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Functional Changes in Astroglial Cells in Epilepsy

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ABSTRACT
Epilepsy comprises a group of disorders characterized by the periodic occurrence of seizures, and pathologic specimens from patients with temporal lobe epilepsy demonstrate marked reactive gliosis. Since recent studies have implicated glial cells in novel physiological roles in the CNS, such as modulation of synaptic transmission, it is plausible that glial cells may have a functional role in the hyperexcitability characteristic of epilepsy. Indeed, alterations in distinct astrocyte membrane channels, receptors and transporters have all been associated with the epileptic state. This review integrates the current evidence regarding astroglial dysfunction in epilepsy and the potential underlying mechanisms of hyperexcitability. Functional understanding of the cellular and molecular alterations of astroglia-dependent hyperexcitability will help to clarify the physiological role of astrocytes in neural function as well as lead to the identification of novel therapeutic targets.

INTRODUCTION

Epilepsy (from Greek *epilambanein,* seize or attack), affecting about 1% of the population, comprises a group of disorders of the brain characterized by the periodic and unpredictable occurrence of seizures. It is clear that epilepsy is a major public health problem in that those affected experience seizures which impair the performance of many tasks and secondarily the procurement and maintenance of steady employment. Elucidating the cellular and molecular mechanisms of seizure generation may lead to novel antiepileptic drug (AED) therapies.

Most current AEDs act on widely expressed ion channels that directly control neuronal excitability (Rogawski and Löscher, 2004). For example, sodium channel blockers (e.g. phenytoin) reduce the rate and/or rise of neuronal action potentials and thus inhibit high-frequency neuronal firing. GABA receptor agonists (e.g. phenobarbital) increase the efficacy of inhibitory synapses, thus attenuating excitability. Existing medications have two major drawbacks. First, even with optimal current AED therapy, ~30% of patients have poor seizure control and become medically refractory. Second, as many of these nonspecific medications act as general CNS depressants and must be taken chronically for seizure suppression, they also have marked inhibitory effects on cognition.

Several recent lines of evidence suggest that glial cells are potential novel targets for the treatment of CNS diseases. First, recent findings now link glial cells to modulation of synaptic transmission (Volterra and Meldolesi, 2005; Volterra and Steinhäuser, 2004). Second, functional alterations of specific glial membrane channels, receptors and transporters have been discovered in several neurological disorders, including epilepsy (de Lanerolle and Lee, 2005; Heinemann et al., 2000; Seifert et al., 2006; Steinhäuser and Seifert, 2002). For example, direct stimulation of astrocytes has been shown to be sufficient for neuronal synchronization in acute epilepsy models (Tian et al., 2005). Thus, if the cellular and molecular mechanisms by which glial cells (especially astrocytes) modulate excitability are better understood, specific antiepileptic therapies based selectively on modulation of glial receptors and channels that are likely to have fewer deleterious side effects can be contemplated. In this review, we describe the evidence to date regarding alterations and functional roles of distinct astrocyte receptors, membrane channels and transporters in epilepsy.

Two basic limitations of the topic covered here should be considered. First, since the physiological consequences of the intriguing bidirectional communication between neurons and glial cells are still incompletely understood, it is often unclear whether the glial changes are causative of the disease or rather represent an accompanying phenomenon. Second, it is becoming clear that different types of cells with astroglial properties exist within a given brain region, and that the properties of these cells vary in different areas. So far, we have only rudimentary understanding of this glial diversity, and most of the previous studies describing astroglial alterations in epilepsy did not identify the specific cell type affected. In this review, we refer to different types of cells with astroglial properties as "astrocytes."

