

REGULATION OF BRAIN AQUAPORINS

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Abstract—Emerging evidence suggests that brain aquaporins (AQP) play important roles for the dynamic regulation of brain water homeostasis and for the regulation of cerebrospinal fluid production. This review deals with the short- and long-term regulation of AQP4 and AQP9, both expressed in astrocytes, and of AQP1, expressed in the choroid plexus. AQP1 and 4 have in other cell types been shown to be regulated by phosphorylation. Phosphorylation affects the gating of AQP4 and the trafficking and insertion into membrane of AQP1. Mercury inhibits the water permeability of AQP1 and AQP9, but not AQP4. The permeability of AQP4 is increased by lead. AQP4 is also regulated by protein–protein interaction. The assembly between AQP4 and syntrophin is required for the proper localization of AQP4 in the astrocyte plasma membrane that faces capillaries. There is evidence from studies on peripheral tissues that steroid hormones regulate the expression of AQP1, AQP4 and AQP9. There is also evidence that the expression of AQP1 can be regulated by ubiquitination, and that osmolality can regulate the expression of AQP1, AQP4 and AQP9. Further insight into the mechanisms by which brain AQPs are regulated will be of utmost clinical importance, since perturbed water flow via brain AQPs has been implicated in many neurological diseases and since, in brain edema, water flow via AQP4 may have a harmful effect. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: aquaporin 1, aquaporin 4, aquaporin 9, astrocytes, phosphorylation, protein kinase C.

Integral membrane proteins with a transporting function, i.e. pumps, channels and co-transporters, are responsible for the distribution of water and ions between extra- and intracellular space and for the maintenance of a proper intracellular environment. From this follows that much of the physiological significance of these transporters lies in their capacity to be regulated. Much of the short-term regulation is mediated via hormone receptors inserted in the plasma membrane. The majority of these receptors belong to the family of G-protein-coupled receptors (GPCR), also called seven transmembrane spanning receptors. Ligands and activators of GPCR include peptide hormones, prostanoids, catecholamines and amino acids.

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Abbreviations: AC, adenylate cyclase; AQP, aquaporin; AVP, vasopressin; CaMK, calcium/calmodulin-dependent protein kinase; cAMP, cyclic AMP; CK, casein kinase; CSF, cerebrospinal fluid; GFP, green fluorescent protein; GPCR, G-protein-coupled receptors; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PP, protein phosphatase.

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Sequences for approximately 1000 GPCR have been identified in humans (Horn et al., 1998) and for many of them a physiological ligand has not yet been identified (known as orphan receptors). The most common intracellular signaling pathways for GPCR are initiated by activation of either adenylate cyclase (AC) or of phospholipase C (PLC).

The endpoint in signaling cascades triggered by activated GPCR is protein phosphorylation/dephosphorylation. Several water channels, including aquaporin (AQP)1, AQP2, AQP4 and AQP5, have been shown to be regulated by reversible phosphorylation. Phosphorylation results in an allosteric change of the transporter protein. This may either trigger a translocation of intracellularly located transporters to the plasma membrane or change the opening state of the transporter already inserted in the plasma membrane. A transporting protein may also be dynamically regulated via phosphorylation of a protein, which interacts with the transporter and modulates its function by protein–protein interaction.

Reversible protein phosphorylation is maybe the most common mechanism by which the function of a protein can be dynamically regulated. There are several ubiquitous protein kinases, i.e. protein kinase A (PKA), protein kinase C (PKC), casein kinase (CK) and calcium/calmodulin-dependent protein kinases (CaMK); and several ubiquitous protein phosphatases (PP) such as PP1, PP2A and the calcium-dependent PP2B, also named calcineurin. In addition a large number of more cell-specific protein kinases and phosphatases have now been identified. Emerging evidence suggests that protein kinases and PPs are often located in a close proximity with the target proteins.

It is well established that heavy metals can directly interact with ion channels thereby affecting their activity. Mercury was found to inhibit rapid transcellular transport in epithelial cells before the water channels were discovered. The activities of most mammalian AQPs have now been shown to be inhibited by mercury. AQP4 is resistant to mercury and the activity of AQP6 is stimulated by this metal (Yasui et al., 1999). Recently it was found that AQP3, an aquaglyceroporin present in kidney, lung and skin, is inhibited by nickel and copper (Zelenina et al., 2003a,b). These heavy metals have however no effect on AQP4, the predominant brain AQP. Nor does zinc, a modulator of many ion channels, have any effect on AQP4 (Tritto, Zelenina, unpublished observations).

Changes in extracellular pH may influence the activity of many transmembrane proteins via protonation of extracellular amino acid residues. AQP3 has been shown to be pH-sensitive (Zeuthen and Klaerke, 1999; Zelenina et al., 2003a), but no such effect has been found for AQP4.

