REVIEW

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Glymphatic system and aquaporin-4 in epilepsy

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Over the past decade glymphatic concept has gained more and more interest. Despite some lacking data regarding structural and functional aspects, glymphatic system is widely considered the main mechanism of water and solutes transport in brain parenchyma, as well as waste clearance from the brain. Glymphatic system modulates the extracellular space volume and is involved in spatial K^+ buffering (*via* influencing Kir4.1 channel functioning), two factors crucial for neuronal excitability and seizure susceptibility, and is itself strongly stimulated during sleep. This review summarizes information regarding the potential role of the glymphatic system in the development and progression of epilepsy, especially the role of the glial water channel aquaporin-4 in modulation of brain excitability and in epilepsy. Data from animal models and human studies are presented.

Key words: AQP4, interstitial fluid flow, perivascular space, astrocytic endfeet

INTRODUCTION

Throughout the body, tissues undergo constant clearance of metabolic waste and excess fluid (Miteva et al., 2010). In most tissues, the interstitial fluid, an ultrafiltrate of plasma penetrating through blood vessel walls, is then drained into lymphatic capillaries and back to blood circulation (Scallan et al., 2016; Breslin et al., 2018). In the brain, however, there is no direct flow between blood and nervous tissue. Blood is separated from the brain by the blood-brain barrier (BBB) in most regions, and from cerebrospinal fluid (CSF) by the CSF barrier (Damkier et al., 2013; Daneman and Prat, 2015; Liddelow, 2015). The waste clearance and homeostasis is supported by brain-specific feature, namely the glymphatic system, and generally consists of the circulation of the CSF between subarachnoid areas through brain parenchyma, mixing with interstitial fluid, then exiting the central nervous system and draining into the lymphatic system together with metabolic waste collected on the way (Iliff et al., 2012). The CSF enters the brain parenchyma through periarterial spaces created by the meninges around penetrating arteries (so-called Virchow-Robin spaces), and flows along narrowing Virchow-Robin spaces. It is not clear how the fluid travels when the periarterial spaces disappear. It has been shown that there exists a residual space between the vascular and glial basement membrane at the capillary level (Hannocks et al., 2018). Possibly, the flow is directed through the basal lamina of arterioles, surrounded by aquaporin-4 (AQP4)-expressing astrocytic endfeet, covering whole vasculature and creating the external boundary of the perivascular space (Wolburg et al., 2009). The coverage of the perivascular space with astrocytic endfeet is thought to be 63-99% (Wolburg et al., 2009; Mathiisen et al., 2010; Korogod et al., 2015) with 20-50 nm uncovered gaps (Mathiisen et al., 2010). However, recent studies using cryofixation showed that the extent of coverage might be much smaller, leaving clefts *ca* 1 µm wide (Korogod et al., 2015).

From the loose matrix of periarterial space, the CSF is transported to much denser brain parenchyma transcellularly, through polarized AQP4 water channels of astrocytic endfeet, or paracellularly, through the clefts, mixing with the extracellular fluid of the brain tissue (Mestre, et al., 2018, Rasmussen et al., 2022). This convective bulk flow is facilitated by pulsation, vasomotion, and respiration (Iliff et al., 2013; Kiviniemi et al.,

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2016; Fultz et al., 2019; Rasmussen et al., 2022). As the extracellular matrix structure is complex and not yet fully described, the biophysics of extracellular fluid flow needs further clarification. The interstitial fluid exits the brain parenchyma along the perivenous spaces, transependymal and subependymal routes, and white matter tracts (Bradbury et al., 1981; Morrison et al., 1994; Cserr et al., 1997; Iliff et al., 2012; 2013; Rangroo Thrane et al., 2013; Lei et al., 2017; Rasmussen et al., 2022). Eventually, the CSF/interstitial fluid drains to the peripheral lymphatic system *via* the lymphatic network located in the meninges (Aspelund et al., 2015; Louveau et al., 2015; 2018; Da Mesquita et al., 2018; Ahn et al., 2019) or along cranial nerves (mainly olfactory nerve) to cervical lymphatic vessels (Kida et al., 1993; Nakao et al., 1997; Zakharov et al., 2003; Ma et al., 2017). This process, however, is still very enigmatic. Further research is needed to reveal the interface between the glymphatic and lymphatic systems.

