Candidate Drug Targets for Prevention or Modification of Epilepsy

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Abstract

Epilepsy is a prevalent neurological disorder afflicting nearly 50 million people worldwide. The disorder is characterized clinically by recurrent spontaneous seizures attributed to abnormal synchrony of brain neurons. Despite advances in the treatment of epilepsy, nearly one-third of patients are resistant to current therapies, and the underlying mechanisms whereby a healthy brain becomes epileptic remain unresolved. Therefore, researchers have a major impetus to identify and exploit new drug targets. Here we distinguish between epileptic effectors, or proteins that set the seizure threshold, and epileptogenic mediators, which control the expression or functional state of the effector proteins. Under this framework, we then discuss attempts to regulate the mediators to control epilepsy. Further insights into the complex processes that render the brain susceptible to seizures and the identification of novel mediators of these processes will lead the way to the development of drugs to modify disease outcome and, potentially, to prevent epileptogenesis.

EPILEPSY

A Babylonian tablet in London's British Museum dating from approximately 1060 BC refers to epilepsy as "the falling disease," with the subjective aura and the subsequent seizures themselves ascribed to the work of childless demons who viewed humans with envy and spite (1). The Greek physician Hippocrates argued around 400 BC that epilepsy is a physical disorder of the brain (2), but he was widely disbelieved. There followed more than two millennia of attempts to treat seizures by bleeding, exorcism, trepanation, and silver nitrate or bromide ingestion. In 1912, phenobarbital was introduced as the first modern antiseizure medication, followed by phenytoin in 1938 and then a raft of other anticonvulsant drugs. These now number over 30,[1] the most recent being the glutamate receptor antagonist perampanel. All these drugs act to suppress seizures in people who have already been diagnosed with epilepsy, yet they are ineffective in more than a third of patients, and there is no successful therapeutic strategy designed to prevent epilepsy in those at risk.

Epilepsy affects 1–2% of the population. Risk factors for epilepsy include stroke or bleeding into the brain, central nervous system (CNS) infections, brain tumors, prolonged febrile seizures (FS), and other occurrences of status epilepticus (SE). Additionally, hundreds of rare, simple Mendelian epilepsies exist, and there is a growing recognition that genetics also plays a role in many if not all acquired epilepsies. The process of converting a normal brain to a brain with epilepsy is known as epileptogenesis. A successful preventive intervention, were it to be developed, would be termed antiepileptogenic. Developing a better understanding of epileptogenesis is currently a major focus of the epilepsy research community; hence, many candidate processes and molecular targets are under intense scrutiny. Below, we review potential epileptogenic processes and the evidence for selected candidate targets. But first, a few words on the relationships among seizures, epilepsy, and epileptogenesis.

A FRAMEWORK FOR UNDERSTANDING EPILEPTOGENESIS

A seizure results from a pathologic, synchronous overactivation of one or more synaptic pathways, typically in the forebrain. Epilepsy is a family of chronic neurological disorders that can be characterized by a persistently lowered seizure threshold in one of these pathways. Under this framework, epileptogenesis is the process by which a persistently lowered seizure threshold is created. Epileptogenic effectors are those ion channels and ligand-gated receptors that together determine or set the moment-to-moment seizure threshold in each synaptic pathway. Epilepsy is often considered to be primarily a channelopathy, a disease caused by dysfunction of these effector ion channels. Indeed, many of the Mendelian epilepsies involve an ion channel mutation, and most of the currently available anticonvulsants directly target one or more effector ion channel proteins. However, for the acquired epilepsies, researchers must search for mechanisms that create ion channel dysfunction. Here we introduce the term epileptogenic mediator to refer to a protein that controls the expression level or functional state of the effector proteins. The decisive concept (if you accept the framework of effector and mediator) is that identification and control of the epileptogenic mediator proteins is the key to interrupting epileptogenesis. The poverty of an early focus on the ion channels and receptor effector proteins themselves is highlighted by the results

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[1]The following are the US Food and Drug Administration–approved drugs for seizures in early 2014: acetazolamide, amobarbital, carbamazepine, clonazepam, clorazepate, diazepam, eslicarbazepine, ethosuximide, ethotoin, ezogabine, felbamate, fosphenytoin, gabapentin, lacosamide, lamotrigine, levetiracetam, magnesium salts, mephobarbital, methsuximide, oxcarbazepine, perampanel, phenobarbital, phenytoin, prednisone, pregabalin, primidone, pyridoxine, rufinamide, tiagabine, topiramate, valproic acid, vigabatrin, and zonisamide.

