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Managing epilepsy by modulating glia

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Abstract

Antiepileptic drugs suppress epileptic seizures and provide a symptomatic control of seizures rather than anti-epileptogenic effects. Evidence about links between glial functions and neuronal signaling has been accumulating and has opened a perspective for the development of anti-epileptogenic approaches for the management of convulsive and non-convulsive forms of epilepsy. Astrocytic excitability and glial transmission have been shown to play critical roles in epileptogenesis and seizure generation. Although glial cells in convulsive types of epilepsy have been widely studied, little work has been done on the contribution of these cells in the non-convulsive epilepsy forms, particularly in absence epilepsy. This review underlies the participation of reactive astrocytes, glia modulating approaches and the roles of inflammatory cytokines in the modulation of convulsive and non-convulsive forms of epilepsy. The understanding of links between astrocyte functions and neuronal signaling in epileptogenesis will be one of the top epilepsy research advances in the future.

Keywords: antiepileptic; astrocyte; epileptogenic; microglia; seizure

Introduction

Modern antiepileptic drugs provide symptomatic control of seizures and suppression of symptoms.[1] The pharmacological treatment of epilepsy with marketed drugs suppresses epileptic seizures rather having an anti-epileptic or anti-epileptogenic effect.[2] In other words, the conventional antiepileptic drugs, used for the management of the acute seizures, or of ictogenesis, are mostly 'anti-seizure or anti-ictogenic drugs' and act directly on existing seizures, whereas anti-epileptic or anti-epileptogenic therapy aims to counteract the progress of epileptogenesis and epilepsy.[3] Epileptogenesis is defined as a dynamic process throughout the critical and latent period that progressively changes neuronal excitability, sets up important interconnections, and possibly requires intricate structural changes before theepilepsy develops. It also requires a progression after the epileptic condition is established.[4] However, none of the existing antiepileptic drugs prevent development of epilepsy in the patients at risk.[2] Therefore, there is a need for the discovery of novel antiepileptic drugs, particularly those effective on epileptogenesis, as well as disease modifying therapeutics aiming at sustained modulatory effects on the underlying epileptic state. There is a challenging need for a safe and effective seizure suppression and anti-epileptogenic approach.[4,5]

Another issue in the treatment of epilepsy is inadequate control of epileptic seizures in epileptic patients despite appropriate drug therapy with antiepileptics.[4] The challenge in one third of the epileptic patients is the therapeutic failure of antiepileptic drugs known as pharmacoresistant epilepsy that is identified with persistent ongoing seizure activity despite appropriate antiepileptic therapy. Moreover, adverse reactions of antiepileptics can diminish drug compliance of the patients or toxic reactions of antiepileptics can increase the risk of mortality and epilepsy related co-morbidities.[6] The use of animal models of pharmacoresistance or toxic reactions to antiepileptic drugs play an important role in the discovery of novel treatment strategies.

Several molecular and cellular alterations have been considered as contributing to the process of ictogenesis and epileptogenesis, by increasing the excitability of the brain and leading to recurrent seizures in animal models. These include neuronal injury and cell death, axonal and dendritic plasticity, presynaptic and postsynaptic modifications, neurogenesis, neuroinflammation, glial cell acti-

vation, vascular damage and angiogenesis, disruption of extracellular matrix integrity and structural and functional changes of ion channels.[5] Clinical and experimental evidences have demonstrated that molecular inflammatory processes of neuronal and non-neuronal cells play a role in epileptic activity.[7] However, efforts to understand the development and manifestations of seizure activity have focused exclusively on the neuronal dysfunction. Thus, the mechanisms of the available antiepileptic drugs have primarily targeted neuronal ion channels, receptors and neurotransmission. Neuronal receptors of voltage-gated sodium or calcium channels on the neuronal membrane, inhibitory neurotransmitter agonists and excitatory neurotransmitter antagonists have been developed for preventing the recurrent epileptic seizures.[4] The generation of action potentials and synaptic transmission have been defined as the principal targets for depressing the spread of aberrant electrical activity of epileptic seizures.[4] However, it has become clear that neuronal function and by extension neuronal dysfunction, are tightly modulated, in fact controlled, by a number of non-neuronal cells. Many of these are glial cells including astrocytes, microglial cells, and oligodendrocytes.