GLIAL MORPHOLOGICAL CHANGES IN TEMPORAL LOBE EPILEPSY

Alterations in astrocytic properties have been best described in the specific case of human temporal lobe

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epilepsy. Temporal lobe epilepsy is the most common form of epilepsy, and the most common pathology in patients found in patients with medically-intractable temporal lobe epilepsy is hippocampal sclerosis, more generally termed mesial temporal sclerosis. Mesial temporal sclerosis is characterized by neuronal cell loss in specific hippocampal areas, gliosis, microvascular proliferation, and synaptic reorganization (Blümcke et al., 1999; Margerison and Corsellis, 1966; Mathern et al., 1997). Early autopsy studies found mesial temporal sclerosis in 30–58% of temporal lobe epilepsy cases (Bratz, 1899; Margerison and Corsellis, 1966; Sommer, 1880); and similar proportions have been found in examination of specimens resected from patients undergoing surgery for medically intractable temporal lobe epilepsy (de Lanerolle et al., 2003; Falconer, 1974; Mathern et al., 1997). One striking hallmark of the sclerotic hippocampus is that while there is a specific pattern of neuronal loss, there is also "reactive gliosis" with hypertrophic glial cells exhibiting prominent GFAP staining and long, thick processes. Only a few studies have attempted to quantitate changes in astrocyte numbers and densities in epileptic tissue (Briellmann et al., 2002; Krishnan et al., 1994; Mitchell et al., 1999; Van Paesschen et al., 1997). Most of the changes in astrocytic channels and transporters described below have been discovered in sclerotic hippocampi from temporal lobe epilepsy patients. However, the cellular and molecular processes leading to astrocytic changes during epileptogenesis are not yet understood.

GLIAL GLUTAMATE RECEPTORS, TRANSPORTERS AND RELATED ENZYMES IN TEMPORAL LOBE EPILEPSY

Dysfunction of Glutamate Transport and Synthesis

Glutamate transporters are expressed by several CNS cell types, but astrocytes are primarily responsible for glutamate uptake. Important studies using mice with deletion (Tanaka et al., 1997) or antisense oligonucleotide-mediated inhibition of synthesis (Rothstein et al., 1996) of the astroglial transporter GLT-1 revealed that this subtype is responsible for the bulk of extracellular glutamate clearance in the CNS (Danbolt, 2001). Several studies have suggested an involvement of glutamate transporters and receptors in seizure development and spread. Increased extracellular levels of glutamate have been found in epileptogenic foci (During and Spencer, 1993; Glass and Dragunow, 1995). GLT-1 knockout in mice caused spontaneous seizures and hippocampal pathology resembling alterations in temporal lobe epilepsy patients with mesial temporal sclerosis (Tanaka et al., 1997). Pharmacological inhibition of GLT-1 reduced the threshold for evoking epileptiform activity (Campbell and Hablitz, 2004); see also (Demarque et al., 2004) but other animal studies were contradictory. Tessler and colleagues (Tessler et al., 1999) investigated transporter expression on the mRNA and protein levels in human temporal lobe epilepsy specimens and found changes neither for GLT-1 nor GLAST. However, two other groups reported decreased GLT-1 protein as well as unchanged (Mathern et al., 1999) or decreased (Proper et al., 2002) GLAST immunoreactivity in the sclerotic human hippocampus. The latter authors also noted an up-regulation of GLT-1 in the non-sclerotic epileptic hippocampus (Table 1). These findings supported the hypothesis that reduced or dysfunctional glial glutamate transporters in the hippocampus may trigger spontaneous seizures in patients with mesial temporal sclerosis (During and Spencer, 1993), yet the underlying mechanisms are unclear. It has been proposed that the role of glutamate transporters in epilepsy may not be related directly to the control of excitation through synaptic glutamate concentration but rather to alterations in glutamate-dependent metabolism (Maragakis and Rothstein, 2004). In this context, the finding of a loss of glutamate synthetase in the sclerotic vs. non-sclerotic hippocampus of temporal lobe epilepsy patients (Eid et al., 2004) deserves further consideration. After uptake of glutamate into astrocytes, this enzyme rapidly converts the transmitter into glutamine that is then transported to neurons, where it may be resynthesised to glutamate. Eid and co-workers did not observe epilepsy-related changes in the expression of GLT-1. They concluded that in the sclerotic tissue, downregulation of glutamate synthetase caused a slowing of the glutamate-glutamine cycling and accumulation of the transmitter in astrocytes and in the extracellular space (Eid et al., 2004). This conclusion was compatible with findings in animal models of epilepsy and earlier data demonstrating slowed glutamate-glutamine cycling in sclerotic human epileptic hippocampus with magnetic resonance spectroscopy (Petroff et al., 2002). Whether activation of glutamate transporters, e.g. through β-lactam antibiotics (Rothstein et al., 2005), might be beneficial in the treatment of epilepsies remains a matter of further investigation.