DISEASE MODIFICATION VERSUS SYMPTOMATIC INTERVENTION

Disease modification refers to treatment approaches that target the underlying disease-promoting mechanisms rather than simply alleviating disease symptoms. Interventions may be considered disease modifying if they provide sustained improvement in functionally relevant biomarkers of disease. In contrast, symptomatic treatments attenuate symptoms of the disease but do not interfere with the underlying disease processes such that when the intervention is discontinued, symptoms return quickly. Disease-modifying strategies are expected to attenuate symptoms during intervention, but if the treatment were discontinued, symptoms would reappear more slowly, if at all. A long-lasting benefit after removal of the therapy implies that changes have occurred in the pathologic process. Complicated clinical trial designs involving delayed or staggered treatment starts for some groups or randomized times of withdrawal have been proposed to reveal disease modification superimposed on symptomatic relief. No US Food and Drug Administration–approved anticonvulsant has yet been demonstrated to be disease modifying; each provides only symptomatic relief.

of 47 clinical trials designed to test the idea that administration of anticonvulsants after an epileptogenic trigger event would prevent the development of epilepsy. All 47 trials failed (3), hence the current focus on control of processes that influence seizure threshold, such as inflammation and neuronal plasticity.

EPILEPTOGENESIS VERSUS DISEASE MODIFICATION

An antiepileptogenic therapy would prevent the frank appearance of epilepsy in an at-risk patient as described above. Disease modification, by contrast, is designed to slow progression, alleviate symptoms of epilepsy, or convert medically intractable epilepsy to drug-responsive epilepsy (see the sidebar entitled Disease Modification Versus Symptomatic Intervention). Whether an anticonvulsant drug is also disease modifying or antiepileptogenic can be determined by observing the response to withdrawing the drug. Seizures will appear immediately upon washout of an anticonvulsant drug, will be delayed substantially beyond washout for a disease modifier, and will be prevented from appearing altogether by an antiepileptogenic treatment. Treatments that reduce the frequency or severity of epileptic seizures or associated problems by attenuating the underlying pathology would also be highly beneficial. For example, the cognitive deficits encountered in some epileptic patients are likely due, in part, to neurodegeneration accompanying some forms of epilepsy. If so, a neuroprotectant administered before neuronal injury peaked would be expected to improve performance on cognitive tasks. Likewise, anti-inflammatory, immunosuppressant, and neuroplasticity approaches are being considered as disease-modifying therapies, as are strategies that convert pharmacoresistant seizures to pharmacosensitive epilepsy (4).

POTENTIAL EPILEPTOGENIC PROCESSES

Researchers have proposed numerous processes that can lower seizure threshold and thus render the brain susceptible to the generation of spontaneous seizures, i.e., epilepsy. Here we examine the rationale for some of these deleterious events in promoting epilepsy.

Microglial Activation

Microglia: resident brain innate immune cells of myeloid lineage; can proliferate, undergo structural modifications, migrate, and phagocytose cells and noncellular debris

Blood-brain barrier: multicellular structure with continuous endothelial cell membrane; regulates transport of cells and blood-circulating molecules into the brain and removal of neurotoxic molecules

In the healthy brain, microglia were once thought of as a static cellular population that passively waited for insults to the CNS. However, advances in two-photon in vivo imaging and generation of sophisticated rodent models have revised this long-held view. Microglia continually palpate their local microenvironment, including astrocytes and other microglia as well as neuronal structures such as synapses, and they respond rapidly to tissue injury (5, 6). Insults to the CNS induce microgliosis, a response typified by microglial proliferation, morphological modifications, and expression and secretion of cytokines, immune molecules that can induce robust changes in cellular metabolism in an autocrine or paracrine manner (7). Microgliosis has been documented in brain tissue resected from epileptic patients (8, 9) as well as rodent models of SE induced by kainic acid or pilocarpine (10–12). In addition, elevated levels of microglia-derived inflammatory cytokines are also encountered in brain material from mice after SE and recurrent seizures as well as from epileptic patients (9, 13). Thus, a rich inflammatory environment is observed in the hyperexcitable brain.