Involvement of Glia as a Target for Therapeutic Strategies in Epilepsy

Recent evidence has improved the understanding of the communication between glial functions and neuronal signaling and has also opened a new perspective on the development of approaches for managing convulsive and non-convulsive forms of epilepsy by modulating astrocytic functions.[7] Astrocytes are specialized glial cells and are widely present throughout the central nervous system (CNS). Contrary to earlier conventional thought which considered glia mainly as nutritional resources and supportive elements for neuronal homeostasis, glial cells actively participate in synapse development, synaptic plasticity and synapse function.[7,8] Astrocytes in the healty brain are considered as a part of the neurovascular unit that controls blood-brain barrier by regulating the ionic environment and interstitial volume. Indeed, by reactive astrogliosis, the process realized with the changes in molecular expression of astrocytes, can have the ability to react against to infection, trauma, ischemia or neurodegenerative diseases of CNS.[8,9] CNS cells and tissue can be protected by reactive astrocytes with different molecular mechanisms such as uptake of potentially excitotoxic glutamate, glutathione production, adenosine release, facilitation of blood brain barrier repair, stabilization of extracellular fluid and ion balance or suppression of the inflammatory cells and infectious agents.[7–9]

Astrocytes contribute to regulation of both neuronal excitability and synaptic transmission through the Ca^{2+} dependent release of neuroactive molecules, called gliotransmitters (**Figure 1**).[8,9] The tripartite synapse concept includes signaling between astrocytes and neurons for active control of astrocytes on neuronal activity and synaptic neurotransmission.[9] In terms of this concept, astrocytes have an ability not only to respond to released neurotransmitters throughout neuronal activity but also to release gliotransmitters with Ca^{2+} elevations such as glutamate, GABA, ATP, D-serine, prostaglandins and also inflammatory agents interleukin-1β (IL-β) and tumor necrosis factor-α (TNF-α) which play role in the neuromodulation and affect neuronal excitability and synaptic transmission.[9,10] Neuronal pools of neurotransmitters sustain synaptic neurotransmission and replenish neurotransmitters by *de novo* synthesis and recycling of previously released neurotransmitters. Released neurotransmitters from the synapse can be directly taken into the presynaptic neurons or the astrocytes can indirectly modulate the recycling of excitatory or inhibitory neurotransmitters, such as glutamate and GABA, by internalizing these transmitters with high affinity transporters, thus prompting glutamine synthesis.[9,10]

Astrocytes mediate glutamatergic neurotransmission. Once glutamate is released into the synapse, it is taken up into astrocytes by the EAAT1 and EAAT2 transporters, which are localized primarily on the astrocytic membranes (GLAST and GLT-1 in rat)[10] and then in the astrocyte, glutamate is converted to glutamine by the astrocyte-specific enzyme, glutamine synthetase and cycled back to neurons (**Figure 1**). Glutamine is a substrate for the production of GABA in inhibitory GABAergic neurons. Thus analogous reactions occur in GABAergic neurons, where glutamine is converted to GABA. This metabolic relationship between astrocytes and neurons is named the glutamine–glutamate–GABA cycle which has critical importance for the stability and continuity of synaptic activity.[9,10] Therefore, the contribution of astrocytes to synaptic activity can be considered to be a target for development of new anti-epileptic drugs relating to modulation of the genesis, maintenance and also the suppression of epileptic seizures.

Approaches for Modulating Glia in Order to Manage Convulsive Forms of Epilepsy

The hyperexcitability of neurons, which makes the neurons prone to epileptic seizures, can be modulated by factors not only linked to neurons such as ion channel mutations or brain injuries but also linked to astrocytes. Several lines of evidence have demonstrated that astro-

Figure 1. Schematic illustration of signaling between astrocytes and neurons for the active control of astrocytes in neuronal activity and synaptic neurotransmission with two active synapses (one glutamatergic-upper and one GABAergic-bottom) and parts of reactive astrocytes in between. Generated action potentials in pre-synaptic glutamatergic neuron, lead to the exocytotic synaptic release of neurotransmitter glutamate (**1**). Glutamate activates AMPA and NMDA receptors in the post-synaptic glutamatergic neuron and excitatory postsynaptic potentials is generated by influx of Na+ and Ca^{2+} (**2**). Glutamate is taken up into astrocytes by the EAAT-1 and EAAT-2 transportes localized on the astrocytic membranes (GLAST and GLT-1 in rat) (**3**) and converted to glutamine by glutamine synthetase (GS) (**4**). Glutamine is taken up by GABAergic neurons (**5**) where it is converted to glutamate by glutaminase and then to GABA *via* glutamic acid decarboxylase (GAD) (**6**). When released from presynaptic vesicles into the synaptic cleft, GABA diffuses across and binds to postsynaptic GABAA and GABAB receptors. It may also bind to presynaptic GABAB receptors. GABA binds to specific recognition sites on the GABAA receptor, this triggers a conformational change leading to opening of the intrinsic anion channel allowing chloride ions to flow through the cell resulting in hyperpolarization of the neurons (**7**). GABA is removed from the synaptic cleft into surrounding astrocytes or the presynaptic terminal by GABA transporters (**8**). GABA is taken up by mitochondria, converted by GABA transaminase (GABA-T) into glutamate in astrocyte (**9**). Neurotransmitters, released by depolarized neurons activate the astrocytic G protein-coupled receptors (GPCRs) (**10**). The GPCRs activate the phospholipase C/inositol 1,4,5-triphosphate (PLC/IP3)-mediated pathway for the release of Ca^{2+}

