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Aquaporins in the brain: from aqueduct to "multi-duct"

Jérôme Badaut · Jean-François Brunet · Luca Regli

Abstract The aquaporin channel family was first considered as a family of water channels, however it is now clear that some of these channels are also permeable to small solutes such glycerol, urea and monocarboxylates. In this review, we will consider AQP4 and AQP9 expressed in the rodent brain. AQP4 is present on astrocytic end-feet in contact with brain vessels and could be involved in ionic homeostasis. However, AQP4 may also be involved in cell adhesion. AQP4 expression is highly modified in several brain disorders and it can play a key role in the cerebral edema formation. However, the exact role of AQP4 in edema formation is still debated. Recently, AQP4 has been shown to be also involved in astrocyte migration during glial scar formation. AQP9 is expressed in astrocytes and in catecholaminergic neurons. Two isoforms of AQP9 are expressed in brain cells, the shortest isoform is localized in the inner membrane of mitochondria and the longest in the cell membrane. The level of expression of AQP9 is negatively regulated by high concentrations of insulin. Taken together, these results suggest that AQP9 could be involved in brain energy metabolism. The induction of AQP9 in astrocytes is observed with time after stroke onset suggesting participation in the clearance of excess lactate in the extracellular space. These recent exciting results suggest that AQPs may not only be involved in water homeostasis in the brain but could also participate in other important physiological functions.

Keywords Water channel · Glycerol · Lactate · Catecholaminergic neurons · Edema · Ischemia

Water is an important molecule involved in several biochemical processes present in living cells. Water was considered for a long time to freely diffuse through the plasma membrane, but this hypothesis was revisited after the discovery of water

J. Badaut (✉) · J.-F. Brunet · L. Regli
Department of Neurosurgery, CHUV-UNIL, 1011 Lausanne, Switzerland
e-mail: jerome.badaut@chuv.ch

channels (Preston *et al.* 1992). The water channel family is still growing with 13 members ubiquitously distributed in mammalian tissues. The importance of these channels for life is supported by the fact that there are more than 150 types of AQPs in microbes, invertebrates, non mammalian vertebrates and plants (Santoni *et al.* 2000).

These channels have been highly conserved throughout evolution and the family is now divided accordingly to sequence homology and permeability into *aquaporins* and *aquaglyceroporins*.

Research in this field has significantly advanced in the last years, and in this review we will illustrate several new potential functional roles for brain AQPs.

Water channels: a general introduction

Aquaporins (AQP) are water channel proteins with a molecular weight of around 30 kDa, and exhibit a common structure of six membrane spanning alpha helical domains with intracellular carboxyl (C) and amino (N) termini. They contain a consensus motif Asn-Pro-Ala (NPA), implied in pore formation (Badaut *et al.* 2002). As mentioned above, the AQP family is now divided into two subgroups based on sequence homology (Amiry-Moghaddam and Ottersen 2003): *aquaporin* and *aquaglyceroporin*.

The subgroup *aquaporin* is composed of AQP0, 1, 2, 4, 5, 6, 8 and is considered to be mainly permeable to water with a high flow rate. A few of these pure water channels are also permeable to anion (AQP6) and volatile solutes such as CO_2 for AQP1 (Cooper *et al.* 2002). Water diffusion through AQPs is inhibited by mercury, except AQP4 which is a mercury-insensitive aquaporin (Amiry-Moghaddam and Ottersen 2003).