Glymphatic system function, regulation, and pathology

The main function of the glymphatic system is the disposal of harmful proteins such as amyloid-β, tau, or α-synuclein, waste metabolites such as lactate and potassium, and the excess of fluid, from the brain parenchyma (Illif et al 2012). While for some of the proteins, more than one disposal mechanism exists (e.g., local degradation, export across BBB), for others glymphatic transport is the only known way of getting rid of it (Rasmussen et al., 2022).

Another function of the glymphatic system, often underappreciated, is its role in delivering various molecules and nutrients to the brain. The glymphatic system probably extends throughout the brain and spinal cord and can be thus considered a supporting system for brain vasculature. While most of the nutrients enter the brain from the blood, across BBB, the CSF transported along periarterial spaces might help distribute these nutrients to cells. Moreover, CSF itself can be considered an alternative source of glucose (Lundgaard et al., 2015). Also, lipids and their transporter, apolipoprotein E, are widely distributed by the glymphatic system (Rangroo Thrane et al., 2013). Some vitamins (folate) are produced exclusively by the choroid plexus and transported with CSF into the brain parenchyma (Grapp et al., 2013).

Importantly, glymphatic function strongly depends on age, with its peak in young adulthood, it substantially deteriorates in the elderly. Age-dependent decrease in the clearance rate of MRI tracers is observed in older patients in comparison to young (Kress et al., 2014; Zhou et al., 2020). A similar phenomenon was observed in rodents, with a 40% reduction in β-amyloid clearance rate in aged brain (Kress et al., 2014). Whether this decrease depends on disorders of the glymphatic system *per se* (e.g., changes in AQP4 expression/localization), or rather is a result of changes in influx/drainage pathways (blood and lymphatic vessels), remains to be elucidated (Albeck et al., 1998; Illif et al., 2013; Mestre et al., 2018; Zeppenfeld et al., 2017). Some data, however, point to the possibility that AQP4 changes are responsible for the worsening of glymphatic function observed in aged animals. AQP4 gene expression was found increased in the cerebellar and cerebral cortexes (Gupta and Kanungo, 2013), and in the CA1 field of the hippocampus (Bronzuoli et al., 2019) of aged mice. It was shown that AQP4 polarization around large penetrating arterioles was lost in ageing mice brains due to decrease of expression in astrocytic endfeet and increase in the processes of astrocytes localized closely to the vessels. This phenomenon was very pronounced in the cortex and in the hippocampus (Kress et al., 2014). Age-dependent increase of AQP4 protein expression was confirmed in human *post mortem* nerve tissue (Zeppenfeld et al., 2017; Owasil et al., 2020). Interestingly, the perivascular endfeet AQP4 localization was preserved in old individuals who remained cognitively intact, and was severely disturbed in Alzheimer brains (Zeppenfeld et al., 2017).

How the glymphatic flow is regulated is not yet thoroughly investigated. Given that perivascular flow is closely apposed to brain vasculature, it seems plausible that it might be regulated jointly. Indeed, cardiovascular pulsation is observed in CSF flow in periarterial spaces (Kiviniemi et al., 2016; Mestre et al., 2018). Dobutamine, a β-adrenergic agonist, increases heart rate and blood flow and also stimulates CSF flow as well as glymphatic influx (Illif et al., 2013). Glymphatic efflux, on the other hand, is tightly dependent on intraventricular CSF flow, which, in turn, depends on the respiratory cycle. Respiratory-driven venous pulsation propagates along veins from the cortical areas to the brain center (Kiviniemi et al., 2016). How exactly the respiratory cycle drive glymphatic efflux is not clear. Venous pulsations create pressure differences between arterial and venous compartments, which possibly drive fluid efflux from the brain in a way similar to the regulation of glymphatic inflow by the cardiovascular cycle (for a thorough review see Rasmussen et al., 2022).