Microgliosis is typified by enhanced expression and secretion of proinflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), and IL-6, discussed further below. The role of cytokines in seizures and epileptogenesis has been studied using genetically modified animal models or pharmacological methods. The injection of IL-1β (14, 15), complement system components (16), or prostaglandins (17) into rodent brain lowers the seizure threshold. The cellular mechanisms that underlie these seizure-promoting effects include upregulation of excitatory glutamatergic transmission and downregulation of inhibitory gamma-aminobutyric acid (GABA)-ergic transmission (18).

A cytokine-mediated inflammatory response has also been suggested to promote blood-brain barrier damage. Dysfunction of the blood-brain barrier can result in ionic imbalances and alterations in neurotransmitter and metabolite distribution, which can culminate in seizures (19). One of the mechanisms by which IL-1β has been proposed to enhance blood-brain barrier dysfunction and permeability is through downregulation of ZO-1 in the vascular endothelium (20, 21).

Finally, it is now becoming clear that microglia mediate developmental elimination of inappropriate synapses. Such synapses are tagged by classical components of the complement cascade and subsequently removed by microglia through complement receptor 3 signaling (22, 23). Microglia-mediated synaptic pruning during critical periods of development appears to be important for neurological function in later life, as increased synaptic spine density leads to decreased functional connectivity in adult mice (24). In addition to early developmental periods, microglia-dependent synaptic elimination has been reported in adult animals (25), and synaptic stripping is elevated after brain injury and in multiple neurodegenerative conditions (26). Thus, an intriguing possibility is that an initial injury or infectious event might lead to microglia-mediated synaptic stripping. If the synapses are inhibitory connections, then the seizure threshold in the affected neuronal circuit could be decreased.

Astrogliosis

Astrocytes are the most numerous cell type in the brain and are responsible for a wide spectrum of functions that promote CNS homeostasis. These roles include structural support, ionic balance maintenance, clearance of neurotransmitters, and maintenance of the blood-brain barrier. Reactive astrocytosis is a term used to describe the structural and metabolic changes in astrocytes that are frequently observed after insults to the brain that cause injury or disease. Astrocytosis is a feature of both mesial temporal lobe epilepsy and animal models of recurrent seizures and is accompanied by ionic and transmitter imbalances that can render the brain susceptible to the generation of spontaneous seizures.

Astrocytes regulate extracellular potassium levels, water flow, and thus volume of the extracellular space (ECS). The ECS volume and extracellular potassium levels both have a powerful influence on neuronal excitability (27). Multiple lines of evidence have pointed to aquaporins and certain potassium channels as key regulators of ECS volume. Aquaporins are a family of integral membrane proteins that mediate the constitutive and regulated transport of water across cellular membranes. In the brain, aquaporin 4 (AQP4) is predominantly expressed by astrocytes, where it facilitates the bidirectional water flow between brain ECS and blood to regulate the osmolarity of the interstitial fluid bathing neurons in the brain (28). Indeed, AQP4 knockout mice have decreased levels of brain water following cerebral focal ischemia (29). Astrocytes also play an important role in maintaining the concentration of extracellular potassium, mainly through the inward rectifier potassium channel Kir4.1 (30, 31).

Finally, astrocytes also impact the excitability of the brain through controlled uptake of extracellular glutamate through glutamate transporters. Glial-specific transporters—excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2)—are expressed by astrocytes (32, 33). The high efficiency of these transporters ensures extracellular glutamate levels remain low, promoting proper synaptic transmission, limiting neuronal depolarization, and preventing excitotoxic cell death (34). However, whether glutamate transporter expression is altered in epilepsy remains unresolved. In one study, glial EAAT1 and EAAT2 expression was decreased in brain tissue from epileptic patients with hippocampal sclerosis compared to nonepileptic controls (35). In contrast, other studies found no difference in transporter expression in tissue from epileptics (36, 37). These conflicting observations might be attributed to differences in neuronal cell number between epileptic and control groups, epileptogenesis development, or tissue processing.

Neuronal Plasticity

One of the most fascinating characteristics of the brain is its ability to undergo structural and functional changes in response to environmental signals. Functional changes can be transient as well as long lasting, persisting for months or longer. Neuronal plasticity is fundamental to the formation of new memories but is also likely to be involved in numerous brain disorders, including epilepsy.