The second subgroup *aquaglyceroporins*, is composed of AQP3, 7, 9, 10 and bacterial glycerol facilitator (Glpf; Badaut and Regli 2004). These channels are permeable to water and glycerol. AQP9, a member of this group, was also surnamed "neutral channel" (Tsukaguchi *et al.* 1998). Indeed, the presence of AQP9 in Xenopus Oocytes or proteoliposomes injected facilitates the diffusion of water, but also polyols (glycerol, mannitol, and sorbitol), purines (adenine), pyrimidines (uracil and chemotherapeutic agent 5-fluorouracil) and monocarboxylates (lactate and β-hydroxybutyrate; Ishibashi *et al.* 1998; Tsukaguchi *et al.* 1998; Ko *et al.* 1999; Tsukaguchi *et al.* 1999; Carbrey *et al.* 2003). However, the osmotic water coefficient for AQP9 is lower than in a pure water channel like AQP4 (Carbrey *et al.* 2003). In addition, AQP9 facilitates metalloid transport further suggesting that APQ9 may be a major route of arsenite uptake into mammalian cells (Liu *et al.* 2002).

Water homeostasis is critical to sustain normal neural activity. An increase in the water content into the brain leads to brain swelling and rapidly becomes deleterious. Obviously, the knowledge of the distribution and regulation of water channels in the brain is important to understand water homeostasis. To date, six aquaporin subtypes (AQP1, AQP3, AQP4, AQP5, AQP8, AQP9 have been described in rodent brain cells. However, only three aquaporins have been clearly identified in brain cells in vivo: AQP1, AQP4 and AQP9 (Badaut *et al.* 2002). AQP1 is expressed in epithelial cells of the choroid plexus (Nielsen *et al.* 1993), and is proposed to be involved in cerebrospinal fluid formation (Brown *et al.* 2004). Expression of AQP1 is seen in

many non-brain endothelia (Nielsen *et al.* 1993) but its expression is suppressed in the specialized endothelial cells of the blood–brain barrier (Dolman *et al.* 2005). Indeed, the presence of astrocytes inhibits the expression of AQP1 in endothelial cells (Dolman *et al.* 2005). In this review, we will focus on distributions and putative roles of AQP4 and 9 in the mammalian brain. Recent reports concerning the level of expression of AQP in brain disorders will be then presented.

AQP4 expression in brain and its several functional roles

Historically, mRNA for AQP4 was first observed by in situ hybridization in the rat brain, with expression of the messenger in the glia limitans, the ependymal lining system, the magnocellular hypothalamic nuclei, the cerebellum, the hippocampus, the neocortex and in the medial habenular nucleus (Jung *et al.* 1994; Venero *et al.* 1999). This regional distribution for AQP4 mRNA was confirmed by several immunohistochemistry studies (Nielsen *et al.* 1997; Badaut *et al.* 2000a, b). AQP4 protein is present on astrocyte endfeet in contact with blood vessels but also on astrocytic processes in contact with the synapses (Nielsen *et al.* 1997; Badaut *et al.* 2000a, b).

Electronic microscopy studies after cryofracture and immunogold techniques have shown that the highest density of AQP4 in astrocyte endfeet was observed in geometric structures named orthogonal arrays of proteins (OAPs; Verbavatz *et al.* 1997; Rash *et al.* 1998, 2004). Interestingly, the ratio between the expression of the long and the short AQP4 splice variant (AQP4m1 and AQP4m23) determines the size of the OAPs (Rash *et al.* 2004). The AQP4m23 isoform stabilizes the structure of the OAPs and an increase of its expression induces an increase in the size of the OAPs (Rash *et al.* 2004). The first functional role of the OAPs has been suggested to be an involvement with the astrocytic potassium-buffering (Grange-Messent *et al.* 1996). To date, functional consequences of the variation of the size of the OAPs are not known. Recently, another physiological role has been suggested for AQP4, in cell adhesion (Hiroaki *et al.* 2006). The involvement of an APQ in cell adhesion has been well described for epithelial cells of the lens, where AQP0 participates in the linkage of cells (Gonen *et al.* 2004). The high level of AQP4 protein in the hypothalamic glia lamellae should facilitate the adhesion between the astrocyte processes (Hiroaki *et al.* 2006). In this situation, AQP4 should not be involved in water diffusion but rather in cell adhesion between astrocytes and possibly to endothelial cells or muscle cells in the perivascular compartment. To support this idea, recent reports showed that the presence of AQP4 of the endfoot membrane is dependent on the presence of proteins in the basal lamina such as agrin, α-dystroglycan and laminin (Guadagno and Moukhles 2004; Warth *et al.* 2004), suggesting an involvement in the ability of astrocytes to maintain the integrity of the blood–brain barrier. In the intracellular region, AQP4 is anchored to several proteins of the astrocytic cytoskeleton such as α1-syntrophin and dystrophin (Frigeri *et al.* 2001; Neely *et al.* 2001; Vajda *et al.* 2002; Amiry-Moghaddam *et al.* 2004). However, AQP4 is also observed outside the OAPs (Warth *et al.* 2004) raising the following questions: is there a water diffusion inside the OAPs? Is there a difference in the capacity of water diffusion inside or outside the OAPs? These questions are