from intracellular calcium stores, such as the endoplasmatic reticulum (ER) resulting in an increase in intracellular Ca^{2+} (**11**), intracellular Ca^{2+} elevations in astrocytes stimulate gliotransmitter release (**12**) that can influence neuronal excitability (**13**). Synaptic adenosine (AD) is taken up into astrocytes by equilibrative nucleoside transporters (ENT) and converted to AMP by adenosine kinase (ADK) (**14**). Astrocytes release ATP *via* vesicular release and/or by direct release through hemichannels and extracellulary ATP is rapidly degraded into AD by a series of ectonucleotidases (**15**). K+ released from neurons enters astrocytes *via* inward rectifying K+ channels (Kir 4.1) and is distributed into capillaries (**16**). The astrocytic water channel aquaporin-4 (AQP4) mediates the flow of water between the extracellular space and the blood to maintain osmotic balance (**17**). Astrocytes are connected to each other *via* gap junction channels composed of connexin 30 (C×30) and connexin 43 (C×43) which mediates spatial buffering of K+ ions (**18**). [Color figure can be viewed in the online issue, which is available at www.anatomy.org.tr]
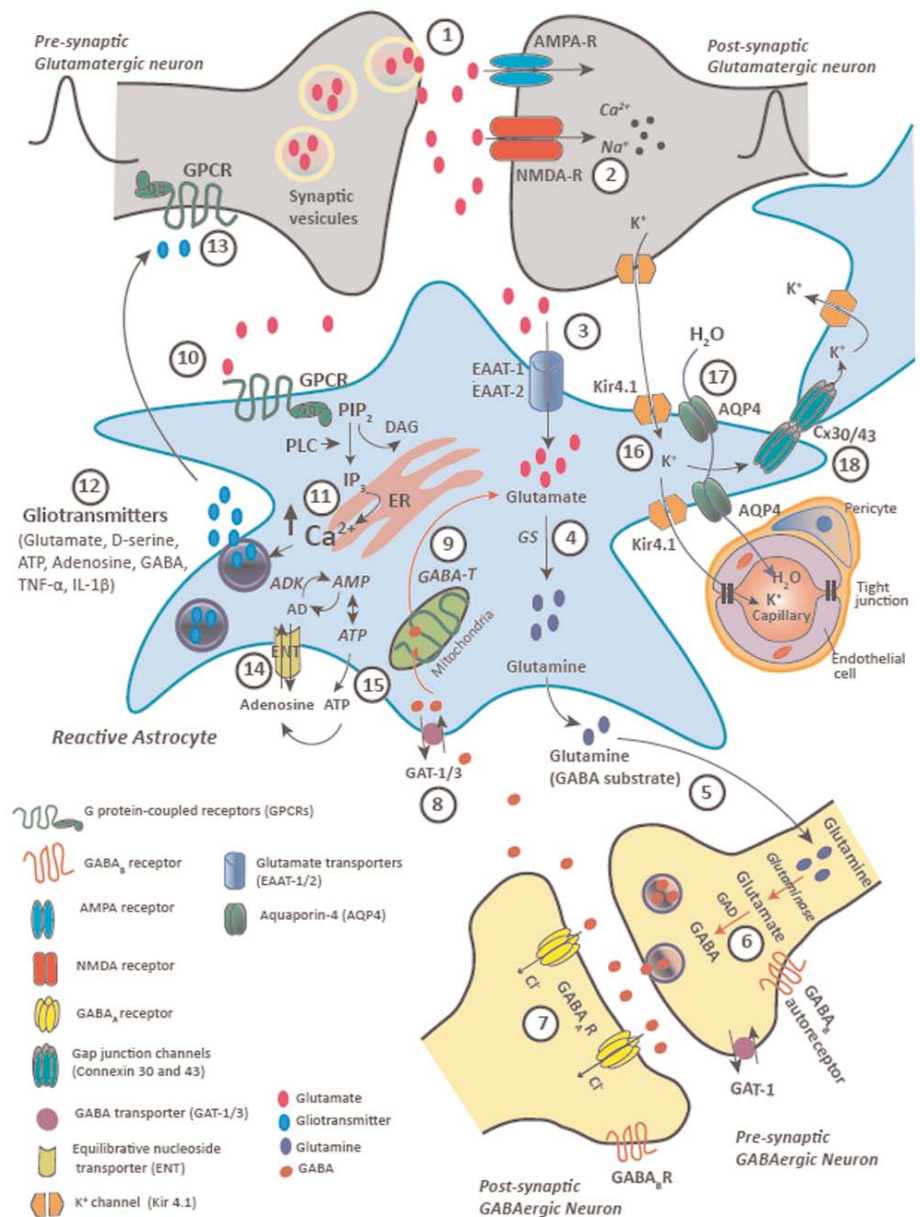
cytes and microglial cells are critical players in seizure initiation and progression. Temporal lobe epilepsy is the most common form of convulsive epilepsies and generally an insult such as status epilepticus, head trauma or febrile seizures trigger temporal lobe seizures that are usually refractory to antiepileptic drugs.[10,11] The spontaneous discharge of neurons in an epileptogenic focus of the lateral or medial temporal lobe results in seizures by interaction of the neuronal networks with limbic structures.[11,12]