Neither AQP1 nor AQP9, the other AQPs that are today known to be expressed in the brain, has, to the best of our knowledge, been examined for pH or nickel, copper or zinc sensitivity.

Methods to study the dynamic regulation of water channels

The choice of strategies to demonstrate a dynamic regulation of a water channel is based on ability of the channel to undergo a regulated subcellular trafficking. The trafficking can be demonstrated by immunohistochemistry (Nielsen et al., 1995), by imaging of green fluorescent protein (GFP)-tagged proteins (Gustafson et al., 1998), and by differential centrifugation and Western blotting (Zelenina et al., 2000). If trafficking of an AQP is established, the regulation of individual channel permeability must be studied in a liposome reconstitution system or in vesicle preparations, where the trafficking component is excluded (Meyer and Verkman, 1987; Lande et al., 1996).

If trafficking of an AQP does not occur or is very slow *in vivo*, studies of the dynamic regulation of the channel permeability can be performed in a whole cell system. *Xenopus laevis* oocyte expression system proved to be extremely useful in AQP studies, beginning with the classic

experiments of Agre and coauthors that demonstrated the water permeability properties of the first AQP (Preston et al., 1992). However, when it comes to the studies of an AQP regulation in its native environment, a mammalian expression system is indispensable. The method for water permeability measurements in individual mammalian cells has been recently established (Zelenina and Brismar, 2000). It is based on confocal laser scanning microscopy of calcein-loaded cells and can be used in any type of adherent cell cultures. The cells can be transiently transfected with a GFP-tagged AQP (Zelenina et al., 2002), in which case the permeability of AQP-positive cells can be directly compared with the permeability of surrounding untransfected cells. Fig. 1 shows a typical experiment performed on an astroglial cell line transfected with GFP-AQP4. To exclude a possibility that GFP-tagging may change the regulation of AQP, the cells can be transfected using constructs expressing AQP and GFP as separate proteins present in the same cell (Zelenina et al., 2003a).

Short-term regulation of AQP1

AQP1 is expressed in most organs. In the brain AQP1 is abundantly expressed in the apical membrane of the choroid plexus epithelium in both humans and rodents. A