crucial because induction of AQP4 has been described in several brain diseases such as ischemia (Badaut *et al.* 2002).

Some new insights were obtained from two mice strains deficient in gene expression of dystrophin (mdx) and α1-syntrophin (a protein linked to dystrophin) which exhibit a marked decrease in AQP4 immunolabeling with swelling of astrocytic end-feet (Frigeri *et al.* 2001; Neely *et al.* 2001; Vajda *et al.* 2002; Amiry-Moghaddam *et al.* 2004). In mdx and syntrophin knock out mice, astrocyte swelling may be due to impaired water elimination resulting from AQP4-disturbed organization at the plasma membrane. Another clue to the function of perivascular AQP4 was obtained by platelet-derived growth factor B (PDGF-B)-knockout mice, which showed abnormal vascular morphogenesis resulting in the absence of pericytes, and the presence of endothelial hyperplasia already at embryonic day 11.5 (Hellstrom *et al.* 2001). These knockout mice showed an increase in AQP4 concentration and swelling of astrocytes which may be a response to vascular abnormalities (Hellstrom *et al.* 2001). Similarly, a significant induction of AQP4 expression was observed on astrocyte endfeet in the ischemic hemisphere, one hour after stroke onset (de Castro Ribeiro *et al.* 2006). These results suggest that the increased AQP4 content of the perivascular space highlights the need for rapid water movements in this region. The pattern of distribution of aquaporins within the perivascular space might be related to the control of the perivascular volume, a function that may be crucial for maintenance of cerebral blood perfusion (Badaut *et al.* 2000b) and facilitation of the water clearance from perivascular space.

In the rodent brain, several regions show a strong AQP4 immunoreactive signal that may be due to the presence of the channel outside the perivascular space on astrocyte processes surrounding neuronal cells (Nielsen *et al.* 1997; Venero *et al.* 1999; Badaut *et al.* 2000b). The cellular distribution of AQP4 protein suggest that it may be involved in potassium homeostasis due to its co-distribution with KIR4.1 (Nagelhus *et al.* 2004). This hypothesis is also supported by functional results obtained from AQP4 KO mice showing that the delay of potassium re-uptake during electrical activity is increased and therefore these mice develop seizures more easily (Binder *et al.* 2006). The perivascular pool of AQP4 anchored by α-syntrophin, seems to have an important role in this spatial potassium buffering because the delay of potassium re-uptake during electrical activity is also increased in α-syntrophin KO mice (Amiry-Moghaddam *et al.* 2003b). Interestingly, observations in sclerotic tissues from patients with mesial temporal lobe epilepsy and hippocampal sclerosis, suggest that the loss of perivascular AQP4 could be secondary due to the absence of brain specific dystrophin in these pathological tissues (Eid *et al.* 2005). This hypothesis suggests that the clearance of extracellular potassium can be compromised which then contributes to the accumulation of extracellular potassium in the brain of these patients. These results support the concept that AQP4 plays an important role in ionic homeostasis by facilitating water diffusion.

