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CRUCIAL ROLE OF ASTROCYTES IN TEMPORAL LOBE EPILEPSY

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Abstract—Astrocytes sense and respond to synaptic activity through activation of different neurotransmitter receptors and transporters. Astrocytes are also coupled by gap junctions, which allow these cells to redistribute through the glial network the K^+ ions excessively accumulated at sites of intense neuronal activity. Work over the past two decades has revealed important roles for astrocytes in brain physiology, and it is therefore not surprising that recent studies unveiled their involvement in the etiology of neurological disorders such as epilepsy. Investigation of specimens from patients with pharmacoresistant temporal lobe epilepsy and epilepsy models revealed alterations in expression, localization and function of astrocytic connexins, K^+ and water channels. In addition, disturbed gliotransmission as well as malfunction of glutamate transporters and of the astrocytic glutamate- and adenosine-converting enzymes – glutamine synthetase and adenosine kinase, respectively – have been observed in epileptic tissues. Accordingly, increasing evidence indicates that dysfunctional astrocytes are crucially involved in processes leading to epilepsy. These new insights might foster the search for new targets for the development of new, more efficient anti-epileptogenic therapies.

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Key words: astrocyte, Kir channel, connexin, water channel, adenosine kinase, antiepileptic drugs.

INTRODUCTION

Epilepsy is a pathological condition of the brain that affects about 2% (Hesdorffer et al., 2011) of the population worldwide. The clinical manifestation of this disorder is the seizure, a sudden and unpredictable transient episode of abnormal electrical activity in the brain that can lead to full body convulsions. From the cellular point of view, the seizure reflects an intense, synchronous discharge that can arise in a large population of neurons from a restricted brain region, i.e. the epileptogenic focus, and then spreads across the cortex by progressively recruiting other neuronal populations (Traub and Wong, 1982; Jefferys, 1990; Avoli et al., 2002; Pinto et al., 2005; Trevelyan et al., 2006). In spite of intense experimental research, our knowledge of the cellular mechanisms leading to seizure generation, propagation and cessation is, however, largely unsatisfactory. Also due to our defective understanding of the pathophysiology of epilepsy, no new, more effective drug therapies have been developed over the last decade. Indeed, in about 70% of patients suffering of medial temporal lobe epilepsy (MTLE) – one of the most common and severe forms of epilepsy – seizures can be poorly controlled by currently available antiepileptic drugs.

This review focuses on the role of astrocytes in the generation of abnormal activity in neurons that drives the brain network to seizures. The role of these glial cells as modulators of epileptogenesis was initially proposed over 20 years ago and was linked to the ability of astrocytes to buffer extracellular K^+ or neurotransmitters released in excess during epileptiform discharges. In the first part of the review, we will summarize the evidence obtained from both animal models and human epilepsy that dysregulations of K^+ channels, glutamate transporters, aquaporins and connexins in astrocytes might predispose to network hyperexcitability and seizures. We will also describe a novel systematic pharmacological approach aimed to identify new inhibitors and activators of a subtype of inwardly rectifying K^+ (Kir) channels, i.e., the Kir4.1 channel that is highly expressed in astrocytes where it contributes to a tight control of extracellular K^+ levels. Consistent with the emerging view that brain function is based on bidirectional signaling between neurons and astrocytes (Araque et al., 2014), in the second part of the review we will discuss further evidence that points to

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Abbreviations: ADK, adenosine kinase; AQP, aquaporin; Cx30, connexin 30; Cx43, connexin 43; dnSNARE, dominant-negative SNARE; ECS, extracellular space; FS, febrile seizures; GJ, gap junction; GS, glutamine synthetase; HS, hippocampal sclerosis; $[K^+]_o$, extracellular K^+ concentration; KCNJ10, Kir4.1 encoding gene; Kir channel, inwardly rectifying K^+ channel; MTLE, mesial temporal lobe epilepsy; P450scc, cytochrome P450 cholesterol side-chain cleavage enzyme; PDS, paroxysmal depolarizing shift; SNP, single nucleotide polymorphism; SE, status epilepticus; SIC, slow inward current; TBOA, DL-threo-benzyloxyaspartate; TTX, tetrodotoxin.

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a direct involvement of astrocytes in the generation of epileptiform activity.

