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Altered structure and function of astrocytes following status epilepticus

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Abstract

Temporal lobe epilepsy (TLE) is a devastating seizure disorder that is often caused by status epilepticus (SE). Temporal lobe epilepsy can be very difficult to control with currently available antiseizure drugs, and there are currently no disease-modifying therapies that can prevent the development of TLE in those patients who are at risk. While the functional changes that occur in neurons following SE and leading up to TLE have been well studied, only recently has attention turned to the role in epileptogenesis of astrocytes, the other major cell type of the brain. Given that epilepsy is a neural circuit disorder, innovative ways to evaluate the contributions that both neurons and astrocytes make to aberrant circuit activity will be critical for the understanding of the emergent network properties that result in seizures. Recently described approaches using genetically encoded calcium-indicating proteins can be used to image dynamic calcium transients, a marker of activity in both neurons and glial cells. It is anticipated that this work will lead to novel insights into the process of epileptogenesis at the network level and may identify disease-modifying therapeutic targets that have been missed because of a largely neurocentric view of seizure generation following SE.

Keywords

Temporal lobe epilepsy; Status epilepticus; Astrocytes; GCaMP

Temporal lobe epilepsy (TLE) is a neurological disorder characterized by spontaneous and recurrent complex partial seizures that originate in one or both of the temporal lobes and can subsequently generalize. Temporal lobe epilepsy is one of the most common acquired seizure disorders and is often resistant to drug therapy [1]. One common CNS insult that results in the development of TLE is status epilepticus (SE). Status epilepticus is defined as

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either continuous seizure activity lasting longer than 10 min or two or more sequential seizures without full recovery between seizures [2]. Patients with TLE have seizures that are prone to increase in severity over time, often present with hippocampal sclerosis, and it is not uncommon for patients to develop cognitive impairments [3]. One of the most robust pathological findings, universally described in human and animal models of TLE, is profound astrogliosis that occurs throughout the temporal lobe [4]. Thus, the last several decades have seen a steady increase in the numbers of studies designed to ascertain the role of astrocytes in the disease process. Indeed, dramatic changes in expression of neurotransmitter receptors, voltage-gated ion channels, inflammatory cytokines, and other proteins have been identified in astrocytes in both animal and human models of TLE [4–7]. As there is still a significant clinical need for the development of therapeutic approaches that can control seizures in patients with existing TLE and that can prevent the development of TLE in patients at risk, targeting changes in astrocytes with novel therapeutics may be an innovative approach for a disease-modifying therapy.

Reactive astrogliosis is characterized by hypertrophy of primary processes, a dramatic increase in the expression of intermediate filament proteins such as glial fibrillary acidic protein (GFAP), a decrease in expression of glutamine synthetase [8–10] and, in some cases, a disruption in domain organization [11]. In addition, we have demonstrated that in the hippocampus (HC), a brain region known to be involved in seizure generation in TLE, there is a dramatic increase in gap junction coupling in reactive astrocytes, glutamate transport becomes more efficient, potassium buffering remains intact [7], and a number of specific subunits of ionotropic kainate receptors (KAR) are found to be expressed in reactive astrocytes soon after kainic acid (KA)-induced SE [12]. However, the functional significance of these and other changes that have been reported to accompany astrogliosis is not clear [13], and it is currently unknown as to what extent reactive astrocytes either contribute to, or prevent, seizure generation in TLE. Indeed, some components of reactive astrogliosis may be critical compensatory mechanisms following injury, resulting in the dampening of excitability, while other changes may in fact contribute to epileptogenesis and/or seizure generation.

Coming to consensus on the effect of astrogliosis on network activity in epilepsy has been difficult and is likely because of the fact that reactive astrogliosis is not an all-or-nothing event [14] and that the tools to study astrocytes within the context of network activity have only recently been developed. There is a spectrum of severity with respect to astrogliosis, and differences in the kinds of reactive astrocytes being examined may be a contributing factor to a diversity of findings in the literature [14,15]. Mild gliosis to moderate gliosis, found early on in many models with SE, typically do not induce the proliferation of new astrocytes [14], and scarring is not generally observed under those conditions. Indeed, moderate gliosis can actually reverse following resolution of an initial insult [14]. In contrast, following severe CNS injury models, astrocytes contribute to scar formation, which functions to seal off areas of injury. The reactive astrocytes that contribute to scar formation, often observed in severe sclerotic tissue that has been resected [16], may behave considerably differently than those astrocytes that exhibit a milder form of reactivity. Thus, it is important that studies of structure and function of reactive astrocytes following SE be

clear regarding the time points of the studies, the types of controls used, the animal model used, and the extent of the injury.

The study of the role of reactive astrogliosis in SE-induced TLE is far behind our understanding of the changes that take place in surviving neurons in seizure generation zones, and therefore, before rational disease-modifying therapies can be identified for the treatment and/or prevention of TLE, a mechanistic understanding of structure and function of reactive astrocytes must be attained. To this end, our group, as well as many others, has begun the process of unraveling the role of astrocytes not only in SE-induced epileptogenesis, but also in established TLE [13]. Indeed, the development of novel anticonvulsants and interventions that disrupt the process of epileptogenesis critically depends on a comprehensive understanding of the emergent dynamics resulting from the circuit function within seizure-generating brain regions [17], and the role of all cell types within a circuit must be considered.