Potassium spatial buffering is facilitated by application of vasopressin (VP), an anti-diuretic hormone, which stimulates V1b receptors in acute cortical slices (Niermann *et al.* 2001). The increase in extracellular potassium clearance after VP application may be due an increase of AQP4 expression (Niermann *et al.* 2001). In contrast to brain, the effect of VP on the level of AQP4 and AQP2 expression is well

described in the kidney (King *et al.* 2000). However, direct regulation of the level of AQP4 expression by VP has never been demonstrated in the central nervous system.

VP is synthesized in the magnocellular and parvocellular neurons of the hypothalamic nuclei, paraventricular and supraoptic nuclei and released into the blood stream in the neurohypophysis. These hypothalamic nuclei are known to be involved in the central osmoreception of variations of plasma osmotic pressure (Wells 1998). The high level of AQP4 and similar distribution have been observed in all osmosensitive brain areas such as the subfornical organ, the supraoptic nuclei, the paraventricular nucleus and accessory nuclei such as the circularis nucleus (Nielsen *et al.* 1997; Badaut *et al.* 2000a). In these brain regions, AQP4 is on plasma membranes facing both capillaries and magnocellular neurons (Nielsen *et al.* 1997; Badaut *et al.* 2000a). The specificity and intensity of AQP4 staining within hypothalamic magnocellular nuclei strongly suggest that water channels allow variations of plasma osmotic pressure to be transferred from blood to osmosensitive neurons. Furthermore, high blood vessel density is another common feature of these nuclei, which contribute to plasma osmolarity detection (Badaut *et al.* 2000a).

AQP9 expression in brain and its functional roles

Aquaglyceroporins facilitate the diffusion of water and several solutes such as glycerol, urea and monocarboxylate. The highest level of expression of AQP9 is in the liver (Tsukaguchi *et al.* 1998; Elkjaer *et al.* 2000) with polarization of the protein to the hepatocytic plasma membrane facing the sinusoids (Elkjaer *et al.* 2000; Nicchia *et al.* 2001; Nihei *et al.* 2001). The expression of AQP9 is also observed in the rodent brain (Badaut and Regli 2004). AQP9 mRNA was first detected in astrocytic cultures (Tsukaguchi *et al.* 1998) and confirmed by immunocytochemical studies in rodent brain (Badaut *et al.* 2001, 2004). To date, AQP9 expression has been observed in three cell types: glial cells, in particular tanycytes and astrocytes (Elkjaer *et al.* 2000; Badaut *et al.* 2001, 2004), endothelial cells of sub-pial vessels (Badaut *et al.* 2004) and neurons (Badaut *et al.* 2004; de Castro Ribeiro *et al.* 2006).

AQP9 expression was found predominantly in one subtype of neuronal cells, the catecholaminergic neurons, characterized by tyrosine hydroxylase expression in rat and mouse brains (Badaut *et al.* 2004; de Castro Ribeiro *et al.* 2006). In agreement with the regional distribution of AQP9 protein by immunohistochemistry, high levels of mRNA for AQP9 was detected in catecholaminergic nuclei confirming the presence of this protein in these nuclei (Badaut *et al.* 2004). Regarding these results, a sole role for AQP9 in water homeostasis should be revised, as to the best of our knowledge, catecholaminergic neurons are not directly implied in the regulation of systemic osmotic pressure, but are rather involved in energy balance (Grill and Kaplan 2002; Penicaud *et al.* 2002). This leads us to postulate that AQP9 could be involved in brain energy metabolism as a metabolite channel. In agreement with this hypothesis, the presence of AQP9 protein was recently demonstrated in mitochondria of astrocytes and dopaminergic neurons (Amiry-Moghaddam *et al.* 2005). Indeed, two isoforms of AQP9 are expressed in brain cells. The shortest isoform of AQP9 (26 kDa) is observed in inner membrane of the mitochondria and the longest isoform (30 kDa) is present in cell membrane (Amiry-Moghaddam *et al.* 2005). It is