Another possible mechanism of glymphatic system regulation is vasomotion, that is, vasoconstriction/dilation cycles of low frequencies observed in arteries and arterioles of the brain, driven by neuronal activity (Iadecola, 2017; Mateo et al., 2017). It is proposed that CSF

flow in the ventricles depends on changes in cerebral blood volume (arterial, capillary, and venous vascular volumes summed) that are temporally correlated to oscillations in neuronal activity (van Veluw et al., 2020; Goodman et al., 2020). A substantial increase in CSF inflow through the fourth ventricle is observed during sleep, specifically in the non-rapid eye movement phase (NREM). It is correlated with the decrease in BOLD signal in fMRI studies, that is, with decreased blood flow (Fultz et al., 2019). It is postulated that decreased blood volume and arterial constriction facilitates CSF influx through perivascular spaces and glymphatic flow, thus increasing clearance (Rasmussen et al., 2022). Also, during sleep, the extracellular volume increases by 60%, resulting in lowering the flow resistance across brain parenchyma in comparison to a wakeful period (Xie et al., 2013). This enhancement of glymphatic function depends on the noradrenergic activity and is observed not only in the natural sleep state but also during anesthesia in animals anesthetized with xylazine/ketamine or isoflurane/dexmedetomidine, and not with isoflurane alone (Xie et al., 2013; Hablitz et al., 2019). Interestingly, CSF influx, glymphatic flow, and interstitial fluid drainage depend not only on arousal state but are regulated by an internal circadian clock. In anesthetized mice, the glymphatic flow was higher when measured during the day than at night and depended on rhythmic alterations in AQP4 polarization in astrocytic endfeet (Hablitz et al., 2020).

Sleep appears to be a potent regulator of waste clearance from the brain. Sleep disturbances and co-occurring glymphatic clearance deficits, are observed in the course of many pathological states, both acute (e.g., stroke or traumatic brain injury), and chronic (Alzheimer's disease, migraine, or epilepsy) (Gaberel et al., 2014, Lucey and Bateman 2014, Hermann and Bassetti 2016, Peng et al., 2016, Cedernaes et al., 2017; Schain et al., 2017; Piantino et al., 2019). Dysfunctions of the glymphatic system have also been shown in several other human conditions, including hydrocephalus, Parkinson's disease, dementia, and multiple sclerosis. For an excellent and thorough review of studies on the relationship of sleep and glymphatic disturbances in brain pathologies see Rasmussen et al., 2018; Christensen et al., 2021; Hanke et al., 2022.

Glymphatic system in epilepsy

The term "epilepsy" covers several common neurological disorders of various etiology, characterized by the occurrence of unprovoked seizures. It is one of the most common neurological diseases affecting 65 million people worldwide (Clossen and Reddy, 2017). According to a Written Declaration on Epilepsy In Europe, 6 million European citizens suffer from epilepsy (EU Written Declaration on Epilepsy 22-2011, https://www.epilepsyallianceeurope.org/european-parliament/eu-written-declaration-on-epilepsy/ accessed 13.10.2023). Despite worldwide efforts towards finding effective therapy, still, 30% of epilepsy cases are resistant to treatment. Epilepsy may be genetically determined or acquired, the latter occurring after such epileptogenic events as traumatic brain injury, *status epilepticus,* infection or stroke (Pitkänen and Lukasiuk, 2011). Interestingly, these events are associated with perturbations in glymphatic system functioning (Toh and Siow, 2021; Lee et al., 2022a; Park et al., 2023). While causes and factors influencing the development of epilepsy are most probably multifactorial, it is plausible that glymphatic system impairment might be one of them. The development of new variants of conventional magnetic resonance imaging (MRI), namely diffusion tensor imaging (DTI) based on water movement in the tissue, and its modification, diffusion tensor image analysis along the perivascular space (DTI-ALPS), made it possible to follow the diffusivity specifically along perivascular spaces, and glymphatic activity (Taoka et al., 2017). However, to date, there is a limited number of studies investigating glymphatic function in epileptic patients (see Table 1). It was shown that brain edema following *status epilepticus* in mice resulted in temporary glymphatic system impairment with p-tau aggregation (Liu et al., 2021). An early study by Betting et al. (2006) showed, among many other MRI abnormalities, prominent perivascular spaces in patients with idiopathic generalized epilepsy. No specific relation between any abnormality and epilepsy onset or severity was observed. In another study, patients with childhood epilepsy with centrotemporal spikes (formerly known as Rolandic epilepsy) were examined. No differences between patients and controls were revealed in perivascular spaces (Boxerman et al., 2007). In this study, however, children with migraine were chosen as controls instead of healthy subjects. A high incidence of dilatated Virchow-Robin spaces was observed in children with epilepsy (Biedron et al., 2014). Recently, Liu et al. (2020) showed that Virchow-Robin spaces are enlarged in a subpopulation of children with idiopathic generalized epilepsy. However, a spatial correlation between reduced perivascular space and the potential epileptic focus was found in patients with focal epilepsy (Feldman et al., 2018). Moreover, fewer enlarged perivascular spaces (EPVS) were observed in TBI patients with epilepsy than in TBI patients without epilepsy (Hlauschek et al., 2023). DTI-ALPS analysis in young adult patients with juvenile myoclonic epilepsy (JME) demonstrated a lower ALPS-index than in healthy controls, which suggested glymphatic system dysfunction (Lee et al., 2022b). In this same study a negative correlation between age and ALPS-index was

