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ORIGINAL PAPER

Astrocytes and Epilepsy

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Abstract
Changes in astrocyte channels, transporters, and metabolism play a critical role in seizure generation and epilepsy. In particular, alterations in astrocyte potassium, glutamate, water and adenosine homeostasis and gap junctional coupling have all been associated with hyperexcitability and epileptogenesis (largely in temporal lobe epilepsy). Distinct astrocytic changes have also been identified in other types of epilepsy, such as tuberous sclerosis, tumor-associated epilepsy and post-traumatic epilepsy. Together, the emerging literature on astrocytes and epilepsy provides powerful rationale for distinct new therapeutic targets that are astrocyte-specific.

Keywords Astrocyte · Epilepsy · Gap junction · Potassium · Glutamate · Adenosine

Introduction

Epilepsy is a common neurological disorder characterized by the occurrence of unprovoked seizures. Epilepsy is a major public health problem, affecting more than 65 million people worldwide [1]. Healthcare cost estimates associated with epilepsy in the United States range from $9.6 billion to $12 billion per year [2]. Temporal lobe epilepsy (TLE) is the most common form of epilepsy with focal seizures and many patients with TLE develop refractory epilepsies that are pharmaco-resistant to currently available antiepileptic drugs (AEDs) [3, 4]. AEDs work primarily by targeting neurons through modulation of ion channels, enhancement of inhibitory neurotransmission or attenuation of excitatory neurotransmission [5, 6]. Modulation of neurotransmission can consequently lead to dose-dependent "neurotoxic" adverse effects which are common undesired effects associated with AED usage. Adverse cognitive and behavioral effects of AEDs have been shown to lead to AED discontinuation in up to one-third of patients [7]. Moreover, current AEDs only dampen hyperexcitability but do not interfere with the epileptogenic process. Therefore, new non-neuronal targets that could potentially have fewer side effects and weaken the development of the disease should be considered and further investigated.

Epilepsy as an "Astrocytopathy"

Astrocytes play an established role in removal of glutamate at synapses and the sequestration and redistribution of K^+ and H_2O during neural activity [8–10] (Fig. 1). Many studies have shown that changes in astrocyte channels, transporters, and metabolism play a direct role in seizure susceptibility and the development of epilepsy [9, 11–17]. Stimulation of astrocytes leads to prolonged neuronal depolarization and epileptiform discharges [14]. Astrocytes release neuroactive molecules and modulate synaptic transmission through modifications of channels, gap junctions, receptors, and transporters [9, 11, 14, 18–23]. In addition, striking changes in astrocyte form and function occur in epilepsy. Astrocytes adopt reactive morphology [12, 24], become uncoupled [25], and lose domain organization [26] in epileptic tissue. These and other changes such as changes in the expression of various astrocytic enzymes, such as adenosine kinase [27] and