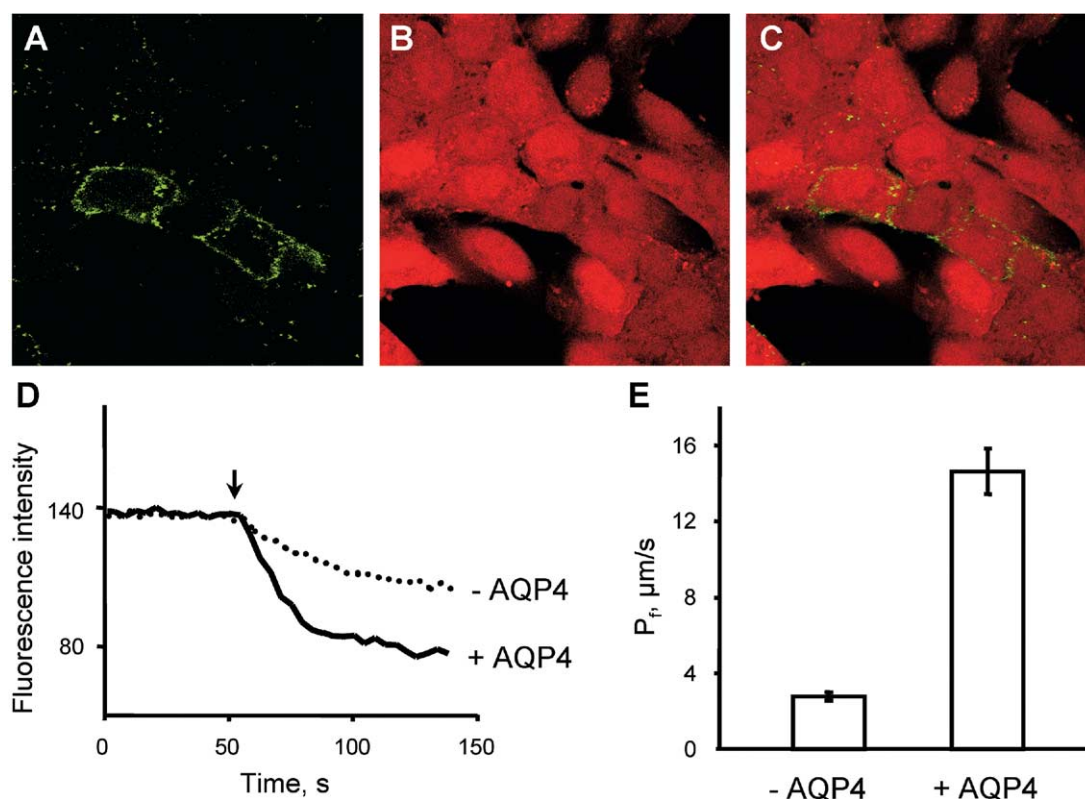


Fig. 1. Water permeability measurement in astrocyte cell line transfected with AQP4. The cells were transiently transfected with cDNA construct encoding the short variant of mouse AQP4 (AQP4.M23) tagged with GFP at NH₂-terminus. AQP4-positive cells were identified by GFP signal in the plasma membrane (A). The cells were loaded with calcein (B), perfused with isotonic PBS and scanned every 2 s in a focal plane in the middle of the cell. After control recording the solution was switched to hypotonic PBS (D, arrow). The decrease in fluorescence after the switch is proportional to the rate of the cell swelling. The image sequences were analyzed off-line using overlay of GFP and calcein images (C). AQP4-positive cell (+AQP4) swells much faster than untransfected (–AQP4). The initial rate of the fluorescence intensity decrease was used to calculate the water permeability (P_f), which in AQP4-positive cells was about four-fold higher than in surrounding untransfected cells (E).

recent immunohistochemical study suggests that low levels of AQP1 may also be expressed in hippocampal region and in ependymal cells in the human brain (Nielsen et al., 1993; Mobasheri and Marples, 2004).

Analysis of human, rat, and mouse AQP1 amino acid sequence using PhosphoBase v. 2.0 (Kreegipuu et al., 1999) indicates that AQP1 contains potential phosphorylation sites for PKA, PKC, and CKII (Fig. 2). Human AQP1 has an additional phosphorylation site for PKC, which is also a potential target for CKI. In line with these predictions, AQP1 water permeability has been shown to be dynamically regulated by several hormones. In *Xenopus* oocyte expression system, water permeability of AQP1 was increased by vasopressin (AVP) and decreased by atrial natriuretic peptide (Patil et al., 1997). The AVP effect was mimicked by forskolin, the activator of AC, and 8-bromo-cyclic AMP (cAMP), a membrane-permeable and metabolically stable cAMP analog. AQP1 was, in an *in vitro* system, shown to be phosphorylated by PKA (Han and Patil, 2000). Incubation of oocytes with 8-bromo-cAMP or AVP was found to increase the abundance of AQP1 in the plasma membrane, indicating that phosphorylation might have an effect on AQP1 trafficking. This possibility was supported by experiments in bile duct epithelial cells that express AQP1 *in vivo* (Marinelli et al., 1997, 1999). Secretin, a hormone that stimulates ductal bile secretion, increased water permeability of the cholangiocytes and caused a redistribution of AQP1 from an intracellular compartment to the apical membrane of cholangiocytes.

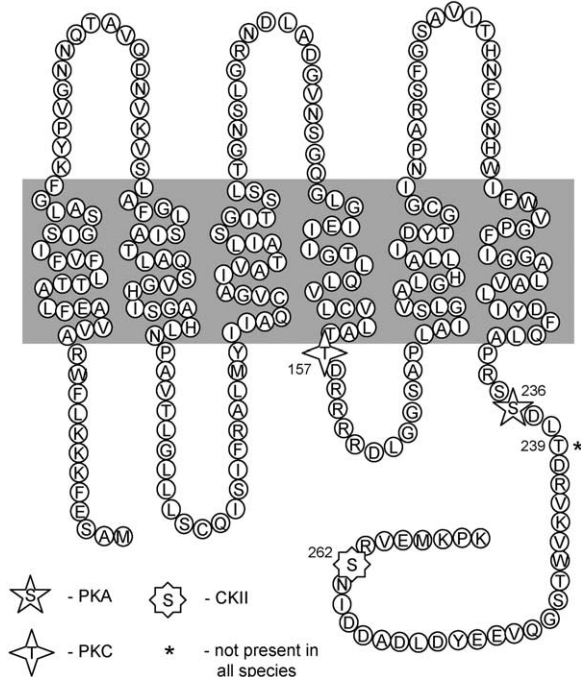


Fig. 2. Putative phosphorylation sites in human AQP1. The sites for PKA (Ser²³⁶), PKC (Thr¹⁵⁷), and CKII (Ser²⁶²) are conserved in human, mouse, and rat AQP1. Another PKC site (Thr²³⁹) is present only in human AQP1; this site can potentially be phosphorylated by CKI if Ser²³⁶ is phosphorylated.