IMPAIRED K^+ BUFFERING IN MTLE

Neuronal activity elicits transient increases in the extracellular K^+ concentration ($[K^+]_o$), which in epilepsy can reach values of up to 10–12 mM (Heinemann and Lux, 1977). Even moderate rises in $[K^+]_o$ may significantly increase neuronal excitability (Balestrino and Somjen, 1986; Walz, 2000), underscoring the necessity of a tight control of K^+ homeostasis for normal brain function. This task is fundamentally accomplished by astrocytes, which display very negative resting potentials and a high resting permeability for K^+. This property is mainly mediated by K^+ channels of the Kir4.1 subtype (Seifert et al., 2009). Astrocytes control $[K^+]_o$ by two mechanisms: K^+ uptake and spatial buffering (for review see Kofuji and Newman, 2004). Net uptake of K^+ is dependent on Na^+/K^+ pumps and $Na^+/K^+/Cl^-$ cotransporters and to a minor extent on Kir4.1 channels (Ransom et al., 2000; D'Ambrosio et al., 2002; Kofuji and Newman, 2004). It is unlikely that this mechanism alone is sufficient for an efficient clearance of $[K^+]_o$ since intracellular K^+ accumulation results in water influx and cell swelling. The spatial buffering model (Orkand et al., 1966) describes another effective mechanism for $[K^+]_o$ regulation which is based on the fact that astrocytes are electrically connected to each other via gap junction (GJ) channels to form a functional network. According to the model, excessive extracellular K^+ is taken up by astrocytes at sites of high neuronal activity and redistributed through the astrocytic network to be released at regions of lower $[K^+]_o$. Here, uptake and release of K^+, which may be accompanied by water movement (Dietzel et al., 1980), occurs via passive diffusion through weakly-rectifying Kir4.1 channels (Fig. 1). These channels are particularly well suited for this task because they possess a high open probability at rest and their conductance increases at high $[K^+]_o$ (Ransom and Sontheimer, 1995). Intercellular K^+ diffusion is energy-independent, driven by the electrochemical gradient between the depolarized resting potential of cells at sites of K^+ entry and the more negative membrane potential of astrocytes distant to the elevated $[K^+]_o$ (Orkand et al., 1966; Walz, 2000; Kofuji and Newman, 2004).

Increased $[K^+]_o$ has been associated with the pathophysiology of epilepsy (Moody et al., 1974; Fisher et al., 1976; Lothman and Somjen, 1976), and is sufficient to trigger epileptiform activity (Zuckermann and Glaser, 1968; Traynelis and Dingledine, 1988). To assess the impact of Kir4.1 channels in K^+ buffering, the effect of Ba^{2+}-induced Kir channel block on stimulus-triggered rises in $[K^+]_o$ or iontophoretically applied K^+ was analyzed in sclerotic and non-sclerotic hippocampal slices from rat and human neurosurgical resections. Ba^{2+} produced enhanced $[K^+]_o$ accumulation under control conditions, but not in sclerotic tissue (Heinemann et al., 2000; Kivi et al., 2000; Jauch et al., 2002). Patch-clamp recordings confirmed these findings and revealed reduced Kir currents in the sclerotic CA1 region of neurosurgical specimens from patients presenting with MTLE (Bordey and Sontheimer, 1998; Hinterkeuser et al., 2000; Schröder et al., 2000). In line with these data, Western blotting found down-regulation of Kir4.1 protein in human hippocampal sclerosis (HS) (Das et al., 2012). In a recent study, Heuser et al. (2012) used immunohistochemistry to examine the distribution of Kir4.1 in hippocampi from MTLE patients. They found significantly reduced astrocytic Kir4.1 immunoreactivity in patients with HS compared to non-HS patients and autopsy controls. The reduction was most pronounced around vessels and might have been caused by disruption of the dystrophin-associated protein complex in astrocytic endfeet (Heuser et al., 2012). Together, these studies imply that impaired K^+ clearance and increased seizure susceptibility in MTLE-HS result from reduced expression of Kir4.1 channels (Fig. 1). However, it remains unclear whether this reduction represents cause, effect or adaptive response. In favor of a causative role for altered Kir channel expression in epilepsy, David et al. (2009) showed in an albumin epilepsy model that Kir4.1 down-regulation occurs before the onset of epileptic activity. Another study, however, reported no changes in astrocytic Kir currents 7–16 days following systemic kainate injection, implying that Kir down-regulation represents a consequential event in epilepsy (Takahashi et al., 2010).

Further support for the crucial role of Kir4.1 in glial K^+ buffering emerged from the characterization of Kir4.1 knockout mice (Kofuji et al., 2000; Djukic et al., 2007). Global deletion of the Kir4.1 encoding gene, *KCNJ10*, resulted in motor impairment and premature death (Neusch et al., 2001). Mice with astrocyte-specific deletion of Kir4.1 (cKir4.1$^{-/-}$ mice) displayed a similarly phenotype, including ataxia, seizures and early lethality. At the cellular level, substantial depolarization of gray matter astrocytes and impaired K^+ and glutamate uptake were observed (Djukic et al., 2007). Similar results were obtained in cultured astrocytes after down-regulation of Kir4.1 by RNAi (Kucheryavykh et al., 2007). Follow-up studies with cKir4.1$^{-/-}$ animals substantiated the crucial role of Kir4.1 channels in K^+ buffering and demonstrated that their loss causes epilepsy (Chever et al., 2010; Haj-Yasein et al., 2011).

Missense variations in the *KCNJ10* gene have been linked to seizure susceptibility in man (Buono et al., 2004), and it has been demonstrated that missense mutations in *KCNJ10* can protrude into an autosomal recessive disorder characterized by seizures, ataxia, sensorineural deafness, mental retardation and tubulopathy (EAST/SeSAME syndrome) (Bockenhauer et al., 2009; Scholl et al., 2009; Reichold et al., 2010; Williams et al., 2010). Patients suffering from this disorder display focal and generalized tonic-clonic seizures since childhood. Heuser et al. (2010) showed that a combination of three single nucleotide polymorphisms (SNPs) in the *AQP4* gene (encoding a water channel) together with two SNPs in the *KCNJ10* gene was associated with MTLE. Association analysis in patients with a history of febrile seizures (MTLE-FS) versus such without FS revealed that a combination of SNPs in *KCNJ10*, *AQP4*, and the area between *KCNJ10* and *KCNJ9* was significantly associated with MTLE-FS (Heuser et al., 2010). Recently,