It is now well established that seizures can induce profound changes in gene expression. Brain expression levels of neuromodulators—proteins that are involved in neural survival, differentiation, growth, and communication—fluctuate after seizures and have been proposed as an epileptogenic process (38). For example, brain levels of brain-derived neurotrophic factor (BDNF) are upregulated in hippocampal tissue resected during epilepsy surgery from individuals with temporal lobe epilepsy (39). The activation of neurotrophic tyrosine kinase receptor, type 2 (TrkB) has been proposed as a necessary event for SE-induced epilepsy (40). However, others have proposed that BDNF signaling reduces spontaneous seizures (41). Recently, Liu and colleagues (42) showed that inhibition of TrkB signaling during the epileptogenic phase after SE can prevent the development of epilepsy as well as associated behavioral phenotypes and neuronal damage, suggesting that TrkB could be an attractive target for epilepsy prevention.

Changes in expression or function of ion channel proteins also underlie neuronal plasticity. In rodents, SE causes aberrant expression of GABA receptor subunits (43, 44) that continues after the animal becomes epileptic (45). Additionally, N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit expression can also be altered, further promoting neuronal excitability and lowering seizure thresholds.

Structural plasticity has long been described as a consequence of seizures. Several animal models of epilepsy, including the kainic acid and pilocarpine models, develop structural changes in the

hippocampus involving growth of new axon collaterals from granule cells of the dentate gyrus (46–48). Mossy fiber sprouting is also observed in epilepsy patients (49–51). SE can induce aberrant sprouting of mossy fibers accompanied by new synaptic connections with both excitatory granule cells and inhibitory interneurons (52–55). Whereas some studies have reported that synaptic reorganization of mossy fibers creates new recurrent excitatory circuits (56, 57), others have shown that reorganization can lead to greater inhibition in the dentate gyrus when sprouting occurs (58). The latter scenario is likely attributed to sprouting and synapse formation on inhibitory interneurons (52).

However, although numerous in vivo studies have reported correlations between the degree of mossy fiber sprouting and seizure frequency in the chronic epileptic phase (59–61), some have not (62). Convincing evidence now exists for one mouse model of epilepsy that mossy fiber sprouting neither promotes nor impedes the development of spontaneous seizures (63).

Blood-Brain Barrier Breakdown

The blood-brain barrier is a multicellular structure that separates the CNS from peripheral circulation. Numerous cell types maintain the blood-brain barrier, including astrocytes, endothelial cells, and pericytes, as well as noncellular elements such as extracellular matrix proteins (19). The blood-brain barrier not only limits entry of blood-derived proteins and peripheral cells but also controls the regulated entry of numerous biologically active molecules necessary for maintaining CNS homeostasis, such as potassium, glucose, and amino acids (64). Compromising the integrity and function of the blood-brain barrier can result in deleterious consequences due to the extravasation of both peripheral molecules and blood cells into the CNS.

A causative relationship between blood-brain barrier breakdown and seizures has been suggested because osmotic opening of the blood-brain barrier results in seizures in rodents (65, 66). Opening of the blood-brain barrier can promote brain hyperexcitability and epileptogenesis through numerous different mechanisms. First, CNS invasion of blood-derived proteins can activate resident brain astrocytes. For example, the blood protein albumin can enter astrocytes in a transforming growth factor-β (TGF-β) receptor–dependent manner, leading to activation and phosphorylation of Smad2, which subsequently downregulates the Kir4.1 potassium channel. As a consequence, an epileptic focus is created (67, 68). Second, pilocarpine-induced SE can enhance the expression of numerous leukocyte adhesion proteins such as intercellular adhesion molecule-1 (Icam-1), vascular cell adhesion protein-1 (Vcam-1), and E- and P-selectin (69). Expression of these adhesion proteins promotes the interaction of blood leukocytes with endothelial cells on the luminal side of the vessel wall and facilitates leukocyte transmigration through the endothelial cell layer. Interestingly, antibody-mediated inhibition of leukocyte-adhesion protein interaction prevented the appearance of spontaneous seizures in the chronic phase of chemoconvulsant-induced SE (69). These findings provide evidence that limiting brain infiltration of blood-borne immune cells can inhibit epileptogenesis.

Finally, a consequence of epileptic activity is upregulation of the endothelial efflux transporter P-glycoprotein, a major molecular gatekeeper of the blood-brain barrier (70). Numerous lines of experimental and clinical evidence suggest that seizure-induced upregulation of P-glycoprotein can impair access of anticonvulsant drugs to the brain (71). Thus, inhibiting the activity or expression of P-glycoprotein might improve antiepileptic drug efficacy, as several widely used antiepileptic drugs are substrates for P-glycoprotein. P-glycoprotein is expressed after epileptic seizures through a pathway that involves endothelial NMDA receptor activation and cyclooxygenase-2 (COX-2) induction (72). Notably, inhibition of COX-2 in chronic epileptic rats maintained control levels of P-glycoprotein and enhanced brain delivery of phenytoin (73), indicating that limiting

the expression level of P-glycoprotein might enhance drug delivery, especially in drug-resistant epilepsy patients, and potentially alleviate symptoms of epilepsy.