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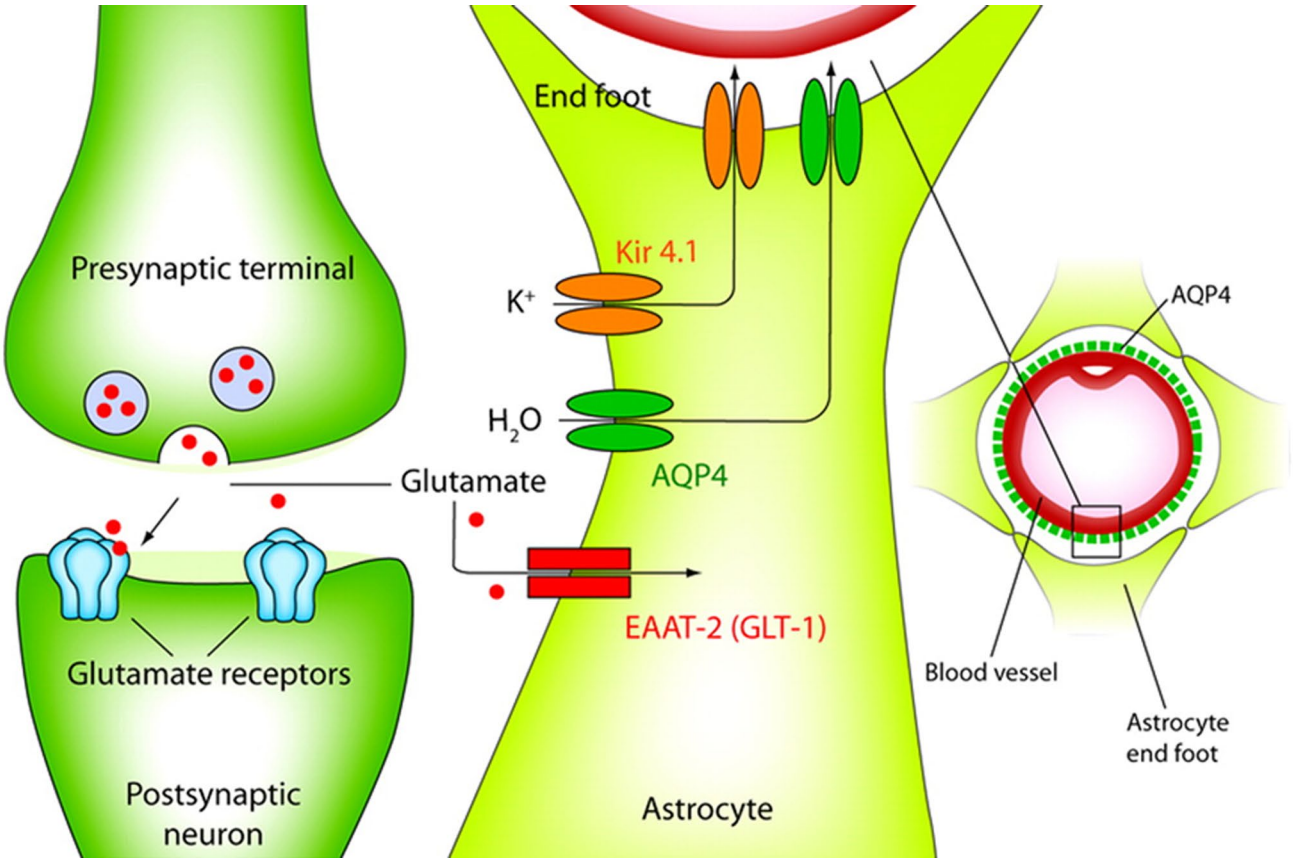

Fig. 1 Astrocyte regulation of water, potassium, and glutamate homeostasis at the tripartite synapse. Colocalization of AQP4, $K_{ir}4.1$ and GLT1 in distinct astrocyte membrane domains (perisynaptic, perivascular) provides the basis for a critical role of astrocytes in control of water, potassium, and glutamate homeostasis. *Reproduced with permission from:* Benarroch E. 2007. Aquaporin-4, homeostasis, and neurologic disease. *Neurology* 69:2266–2268 (Fig. 1)

glutamine synthetase [28], astroglial proliferation, dysregulation of water and ion channel and glutamate transporter expression [9, 29, 30], alterations in secretion of neuroactive molecules, and increased activation of inflammatory pathways [9, 12, 16, 24, 31–34] may all contribute to hyperexcitability and epileptogenesis. Various forms of "astrocytopathy" (reactivity, remodeling, and deleterious alteration in function) have been implicated in neurological diseases other than epilepsy as well [35, 36].

Alterations in Astrocyte Channels, Transporters, and Enzymes

K_{ir} Channels

During neuronal hyperactivity, K^+ released by active neurons is thought to be primarily taken up by astrocytes. Any impairment of astrocyte K^+ uptake would be expected to be proconvulsant: in the hippocampus, millimolar and even submillimolar increases in extracellular K^+ concentration powerfully enhance epileptiform activity. A primary mechanism for K^+ reuptake is via glial inwardly rectifying K^+ channels (K_{ir} channels). Several studies have indicated downregulation of K_{ir} currents in specimens from patients with TLE. Using ion-sensitive microelectrodes, Heinemann's group compared glial Ba^{2+}-sensitive K^+ uptake in the CA1 region of hippocampal slices obtained from patients with or without mesial temporal sclerosis (MTS) [34, 37]. Ba^{2+}, a blocker of K_{ir} channels, augmented stimulus-evoked K^+ elevation in non-sclerotic but not in sclerotic specimens, suggesting impairment in K^+ buffering in sclerotic tissue. Direct evidence for downregulation of K_{ir} currents in the sclerotic CA1 region of hippocampus came from a comparative patch-clamp study in which a reduction in astroglial K_{ir} currents was observed in sclerotic compared to non-sclerotic hippocampi [33]. These data indicate that dysfunction of astroglial K_{ir} channels could underlie impaired K^+ buffering and contribute to hyperexcitability in epileptic tissue [38].