possible that the presence of AQP9 facilitates the diffusion of glycerol and monocarboxylates, which serve as energy substrates for neurons (McKenna *et al.* 1986; Magistretti *et al.* 1999; Nguyen *et al.* 2003). Interestingly, utilization of glycerol by neuronal cells in vitro was inhibited by mercury, suggesting transport by an AQP (Nguyen *et al.* 2003). In the "lactate shuttle" model, glucose is transformed by astrocytes into lactate and diffuses from astrocytes to neurons using the monocarboxylate transporters (Magistretti and Pellerin 1999). Therefore, presence of AQP9 in astrocytes suggests that it may facilitate the diffusion of lactate to neuronal cells in conjunction with the monocarboxylate transporters.

In addition, some catecholaminergic neurons are known to be sensitive to variations in glucose levels and recently, lactate and glycerol have been also shown to be potential activators of these neurons (Yang *et al.* 1999; Ainscow *et al.* 2002; Penicaud *et al.* 2002). These neurons are located in the same brain areas as AQP9-positive neurons (Badaut and Regli 2004). Taken together, these results raise the hypothesis that neuronal AQP9 plays a role in energy balance as a glycerol-lactate-channel, but this awaits functional proof.

In liver, AQP9 expression has been shown to be regulated by the physiological feeding state mediated by the insulin response element (IRE) in the promoter of the gene (Kuriyama *et al.* 2002). High plasma insulin suppresses glycerol uptake into hepatocytes which participates in neoglucogenosis to replenish blood glucose concentrations. AQP9 is down regulated by high insulin concentration suggesting that AQP9 may play a key role in cellular energy balance as a glycerol channel in the liver (Kuriyama *et al.* 2002; Carbrey *et al.* 2003). In vivo, the expression of AQP9 is increased after fasting and returns to basal levels upon refeeding (Carbrey *et al.* 2003). AQP9 expression in liver is dramatically increased in models of diabetes induced by streptozotocin (STZ) injection (Carbrey *et al.* 2003) and in a mouse model of insulin resistance (Kuriyama *et al.* 2002). As mentioned above, energy balance is regulated by detection of glucose levels in the periphery as well as in the central nervous system (Levin *et al.* 1999). The feeding state, set by blood insulin concentration, also influences the glucose sensitive areas of the brain. Interestingly, AQP9-positive neurons are present in brain regions known to be glucose sensitive or implicated in the feeding state (Badaut *et al.* 2004); systemic insulin has been reported to cross the BBB, and insulin receptors are expressed in catecholaminergic neurons (Kyriaki 2003; Unger *et al.* 1991 #211). These data lead us to investigate whether brain AQP9 expression is regulated by insulin. The first in vitro results on brain stem slices containing catecholaminergic neurons showed a decrease in AQP9 protein levels 6 h after insulin application (Badaut *et al.* 2005). In vivo, the level of AQP9 was increased in liver and in the NTS of rats treated with STZ injections to induce diabetes (Badaut and Regli 2004; Badaut *et al.* 2005). Therefore, it would appear that brain AQP9 expression appears is regulated by insulin concentrations, supporting the hypothesis that AQP9 is involved in brain energy metabolism.

AQP9 was also observed in endothelial cells of the pial vessels, and in the intraparenchymal vessels (Badaut *et al.* 2004; Amiry-Moghaddam *et al.* 2005). interestingly in mice, AQP4 expression was observed by immunogold labeling in endothelial cells at their luminal, as well as abluminal membranes, but at much lower levels than observed on astrocytic endfeet (Amiry-Moghaddam *et al.* 2004). The blood–brain barrier (BBB) is known to be highly permeable to water (Oldendorf

1970) and therefore, AQP4 and AQP9 may facilitate the water flow through endothelial cells. AQP9 could also participate in diffusion of monocarboxylate through the BBB which is mainly carried out by the monocarboxylate transporter, MCT1, highly expressed in endothelial cells (Gerhart *et al.* 1997; Pierre *et al.* 2000; Bergersen *et al.* 2002).