Given the complexity of the brain, these aforementioned epileptogenic processes are probably not mutually exclusive in epileptogenesis. Rather, a trigger event can likely initiate multiple epileptogenic processes because some of the signaling molecules are shared. Thus, targeting these molecules might be an efficacious strategy for preventing or slowing the onset of epilepsy.

MOLECULAR TARGET CANDIDATES

We now examine selected putative mediators of epileptogenesis. These mediators are potential candidates for therapeutic intervention to inhibit epileptogenesis or modify disease outcomes because they are responsible for controlling the expression levels and functional state of the effector proteins that set the seizure threshold (see **Figure 1**). Thus, controlling the expression of epileptogenic mediators could have profound consequences on the excitability of the brain.
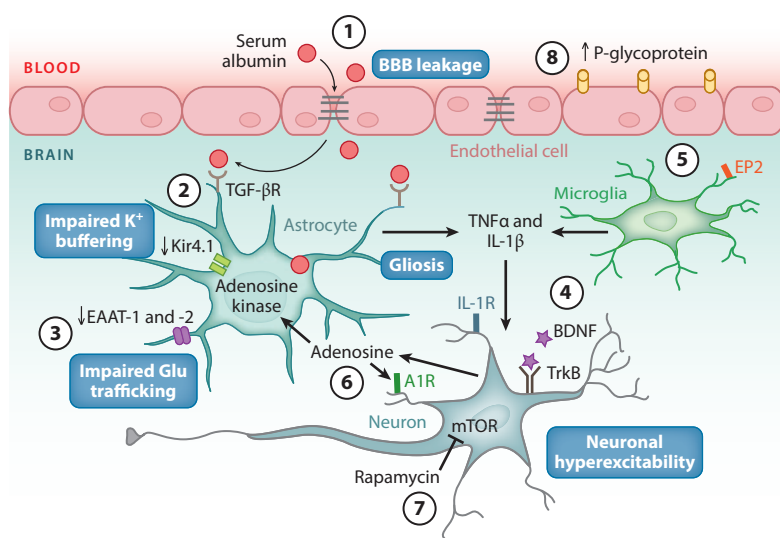

Figure 1

Potential epileptogenic mechanisms and epileptic mediators. ① A defective blood-brain barrier (BBB) allows serum albumin to enter the brain. ② Albumin binds to astrocyte transforming growth factor-β (TGF-β) receptors, resulting in downregulation of the potassium channel Kir4.1. ③ Decreased expression of glutamic acid (Glu) transporters, EAAT-1 and -2, occurs in brain tissue after seizures. These events cause neuronal hyperexcitability through loss of K^+ buffering and Glu trafficking. ④ Seizure-induced inflammation can be propagated by cytokine production by astrocytes and microglia. Elevated levels of inflammatory cytokines as well as brain-derived neurotrophic factor (BDNF) can also sustain neuronal hyperactivity. ⑤ Inhibiting inflammatory pathways with E prostanoid 2 (EP2) antagonists results in reduced cytokine levels, delayed mortality, rescue of BBB dysfunction, and less neuronal injury after status epilepticus. ⑥ Astrocytic adenosine kinase is the main route for degradation of adenosine, a neuron-derived anticonvulsant. Inhibition of adenosine kinase–mediated degradation of adenosine or mammalian target of rapamycin (mTOR) inhibition with rapamycin ⑦ represents two disease-modifying strategies. ⑧ Seizure-induced elevated expression of endothelial P-glycoprotein can limit effective therapy by increasing efflux of drugs out of the brain. Other abbreviations: A1R, adenosine A1 receptor; EAAT, excitatory amino acid transporter; IL-1, interleukin-1; IL-1R, interleukin-1 receptor; TNFα, tumor necrosis factor-α; TrkB, neurotrophic tyrosine kinase receptor, type 2.