Several pharmacological agents have been tested as potential regulators of AQP1. Acetazolamide, a carbonic anhydrase inhibitor, was found to inhibit AQP1 water permeability in oocytes (Ma et al., 2004). Tetraethylammonium, an inhibitor of voltage-dependent potassium channels, was found to inhibit water permeability of AQP1 expressed in oocytes and MDCK cells and of native AQP1 in rat renal descending thin limbs of Henle's loops (Brooks et al., 2000; Yool et al., 2002). So far nothing is known about the short-term hormonal regulation of AQP1 in brain cells.

Short-term regulation of AQP4

AQP4 is expressed in several organs including brain, lung and kidney. In the brain AQP4 is expressed in astrocytes and ependymal cells. It is strongly enriched in those astrocyte membrane domains that form the interface between brain neuropil and extracerebral spaces. AQP4 is also abundant in osmosensory areas, including the supraoptic nucleus and subfornical organ (Nielsen et al., 1997).

It is well established that AQP4 water permeability can be regulated by reversible protein phosphorylation. Sequence analysis of the AQP4 molecule (Kreegipuu et al., 1999) reveals several potential phosphorylation sites for PKA, CaMKII, PKC, and CKII (Fig. 3). Activation of PKC by phorbol esters downregulates AQP4 water permeability

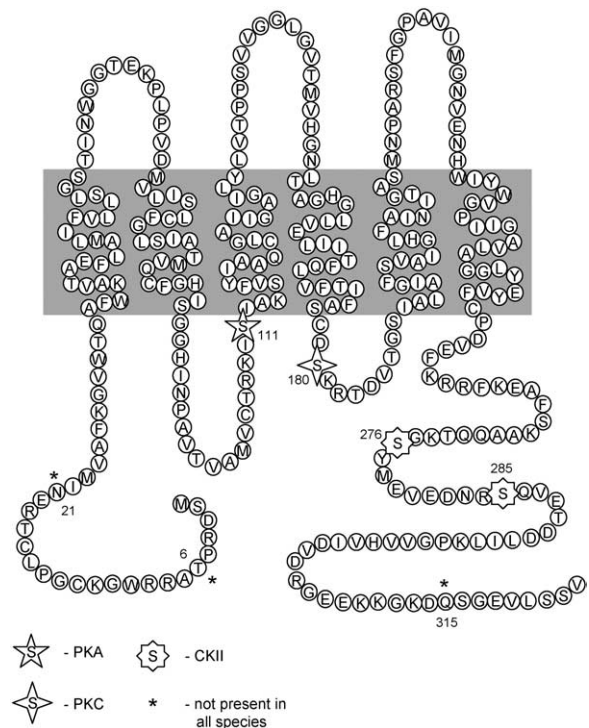


Fig. 3. Putative phosphorylation sites in human AQP4. The sites for PKA (Ser¹¹¹), PKC (Ser¹⁸⁰), and CKII (Ser²⁷⁶ and Ser²⁸⁵) are conserved in human, mouse, and rat. Except PKA, Ser¹¹¹ is also potential phosphorylation site for CaMKII. Another PKA site at position 21 and additional CKII site at position 315 are present only in the rodents. Thr⁶ may potentially be phosphorylated by PKA, PKC, and CKII and is present only in human AQP4.

both in *Xenopus* oocyte expression system and in cultured kidney epithelial cells (Han et al., 1998; Zelenina et al., 2002). This effect is mediated via phosphorylation of Ser¹⁸⁰, a consensus site for PKC phosphorylation. The PKC-triggered downregulation of AQP4 water permeability is likely due to a gating effect. Expression of AQP4 in the cytosolic compartment is negligible both under basal conditions and in hormone-stimulated cells (Zelenina et al., 2002). Activation of PKA was found to cause an increase of water permeability of AQP4 expressed in a renal cell line (Zelenina et al., 2001). Agents that stimulate cAMP production including forskolin, AVP, V2 receptor agonist and β -adrenergic receptor agonist have been found to increase the water permeability in a renal cell line transfected with AQP4.