Table 1. Human epilepsies with described glymphatic system disturbances.

Type of epilepsy	Glymphatic disturbance	References
Temporal lobe epilepsy with hippocampal sclerosis	DTI-ALPS index in TLE patients with hippocampal sclerosis lower than in healthy controls	Lee et al., 2022c
	Increased AQO4 levels in sclerotic hippocampal tissue	Lee et al., 2004
Temporal lobe epilepsy	Decreased DTI-ALPS index	Zhao et al., 2023
	Decreased ALPS indices observed in a manner dependent on the side of the brain affected	Zhang et al., 2023
Juvenile myoclonic epilepsy	Lower DTI-ALPS-index in epileptic patients than in healthy controls	Lee et al., 2022b
TBI-induced epilepsy	Fewer enlarged perivascular spaces (EPVS) in TBI patients with epilepsy than in TBI patients without epilepsy	Hlauschek et al., 2023
Idiopathic generalized epilepsy	Increased perivascular spaces	Betting et al., 2006, Liu et al., 2020
Childhood epilepsy with centrotemporal spikes	Unchanged perivascular spaces (compared to children with migraine)	Boxerman et al., 2007
Focal epilepsy	Correlation between reduced perivascular space and the potential epileptic focus	Feldman et al., 2018

shown, demonstrating an age-dependent decline of glymphatic activity in JME patients. Decreased ALPS-index and glymphatic system perturbations were also reported in temporal lobe epilepsy (TLE) with hippocampal sclerosis, in occipital lobe epilepsy, and in patients with *status epilepticus* (Lee et al., 2022a; 2022c; Kim et al., 2023), but not in newly diagnosed TLE patients (Lee et al., 2022d). In another study, decreased ALPS indices were observed in TLE patients in a manner dependent on the side of the brain affected. In right-TLE patients, both left and right ALPS indices were decreased, while in left-LTE patients only the left one was reduced. Interestingly, glymphatic system asymmetry was present in healthy persons, with left ALPS higher than right APLS (Zhao et al., 2023). A convincing argument in favor of the hypothesis of the interdependence of epilepsy and glymphatic system was provided by Zhang et al. (2023). In their study, DTI ALPS indices were assessed in TLE patients before and after a standard resection of the left anterior temporal lobe, bearing the epileptogenic focus. Before surgery, left ALPS index was decreased compared to the right one and also to the left index of healthy controls, suggesting impaired glymphatic clearance, while after resection it returned to normal levels. There is a colorable discrepancy between results showing EPVS and decreased ALPS-indices in epileptic patients. Enlarged perivascular space intuitively should lead to increased diffusivity and facilitated glymphatic flow, while decreased ALPS points to the opposite. Clinical data showed that EPVS are present in small vessel disease-affected brains and co-occur with glymphatic clearance disturbance (Mestre et al., 2017). Moreover, EPVS are a typical sign of ageing (Chen et al., 2011) and are observed also in preclinical phase of Alzheimer's disease (Lynch et al., 2022), both states characterized by reduced glymphatic clearance. These features suggest that EPVS may be a sign of glymphatic fluid stasis, possibly due to overload of the perivascular drainage system (Weller et al., 2009).