Over the past several years, a large and growing body of evidence has identified a complex interaction between neuroinflammatory processes and the hyperexcitable brain. Activated microglia and elevated levels of the cytokine group of immune molecules are encountered in surgical brain tissue obtained from epileptic patients (9, 74) and in animal models after SE (9, 11, 75). Intraperitoneal injection of lipopolysaccharide (LPS), a component of the bacterial cell wall, induces robust expression of multiple cytokines accompanied by reduced seizure threshold in the mouse pentylenetetrazol model. Notably, a cyclooxygenase inhibitor can prevent the enhanced seizure susceptibility produced by LPS (76). Taken together, these findings suggest that inflammatory cytokines may play a role in the development of epilepsy. We now discuss the current evidence for this hypothesis.

Interleukin-1β Convertase and the Interleukin-1 Receptor

The interleukin-1 (IL-1) family is comprised of three endogenous ligands: IL-1α, IL-1β, and IL-1Ra, all of which bind to the IL-1 receptor (IL-1R). Whereas IL-1β is secreted predominately by both microglia and astrocytes, IL-1α is mainly membrane bound. Prior to cellular secretion, pro-IL-1β must first be activated through proteolytic cleavage by IL-1β convertase (ICE), also known as caspase 1. An endogenous IL-1R antagonist (IL-1Ra) can inhibit IL-1 signaling by binding to IL-1R (77). Thus, the biological activity of the IL-1 family of cytokines is under considerable endogenous regulation. IL-1 cytokines are expressed in the healthy brain at very low levels (78), but increased expression of numerous cytokines, including IL-1β, is observed after seizure activity (79).

Support for a role for IL-1β signaling in epileptogenesis stems, in part, from animal models of FS. IL-1R-deficient mice require higher fever to induce FS than wild-type control mice. Additionally, exogenous IL-1β reduces seizure threshold in wild-type but not IL-1R-deficient mice, indicating that IL-1β is proconvulsant and contributes to the underlying hyperexcitability during FS (80). IL-1β levels are elevated not only during FS but also in the following 48 h. Interestingly, animals that become epileptic after FS exhibit higher hippocampal IL-1β levels than animals subject to FS that did not become epileptic (81). Although these findings suggest that IL-1β may be involved in the epileptic process, they are not conclusive, as elevated IL-1β levels in the epileptic animals could result from spontaneous seizures.

The IL-1β signaling pathway has also been targeted to decrease seizure severity. Intracerebral injections of IL-1Ra during electrical stimulation result in anticonvulsant effects (82). Moreover, transgenic overexpression of astrocytic IL-1Ra inhibits motor and electroencephalographic seizures (15). Similar results have been obtained in ICE knockout animals as well as when ICE is pharmacologically inhibited (74, 78, 83). These findings provide evidence that targeting IL-1β biosynthesis may have therapeutic value as a disease modifier in epilepsy. Indeed, a Phase II clinical trial has been initiated for treatment of medication-resistant partial epilepsy with an ICE inhibitor (**http://clinicaltrials.gov/ct2/show/results/NCT01048255**).

Prostaglandin E2/E Prostanoid 2 Receptor Signaling Pathway

The inducible isozyme COX-2 is rapidly upregulated in some glutamatergic neurons after a seizure or cerebral ischemia. The induction of COX-2 contributes to brain inflammation and injury and perhaps epileptogenesis and cognitive deficits following prolonged seizures (84, 85). However, the significant cerebrovascular adverse effects from long-term use of COX-2 inhibitors suggest that some COX-2 downstream prostaglandin signaling pathways might be more amenable therapeutic targets than blocking the entire COX-2 cascade. As a dominant product of COX-2 within the brain, prostaglandin E2 (PGE_2) can activate four G protein–coupled receptors: E prostanoid 1

(EP1), EP2, EP3, and EP4. PGE$_2$ signaling via EP2 mediates some beneficial effects in the CNS, including neuroprotection in acute models of excitotoxicity (86, 87), neuroplasticity, and spatial learning (88, 89). However, recent studies show that EP2 receptor activation can also promote oxidative damage and neurotoxicity in models of chronic inflammation and neurodegeneration, accompanied by induction of a host of proinflammatory mediators, mostly in activated microglia (75, 90–93).