Aquaporin-4 Water Channels

Alterations in astroglial water regulation could also powerfully affect excitability [9]. Brain tissue excitability is exquisitely sensitive to osmolarity and the size of the extracellular space (ECS) [39]. Decreasing ECS volume produces hyperexcitability and enhanced epileptiform activity; conversely, increasing ECS volume with hyperosmolar medium attenuates epileptiform activity. These experimental data parallel extensive clinical experience indicating that hypo-osmolar states such as hyponatremia lower seizure threshold while hyperosmolar states elevate seizure threshold [40].

The aquaporins (AQPs) are a family of membrane proteins that function as "water channels" in many cell types and tissues in which fluid transport is crucial [41]. Aquaporin-4 (AQP4) is expressed ubiquitously by glial cells, especially at specialized membrane domains including astroglial end feet in contact with blood vessels and astrocyte membranes that ensheathe glutamatergic synapses. Mice deficient in AQP4 have markedly decreased accumulation of brain water (cerebral edema) following water intoxication and focal cerebral ischemia [42] and impaired clearance of brain water in models of vasogenic edema [43], suggesting a functional role for AQP4 in brain water transport. In addition, $AQP4^{-/-}$ mice show facilitated hippocampal K^+ spatial buffering [44] but impaired overall K^+ clearance and prolonged seizures in response to in vivo hippocampal stimulation [45]. These data suggest that AQP4 downregulation may trigger hyperexcitability.

Alteration in the expression and subcellular localization of AQP4 has been described in sclerotic hippocampi obtained from patients with MTS. Using immunohistochemistry, rt-PCR and gene chip analysis, Lee et al. demonstrated an overall increase in AQP4 expression in sclerotic hippocampi [46]. However, using quantitative immunogold electron microscopy, the same group found mislocalization of AQP4 in the human epileptic hippocampus, with reduction in perivascular membrane expression [47]. The authors hypothesized that the loss of perivascular AQP4 perturbs water flux, impairs K^+ buffering, and results in an increased propensity for seizures.

Subsequently, very similar AQP4 dysregulation has been confirmed in animal models of epilepsy. In particular, downregulation and/or mislocalization of AQP4 occur during the early epileptogenic phase in the rat pilocarpine [48, 49], rat kainic acid [50] and mouse kainic acid [29, 30] models of epilepsy. Based on these data, restoration of AQP4 homeostasis may represent a novel antiepileptogenic strategy [9].

Glutamate Transport and Metabolism

Astrocytes are primarily responsible for glutamate uptake from the extracellular space via glutamate transporters. These are Na^+-dependent plasmalemmal transporters and as such are dependent on Na^+ concentration and Na^+ fluxes induced by activity [51]. Studies using mice with deletion [52] or antisense oligonucleotide-mediated inhibition of synthesis [53] of the astroglial transporter GLT-1 (also called EAAT2) revealed that this subtype is responsible for the bulk of extracellular glutamate clearance in the brain [54]. Several studies have suggested an involvement of glutamate transporters in seizure development. GLT-1 knockout mice exhibit spontaneous seizures and hippocampal pathology resembling alterations in TLE patients with MTS [52]. A more recent follow-up paper by the same group using region-specific GLT-1 knockouts confirmed spontaneous seizures when GLT-1 was deleted from forebrain astrocytes [55].

What is the evidence for alteration in astrocyte glutamate transporters in human epilepsy specimens and in animal models? Decreased GLT-1 immunoreactivity has been reported in the sclerotic human hippocampus, although GLAST immunoreactivity was reported as unchanged [56] or decreased [57]. These findings support the hypothesis that reduced or dysfunctional glial glutamate transporters in the hippocampus may trigger spontaneous seizures in patients with MTS [58]. In animal models, GLT-1 protein levels have been shown to be downregulated during the development of epilepsy. A study in the mouse intrahippocampal kainic acid model found a significant initial increase in hippocampal GLT-1 immunoreactivity and protein levels 1 day after status epilepticus followed by a marked downregulation at 4 and 7 days post-status epilepticus, a time period during which spontaneous seizures arise in this model [29]. A follow-up study by the same group found that synaptosomal GLT-1 levels, which include components of the tripartite synapse, are also reduced by nearly 80% 1 week following intrahippocampal kainate-induced SE [59]. Another group also found that perisynaptic GLT-1 at the plasma membrane in astrocytes is significantly reduced around CA3-CA1 synapses during the latent period following systemic kainate-induced status epilepticus (SE) [60]. Together, these data suggest that reduction of the pool of GLT-1 transporters available for glutamate uptake at excitatory synapses may precede spontaneous seizures and contribute to epileptogenesis. The therapeutic potential of GLT-1 upregulation in epilepsy remains to be fully investigated.