Several lines of evidence suggest that the activity of AQP4 in brain cells is stimulated by AVP. In primary cultures of astrocytes, where endogenous AQP4 expression is presumably preserved, AVP was reported to increase the rate of cell swelling in hypotonic medium (Sarfaraz and Fraser, 1999). In cortical slices AVP was found to facilitate radial water flux generated by evoked neuronal activity (Niermann et al., 2001). The recorded change in water flux was interpreted as an index of increased water flow via AQP4 in astrocytes. AVP can activate two types of receptors, V1 and V2 (Fig. 4). V1 receptors are coupled to PLC, and activation of these receptors leads to release of calcium from intracellular stores and to activation of several PKC isoforms. V2 receptors are coupled to AC and trigger cAMP production and activation of PKA. In the studies mentioned above the AVP-induced changes in water transport were mediated by V1 receptors. The Ser¹¹¹ residue of AQP4 is a potential site for both calcium-dependent

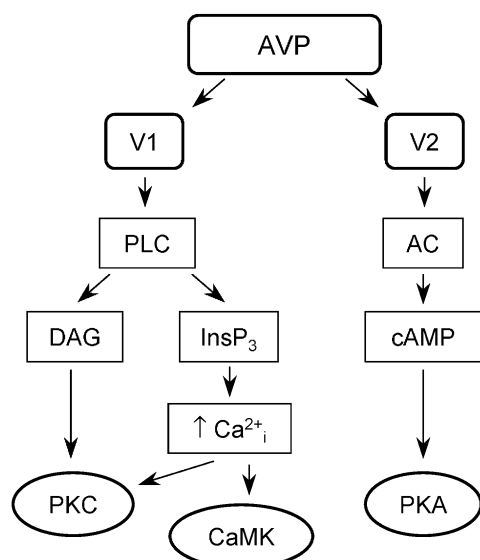


Fig. 4. AVP acts via V1 and V2 receptors. V1 receptors activate PLC, which produces diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (InsP₃). DAG and InsP₃-activated increase of intracellular Ca²⁺ level (Ca²⁺_i) activate various forms of PKC. In the brain, V1 receptors can also activate calcium/calmodulin-dependent protein kinase (CaMK). V2 receptors activate AC. cAMP that is produced by AC activates cAMP-dependent PKA.

CaMKII phosphorylation and for PKA phosphorylation (Fig. 3). It is tempting to speculate that Ser¹¹¹ is phosphorylated by CaMKII in astrocytes and by PKA in kidney cells and that in both cases phosphorylation leads to increased permeability of AQP4. It should be noted that activation of CaMKII by V1 receptors was recently demonstrated in astrocytes (Zhao and Brinton, 2003).

Activation of PKC will downregulate activity of AQP4 expressed in oocytes and renal epithelial cells, an effect that appears to be mediated via phosphorylation of the Ser¹⁸⁰ residue (see above). In the study where radial water flux was recorded, the stimulatory effect of AVP was found to be blocked by PKC inhibitors. This apparent controversy may, however, be explained by the fact that AQP4 may interact with different proteins in different tissues. It is known that AQP4 interacts with syntrophin in astrocytes and that loss of syntrophin interaction will result in displacement of AQP4 in the brain, but not in kidney (Neely et al., 2001). It is possible that the AQP4-syntrophin interaction in astrocytes may prevent PKC-dependent phosphorylation of Ser¹⁸⁰ site, and favor phosphorylation of Ser¹¹¹ by CaMKII.

It remains to be elucidated whether the effect of other hormones on AQP4 activity might also differ in brain and in peripheral tissues. For example, dopamine has been found to downregulate AQP4 water permeability in kidney epithelial cells (Zelenina et al., 2002), but the type of dopamine receptor mediating this dopamine effect has not yet been established.

AQP4 is insensitive to mercury compounds. However, using astrocyte cell transfected with AQP4 as well as primary culture of astrocytes from hippocampus, we have recently found that another heavy metal, lead, causes an increase in AQP4 water permeability (Gunnarson et al., 2003). This effect may have important clinical implications, since acute lead intoxication is associated with brain edema.

Short-term regulation of AQP9

The presence of AQP9 in rodent brain has been documented with immunostaining (Elkjaer et al., 2000; Badaut et al., 2001). Strong immunosignals were observed in astrocytes in the white matter, hippocampus, hypothalamus, and lateral septum, as well as in cells bordering the subarachnoid space and ventricles. Little is known about short-term regulation of AQP9. Analysis of human AQP9 amino acid sequence using PhosphoBase v. 2.0 (Krengli-puu et al., 1999) reveals two potential phosphorylation sites, Thr²⁶ for CKII and Ser¹¹ for PKC, but only CKII site is conserved among humans and rodents (Fig. 5).

AQP9 is, in addition to water, also permeable for glycerol, urea, and β -hydroxybutyrate. Low pH increases β -hydroxybutyrate permeability (Carbrey et al., 2003). It is therefore possible that water permeability of AQP9 may also be regulated by extracellular pH.