In mice, systemic administration of brain-permeable EP2 antagonists commencing hours after pilocarpine-induced SE reduced delayed mortality, accelerated weight regain and functional recovery, reduced the formation of inflammatory cytokines and gliosis in hippocampus, maintained the integrity of the blood-brain barrier, and reduced delayed neurodegeneration in hippocampus (75, 94). Intriguingly, behavioral seizure scoring and cortical electroencephalographic recording demonstrated that pharmacological inhibition of EP2 did not have an acute antiseizure effect in the same SE model, suggesting that the beneficial effects from EP2 antagonism might be caused through an anti-inflammatory mechanism (75). It will be critical to address whether EP2 receptors are involved in epileptogenesis and whether these EP2 antagonists are useful to treat chronic epilepsy. In this regard, EP2 receptor antagonism might represent an adjunctive therapy for the treatment of SE, along with antiepileptic drugs.

Adenosine Kinase

Adenosine, through its G$_{i/o}$-coupled A1 receptor, has long been recognized as a powerful endogenous anticonvulsant neuromodulator (95). Adenosine is released directly from activated neurons (96) and metabolically cleared by astrocytic adenosine kinase (97). Intraventricular infusion of adenosine is anticonvulsant in the rat kindling model (98), and conditional forebrain ablation of adenosine kinase attenuates seizures following intra-amygdala injection of kainate (99). Conversely, overexpression of this enzyme in the hippocampal CA3 region induces spontaneous focal seizures (99), which is significant because adenosine kinase is itself upregulated in activated astrocytes (100). Importantly, adenosine released into rat ventricles for 10–18 days, beginning 9 weeks after kainate-induced SE, produced a long-lasting ~80% reduction in spontaneous seizure frequency and intensity (101). This represents a profound disease modifying effect of adenosine. Although adenosine kinase inhibitors have been described (102), systemic toxicity in preclinical studies has limited enthusiasm for clinical development. Direct administration of an adenosine kinase inhibitor into the brain represents a potential future clinical direction.

Mammalian Target of Rapamycin

The mammalian target of rapamycin (mTOR) complex acts as a serine/threonine kinase that regulates the translation of a class of proteins involved in synaptic plasticity, among other functions. When bound to the FK506 binding protein-12 (FKBP12) protein, the macrolide sirolimus (rapamycin) inhibits the mTOR complex-1 (mTORC1). Sirolimus is an effective anticonvulsant in children suffering from medication-resistant epilepsy associated with tuberous sclerosis complex (TSC) (103). Does rapamycin exert a disease-modifying or antiepileptogenic effect in TSC in addition to its anticonvulsant action? Phosphatase and tensin homolog (PTEN) is an upstream inhibitor of mTORC1; conditional knockout of PTEN from a subset of cortical neurons creates the NS-PTEN mouse model of TSC. A 2-week course of rapamycin treatment suppressed abnormal electroencephalographic activity in the NS-PTEN mouse, but epileptiform activity reappeared 4–7 weeks after discontinuation of rapamycin (104). Intermittent rapamycin treatment (2 weeks on, 4 weeks off) provided continued protection from spontaneous seizures.

The long-lasting antiseizure effect of rapamycin after washout points to a disease-modifying action in this mouse model; researchers have yet to determine whether continued, intermittent, pulsatile treatment can forever prevent the occurrence of epilepsy in this mouse model and in children.

In contrast to the beneficial effects of rapamycin in TSC, the effects of this drug in rodent models of temporal lobe epilepsy have been mixed. In a well-powered experiment, daily high-dose rapamycin treatment in mice begun 1 day after treatment with pilocarpine to induce SE had no effect on spontaneous seizure frequency assessed 1 month later (63), suggesting that rapamycin is neither anticonvulsant nor antiepileptogenic in this mouse model. By contrast, rapamycin treatment suppressed spontaneous seizures in pilocarpine-treated rats, but seizures reappeared within 3 weeks after cessation of treatment (105), suggesting that rapamycin exerts an anticonvulsant effect in this rat model. In a different study, however, pretreatment with rapamycin nearly eliminated the development of spontaneous seizures in rats administered kainate (106). Clearly, much remains to be done to reconcile the different findings in mouse and rat epilepsy models produced by different chemoconvulsants and different rapamycin treatment protocols.