Changes in the activation of astrocytes and microglia have been reported in human and experimental models of convulsive seizures and epilepsy.[12–14] After kainic acid-induced status epilepticus (SE), which is an experimental model of temporal lobe epilepsy, glial activation was reported in the hippocampus within four hours.[12] In another model of temporal lobe epilepsy, produced by pilocarpine injections in rats, activation of astrocytes and microglial cells was detected throughout the CA1 and CA3 regions of the hippocampus as well as in the dentate gyrus over the first 5 days. The microglia and astrocytes remained in an activated state for at least 3–5 days after the initial convulsive insult. The typical resting microglia was shown to take on an activated appearance with its cell body elongated, and its processes thickened and increased in number of branches.[13] In another study, neuropathological changes including neuronal damage and gliosis were investigated in hippocampal regions of mice after the onset of pilocarpine-induced SE. Glial fibrillary acidic protein (GFAP), the first sign of reactive gliosis, was demonstrated for the pyramidal cells in the CA3 and CA1 hippocampal regions and they expressed the highest presence at 1 and 3 weeks after SE onset.[14] Increase in GFAP immunoreactivity was observed also in the hippocampus of rats following kainic-acid induced seizures. Reactive astrocytes were shown 30 min after the kainic acid-induced seizure onset and reached a maximum at 1 h whereas neuronal activity increased after the reactive gliosis, starting at 1 h after the onset and reaching a peak at 2 h. Thus astrocytes and neurons were found to be active during seizures.[13]

Interestingly, these astrocytic and microglial transformations are not always restricted to areas at the site of insult or the injection of the chemical agent but are also observed in many brain regions such as the frontoparietal cortex. This has been demonstrated in a model of self-sustained limbic SE, which is characterized by electrical stimulation of the limbic seizure circuit to induce SE. Reactive microglia and GFAP positive astrocytes were detected in the frontoparietal cortex and CA3 pyramidal layer of the hippocampus in the rats following pilocarpine-induced SE at various times resembling acute phase, epileptogenesis and chronic phase. Activated microglia and astrocytes were shown in whole periods of SE.[15] Another study reported reactive astrocytes and microglia in the hippocampus and piriform cortex of rats after lithium-pilocarpine-induced SE. Particularly the CA1 subfield of hippocampus and layer 1 of the piriform cortex showed reactive gliosis which was correlated with seizure stages according to the Racine scale.[16]

Key factors for participation of reactive astrocytes in the modulation of epileptic seizures can be considered on the basis of the effects of astrocytes on extracellular glutamate

and potassium levels or by looking at the transmitter release from astrocytes themselves.[8,10] Elevated extracellular excitatory glutamate concentrations in the brain have ability to enhance the hyperexcitability of neurons and to stimulate the initiation and progression of seizures.[10] Released glutamate with proliferation of reactive astrocytes, change in glutamate metabolism or dysfunction of glial glutamate transporters can lead to elevated glutamate concentration and can be associated with temporal lobe epileptic seizures.[8,17] Proliferated astrocytes have been defined to be a marker for astrocytic glutamate. Released glutamate from astrocytes potentiates neuronal excitability and elicits neuronal synchrony.[17] A marked proliferation of reactive astrocytes was detected in the hippocampal CA1 and CA3 regions and the dentate gyrus in the temporal lobe of epilepsy patients with hippocampal sclerosis after anteromedial temporal lobectomy and amygdalohippocampectomy operations. One other important finding of this study was the correlation between not only of the mean density of GFAP immunopositive cells and seizure frequencies in patients, but also neuronal cell loss and astroglial GFAP expression in the same patients.[18] Astrocytes take up synaptic glutamate and glial glutamine synthetase (GS) converts glutamate to glutamine (**Figure 1**). Therefore, this enzyme is functionally critical for decreasing both intracellular and extracellular glutamate concentrations by regulating glutamate metabolism.[10,17] Reduced glial GS has been detected in the CA1 and CA4 hippocampal regions of temporal lobe epilepsy patients with hippocampal sclerosis.[19] Reactive astrogliosis was also shown in the same hippocampal subfields. The results of this study suggested that glial changes in the epileptogenic regions are involved in the pathological mechanism producing epilepsy rather than that seizures and reduction of GS contribute to the underlying process of epilepsy by diminishing glutamate clearance.[19]