Another potential mechanism for glutamate dysregulation is loss of the astrocyte enzyme glutamine synthetase (GS) in the sclerotic *vs.* non-sclerotic hippocampus of TLE patients [61]. After uptake of glutamate into astrocytes, this enzyme rapidly converts glutamate into glutamine. In sclerotic TLE hippocampus, downregulation of GS causes slowing of the glutamate-glutamine cycling and accumulation of glutamate in astrocytes and in the extracellular space [61, 62]. In animal models, inhibition of GS via methionine sulfoximine

(MSO) infusion [63] or genetic targeting [64] is sufficient for epileptogenesis. Thus, modulation of GS may represent another antiepileptogenic target [65].

Gap Junctions

Functional coupling analysis, obtained by patch-clamping astrocytes and filling the astrocyte syncytium with a tracer to quantitatively measure astrocyte-astrocyte gap junction coupling, has led to recent seminal findings of astrocyte "uncoupling" in human and animal TLE [25]. In this study, the gap junctional connectivity of astrocytes from 119 specimens from patients with mesial TLE (MTLE) with and without sclerosis were examined. In MTLE specimens with typical hippocampal sclerosis, there is a complete absence of typical "classical" astrocytes and astrocyte gap junctional coupling. In contrast, coupled astrocytes were abundant in non-sclerotic hippocampus. In the intracortical kainic acid model of TLE, mice exhibited decreased astrocytic coupling already 4 h post-injection in the ipsilateral hippocampus, accompanied by impaired K^+ clearance, and completely lacked coupling 3 and 6 months after status epilepticus [25]. In the contralateral, non-sclerotic hippocampus, however, coupling remained intact. Interestingly, decreased astrocyte coupling preceded apoptotic neuronal death and the onset of spontaneous seizures. The authors found that pro-inflammatory cytokines induced the uncoupling of hippocampal astrocytes in vivo [25], which agreed with similar in vitro findings that proinflammatory cytokines have an inhibitory effect on astrocytic gap junctional coupling [66]. Their data suggest that inflammation may contribute to rapid uncoupling of astrocytes and the loss of coupling of astrocytes may be involved in, or even initiate, epileptogenesis. Notably, lack of astrocytic coupling was not due to loss of gap junction proteins because unchanged or even increased levels of Cx43 and Cx30 were found in both human and mouse chronic sclerotic hippocampi [67].

How general is the mechanism of astrocytic uncoupling for other forms of epilepsy? In a follow-up paper from the same group, uncoupling of astrocytes was also observed in a febrile seizure model [68]. Furthermore, constitutive deletion of astrocytic connexins aggravates kainate-induced epilepsy [69]. This body of work suggests that prevention of uncoupling or restoration of gap junctional coupling in astrocytes, perhaps via modulation of the TLR4 pathway, may represent a novel anti-epileptogenic therapeutic strategy.

Adenosine

Adenosine exerts a powerful inhibitory effect on excitatory synaptic transmission primarily through its interaction with presynaptic A_1 adenosine receptors (A_1Rs) to suppress neurotransmitter release. Once released from neurons and astrocytes, ATP is rapidly converted into adenosine monophosphate (AMP) and then into adenosine by extracellular nucleotidases. The reuptake of adenosine occurs through equilibrative nucleoside transporters, and phosphorylation by the astrocyte-specific enzyme adenosine kinase (ADK) breaks down adenosine and therefore clears excess adenosine from the extracellular space. Therefore, alterations in ADK are especially relevant to the generation of seizures. Increased ADK expression has been linked to seizure activity in both human tissue and experimental models of epilepsy [27, 70–72].

Collectively, the above findings support the ADK hypothesis of epileptogenesis [70, 71], including the dysregulation of ADK and its contribution to the epileptogenic cascade. Adenosine, adenosine receptor agonists, and ADK inhibitors have well established anticonvulsant efficacy [73–76]. Intracranial injection of adenosine prevents seizures in rats [77]. In addition, the use of transgenic mice revealed that reduced forebrain ADK protects against epileptogenesis [78]. Other studies involving adenosine augmentation therapies include a silk protein-based release system for adenosine [79] and the local release of adenosine from grafted cells [80], both of which resulted in seizure suppression. Focal adenosine delivery, such as slow-release polymers, cellular implants, gene therapy, or pump systems, has been suggested as a new pharmacological tool to treat refractory epilepsy with minimal side effects [81].