The mechanisms of glymphatic system impairment in epilepsy are elusive. It is postulated that cerebrovascular damage and BBB dysfunction is an important causative factor underlying both seizure activity and disturbances in interstitial fluid (ISF) composition and circulation (van Vliet et al., 2007; 2014; Janigro and Walker, 2014; Marchi et al., 2016). Animal MRI studies revealed gadolinium contrast leakage into brain parenchyma after kainic acid-induced chronic seizures (van Vliet et al., 2014). Interestingly, acute seizures were observed after BBB disruption (Marchi et al., 2016), suggesting reciprocal causative relation between seizures and BBB dysfunction. It is conceivable that initial rupture of BBB, resulting in serum protein extravasation and alteration of ionic balance in ISF, disturbs ISF flow and reduces brain clearance. This perturbation, however, may persist beyond the strict time window of the leakage, due to prolonged time of waste or ion disposal. The inefficient clearance may result in occurrence of the seizures, which, in turn worsen the glymphatic system dysfunction, possibly leading to a vicious cycle.

Another factor linking epilepsy to glymphatic system functioning is its dependence on the sleep-wake cycle. Sleep deprivation increases cortical excitability and thus has been suggested as a risk of seizure in epileptic patients (Mattson et al., 1965; Pratt et al., 1968; Mallow, 2004; Badaway et al., 2006, for a review see Dell'Aquila and Soti, 2022). Some patients, e.g., with ju-

venile myoclonic epilepsy, are very sensitive to sleep deprivation (Sousa et al., 2005; Xu et al., 2018). Clinically, deprivation from sleep has been used as a method to diagnose early onset epilepsy in children, otherwise having normal awake EEG recordings (Kubicki et al., 1991; Shahar et al., 2010). Different seizure types in pediatric patients tend to occur preferentially during sleep or during wake, with tonic and tonic-clonic seizures more frequently seen in sleep and other generalized seizure types occurring more frequently during wakefulness (Loddenkemper et al., 2011). According to another study, 80-92% of examined patients with epilepsy showed circadian (24 h) modulation of their seizure rates (Karoly et al., 2018). Long-term EEG monitoring studies revealed that interictal epileptiform discharges (IEDs, isolated electrophysiological events that occur between seizures) occur in a time-specific manner. A meta-analysis based on ten publications demonstrated the preferential appearance of IEDs during NREM phases of sleep (with a higher incidence in stage 3 of NREM) and their repression in rapid eye movement (REM), except the transition to wakefulness phase (Bazil, 2003; Ng and Pavlova, 2013; Ng, 2017). While these observations may be possibly explained by wave synchronization typical for NREM, it cannot be excluded that impaired glymphatic activity and waste clearance can attribute to these phenomena. There is no undisputable proof of the causal link between the glymphatic system and epilepsy; however, the complicated and mutual relationship between sleep and seizures points to such a possibility. Sleep problems are common in epilepsy patients. Aside from the factors that are common for the whole population, seizures themselves can disrupt sleep, even when they occur during wakefulness (Bazil, 2003). Increased frequency of seizures affects overall brain activity and sleep architecture, decreasing the proportion of NREM sleep (Bruni et al., 2010), and possibly affecting glymphatic function. Sleep deprivation impairs the clearance of misfolded proteins like α-synuclein, amyloid-β, and tau (reviewed in Bishir et al., 2020). Importantly, depositions of p-tau and α-synuclein have been observed in temporal lobe epilepsy patients suggesting cerebral waste accumulation and glymphatic system impairment (Yang et al., 2006; Tai et al., 2016; Paudel et al., 2020).

Despite the apparent interrelation between epilepsy, sleep and glymphatic system, still, the mechanism explaining how these phenomena are intertwined together, is unclear. Recently, brain orexinergic system has been proposed as a link. Orexinergic neurons, residing in the lateral hypothalamic area, are responsible for regulating sleep/wakefulness phases (Scammell and Winrow, 2011). They project widely to both subcortical areas and the cortex, secreting two neuropeptides, orexin A and orexin B (also known as hypocretin-1 and hypocretin-2, respectively), which activate two receptors: OX1R and OX2R (Peyron et al., 1998; Sakurai et al., 2010). Orexinergic phasic activity (orexin bursts) promotes arousal and is observed in state transitions; tonic activity is highest in awake states, low in NREM phase of sleep, and no activity is observed in REM phase (Tsujino and Sakurai, 2009; Schwartz and Kilduff, 2015). In line with these observations, in feline model of kindling REM is the most difficult phase during which the seizures can be induced (Calvo et al., 1982; Shouse et al., 1986; Calvo 1991). According to a systematic review of 42 distinct studies, only 1% out of 2000 seizures analyzed were observed during REM phase with strong cortical desynchronization (Ng and Pavlova, 2013). In contrast, weak tonic orexinergic activity promoting slow wave sleep (NREM phase) increases cortical synchronization and seizure incidence (Ng and Pavlova, 2013; Kohyama et al., 2000). Apparently, lack of orexinergic activity, associated with cortical desynchronization is protective against seizures (Ng, 2017).