Alterations of Ionotropic Glutamate Receptors

A few studies have addressed the potential involvement of ionotropic glutamate receptors in seizure generation. Astrocytes abundantly express receptors of the AMPA subtype composed of the subunits GluR1 to GluR4 (reviewed by Verkhratsky and Steinhäuser, 2000). Mouse mutants with deficient GluR2 Q/R editing developed early-onset epilepsy with spontaneous and recurrent seizure activity, suggesting that enhanced Ca^{2+} influx through the Q form of the GluR2 subunit of AMPA receptors reduces seizure threshold (Brusa et al., 1995). Astrocytes also carry the GluR2 subunit, but altered glial GluR2 editing seems not to play a role in human temporal lobe epilepsy. Rather, combined functional and single-cell transcript analyses put forward the idea that enhanced expression of GluR1 flip variants accounts for the prolonged receptor responses observed in hippocampal astrocytes of epilepsy patients with mesial temporal sclerosis (Seifert et al., 2002; Seifert et al.,

TABLE 1. Involvement of Astroglial Membrane Channels, Transporters, and Receptors in Specific Epilepsy Syndromes

Epilepsy syndrome	Astroglial molecule	Effect	Species	Methods	Reference(s)
Temporal lobe epilepsy	GLT-1	No change	Human	IHC, WB, ISH	(Tessler et al. 1999)
	GLAST	No change			
Temporal lobe epilepsy	GLT-1	↓	Human	IHC	(Mathern et al. 1999)
	GLAST	No change			
Temporal lobe epilepsy	GLT-1	↓	Human	IHC, ISH	(Proper et al. 2002)
	GLAST	↓			
Temporal lobe epilepsy	GLT-1	No change	Human	IHC, WB,	(Eid et al. 2004)
	(Glutamine synthetase)	(↓)		enzyme activity	
Temporal lobe epilepsy	GluR1 ("flip" variant)	↑	Human	PC, pharmacology (CTZ, PEPA), single-cell rtPCR, RA	(Seifert et al. 2002; Seifert et al. 2004)
Temporal lobe epilepsy	mGluR2/3	↑	Human	IHC	(Notenboom et al. 2006; Tang and Lee 2001; Tang et al. 2001)
	mGluR5	↑			
	mGluR8	↑			
Focal cortical dysplasia	mGluR2/3	↑	Human	IHC	(Aronica et al. 2003b)
	mGluR5	↑			
Temporal lobe epilepsy	Kir channel	↓	Human	PC	(Bordey and Sontheimer 1998b)
Temporal lobe epilepsy	Kir channel	↓	Human	ISM, Ba^{2+}	(Heinemann et al. 2000; Kivi et al. 2000)
		↓	Rat (pilocarpine)		
Temporal lobe epilepsy	Kir channel	↓	Human	PC, Ba^{2+}, single-cell rtPCR	(Hinterkeuser et al. 2000; Schröder et al. 2000)
Temporal lobe epilepsy	AQP4	↑ overall ↓ perivascular	Human	IHC, rtPCR, gene chip, EM	(Lee et al. 2004; Eid et al. 2005)
Tuberous sclerosis	GLAST	↓	*Tsc1*^{GFAP}CKO mouse	WB, PC	(Wong et al. 2003)
	GLT-1	↓			
Tuberous sclerosis	Kir	↓	*Tsc1*^{GFAP}CKO mouse	PC, WB, Ba^{2+}, mRNA analysis	(Jansen et al. 2005)
Tumor-associated epilepsy	GluR2	↓ Q/R editing	Human glioma	rtPCR, sequencing	(Maas et al. 2001)
Tumor-associated epilepsy	GLT-1	↓	Human glioma	IHC	(Ye et al. 1999)
	GLAST	Mislocalized			
Tumor-associated epilepsy	Kir channel	↓	Human glioma	PC	(Bordey and Sontheimer 1998a; Olsen and Sontheimer 2004)
		Mislocalized		WB, IHC	
Post-traumatic epilepsy	Kir and Kv channels	↓	Rat (fluid-percussion injury)	PC, ISM	(D'Ambrosio et al. 1999)
Post-traumatic epilepsy	GLT-1	↓	Rat (ferrous chloride)	WB	(Samuelsson et al. 2000)
	GLAST	No change			