Another probable cause for an excessive amount of extracellular glutamate is the dysfunction of glutamate transporters, responsible for the re-uptake of glutamate by astrocytes.[17] Five glutamate transporter isoforms are present in the brain, and two of them, GLAST and GLT-1 (EAAT1 and EAAT2, in humans) are mainly expressed in astrocytes (**Figure 1**).[10] In the neurons of the CA1 and CA2 regions of a sclerotic hippocampus of patients with temporal lobe epilepsy, the levels of the EAAT2 protein, a glial glutamate transporter, were found to be lower than in temporal epilepsy patients with no hippocampal sclerosis. Interestingly, patients without hippocampal sclerosis had higher EAAT2 immunoreactive levels than non-epileptic controls, indicating an upregulation for EAAT2 protein expression in the hippocampal CA1 and CA2 subfields of epileptic patients without hippocampal sclerosis.[20] In accordance with these findings, enhanced glutamate uptake by increased EAAT2 expression was shown to

reduce seizure frequencies and mortalities in mice with pilocarpine-induced SE, suggesting the protective effect of astrocytic transporters against SE induced mortality and morbidities.[21] Supporting these, GLT knockout mice showed lethal spontaneous seizures and increased seizure susceptibility that is attributed to increased elevated levels of synaptic glutamate.[22] In GLAST deficient mice, amygdala kindling seizures have been found more prolonged and these mice showed more severe pentylentetrazol induced seizures.[23] All these findings suggest that astrocytic glutamate uptake plays a key role for seizure susceptibility.[8,17]

Besides glutamate, water and potassium buffering regulated by astrocytes can impact neuronal excitability and play an important role in seizure susceptibility.[24] Astrocytes regulate K^+ levels by K^+ uptake and buffering (**Figure 1**). Inwardly rectifying K^+ channels (Kir4.1) are known to mediate K^+ spatial buffering.[25] Patients with temporal lobe epilepsy showed reduced immunoreactivity for astrocytic Kir4.1 specifically in the hilar and CA1 regions of sclerotic hippocampus, suggesting a role of astrocytic K^+ homeostasis in the epileptogenic properties of hippocampal sclerosis.[26] Further support for the importance of Kir4.1 in epilepsy pathophysiology emerged from Kir4.1 knockout mice studies which clearly demonstrated that the deletion of astroglial Kir4.1 encoding gene *KCNJ10* induces epilepsy in laboratory animals.[27] Moreover, human mutations of *KCNJ10* which encodes the astroglial Kir4.1 channel are linked to seizure susceptibility.[28] The astrocytic water channel aquaporin-4 (AQP4) that regulates interstitial fluid osmolarity is also implicated in the seizure susceptibility and provides a new potential therapeutic target (**Figure 1**).[24,29] In kainic acid model, AQP4 is significantly reduced and recently in human mesial temporal lobe epilepsy, the dislocation of AQP4 preceding the chronic phase of epilepsy has been demonstrated.[30,31]

Each of the features associated with neuron-glia communication represents fundamental approaches relevant for the design of innovative therapeutics. In the light of these studies, astrocytic and microglial changes contributing to the underlying mechanisms of seizure activity can be considered to be novel neuromodulatory targets for development of disease-modifying medications for patients with convulsive epilepsies.