The majority of the animal studies confirm the detrimental role of orexin in the pathology of epilepsy. Intracortical injections of orexin A and B increase epileptic activity in the rat model of penicillin-induced seizures (Kortunai et al., 2012). Also, intracortical injection of orexins alone induces epileptic seizures observed both in EEG and behaviorally (Erken et al., 2012). Moreover, administration of orexin A directly to the hippocampus results in increasing of the firing rate of CA1 neurons (Chen et al., 2017). Consistently, orexinergic antagonists have been shown to be preventive against seizures, e.g., in animal PTZ models (Ni et al., 2014; Goudarzi et al., 2015; Socała et al., 2016; Kordi et al., 2017). Few studies show opposite results (Doreulee et al., 2010; Zhao et al., 2014). Human studies concerning orexin in epileptic patients are scarce and based on CSF measurements of orexin level. One large case-control study, primarily focused on narcolepsy type-1 patients, revealed decreased CSF orexin A levels in epileptic patients after generalized tonic-clonic seizures (Ripley et al., 2001). Similarly, lower levels of orexin A were observed in patients 48 h after single or repetitive seizures, compared to controls (Rejdak et al., 2009). Lowered levels of orexin A were also observed in patients with generalized convulsive *status epilepticus*, 3-10 days after the last seizure (Samzadeh et al., 2020). Few clinical studies analyzed orexin A levels in blood of epileptic patients. Interestingly, it appeared that blood levels of orexin A were increased seizures when measured 4 h after seizures, possibly due to the leakage of orexins to the circulation across compromised BBB (Kaciński et al., 2012; Çikriklar et al., 2020). While

orexin activity clearly shapes brain excitability, which in turn, has the ability to modify the ISF flow, experimental data concerning direct involvement of orexins in regulating the glymphatic system functionality are yet unavailable. The idea of glymphatic impairment in epilepsy is supported by the observation that low molecular weight insoluble tau levels are increased in neurons, perivascular regions around penetrating pial vessels and meninges in epilepsy patients (Marchi et al., 2016; Puvenna et al., 2016). On the other hand, sleep disturbances, often reported in epilepsy, result in significant increases in CSF tau and phospho-tau levels (Sato et al., 2019; Barthélemy et al., 2020). Altogether, given the apparent relationship between disturbed sleep, proconvulsive effect of orexin and increased deposition of tau in epileptic brain, it seems plausible that orexinergic system does play a role in epilepsy pathology.

Aquaporin-4 in epilepsy

Aquaporins are a family of water-permeable integral membrane proteins comprising 13 (AQP4-AQP12) members, with AQP1, AQP4, and AQP9 expressed in the brain (Oshio et al., 2004; 2005; Zelenina, 2010). AQP4 is a predominant water channel in the brain and is highly enriched in astrocytic endfeet in contact with cerebral blood vessels and in astrocytic membranes ensheathing glutamatergic synapses (Rash et al., 1998; Oshio et al., 2004; Nagelhus and Ottersen, 2013). This distinctive, non-uniform pattern of AQP4 expression in the plasma membrane of astrocytes is observed throughout most brain regions and is often referred to as AQP4 polarization (Nielsen et al., 1997; Rash et al., 1998). Within areas of high density, AQP4 reveals the tendency to aggregate into larger complexes, tetramers forming additional central pores, which in turn are incorporated into higher order assemblies termed orthogonal arrays of particles (OAPs) (Neely et al., 1999; Jin et al., 2011). Of nine known AQP4 isoforms, two, namely AQPc (M23) and AQPa (M1), are most frequently found in the complexes localized to astrocytic endfeet (for a comprehensive review see Jorgacevski et al., 2020). The localization and positioning of AQP4 OAPs or monomers in the astrocytic plasma membrane is mediated indirectly by several extracellular or membrane proteins, namely agrin, laminin, α- and β-dystroglycan, dystrophin and α-dystrobrevin, and directly through interaction of AQP4c (M23) C-terminus with α-syntrophin (Jorgacevski et al., 2020). The role of AQP4 in water transport and the regulation of glymphatic flow has been recently established (Gomolka et al., 2023), the function of OAPs, however, still needs elucidation. It's been proposed that higher-order organization of AQP4 in OAPs increases single-channel osmotic water permeability, enables oxygen exchange, or facilitates clearance of harmful molecules from brain parenchyma, but these hypotheses still await confirmation (Potokar et al., 2016).