AQP expression in brain disorders

As mentioned previously, AQPs in rodent brain as water-channels are likely to play an important role in extracellular homeostasis, and thus may sustain normal neuronal activity (Badaut *et al.* 2002). A profound perturbation of the brain environment usually induces a regional cerebral edema, as observed in ischemia. Brain edema which leads to an expansion of brain volume, has a crucial impact on morbidity and mortality after stroke as it increases intracranial pressure, favours herniations, and contributes to additional ischemic injuries (Klatzo 1985). Despite its complexity, brain oedema has been defined as an increase in net brain water content which leads to an increase in tissue volume (Pappius 1974). The two major types of brain oedema, cytotoxic and vasogenic oedema, both occur after brain ischemia. Cytotoxic oedema is characterized by intracellular water accumulation involving both astrocytes and neurons and depending mainly on the perturbation of ionic gradients (Kimelberg 2004). Vasogenic oedema is characterized by a protein rich exudate derived from plasma, as a result of an increased permeability of the capillary endothelial cells to albumin and other plasma proteins (Unterberg *et al.* 2004).

As mentioned previously, three AQPs could be involved in water movements occurring during formation and resolution of cerebral edema after ischemia. Astrocytic AQP1 expression is induced in human brain tissues after subarachnoid hemorrhage (SAH; Badaut *et al.* 2003) but modification in expression has not yet been described in rodent models of human brain disorders (de Castro Ribeiro *et al.* 2006). The level of AQP4 expression is regulated in several brain disorders, such as trauma (Ke *et al.* 2001; Kiening *et al.* 2002; Sun *et al.* 2003), ischemia (Taniguchi *et al.* 2000; Amiry-Moghaddam *et al.* 2003a; Meng *et al.* 2004; de Castro Ribeiro *et al.* 2006) and human SAH (Badaut *et al.* 2003). To better understand the roles of AQP4 and AQP9 in edema, the expression profiles were recently characterized at various time points after transient cerebral ischemia in mice. Two peaks of AQP4 expression were observed 1 h and 48 h after stroke, coinciding with the two peaks of maximal hemispheric swelling (de Castro Ribeiro *et al.* 2006). This temporal expression of AQP4 differs with the result from brain trauma where there is an initial decrease in AQP4 levels within 48 hours, followed by an increase (Ke *et al.* 2001, 2002; Kiening *et al.* 2002). The difference in expression between the two models suggests that the role of AQP4 in edema formation and resolution is complex. In contrast to AQP4, AQP9 showed a significant induction at 24 h, that increased gradually with time, without correlation to swelling (de Castro Ribeiro *et al.* 2006), suggesting that AQP4 but not AQP9 plays a role in edema formation after transient cerebral ischemia in the mouse. Interestingly, AQP4 expression is rapidly regulated with a major induction at 1 h after stroke onset on astrocyte endfeet, suggesting that we need to consider early time points after brain disorder onset (de Castro Ribeiro *et al.*

2006). To date, functional consequences of the early AQP4 induction on astrocyte endfeet have not yet been determined. It is possible that the presence of AQP4 in cytotoxic edema formation is deleterious (Manley *et al.* 2000). Indeed, edema is lower in AQP4-KO mice compared to wild type after permanent ischemia and acute water intoxication (Manley *et al.* 2000). On the other hand, AQP4 was shown to be important in water clearance in vasogenic edema (Papadopoulos *et al.* 2004). Perhaps, AQP4 plays a dual role in edema evolution. However, a treatment with sulforaphane which enhances AQP4 expression, induces a decrease in edema following traumatic brain injury (Zhao *et al.* 2005), suggesting that induction of AQP4 expression could facilitate the water clearance to decrease edema.

As mentioned previously, the role of AQP4 in brain disorders is complex. Indeed, AQP4 has been recently shown to be involved in glial scar formation due to its involvement in astrocyte migration towards the lesion (Saadoun *et al.* 2005). This new role for AQP4 illustrates well that one AQP could be involved in several cellular functions in one organ and in one cell type, depending on the environment.