Approaches for Modulating Glia in Order to Manage Non-Convulsive Forms of Epilepsy

Although the role of glial cells in convulsive seizures has been widely studied, little work has been performed on the contribution of these cells to the non-convulsive seizures. Absence epilepsy is one of the idiopathic generalized epilepsies and is a well-known non-convulsive form of epilepsy identified mostly during childhood.[32] Typical absence seizures are associated with intermittent impairment of consciousness characterized by a brief interruption of the ongoing activity and bilateral, synchronous, and symmetrical 2.5–4 Hz spike-and-wave discharges (SWDs) on the EEG. Genetic Absence Epilepsy Rats from Strasbourg (GAERS), as a well-validated animal model for absence epilepsy, display SWDs accompanied by a decrease in consciousness, behavioral arrest and absence of response to external stimuli.[33] In this multigenic model, occurrence of SWDs starts at about postnatal day 30 and reaches to a mature pattern in 3-4 month old rats,[34] giving opportunity to explore the underlying mechanisms of absence epilepsy by investigating the onset and maturation of SWDs.

Rhythmogenic interplay of cortex and thalamus is considered to lead to absence seizures and GABA and glutamate are suggested to play main roles in the initiation and generation of SWDs.[32] Recent findings suggest that GABAergic transmission in the thalamocortical network plays important roles in the pathophysiology of absence epilepsy.[32,35] It has been established that enhancement of GABAergic transmission inhibits convulsive epileptic seizures;[36] however, systemic administration of GABA-mimetic antiepileptic drugs exacerbates absence seizures in animal models of absence epilepsy.[37] In contrast, local administration of GABA-mimetic agents into the cortex or hippocampus inhibits SWDs of genetic absence epileptic rats.[38] Therefore, astrocytic GABA transporters and astrocyte neuron signaling are considered to be the main glial factors involved in absence seizure activity.[35]

Signaling between neurons and glia by glutamate or GABA has been studied in GAERS by several investigators who examined glutamate uptake in the cortex and thalamus of GAERS and non-epileptic control (NEC) rats.[39,40] A significant decrease was found in glutamate uptake in the cortex of adult GAERS compared to NEC.[39] The lower glutamate uptake in the cortex of GAERS may be due to alterations of glutamate transporters in astrocytes, since these are the major contributors to clearance of extracellular glutamate. Dutuit et al.[40] showed decreased amounts of glutamate transporters in 30-day old GAERS compared to adult GAERS and NEC. A decreased expression of GLAST mRNA in the cortex of adult GAERS, decreased levels of GLT1 and GLAST in the cortex of 30-day-old GAERS and also a lower expression of GLT1 and GLAST in cortical primary astrocytes obtained from 1-day-old GAERS suggest an impairment of turnover of transporter proteins. The change in

GLAST and GLT1 can lead to lower levels of glutamate uptake.[40] Another observation is the increased expression of GFAP as the first sign of reactive astrocytes before the occurrence of absence seizures in young, as well as adult GAERS compared to NEC. Reactive astrocytes were investigated in the 30-day-old and adult GAERS, and increased amounts of GFAP were shown in both age groups of GAERS, suggesting the presence and involvement of reactive astrocytes in the onset of the absence seizures.[41]

Neuronal and astrocytic glutamate metabolism was examined by nuclear magnetic resonance spectroscopy which was used to analyze neuronal glial interactions and to study cerebral metabolism.[42] An increased cycling of glutamate and glutamine between astrocytes and glutamatergic neurons in the cortex of 5-month-old GAERS relative to NEC was shown by this method. The increased glutamate and glutamine metabolism can be considered to indicate a dysregulation of astrocyte-neuron interactions in the thalamo-cortical loop.[42] The metabolic dysregulation was also observed in immature 1-month-old GAERS. Mitochondrial metabolism in cortical neurons of both immature and adult GAERS was higher than in the NEC animals. Although the interaction between glutamatergic neurons and astrocytes was normal in the immature GAERS, astrocytic metabolism was found to be increased in adult GAERS.[43] These findings highlight how neuroglial relationships relating to glutamatergic transmission are affected in an age-dependent manner in experimental absence epilepsy. A dysregulation of glutamate metabolism and an increase in cerebral glucose utilization and energy metabolism throughout the whole brain have been reported in absence epileptic rats by several investigators.[39,42,44] Nehlig et al.[44] demonstrated an overall consistent increase in local cerebral metabolic rates for glucose in absence epileptic rats compared to NEC animals. However, there was no direct relationship between high cerebral energy metabolism and the occurence of SWDs in the epileptic rats. Although local cerebral glucose metabolism was higher in most of the brain regions in adult GAERS compared to NECs, immature GAERS without any SWDs showed no increase in glucose metabolism particularly in the thalamo-cortical regions, critical for the occurrence of SWDs.[45]

