled current, does not lead to an instant water accumulation in the same GAT-1 expressing oocytes (Fig. 1B). Third, cotransport of water is independent of external osmotic gradients. This

Table 1. Water transport properties of brain aquaporins, cotransporters and uniporters

Protein	Substrates	Unit L_p (10^{-14} cm^3 s^{-1})	Water molecules transported	References
KCC4	K^+/Cl^-	NA	500	Zeuthen, 1991b, 1994
NKCC1	$Na^+/K^+/Cl^-$	NA	590[a]	Hamann et al., 2005; Hamann et al., unpublished observations
MCT1	H^+/lactate	NA	500	Zeuthen et al., 1996; Hamann et al., 2000, 2003
GAT-1	Na^+/Cl^-/GABA	0.7	330	Loo et al., 1999; MacAulay et al., 2002b
EAAT1	Na^+/glutamate	0.2	425	MacAulay et al., 2001, 2002a
GLUT1	Glucose	0.2[b]	40	Zeuthen et al., unpublished observations
AQP4	H_2O	24	NA	Yang and Verkman, 1997
AQP1	H_2O	4	NA	Zeidel et al., 1992

ND, not determined; NA, not applicable.

[a] The coupling ratio for the NKCC1 was determined by comparing two different situations which give the same rate of water transport. In the pigmented epithelium of the cilliary body of the eye, hyperosmolar addition of NaCl to the outside solution did not alter the transport rate for water by the NKCC1.

[b] Calculated from the glucose uptake, the glucose permeability P_s (1.5×10^{-6} cm s^{-1}), and the water permeability L_p (6.5×10^{-5} cm s^{-1}) for human GLUT1 expressed in *Xenopus* oocytes at room temperature. The turnover rate was 151 s^{-1} at room temperature (Simpson et al., 2007). The measurements were performed in analogy to the study of GLUT2 (Zeuthen et al., 2007).