Abbreviations: CTZ, cyclothiazide; EM, electron microscopy; IHC, immunohistochemistry; ISH; in situ hybridization; ISM, ion-sensitive microelectrodes; PC, patch clamp; PEPA, 4-[2-(phenylsulfonylamino)ethylthio]-2,6-difluoro-phenoxyacetamide; RA, restriction analysis; rtPCR, reverse transcriptase polymerase chain reaction; WB, Western blot.

2004) (Table 1). This alteration in the splicing status of AMPA receptors predicts enhanced depolarization upon activation by endogenously released glutamate. Actually, the GluR1 flip variant, if co-expressed with GluR2 that is most abundant in astroglial cells of rodent and human hippocampus (Seifert et al., 1997), produces more incomplete receptor desensitization than GluR1 flop (Mosbacher et al., 1994). Prolonged receptor opening will promote influx of Ca^{2+} and Na^{+} ions, and the latter block astroglial Kir channels (Schröder et al., 2002) which will further strengthen depolarization and reduce the K^{+} buffering capacity of astrocytes. It is yet unknown whether the changes in glial receptor function are causative of, or result from, the epileptic condition. Also, to what extent alterations in glial GluR1 splicing contribute to seizure generation or spread requires further investigation. Astrocytes cultured from patients with Rasmussen's encephalitis, a rare form of childhood epilepsy, showed spontaneous Ca^{2+} oscillations that were dependent on transmembrane influx of Ca^{2+} (Manning and Sontheimer, 1997). The authors speculated that these responses might promote neuronal hyperactivity, possibly due to autocrine ionotropic glutamate receptor stimulation by glutamate released from astrocytes. Another study suggested that the destruction of astrocytes by GluR3 antibodies plays a critical role in the progression of this autoimmune disorder (Whitney and McNamara, 2000).

Metabotropic Glutamate Receptors and Astroglial Ca^{2+} Signalling in Epilepsy

Under normal conditions, mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor subtypes expressed by glial cells. Activation of these receptors affects cAMP accumulation and leads to an increase in intracellular Ca^{2+}, respectively. Group II mGluRs (mGluR 2, 3) have been shown to be negatively coupled to cAMP levels in cultured astrocytes (Wroblewska et al., 1998) although other studies reported increases in cAMP levels (Moldrich et al., 2002; reviewed by Winder and Conn, 1996). The Ca^{2+} rise may oscillate and initiate Ca^{2+} wave propagation within the astrocyte network, activate Ca^{2+}-dependent ion channels and induce glutamate release from astrocytes (Volterra and Meldolesi, 2005). In experimental epilepsy, reactive astrocytes of the hippocampus persistently upregulate mGluR3, mGluR5 and mGluR8 protein (Steinhäuser and Seifert, 2002). Electron-microscopic and immunohistochemical inspection of hippocampal tissue from temporal lobe epilepsy patients revealed expression of mGluR2/3, mGluR4, mGluR5 and mGluR8 in reactive astrocytes, suggesting an involvement of these receptors in gliosis (Notenboom et al., 2006; Tang and Lee, 2001; Tang et al., 2001). Upregulation of astroglial mGluR2/3 and mGluR5 was also observed in epileptic specimens from patients with focal cortical dysplasia (Aronica et al., 2003b) (Table 1). Whether these changes affect the activity of glial glutamate transporters is currently under discussion (Aronica et al., 2003a).

ASTROCYTIC GLUTAMATE RELEASE IN EPILEPSY

Over the past few years, Ca^{2+} signalling mechanisms in astrocytes have received considerable attention. Of particular importance is the novel observation that astrocytes exhibit Ca^{2+}-induced release of glutamate which provides direct excitation to neighboring neurons (Volterra and Meldolesi, 2005). Because of the observed changes in protein expression within astrocytes following injuries that lead to the development of epilepsy, it is tempting to speculate that alterations in this glial-derived excitatory pathway in coordination with reductions in glutamate uptake might provide an excitatory drive underlying seizure disorders.