Astrocytes also play an important role in mediating GABAergic transmission. Activation of GABA_A receptors (GABA_ARs) produce two forms of inhibition: 'phasic' inhibition generated by rapid transient activation of synaptic GABA_ARs (sGABA_ARs), and tonic inhibition generated by activation of peri- or extrasynaptic GABA_ARs (eGABA_ARs) by ambient GABA which causes a persistently active, or tonic current.[35] GABA transporters located on astrocytic membranes, GAT-1 and GAT-3,[46] play a pivotal role in regulating GABA_A-mediated tonic inhibition. GAT-1 proteins are expressed in presynaptic neurons and GAT-3 proteins are expressed in astrocytes.[10,25] However, this expression pattern is not consistent throughout all brain regions. For example, in thalamus GAT-1 and GAT-3 are mainly expressed in astrocytes, but not in presynaptic neurons.[46] Thus, the regulatory action of ambient GABA can differ between brain regions. Recently, dysfunction of glial GATs and enhanced tonic GABA_A inhibition have been shown to underlie the pathophysiology of absence epilepsy.[47] An increased tonic inhibition mediated by GABA_ARs was present in thalamocortical neurons of GAERS. GAT-1 in thalamic astrocytes was reported to be crucial in controlling the generation of SWDs rather than GAT-3. It was suggested that dysfunction of GAT-1 in thalamic astrocytes of GAERS can trigger GABA_A inhibition by enhanced activity of GABA_ARs.[47] GAT-1 GABA transporter currents in astrocytes of ventrobasal thalamus were investigated and found to be functionally deficient in GAERS, showing an abnormal astrocytic modulation of peri- and extrasynaptic GABA concentrations in the thalamus. GABA transporter currents were unaffected by blocking GAT-1 whereas they were abolished by blocking GAT-3. This finding indicates a role of abnormal GAT-1 functions in experimental absence epilepsy.[48]

Recently, neuroprotective effects of ONO-2506 (arundic acid), a glial modulating agent, have been demonstrated in Cacna1a^tm2Nobs/tm2Nobs mice, a genetic animal model of absence epilepsy.[49] ONO-2506 inhibits the production and release of a calcium-binding protein, S100B, from astrocytes. ONO-2506 inhibited spontaneous absence epileptic seizures of Cacna1a^tm2Nobs/tm2Nobs mice without affecting maximal electroshock (MES) or pentylentetrazole (PTZ) tests.[49] Therefore, ONO-2506 is not an anticonvulsant, but an antiepileptic drug. This antiepileptic profile of ONO-2506 is similar to that of levetiracetam.[50] ONO-2506 increases the basal release of inhibitory transmitters, GABA and kynurenic acid in the medial prefrontal cortex in a dose-dependent manner. This novel potential glial targeting agent inhibited tripartite transmission during the hyperactive stage of absence seizures.[49]

The Role of Inflammatory Cytokines in Convulsive and Non-Convulsive Forms of Epilepsy

There are several reports supporting the involvement of inflammatory processes in the pathological mechanisms of epileptic seizures, particularly those crucial for the generation of seizures.[51] Astrocytes and microglia are the main source of inflammatory molecules in the brain and are the

first cells to produce inflammatory cytokines during epileptic activity. Astrocytes have dynamic functions and can produce many pro- and anti-inflammatory molecules such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, transforming growth factor beta (TGF)-β, as well as chemokines.[52] Resting astrocytes and microglial cells are activated at an early stage in response to insults or injuries associated with the break-down of the blood-brain barrier or with changes in blood flow and extravasation of molecules such as albumin. Reactive astrocytes and microglia up-regulate the expression of surface molecules, such as complement receptors and release a variety of pro-inflammatory and cytotoxic soluble factors, such as IL-1β. The production of TNF-α and IL-1β by astrocytes can lead to beneficial or detrimental outcomes depending on which receptors are activated and the timing of the expression.[51,52] Among pro-inflammatory cytokines, IL-1β which has been reported to be rapidly synthesized by glia after the induction of seizures, significantly affects neuronal excitability by the release of neurotransmitters, neuropeptides or neurotrophic factors, and also by its actions on synaptic transmission and control of ionic currents.[53]