Particularly exciting is the recent finding that even a transient adenosine augmentation may have longer-lasting epigenetic effects that are antiepileptogenic [82, 83]. Probably the most effective treatment would be a brain-permeant peripherally-administered small molecule inhibitor of ADK. This would potentially obviate systemic side effects observed with direct adenosine delivery. If effective in triggering long-lasting antiepileptogenesis, such a drug would ideally need to be given only during an isolated therapeutic window just after an epileptogenic stimulus.

Examples of Astrocyte Dysfunction in Other Specific Epilepsy Syndromes

Tuberous Sclerosis

Tuberous sclerosis (TS) is a multisystem genetic disorder resulting from autosomal dominant mutations of either the TSC1 or TSC2 genes. The TSC1 gene encodes the protein hamartin and TSC2 encodes tuberin, which are thought to be regulators of cell signaling and growth. Epilepsy occurs in 80–90% of cases of TS, frequently involves multiple seizure types and is often medically refractory. Cortical tubers represent the pathologic substrate of TS, and microscopically consist of a specific type of dysplastic lesion with

astrocytosis and abnormal giant cells. While this suggests that astrocytes are involved in the pathologic lesion, in itself this is not evidence for a causative role of astrocytes in TS epileptogenesis. However, evidence using astrocyte-specific TSC1 conditional knockout mice has provided insight into a potential role of astrocytes in the etiology of TS. These mice, which have conditional inactivation of the TSC1 gene in GFAP-expressing cells (*Tsc1*^{GFAP}cKO mice), develop severe spontaneous seizures by 2 months of age and die prematurely [84]. Intriguingly, the time point of onset of spontaneous seizures in these mice is concordant with increased astroglial proliferation. Furthermore, two functions of astrocytes—glutamate and K^+ reuptake—are impaired in these mice. These mice display reduced expression of the astrocyte glutamate transporters GLT-1 and GLAST [85]. In addition, astrocytes from *Tsc1*^{GFAP}cKO mice exhibit reduced K_{ir} channel activity, and hippocampal slices from these mice demonstrated increased sensitivity to K^+-induced epileptiform activity [86]. A more recent inducible Tsc1 knockout mouse in which Tsc1 gene inactivation in GFAP-expressing cells was done at 2 weeks of age was sufficient to cause astrogliosis and mild epilepsy (but the phenotype was less severe than prenatal Tsc1 gene activation) [87]. Together, these studies demonstrate that in this model, changes in glial properties may be a direct cause of epileptogenesis.

Tumor-Associated Epilepsy

Tumor-associated epilepsy is an important clinical problem, seen in approximately one-third of tumors. Surgical removal of tumors usually results in seizure control, but many tumors cannot safely be resected, and tumor-associated seizures are often resistant to anticonvulsant therapy. Classic epilepsy-associated brain tumors include astrocytoma, oligodendroglioma, ganglioglioma, dysembryoplastic neuroepithelial tumor, and pleomorphic xanthoastrocytoma [88]. Microdialysis studies of gliomas have revealed reduced glutamate in the tumor compared to peri-tumoral tissue [89]. A "glutamate hypothesis" of tumor-associated epilepsy has been advanced which suggests that tumors excite surrounding tissue by glutamate overstimulation. Two lines of evidence are relevant to this hypothesis. First, the glutamate receptor subunit GluR2 has been found to be underedited at the Q/R site in gliomas, which would increase AMPA receptor Ca^{+2} permeability and potentially result in increased glutamate release by glioma cells [90]. Second, Sontheimer's group found that glioma cells release larger than normal amounts of glutamate in vitro [91]. The release of glutamate was accompanied by a marked deficit in Na^+-dependent glutamate uptake, reduced expression of astrocytic glutamate transporters, and upregulation of cystine-glutamate exchange [92]. Hence, glioma cell glutamate release at the margins of the tumor may initiate seizures in peritumoral neurons. A distinct potential mechanism underlying tumor-associated epilepsy is altered K^+ homeostasis. In support of this hypothesis, both reduced K_{ir} currents [93] and mislocalization of K_{ir}4.1 channels [94] have been found in malignant astrocytes. Recent studies have shown that a hypothesized glutamate release pathway, cysteine-glutamate transporter (SXC), is active in a subset of gliomas [95]. SLC7A11/xCT, the catalytic subunit of SXC, demonstrated elevated expression in about 50% of patient tumors. Compared with tumors lacking this transporter, SLC7A11-positive tumors were associated with faster growth, peritumoral glutamate excitotoxicity, seizures, and decreased survival. In a translational pilot study, use of the FDA-approved SXC inhibitor sulfasalazine in nine patients with biopsy-proven SXC expression led to inhibition of glutamate release from the tumor in vivo as assessed by magnetic resonance (MR) spectroscopy [95]. This exciting study demonstrates that phenotyping tumors for glial-associated transport molecules will lead to selective pharmacological targeting to prevent or ameliorate tumor-associated epilepsy, and addresses the pathologic mechanism of glutamate release from tumor cells rather than standard antiepileptic drug approaches of globally suppressing synaptic transmission.