While the main role of AQP4 is water transport through membranes and regulation of water content in the brain, it has been also suggested in the regulation of K^+ homeostasis. Electron microscopic studies revealed precise colocalization of AQP4 and inwardly rectifying K^+ channel Kir4.1 in astrocytic endfeet membranes (Naghellus et al., 2004). Astrocytic Kir4.1 is thought to be involved in maintaining normal neuronal excitability and mutations in the human *KCNJ10* gene encoding Kir4.1 were reported to cause epileptic disorders (Bockenhauer et al., 2009; Reichold et al., 2010). Interestingly, α-syntrophin knockout resulting in AQP4 mislocalization displayed a delayed clearance of K^+ following high-frequency synaptic stimulation in hippocampal slices (Neely et al., 2001; Amiry-Moghaddam et al., 2003a; Strohschein et al., 2011). Moreover, the same result was observed *in vivo*, in AQP4 knockout mice, following electrical stimulation or during the experimental spreading depression (Padmawar et al., 2005; Binder et al., 2006). The mechanism of this AQP4 coupling to K^+ clearance is, so far, unclear. The expression of Kir4.1 in AQP4 knockout mice is unchanged, so are its basic electrophysiological properties (Binder et al., 2006; Zhang and Verkman, 2008; Hsu et al., 2011). Soe et al. (2009) proposed that Kir4.1 channel is stretch-sensitive and AQP4 affects Kir4.1 function by regulating volume changes resulting in membrane stretch.

Although the most interest of epilepsy researchers is focused on synaptic mechanisms of epilepsy, some studies suggested non-synaptic mechanisms of interactions between neurons involving ephaptic transmission (Jefferys et al., 2012). This kind of transmission refers to communication between adjacent neuronal cells *via* electrical conduction through the surrounding extracellular space and depends on extracellular space (ECS) volume. Alterations in water content and ECS volume strongly influence neuronal activity in experimental models. Increasing ECS volume with hyperosmolar treatment decreases epileptiform activity, and contrarily, lowering osmolality, and subsequent shrinkage of the extracellular space causes enhanced epileptiform bursting (for review see Binder et al., 2012). Similarly, rapid lowering of plasma osmolality (hydration) in clinical situations can promote the hyperexcitability associated with generalized tonic-clonic seizure (Andrew, 1991; Maa et al., 2011). These observations implicate AQP4 as a potential factor regulating brain excitability and epilepsy. Data from

animal models of epilepsy are scarce and incoherent. AQP4 knockout mice exhibit increased seizure threshold in PTZ (pentylenetetrazole) or electrical stimulation models of seizures (Binder et al., 2004; 2006). However, these mice exhibit an increase in seizure duration (Binder et al., 2006; Szu et al., 2020a). In mice that developed epilepsy after traumatic brain injury in the frontal cortex, AQP4 expression was increased and shifted from perivascular endfeet towards neuropil in the region of impact, compared to mice that did not develop seizures (Szu et al., 2020b). Contrastingly, in mice after *status epilepticus* evoked by intrahippocampal injection of kainic acid (KA), AQP4 protein was transiently downregulated in the dorsal hippocampus and significantly increased at chronic time points (Lee et al., 2012; Hubbard et al., 2016). Another study employing systemic KA injection as a model of mesial temporal lobe epilepsy (MTLE) showed that AQP4 mislocalization was observed already in the latent phase (14 days) suggesting a role in epileptogenesis (Alvestad et al., 2013). In this same study, a reduction of α-syntrophin and a marked increase in M1 isoform of AQP4 was observed in the hippocampi of KA-injected rats. Reduction of AQP4 expression was observed also in rats as early as 12 hours after pilocarpine-evoked *status epilepticus* (Kim et al., 2010).