Astrocytes are capable of releasing glutamate through a Ca^{2+}-dependent process, which might be involved in seizure generation (Kang et al., 2005). In chemically-induced, acute epilepsy models, astrocytes were reported to contribute to the generation of synchronized epileptiform activity (Tian et al., 2005). In these studies, epileptiform discharges were provoked through the application of 4-aminopyridine, $GABA_A$ receptor antagonists, or bath solutions containing low concentrations of divalent cations. Surprisingly, and in contrast to several previous findings, the paroxysmal neuronal depolarization shifts (PDSs) provoked under these conditions were largely insensitive to TTX and also occurred after suppressing synaptic activity. The authors then showed that astrocytic increase in $[Ca^{2+}]_i$ is sufficient to stimulate release of glutamate from glial cells, which was critical for the generation of PDSs. This is also surprising because eliciting astrocyte Ca^{2+} signals in numerous other studies did not produce seizure discharges. In addition, in vivo imaging showed that some antiepileptic drugs suppressed astrocytic Ca^{2+}-signalling. It has to be mentioned here that human epilepsy is associated with significant morphological alterations (Blümcke et al., 2002; de Lanerolle and Lee, 2005; Kim, 2001) that are absent in the acute models. Certainly, more experimentation is needed to figure out whether astrocytes represent targets for the development of anti-epileptic treatments.

ASTROCYTE POTASSIUM AND WATER CHANNELS

Since both extracellular K^{+} concentration and osmolarity have been shown to dramatically modulate neural excitability, it is plausible that changes in astrocytic K^{+} or water channels could contribute to hyperexcitability in epilepsy. Indeed, recent studies have found changes in astroglial Kir channels and AQP4 water channels in temporal lobe epilepsy specimens.

K^+ Channels

During neuronal hyperactivity, extracellular [K^+] may increase from ~3 mM to a ceiling of 10–12 mM; and K^+ released by active neurons is thought to be primarily taken up by glial cells (Ballanyi et al., 1987; Heinemann and Lux, 1977; Somjen, 2002; Xiong and Stringer, 1999). Any impairment of glial K^+ uptake would be expected to be proconvulsant based on many previous studies. In the hippocampus, millimolar and even submillimolar increases in extracellular K^+ concentration powerfully enhance epileptiform activity (Feng and Durand, 2006; Rutecki et al., 1985; Traynelis and Dingledine, 1988; Yaari et al., 1986). High-K^+ also reliably induces epileptiform activity in hippocampal slices from human patients with intractable temporal lobe epilepsy and hippocampal sclerosis (Gabriel et al., 2004).

A primary mechanism for K^+ reuptake is thought to be via glial inwardly rectifying K^+ channels (Kir channels). Glial Kir channels may contribute to K^+ reuptake and spatial K^+ buffering (Orkand et al., 1966; Ransom, 1996), which has been most clearly demonstrated in the retina (Newman and Karwoski, 1989; Newman et al., 1984; Newman, 1986; Newman, 1993). While multiple subfamilies of Kir channels exist (Kir1-Kir7) differing in tissue distribution and functional properties, in brain astrocytes the expression of Kir4.1 has been investigated most thoroughly (Hibino et al., 2004; Higashi et al., 2001). Pharmacological or genetic inactivation of Kir4.1 leads to impairment of extracellular K^+ regulation (Kofuji and Newman, 2004; Kofuji et al., 2000; Neusch et al., 2006). However, members of the strongly rectifying Kir2 family may also contribute to astroglial K^+ buffering (Butt and Kalsi, 2006; Neusch et al., 2003).