Post-Traumatic Epilepsy

Post-traumatic epilepsy (PTE) refers to a recurrent seizure disorder caused by traumatic brain injury (TBI). PTE develops in a variable proportion of TBI survivors depending on the severity of the injury and the time after injury [96, 97]. Anticonvulsant prophylaxis is ineffective at preventing the occurrence of late seizures [98–100]. Various animal models of PTE have demonstrated characteristic structural and functional changes in the hippocampus, such as death of dentate hilar neurons and mossy fiber sprouting [101–103]. Earlier studies have also implicated altered astrocyte function in PTE models. Recordings from glial cells in hippocampal slices 2 days after fluid-percussion injury demonstrated reduction in transient outward and inward K^+ currents, and antidromic stimulation of CA3 led to abnormal extracellular K^+ accumulation in post-traumatic slices compared to controls [104]. This was accompanied by the appearance of electrical after discharges in CA3. This study suggests impaired K^+ homeostasis in posttraumatic hippocampal glia. Another study demonstrated reduction in expression of the astrocyte glutamate transporter GLT-1 in a PTE model induced by intracortical ferrous chloride injection, suggesting impaired glutamate transport [105]. Further studies of the role of glial cells in PTE appear warranted now that reliable PTE animal models have been developed [106, 107]. In particular, long-term changes in astrocyte channels and transporters after TBI that may correlate with PTE should be investigated. Interestingly, the latter study found that AQP4

was selectively mislocalized in mice that developed PTE after TBI [107].

Since TBI is associated with breakdown of the blood-brain barrier (BBB) at the time of the initial event, studies of BBB disruption-induced epileptogenesis are also relevant to mechanisms of PTE. Indeed, transient opening of the BBB is sufficient for focal epileptogenesis [108]. Extravasated albumin can be taken up by astrocytes which activates the transforming growth factor-β (TGF-β) pathway leading to focal epileptogenesis [109]. This mechanism provides an astrocytic basis for BBB disruption-induced epileptogenesis and suggests antiepileptogenic therapeutic approaches (TGF-β inhibition). Indeed, losartan, a TGF-β inhibitor and FDA-approved antihypertensive medication, was found to exert antiepileptogenic effects in these BBB disruption models [110, 111]. It will be of interest in the future to test similar strategies in PTE models for antiepileptogenic efficacy.

Conclusions

An understanding of the various structural and functional changes in astrocytes that occur during epileptogenesis is gradually emerging. Based on the role of astrocytes in K^+, glutamate, and water homeostasis at the synapse (Fig. 1), alterations of these and closely related metabolic mechanisms could lead to multiple astrocytic sources of hyperexcitability [112].

While animal models and human tissue studies have demonstrated astrocytic involvement in epilepsy, both levels of investigation have certain limitations. Animal studies may not accurately represent the disease progression as seen in humans; there are many forms of epilepsy in both animals and humans; and human tissue obtained from resected specimens does not allow determination whether observed cellular and molecular changes are a cause or a consequence of epilepsy. Future studies should focus on characterizing astrocyte alterations that occur prior to spontaneous seizure onset (*i.e.* during early epileptogenesis) in distinct models of epilepsy, as this could lead to a greater understanding of disease pathogenesis. The term "reactive gliosis" is too descriptive and should be replaced by careful morphological, biochemical, and electrophysiological studies of identified glial cell subtypes in human tissue and animal models, paying particular attention to astrocyte heterogeneity [113–115]. In addition to changes in preexisting glial cell populations, newly-generated glial cells with distinct properties may contribute to enhanced seizure susceptibility [116, 117]. The available data likely represent only the "tip of the iceberg" in terms of the functional role of astroglial cells in epilepsy (for more information please see [118]). Further study of astrocyte alterations in epilepsy should open new avenues for the development of anti-epileptogenic, *i.e.* disease-modifying therapies.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest in the preparation of this manuscript.

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