Long-term regulation of brain AQPs

Developmental regulation. There is at the present time no indication of a developmental regulation of brain

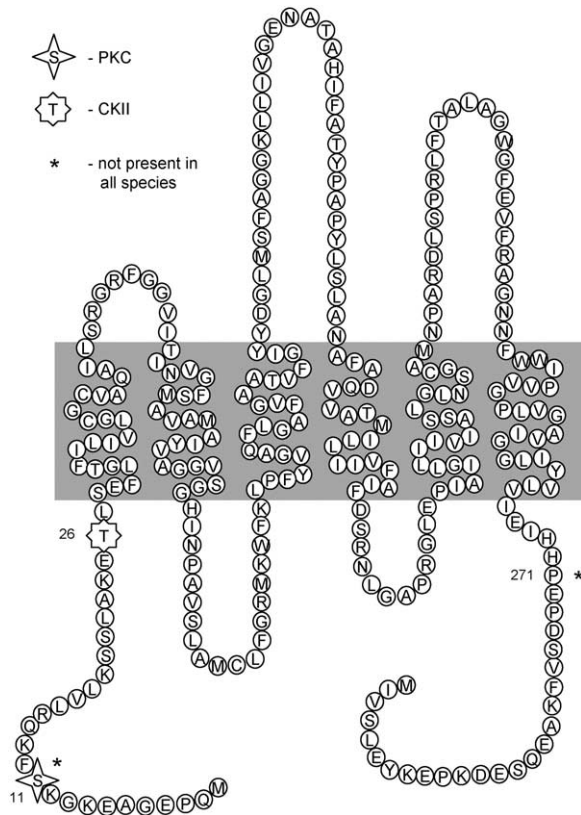


Fig. 5. Putative phosphorylation sites in human AQP9. Only the site for CKII (Thr²⁶) is conserved in human, mouse, and rat. The PKC site at Ser¹¹ is present only in human AQP9, and the CKII site at position 271 is present only in the rodents.

AQP1. AQP1 mRNA appears to be abundantly expressed in choroid plexus throughout fetal life and remains high in the mature animal (Bondy et al., 1993).

The developmental pattern of AQP4 protein expression in the brain has been studied in rat cerebellum (Wen et al., 1999). AQP4 protein immunosignals were first detected in the second week of postnatal life and increased gradually till adulthood.

AQP4 has two isoforms that differ in the length of NH₂-terminus, the longer AQP4.M1 and the shorter AQP4.M23 (Jung et al., 1994; Lu et al., 1996). Studies in mice have revealed the presence of at least two mRNAs that encode the M23 protein isoform, AQP4.M23 and AQP4.M23X mRNA (Zelenin et al., 2000). This opens the possibility that the transcriptional regulation of AQP4 protein can be tissue-specific. Indeed, the AQP4.M23X mRNA was found to be predominantly expressed in the brain and to be developmentally regulated with higher levels in the adult than in infant mouse.

There have been no reports on the developmental profile of AQP9.

Hormonal regulation of AQP expression. Two glucocorticoid response elements have been identified in AQP1 promoter region, and corticosteroid exposure has been shown to induce AQP1 protein expression in mouse

erythroleukemia cells (Moon et al., 1997). In sheep fetal lung, prenatal cortisol infusion significantly increased AQP1 mRNA levels (Liu et al., 2003). AQP1 mRNA expression was also shown to be upregulated by corticosteroids in rat peritoneum (Stoenoiu et al., 2003). Hypothyroidism in rabbits increased the expression of AQP1 and the water permeability in the developing renal brush border (Mulder et al., 2003). In renal inner medullary collecting duct cell line, expression of AQP1 was induced by AVP in the presence of hypertonic medium (Jenq et al., 1998). AVP did not have such an effect in proximal tubular cells (Jenq et al., 1999). The effect of corticosteroids or other hormones on AQP1 expression in choroid plexus has not yet been reported.

The effect of corticosteroids on AQP4 expression is somewhat controversial. In fetal rat lung glucocorticoids and β -adrenergic agonist increase the AQP4 mRNA levels (Umenishi et al., 1996; Yasui et al., 1997). When cortisol was prenatally infused in sheep (Liu et al., 2003), any significant increase in AQP4 mRNA expression could not be detected in lung. Corticosteroids had no effect on AQP4 mRNA and protein abundance in astrocyte primary culture (Gu et al., 2003). In a study of human edematous brain tumors in adults, dexamethasone treatment did not seem to alter the AQP4 protein expression in surrounding non-neoplastic brain tissue (Saadoun et al., 2002).

It is well known that the capacity of corticosteroids to enhance protein expression is particularly high in the perinatal period. So far there is no report of the effect of corticosteroid on AQP expression in perinatal or infant brain. Nor has the effect of β -adrenergic agonists on brain AQP4 mRNA and protein been reported yet.