Recent work from our collaborative research team has focused on developing novel molecular tools for the study of the normal function of astrocytes within a network as well as changes that occur following SE and during the process of epileptogenesis [18,19]. While astrocytes are not electrically excitable like neurons, one mode of prominent activity of astrocytes is the release of calcium from internal stores following activation of a variety of g-protein coupled receptors. Many groups have hypothesized that calcium signaling in astrocytes underlies the release of many signaling molecules, termed ‘gliotransmitters’, which can directly impact neural circuit excitability [20]. While dynamic calcium transients have been readily observed in the somas and proximal thick processes of astrocytes maintained in culture and/or in brain slices prepared from very young animals, synthetic calcium-indicating dyes have been difficult to deliver in either adult or sclerotic tissue. Furthermore, visualization of thin astrocytic processes, where important neuronal–glial interactions most likely occur, has been impossible until the recent development of genetically encoded calcium indicator (GECI) technology [21–24]. Thus, we have developed several tools that will enable us to readily monitor Ca^{2+} activity in the processes of astrocytes in a variety of rodent models of epilepsy. The GFP-derived, circularly permuted family of indicators known as the GCaMPs has been particularly successful GECIs and allows for an outstanding signal-to-noise ratio throughout the fine processes of both astrocytes and neurons [25]. Therefore, we have used these GECIs to begin to investigate neural/glial interactions in seizure-generating brain regions such as the hippocampus and cortex.

The first strategy our group adopted was to develop a reporter mouse, called the PC::G5-td (Polr2a, CAG, GCaMP5G, tdTomato) which reliably reports calcium transients when crossed to Cre drivers specific for astrocytes, such as the *GFAP*-CreER mouse [18]. Both evoked and spontaneous calcium transients in the fine processes of astrocytes can be studied in acutely prepared brain sections or even *in vivo* with this mouse. The ability to image the fine processes of astrocytes will be key to our understanding of the role of astrocytes at the so-called tripartite synapse during the process of epileptogenesis. Recent work from several groups, including ours, using GECIs suggests that spontaneous activity in the fine processes of astrocytes is considerably more frequent and robust than previously thought based on

older work using synthetic calcium imaging dyes [18,26]. Astrocytes ensheath, often in an activity-dependent fashion, both pre and postsynaptic neuronal elements and, because of the expression of a variety of g-protein coupled receptors, are poised to respond to synaptic transmission. It is now well described that, in particular, the metabotropic glutamate receptor 5 (mGluR5) is expressed in astrocytes in a developmental fashion. Activated mGluR5 receptors induce a robust calcium signal in the processes of astrocytes in young animals, but this receptor is not generally expressed in astrocytes in adult rodents [27]. However, reactive astrocytes, such as those observed following SE in rats and in resected TLE tissue from human patients, begin to robustly express mGluR5 [28,29]. Whether or not these aberrantly expressed mGluRs, or the newly expressed kainate receptors (KARs) we have observed to be expressed in reactive astrocytes contribute to enhanced signaling of astrocytes in the epileptic network is currently unknown [12]. However, given the advances in GECIs and two-photon imaging, this question and many others are now well within experimental reach using the versatile PC::G5-td mouse.

While the PC::G5-td mouse will provide an important tool to the epilepsy research community, there is a wealth of information on the effects of SE on astrocytes derived from chemoconvulsant and electrical stimulation models in the rat. Therefore, we set out to find ways to express GCaMPs in rat brain regions likely responsible for seizure generation without the use of viral delivery methods. Viral delivery methods have several limitations, including the need for repeated penetration of the brain with injection needles in order to achieve widespread expression, the potential of the viruses themselves to contribute to inflammation and cytotoxicity [8], and limitations regarding the packaging capability of viral vectors [30]. Therefore, we have adopted the routine use of *in utero* electroporation (IUE) techniques to stably transfect neurons and astrocytes in developing rodent brains with a variety of innovative plasmids featuring several different iterations of GCaMP. Using the piggyBac transposon system, plasmids containing inverted terminal repeats that are recognized by the piggyback transposase enzyme are integrated into genomic DNA and can be stably expressed well into adulthood [19,31]. This is especially important for astrocytes, as they continue to proliferate well after birth in rodents, and episomal DNA would eventually be lost in daughter cells. In addition, given the wealth of information regarding SE-induced changes in adult astrocytes, achieving stable expression in adult cells was a critical component of the approach. Using *IUE*, we have been quite successful in targeting the hippocampus and cortex and can now perform calcium imaging experiments in adult animals with epilepsy. We anticipate that the high resolution provided by the expression of CGaMPs and imaging with two-photon microscopy will lead to an important new understanding of the role of astrocytes in network activity following SE.

Ideally, understanding the wide array of astrocyte functions and changes that also take place at the structural and ultrastructural level following SE will give us clues as to how to exploit those processes that dampen excitability, such as glutamate uptake, gap junctions, and potassium buffering, and how to minimize those processes that contribute to network excitability, such as secretion of cytokines and gliotransmitters [13]. In addition, the development of new tools for wide scale imaging of populations and circuits of neurons and astrocytes, using genetically encoded calcium indicators, will undoubtedly contribute to new

understanding so that new therapies for both disease modification and prevention of acquired epilepsy can be attained.

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