Downregulation of astroglial Kir channels has been found in the injured or diseased CNS. Kir currents are reduced following injury-induced reactive gliosis in vitro (MacFarlane and Sontheimer, 1997), entorhinal cortex lesion (Schröder et al., 1999), freeze lesion-induced cortical dysplasia (Bordey et al., 2000; Bordey et al., 2001), and traumatic (D'Ambrosio et al., 1999) and ischemic (Köller et al., 2000) brain injury. In addition, several studies have indicated downregulation of Kir currents in specimens from patients with temporal lobe epilepsy (Bordey and Sontheimer, 1998b; Hinterkeuser et al., 2000; Kivi et al., 2000; Schröder et al., 2000) (Table 1). 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 (Heinemann et al., 2000; Kivi et al., 2000). Ba^{2+}, a blocker of Kir channels, augmented stimulus-evoked K^+ elevation in non-sclerotic but not in sclerotic specimens, suggesting an impairment in K^+ buffering in sclerotic tissue. Direct evidence for downregulation of Kir currents in the sclerotic CA1 region of hippocampus came from a comparative patch-clamp study in which a reduction in astroglial Kir currents was observed in sclerotic compared with non-sclerotic hippocampi (Hinterkeuser et al., 2000). These data indicate that dysfunction of astroglial Kir channels could underlie impaired K^+ buffering and contribute to hyperexcitability in epileptic tissue (Steinhäuser and Seifert, 2002). When and how this dysfunction develops during epileptogenesis is not yet clear.

Water Channels

Alterations in astroglial water regulation could also powerfully affect excitability. Brain tissue excitability is exquisitely sensitive to osmolarity and the size of the extracellular space (ECS) (Schwartzkroin et al., 1998). Decreasing ECS volume produces hyperexcitability and enhanced epileptiform activity (Chebabo et al., 1995; Dudek et al., 1990; Pan and Stringer, 1996; Roper et al., 1992); conversely, increasing ECS volume with hyperosmolar medium attenuates epileptiform activity (Dudek et al., 1990; Haglund and Hochman, 2005; Pan and Stringer, 1996; Traynelis and Dingledine, 1989). These experimental data parallel extensive clinical experience indicating that hypo-osmolar states such as hyponatremia lower seizure threshold while hyperosmolar states elevate seizure threshold (Andrew et al., 1989).

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 (Verkman, 2005). There is increasing evidence that water movement in the brain involves aquaporin channels (Amiry-Moghaddam and Ottersen, 2003; Manley et al., 2004). Aquaporin-4 (AQP4) is expressed ubiquitously by glial cells, especially at specialized membrane domains including astroglial endfeet in contact with blood vessels and astrocyte membranes that ensheath glutamatergic synapses (Nagelhus et al., 2004; Nielsen et al., 1997). Activity-induced radial water fluxes in neocortex have been demonstrated that could be due to water movement via aquaporin channels in response to physiological activity (Holthoff and Witte, 2000; Niermann et al., 2001). Mice deficient in AQP4 have markedly decreased accumulation of brain water (cerebral edema) following water intoxication and focal cerebral ischemia (Manley et al., 2000) and impaired clearance of brain water in models of vasogenic edema (Papadopoulos et al., 2004), suggesting a functional role for AQP4 in brain water transport. Similarly, mice deficient in dystrophin or α-syntrophin, in which there is mislocalization of the AQP4 protein (Frigeri et al., 2001; Neely et al., 2001; Vajda et al., 2002), also demonstrate attenuated cerebral edema (Amiry-Moghaddam et al., 2003a; Vajda et al., 2002).

Alteration in the expression and subcellular localization of AQP4 has been described in sclerotic hippocampi obtained from patients with mesial temporal sclerosis (Table I). Using immunohistochemistry, rt-PCR and gene chip analysis, Lee et al. demonstrated an overall increase in AQP4 expression in sclerotic hippocampi (Lee et al., 2004). However, using quantitative immunogold electron microscopy, the same group found that there was mislocalization of AQP4 in the human epileptic hippocampus, with reduction in perivascular membrane

expression (Eid et al., 2005). The authors hypothesized that the loss of perivascular AQP4 perturbs water flux, impairs K^+ buffering, and results in an increased propensity for seizures.