Production of IL-1β is rapidly induced during the acute state of convulsive seizures in glia and neurons. Glial activation induced by seizures and up-regulation of pro-inflammatory cytokines directly can trigger neuronal excitability and neuronal injury by interplaying with glutamatergic transmission, or indirectly, by activating gene transcription. Apart from this, there is evidence to suggest that IL-1β also plays role in experimental epilepsy and in epilepsies in humans that are not associated with neuronal injury and changes in excitability. In an experimental model of convulsive epilepsy, hippocampal production of IL-1β was found to increase in microglia-like cells following kainic acid induced seizures.[54] The same study also showed a prolongation of the effects of exogenous application of IL-1β on hippocampal EEG seizures. These were blocked by IL-1Ra, a natural antagonist of IL-1.[54] Work by Vezzani et al. [53] demonstrated that seizures rapidly induce the production of both IL-1β and IL-1Ra in astrocytes. They also lead to transgenic overexpression of IL-1Ra in astrocytes, significantly delaying the onset of seizures and reducing the duration of generalized convulsive seizures induced by bicuculline in mice. These effects of IL-1Ra were absent in the IL-1 receptor type 1 knockout mouse, implying that activation of IL-1 receptor type 1 by IL-1β can reduce seizure thresholds.[53] In addition to these, SE induced by electrical stimulation of the ventral hippocampus of rats was found to induce a time dependent neuronal and astrocytic expression of IL-1 receptor type 1 in the limbic system.[55] Firstly hippocampal neurons were immunoreactive for IL-1 receptor type I and several

hours later astrocytes localized in limbic and extralimbic cortical areas were found to have induced expression of this receptor.[55] Antiepileptogenic effects mediated by inhibition of a biologically active form of IL-1β have been reported by Ravizza et al.[56] VX-765, a selective inhibitor of interleukin converting enzyme (ICE) was used with an experimental model of temporal lobe epilepsy to show that kindling development in rats could be blocked by preventing IL-1β increases in forebrain astrocytes by selective inhibition of ICE. This molecule provides the biologically active form of IL-1β, whereas VX-765 had no effect on seizures or afterdischarge duration in fully kindled rats.[56] IL-1β was expressed by activated microglia and astrocytes within 4 h following the onset of SE in the forebrain areas of rats whereas in rat and human chronic epileptic tissue, IL-1β and IL-1 receptor type 1 was expressed broadly by astrocytes, microglia and neurons.[57]

Although the role of the astrocytes and IL-1β in the pathophysiology of convulsive seizures have been widely investigated, little is known about the contribution of these cells to non-convulsive forms of epilepsy, such as absence epilepsy. IL1-β expression and glial activation were studied in the forebrain of GAERS at postnatal days 14, 20 and 90.[58] The aim of this study was twofold: (1) to show whether glial cells-again primarily astrocytes and microglia- might also take part in absence seizure mechanisms, and (2) to determine whether there are age related changes that parallel seizure initiation in the absence epilepsy model. IL-1β was found to be significantly induced in reactive astrocytes in the somatosensory cortex of GAERS, and was involved in SWD generation in an age-dependent manner as follows: partial at postnatal day 20 GAERS and fully in all adult GAERS when SWDs are completely developed.[59,60] The expression of the cytokine was associated with the development of SWDs. Both SWD number and duration were shown to reduce by inhibition of IL-1β biosynthesis in adult GAERS.[58] These results show that key contributors to the pathophysiology of absence epilepsy include not only neuronal changes, such as the imbalance between excitatory and inhibitory neurotransmission in particular brain areas, but also glial changes including an increased reactivity of astrocytes, as well as the induction of IL-1β. The experimental findings of this study showed the association between SWD development and IL-1β production for the first time and demonstrated not only the contribution of IL-1β to SWD activity, but also a new perspective for the development of novel specific anti-inflammatory agents for absence epilepsy acting by a block of IL-1β biosynthesis. Finally, astrocytic gap junctions formed by connexin (Cx) 30 and Cx43 (**Figure 1**) are also likely to play a role in absence epilepsy pathogenesis as the gap junction blocker car-

benoxolone reduced absence seizures *in vivo* in both rat and mouse models of absence epilepsy.[27,61]

The association between brain inflammation and seizures of limbic epilepsy and absence epilepsy show that both recurrent limbic seizures and absence epileptic seizures in the experimental models lead to an increased production of IL1-β in reactive glial cells primarily in the brain regions where seizures originate and spread. Additionally, risk factors leading to development of epilepsy such as neurotrauma, infection, febrile seizures or SE are known to have the ability to trigger inflammation in the brain and any epileptogenic event can initiate a chronic inflammatory process in the central nervous system involved in the onset of epilepsy.[51,62]

Conclusion

The studies summarized here open a perspective on the development of specific astrocyte modulating approaches for managing convulsive and non-convulsive forms of epilepsy. The contribution of astrocytes and microglia to the generation and spread of convulsive and non-convulsive epileptic seizures and the role of inflammatory cytokines in seizure activity highlight new therapeutic approaches for developing more effective antiepileptogenic agents. Glia may also provide a biomarker of epileptogenesis that can be detected by neuroimaging tools for the diagnosis and treatment of epilepsy and its associated syndromes. The understanding of links between astrocytic functions and neuronal signaling in epileptogenesis will be one of the top epilepsy research advances in the future.

Acknowledgements

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