AQP9 protein is up-regulated on reactive astrocytes in the border of the infarct after transient middle cerebral artery occlusion in mice (Badaut *et al.* 2001; de Castro Ribeiro *et al.* 2006). This up-regulation of AQP9 in reactive astrocytes was also observed in the border zone not influenced by the middle cerebral artery territory (de Castro Ribeiro *et al.* 2006). AQP9 permeability to water, glycerol and lactate may be important under pathological conditions such as brain ischemia (Bertrand *et al.* 1992; Schulz *et al.* 2000; Frykholm *et al.* 2001; Kuo *et al.* 2003) and interestingly, AQP9 permeability to lactate increases fourfold when the pH decreases to 5.5 (Tsukaguchi *et al.* 1998). Lactic acidosis during ischemia may increase the permeability of AQP9 and enable uptake of excess lactate by astrocytes. In this way AQP9 could favor lactate and glycerol clearing from the extracellular space during ischemia. Lactate and glycerol could then be used as energetic substrates, lactate, for example, has been shown to facilitate the recovery of neurons after ischemic insults (Schurr 2002).

To date, molecular pathways involved in AQP4 and 9 regulation have not yet been studied in vivo. However several groups have investigated the regulation of AQP expression in primary astrocyte cultures. Several pathways leading to regulation of AQP expression were identified including protein kinase A (PKA) and C (PKC; Yamamoto *et al.* 2001, 2002). Stimulation of the PKC pathway is known to reduce AQP4 mRNA expression in astrocyte cultures (Nakahama *et al.* 1999) and furthermore, PKC phosphorylation at consensus sites in the AQP4 protein acts to reduce water influx through the channel (Han *et al.* 1998; Vajda *et al.* 2000). AQP9 mRNA and protein were also down-regulated by stimulation of the PKC pathway which did not require de novo protein synthesis (Yamamoto *et al.* 2001). Despite the presence of consensus sites for phosphorylation by PKC in the AQP9 protein, direct regulation of this channel by phosphorylation has not yet been observed (Yamamoto *et al.* 2001). Activation of PKA by dibutirylcAMP induces an increase in AQP9 mRNA and protein expression in contrast to AQP4 in astrocytic cultures (Yamamoto *et al.* 2002). Recently, P38 MAP-kinase was shown to be involved in an increase of AQP4 and AQP9 expression after osmotic stress (Arima *et al.* 2003). MAP-kinase pathways is activated in ischemia and then could be also involved in the increase of astrocytic AQP9 expression at the border of the ischemic infarct (Badaut *et al.* 2001; de Castro Ribeiro *et al.* 2006).

Conclusion

Regarding to recent advances, a unique role of AQP in brain water homeostasis needs to be reconsidered. AQP4 is involved in ionic homeostasis of the brain by facilitation of water diffusion through the cell membrane. An absence of AQP4 in astrocyte endfeet modifies the clearance of potassium during neuronal activity and leads to an increase in the susceptibility of animals to seizure. In pathological conditions, expression of AQP4 is rapidly increased and correlated with edema formation. However, the exact role of AQP4 in edema formation is still debated. Furthermore, the induction of AQP4 in reactive astrocytes after traumatic brain injury has been recently associated with astrocyte migrations towards the glia scar.

AQP9 is expressed in astrocytes and catecholaminergic neurons and may be involved in brain energy metabolism by facilitating the diffusion of solutes such as glycerol and monocarboxylates. Furthermore, the level of AQP9 expression is dependent on the concentration of insulin, supporting the idea that AQP9 is involved in brain energy metabolism. In ischemia, the AQP9 expression is induced over time and could participate in the reuptake of excess glycerol and lactate after stroke onset. Recent reports on AQP9 suggest that the AQP is not only an aqueduct but a metabolite pipeline.

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