Several lines of evidence support the hypothesis that AQP4 and Kir4.1 may act in concert in K^+ and H_2O regulation (Simard and Nedergaard, 2004). First, K^+ reuptake into glial cells could be AQP4-dependent, as water influx coupled to K^+ influx is thought to underlie activity-induced glial cell swelling (Walz, 1987; Walz, 1992). Second, studies in the retina have demonstrated subcellular co-localization of AQP4 and Kir4.1 via both electron microscopic and co-immunoprecipitation analyses (Connors et al., 2004; Nagelhus et al., 2004). Third, Kir4.1$^{-/-}$ mice, like AQP4$^{-/-}$ mice (Li and Verkman, 2001; Li et al., 2002), have impaired retinal and cochlear physiology presumably due to altered K^+ metabolism (Marcus et al., 2002; Rozengurt et al., 2003). Fourth, AQP4$^{-/-}$ mice have remarkably slowed K^+ reuptake in models of seizure and spreading depression in vivo (Binder et al., 2006; Padmawar et al., 2005) associated with a near-threefold increase in seizure duration (Binder et al., 2006). Fifth, afferent stimulation of hippocampal slices from α-syntrophin-deficient mice demonstrates a deficit in extracellular K^+ clearance (Amiry-Moghaddam et al., 2003b). These data are consistent with the idea that AQP4 and Kir4.1 participate in clearance of K^+ following neural activity. However, further studies are required to clarify the expression and functional interaction of AQP4 and Kir4.1 in the hippocampus and their changes during epileptogenesis.

ASTROCYTE DYSFUNCTION INVOLVED IN OTHER 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 (Au et al., 2004). Epilepsy occurs in 80–90% of cases of TS, frequently involves multiple seizure types and is often medically refractory (Thiele, 2004). Cortical tubers represent the pathologic substrate of TS, and microscopically consist of a specific type of dysplastic lesion with astrocytosis and abnormal giant cells (Trombley and Mirra, 1981). 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, recent 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 (Uhlmann et al., 2002). 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 GLT1 and GLAST (Wong et al., 2003) (Table 1). In addition, recent evidence indicates that astrocytes from *Tsc1*^{GFAP}CKO mice exhibit reduced Kir channel activity, and hippocampal slices from these mice demonstrated increased sensitivity to K^+-induced epileptiform activity (Jansen et al., 2005) (Table 1). 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 cases (Ettinger, 1994; Rasmussen, 1975). 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 (Luyken et al., 2003). Microdialysis studies of gliomas have revealed reduced glutamate in the tumor compared with peri-tumoral tissue (Bianchi et al., 2004). 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 (Maas et al., 2001) (Table 1). Second, Sontheimer's group found that glioma cells release larger than normal amounts of glutamate in vitro (Ye and Sontheimer, 1999). The release of glutamate from glioma cells was accompanied by a marked deficit in Na^+-dependent glutamate uptake, reduced expression of astrocytic glutamate transporters (Table 1), and upregulation of cystine-glutamate exchange (Ye et al., 1999). 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 Kir currents (Bordey and Sontheimer, 1998a) and mislocalization of Kir4.1 channels (Olsen and Sontheimer, 2004) have been found in malignant astrocytes (Table 1).

Post-Traumatic Epilepsy

Post-traumatic epilepsy refers to a recurrent seizure disorder whose cause is believed to be traumatic brain injury. It is a common and important form of epilepsy (Frey, 2003; Garga and Lowenstein, 2006), and develops

in a variable proportion of traumatic brain injury survivors depending on the severity of the injury and the time after injury (Annegers et al., 1998; Caveness et al., 1979). Anticonvulsant prophylaxis is ineffective at preventing the occurrence of late seizures (D'Ambrosio and Perucca, 2004; Temkin et al., 1990; Temkin et al., 1999). Weight-drop and fluid-percussion injury animal models of post-traumatic epilepsy have demonstrated characteristic structural and functional changes in the hippocampus, such as death of dentate hilar neurons and mossy fiber sprouting (Golarai et al., 2001; Lowenstein et al., 1992; Santhakumar et al., 2001). Recently, studies have also implicated altered astrocyte function in post-traumatic epilepsy 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 with controls (D'Ambrosio et al., 1999) (Table 1). This was accompanied by the appearance of electrical afterdischarges in CA3. Thus, this study suggests impaired K^+ homeostasis in post-traumatic hippocampal glia. Another study demonstrated reduction in expression of the astrocyte glutamate transporter GLT1 in a post-traumatic epilepsy model induced by intracortical ferrous chloride injection, suggesting impaired glutamate transport (Samuelsson et al., 2000) (Table 1). Further studies of the role of glial cells in post-traumatic epilepsy appear warranted now that reliable post-traumatic epilepsy animal models have been developed (D'Ambrosio et al., 2004).