The promoter region of AQP9 contains putative glucocorticoid response element, and a negative insulin response element (Tsukaguchi et al., 1999; Kuriyama et al., 2002). AQP9 mRNA expression in rat epididymis has been shown to be modulated by androgens (Pastor-Soler et al., 2002). Insulin was found to downregulate AQP9 mRNA in cultured hepatocytes, while insulin deficiency resulted in increased hepatic AQP9 mRNA expression (Kuriyama et al., 2002). No data are available so far on hormonal regulation of brain AQP9.

Regulation via interacting proteins. The polarized AQP4 expression in astrocytes is dependent on the interaction of AQP4 with the dystrophin complex. MDX mice, lacking the dystrophin complex, demonstrated a strong reduction in the expression of AQP4 protein in astrocyte perivascular endfeet (Frigeri et al., 2001). In mice lacking α -syntrophin, the astrocyte perivascular endfeet lack AQP4. In contrast, AQP4 is preserved or even upregulated in other regions of the astrocyte plasma membrane (Neely et al., 2001; Amiry-Moghaddam et al., 2004). Lack of syntrophin does not affect AQP4 expression in peripheral tissues. The presence of an interacting protein may have an important influence on the regulation of activity and localization of the water channel. Therefore it will be important to identify what other proteins may be assembled in AQP4-containing microdomains.

Regulation by hypoxia. We have in ongoing studies identified a hypoxia inducible factor binding motif in the promoter region of AQP4 gene (Zelenin, unpublished observations). In astrocyte cultures, hypoxia was shown to down-regulate mRNA and protein content for AQP4 and AQP9 (Yamamoto et al., 2001; Fujita et al., 2003). Reoxygenation resulted in a rapid restoration of the AQP levels. In rats exposed to focal cerebral ischemia, the expression of AQP4 and AQP9 mRNA was increased in the peri-infarcted cortex (Taniguchi et al., 2000; Badaut et al., 2001). In human brain with cerebral infarction, AQP4 immunoreactivity was also found to be increased (Aoki et al., 2003). It is possible that the switch from hypoxia to normoxia that occurs at birth may trigger transcription of AQP4.

Regulation by osmolality. The AQP1 promoter contains a hypertonicity response element (Umenishi and Schrier, 2002). Exposure to hypertonicity increases AQP1 expression in peritoneal tissues (Ota et al., 2002), in mouse fibroblasts (Leitch et al., 2001) and in cultured renal proximal and inner medullary cells (Jenq et al., 1998, 2002; Umenishi and Schrier, 2003). The effect of hypertonicity may be mediated by promoter-mediated activation of AQP1 synthesis as well as by inhibition of AQP1 protein degradation (Leitch et al., 2001).

Hyperosmotic stress induced by mannitol increased the expression of AQP4 and AQP9 in cultured rat astrocytes, and intraperitoneal infusion of mannitol increased AQP4 and AQP9 expression in the rat brain cortex (Arima et al., 2003). Both AQP4 and AQP9 immunosignals were increased under the pial surface and along the blood vessels. The promoter region of AQP9 has been shown to contain putative hypertonicity response element (Tsukaguchi et al., 1999), but no such data are available so far for AQP4.

Regulation by ammonia. Elevated ammonia levels are observed in fulminant hepatic failure, a condition that is associated with brain edema. Exposure of cultured astrocytes and brain slices to high concentrations of ammonia results in cell swelling (reviewed in Rama Rao et al., 2003). Similarly, studies in animals with acute hepatic encephalopathy have shown swelling of astrocytes. The mechanism by which ammonia induces astrocyte swelling is not completely understood, but a recent study suggests that upregulation of AQP4 protein expression may play a role in this process (Rama Rao et al., 2003).

Regulation by ubiquitination. Ubiquitination is a versatile tool used by eukaryotic cells to control stability, function, and intracellular localization of proteins (Horak, 2003; Schwartz and Hochstrasser, 2003). Ubiquitin is a 76-amino acid polypeptide that is highly conserved and expressed in all eukaryotic cells. When conjugated to proteins, it serves to target them for degradation by cytosolic proteasome complex. Ubiquitination of certain plasma membrane proteins can also promote their internalization via endocytotic pathway, followed by their degradation in lysosomes. It was recently demonstrated that water channels can undergo ubiquitination (Leitch et al., 2001). Mono- and polyubiquitinated species of AQP1 were detected in

AQP1-expressing cells. Exposure of cells to hypertonic medium, which increases AQP1 abundance, resulted in a decrease in AQP1 ubiquitination and increase in AQP1 protein half-life time.