Several human studies also reported alterations in AQP4 expression in epilepsy patients. Increased AQP4 expression in sclerotic tissue of the epilepsy hippocampus was demonstrated using immunohistochemistry and rtPCR. Increased AQP4 expression was correlated with a decrease in expression of the dystrophin gene (Lee et al., 2004). Overall AQP4 protein levels were elevated by 360% in the hippocampi of MTLE compared to non-MTLE patients, however, the density of AQP4 along the perivascular membrane of astrocytic endfeet was decreased by 44% (Eid et al., 2005; Mhatre et al., 2021).

Since AQP4 OAPs are organized by dystrophin-associated protein complex (DAPC) and mislocalization of AQP4 seems to be an important pathogenic factor in epilepsy, it seems plausible that DAPC plays an important role AQP4 functioning and epilepsy pathology. Mice deficient in α-syntrophin showed marked loss of AQP4 from perivascular and subpial membranes and intensified hyperthermia-induced seizures, as well as delayed K^+ clearance (Amiry-Moghaddam et al., 2003b). Likewise, dystrophin-deficient mice exhibit a dramatic reduction of AQP4 in astroglial endfeet which is not accompanied with a decrease in total AQP4 expression (Vajda et al., 2002). Decreased α-syntrophin and dystrophin, but increased AQP4 protein levels were observed in resected human epileptic hippocampi (Das et al., 2012). Dystrophin-deficient mice showed shorter latency and enhanced seizure severity in PTZ kindling (De Sarro et al., 2004). Beta-dystroglycan (βDG) protein was down regulated by acute seizures in hippocampal slices *ex vivo* treated with 0 Mg^+ artificial CSF. This down regulation was accompanied by a decrease in AQP4 protein expression (Gondo et al., 2014). Altogether, these findings, often contradictive, suggest that AQP4 is redistributed in the epileptic brain, the mechanisms of this redistribution, however, need to be further investigated.

So far, no studies combining investigations of AQP4 expression with glymphatic system functioning (e.g., ALPS index or perivascular space MRI visualization) in epilepsy are available in a single experimental or clinical setting. Some information concerning the correlation between the two parameters was acquired from experiments employing mice devoid of aquaporin-4 gene. It appeared that AQP4 KO mice had enlarged interstitial spaces which coincided with reduced glymphatic influx (Gomolka et al., 2023). Also, *AQP4* gene deletion resulted in increases in Aβ accumulation and exacerbation of cognitive deficits of 12-month old APP/PS1 mice (Xu et al., 2015). Some data confirming spatiotemporal concomitance of AQP4 expression decrease with glymphatic disturbance were derived from the experimental hepatic encephalopathy studies (Hadjihambi et al., 2019). In humans, an increase in enlarged perivascular spaces (EPVS) was correlated with high AQP4 CSF levels in patients with dementia. Moreover, both parameters were associated with total tau levels in CSF (Sacchi et al., 2023). The hypothesis of glymphatic system involvement in the pathology of epilepsy drew much attention. There have been a considerable interest in potential antiepileptic drugs targeting AQP4, but no breakthrough though. Several compounds, including tetraethylammonium (TEA^+), bumetanide or carbonic anhydrase inhibitors acetazolamide (AZA) and ethoxyzolamide (EZA) and TGN-020, inhibit AQP4-mediated water transport *in vitro* in *Xenopus* oocytes (Detmers et al., 2006; Huber et al., 2007; Huber et al., 2009; Migliati et al., 2009; Tanimura et al., 2009). Interestingly, some antiepileptic drugs, namely topiramate, lamotrigine, phenytoin and zonisamide were found to be AQP4 inhibitors (Huber et al., 2009). These results, however, were questioned by Yang et al. (2006) as inhibitory role of antiepileptics was not confirmed in AQP4-transfected mammalian cells and primary cultures of brain glial cells.

CONCLUSIONS

The involvement of the glymphatic system in acquired epilepsy pathology seems to be well documented, still, some questions need answering. Most import-

ant, it remains unclear whether observed changes in the glymphatic system or AQP4 polarization are a cause or consequence of the disease. Disturbed K^+ homeostasis and epileptic phenotype in AQP4 knockout mice, as well as early changes in AQP4 expression and localization, preceding the onset of epilepsy in animal models, suggest the first possibility. Establishing causality will be a challenging task, requiring the development of new pharmacological tools targeting the glymphatic system specifically, and new methods of assessment of glymphatic system functioning, possibly in awake, epileptic animals.

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