PERSPECTIVES AND FUTURE DIRECTIONS

Astrocytes undergo cellular and molecular changes in epilepsy, including alteration in glutamate transporters and receptors as well as Kir channels and water channels. So far, most of these changes have been demonstrated in sclerotic hippocampi from patients with temporal lobe epilepsy or animal models resembling this particular human condition. However, the various functions of astrocytes in modulation of synaptic transmission and glutamate, K^+ and H_2O regulation suggest that astrocyte dysfunction could also be part of the pathophysiology of other forms of epilepsy.

One important recent development is the recognition of structural and functional heterogeneity of cells with astroglial properties. It is clear that a subset of hippocampal astroglial cells ("classical" astrocytes or GluT cells) expresses glutamate transporters and not ionotropic glutamate receptors and another (NG2 glia or GluR cells) expresses ionotropic glutamate receptors but not glutamate transporters (Matthias et al., 2003; Nishiyama et al., 2005). However, the lineage relationship of NG2 glia/GluR cells and the relative roles of bona fide astrocytes versus NG2 glia/GluR cells in epilepsy still remain unclear. In addition, the functional roles of ionotropic glutamate receptors, Kir and AQP4 channels in these subsets of glial cells in the hippocampus, are not yet understood. It is worth mentioning in this context that hippocampal NG2 glia/GluR cells lack gap junctional coupling but receive direct synaptic input from GABAergic and glutamatergic neurons (Bergles et al., 2000; Jabs et al., 2005; Lin and Bergles, 2004; Wallraff et al., 2004). Gap junctions may also regulate excitability, although available data are inconsistent and do not allow to estimate the impact of their altered expression on epileptogenesis (Nemani and Binder, 2005; Steinhäuser and Seifert, 2002). The availability of mice with genetically uncoupled astrocytes (Wallraff et al., 2006) will allow examination of this question, by separating the effects produced by alterations of neuronal versus glial gap junctions. It will be important in future studies to examine the cellular and molecular properties of subsets of hippocampal glial cells in human epileptic tissue and unravel the course of their functional alterations during epileptogenesis in appropriate animal models.

Another recent focus in astrocyte biology that may become important for epilepsy research is the "gliovascular junction" (Simard et al., 2003). Microvascular proliferation in the sclerotic hippocampus was noted as early as 1899, but the role of the vasculature and the blood-brain barrier in epilepsy is not yet clear. The intimate relationship between astroglial endfeet ensheathing blood vessels, the targeted expression of AQP4 and Kir4.1 on astroglial endfeet, and the role of astrocytes in blood-brain barrier permeability (Abbott, 2002) and control of microcirculation (Metea and Newman, 2006; Mulligan and MacVicar, 2004; Takano et al., 2006; Zonta et al., 2003) have only recently been appreciated. Local pathological alterations in the gliovascular junction could perturb blood flow, K^+ and H_2O regulation and constitute an important mechanism in the generation of hyperexcitability. Indeed, a recent study suggests that transient opening of the blood-brain barrier is actually sufficient for focal epileptogenesis (Seiffert et al., 2004). The cellular and molecular roles of the gliovascular junction in metabolic homeostasis and changes during epileptogenesis are only beginning to be explored.

In conclusion, the exact changes taking place in astroglial functioning during epilepsy are still poorly understood. 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. In addition to changes in preexisting glial cell populations, newly-generated glial cells with distinct properties may migrate into the hippocampus and contribute to enhanced seizure susceptibility (Hüttmann et al., 2003; Parent et al., 2006). The available data likely represent only the "tip of the iceberg" in terms of the functional role of astroglial cells in epilepsy. In view of the many physiologic functions of astrocytes that have been elucidated within the past decade, it can be expected that the next few years will yield evidence of similar important roles for glial cells in pathophysiology. Further study of astrocyte alterations in epilepsy should lead to the identification of novel molecular targets that might open new avenues for the development of alternative antiepileptic therapies.

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