Regulators of brain AQPs as putative therapeutic tools in brain edema and other diseases

Evidence from studies of transgenic mice suggests that water flow via astrocyte AQPs is beneficial under normal conditions but may be harmful under certain pathological conditions. On one hand AQP4 is required for optimal buffering of extracellular potassium (Amiry-Moghaddam et al., 2003b). The outflow of potassium into the interstitial space, which occurs in association with an action potential, is rapidly cleared by uptake into astrocytes facing the microvessels. A concomitant water flow via AQP4 appears to facilitate this buffering process. Studies on transgenic mice have indicated that low expression of AQP4 attenuates the potassium buffering capacity and increases the susceptibility to epileptic seizures. On the other hand there is substantial experimental evidence that in brain edema the presence of AQP4 may actually worsen the condition. Mice lacking AQP4, or lacking syntrophin, the AQP4 anchoring protein, are significantly less prone to develop brain edema triggered by hyponatremia or ischemia (Manley et al., 2000; Vajda et al., 2002; Amiry-Moghaddam et al., 2003a). Brain edema is a common complication to a variety of diseases, such as infections, hyponatremia, ischemia and trauma. It is harmful to neurons and is a common cause of permanent brain damage. Children are particularly prone to diffuse brain swelling, e.g. after trauma. Treatment of brain edema rests on empirical grounds and has changed little during the last three decades. Several lines of evidence suggest that AVP acting on V1 receptors can enhance water uptake in AQP4 expressing astrocytes (see above). This raises the question whether AVP V1 antagonists or inhibitors of intracellular signaling molecules activated by AVP V1 receptors should be used therapeutically to down-regulate the activity of AQP4 in brain edema. As discussed above, the V1 receptor activation effect on AQP4 function may be either direct, i.e. via phosphorylation of AQP4, or indirect, via an interaction between AQP4 and α -syntrophin or other, yet to be defined, proteins.

The fact that AQP4 carries consensus sites for PKA, PKC and CaMKII, makes it also an urgent question to examine how other first messengers may influence the activity and intracellular localization of AQP4. Of great interest are the metabotropic glutamate receptors mGlu1 and 5 that can initiate a release of calcium from intracellular stores. It will also be an important task to examine if regulators of whole body volume homeostasis may have an effect on fluid homeostasis in the brain and in particular the water permeability of AQP4. Atrial natriuretic peptide, dopamine and angiotensin belong to this group of first messengers.

Glucocorticoids have frequently been given to patients with hypoxia or tumor-associated brain edema. The effect has at best been considered moderate. The effect of glucocorticoids on AQP4 is still controversial. If glucocorticoids would stimulate the expression of AQP4 in brain, their use in brain edema might for the reasons given above

be contraindicated. Glucocorticoids are known to enhance the transcription of many enzymes and transporting proteins in newborn infants. Often the sensitivity to glucocorticoids diminishes during maturation. It is therefore of importance to examine possible effects of glucocorticoids on brain AQP4 in the perinatal period and to consider their possible adverse effects in posthypoxic infants, which often develop some degree of brain edema.

As mentioned above, under normal conditions AQP4 is considered to be required for optimal buffering of potassium. There is evidence that low expression of AQP4 in astrocyte membranes facing microvessels may predispose to epileptic seizures. Genetic studies of AQP4 polymorphism in patients with undefined epilepsy are not yet available, which at the present time precludes speculations about new therapeutic strategies in putative conditions associated with reduced expression of functional AQP4 in astrocyte membranes facing microvessels.

High expression of AQP4 is found in the supraoptic nucleus and other osmosensor regions of the brain (Nielsen et al., 1997). The molecular mechanisms behind the function of brain osmosensors remain to be elucidated and this is an important area for future studies. The cause of inappropriate AVP release in many acute conditions including meningitis is obscure. This inappropriate secretion can result in life-threatening water retention and hyponatremia. From this follows that the question whether and to which extent water transport via AQP4 might contribute to the osmosensor function of the brain should be of high priority.

The involvement of the choroid plexus in conditions such as nonobstructive hydrocephalus is incompletely understood. Inhibition of transporting proteins in this organ has empirically been used as a strategy for symptomatic treatment. Since AQP1 is present in the apical membrane of epithelial cells in the lateral and fourth ventricle choroid plexus, it has been suggested to contribute to water transport across this membrane during the secretion of cerebrospinal fluid (CSF). The finding that CSF production is impaired in AQP1-null mice (Oshio et al., 2003) supports this concept. Inhibitors of AQP1 activity or downregulation of AQP1 protein expression may offer an interesting alternative way of treating hydrocephalus. AQP9 appears to be mainly expressed in cells surrounding the cerebral ventricles, including the ependymal cells and the tanycytes of the mediobasal hypothalamus. Thus, the activity of AQP9 may also influence the production of CSF. More knowledge about the regulation of AQP9 activity and expression may therefore lead to new possibilities to symptomatically treat hydrocephalus.

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