Neurotrophic Tyrosine Kinase Receptor, Type 2

TrkB is activated by three neurotrophic factors—BDNF, neurotrophin-3, and neurotrophin-4—to influence multiple processes including neuronal differentiation and survival, long-term potentiation, learning, and memory (107). Enhanced TrkB activation in the mature brain may be requisite for limbic epileptogenesis following prolonged seizures, based on the following findings. BDNF and TrkB are upregulated after epileptic seizures in both experimental animals and temporal lobe epilepsy patients (13, 108–110), and increased TrkB signaling is observed in the kindling model (111). Intraventricular infusion of TrkB—but not TrkA or TrkC—antibodies inhibited the development of SE in the rat kindling model (112). Interestingly, conditional deletion of TrkB prevented limbic epileptogenesis in the mouse kindling model (113), suggesting TrkB and downstream signaling pathways are critical for the development of chronic recurrent seizures. TrkB activation by BDNF leads to phosphorylation of Y515 and Y816 in the intracellular domain of the receptor to initiate downstream Shc/Ras/mitogen-activated protein kinase (MAPK) and phospholipase Cγ1 (PLCγ1) signaling pathways, respectively.

A recent study showed that the PLCγ1 signaling pathway is essential for BDNF/TrkB-mediated limbic epileptogenesis because mice with the TrkB Y816F mutation, which interferes selectively with coupling of PLCγ1 but not Shc to TrkB, could not kindle (114). More recently, TrkB inhibition—beginning after SE induced by intra-amygdala kainate injection and continuing for 2 weeks—nearly eliminated spontaneous recurrent seizures and reduced anxiety-like behavior and neuronal loss in mice when tested weeks to months later (42). In sum, TrkB receptor and its downstream effector PLCγ1 provide attractive molecular targets for developing novel therapeutics to prevent epilepsy. However, off-target effects and inadequate pharmacokinetic profiles of current TrkB and PLCγ1 inhibitors hinder their development for therapeutic use. Thus, identification of novel small molecules with new pharmacophores as selective inhibitors for these two potential drug targets represents the next effort in this area.

Janus Kinase/Signal Transducer and Activator of Transcription-3 (JAK/STAT3) Signaling Pathway

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway regulates gene transcription by transducing extracellular information into the nucleus. The JAK/STAT signaling system is comprised of three components: a transmembrane receptor, JAK,

and STAT (115). The receptors are often activated by inflammatory cytokines, growth factors, or other chemical messengers and then recruit and activate JAK to initiate the signal transduction cascade (116). In mammals, JAK protein has four forms: JAK1, 2, 3, and tyrosine kinase 2 (TYK2). Once activated, it can phosphorylate STAT, which has seven family members: STAT1, 2, 3, 4, 5A, 5B, and 6. Phosphorylated STATs then form dimers and translocate to the nucleus, where they bind specific DNA regulatory sequences of target genes to increase their transcription (117). Although the JAK/STAT signaling pathway has been conventionally thought to be involved in the regulation of the immune system, recent studies revealed that it is often upregulated in the hippocampus following brain insults in both experimental animals and patients after prolonged seizures (118), traumatic brain injury (119), and ischemia (120), all of which could result in epilepsy.

The propenamide analog WP1066 is a weak JAK2 inhibitor and also facilitates JAK degradation. When systemically administered within the first hour after the onset of pilocarpine-induced SE, WP1066 transiently inhibited phosphorylation of STAT3, a JAK target, and reduced transcription of downstream STAT3 targets including cyclin D1 and Mcl-1, which are important for cell cycle progression and cell survival. Importantly, early WP1066 administration also reduced the frequency and severity of spontaneous recurrent seizures over a period of 2 weeks after SE but did not affect the intensity or duration of SE or the associated acute neuronal death (121). Given the relatively short plasma half-life and low brain penetration of WP1066 after systemic administration in rats and its potential off-target activities, a JAK inhibitor with a better pharmacokinetic profile and more specificity might yield improved therapeutic effects. Thus, JAK or STAT3 inhibition should be further investigated as a potential antiepileptogenic therapy for epilepsy treatment.

Transforming Growth Factor-β Receptor Kinase Activity

TGF-β is a multifunctional cytokine that mediates important roles in cell growth and differentiation, embryogenesis, and inflammatory responses (122). Signal transduction is initiated by TGF-β binding to one of two serine threonine kinase receptors, TGFβRI or TGFβRII, which in turn activate intracellular Smad protein complexes as well as the p38 MAPK pathway.

Recent reports provide evidence that a dysfunctional blood-brain barrier can lead to inappropriate activation of TGF-β signaling pathways. Serum albumin can enter the brain if the blood-brain barrier is compromised and subsequently increases excitability and promotes epileptogenesis (123). The mechanisms of this involve albumin transport into astrocytes, which initiates intracellular signaling pathways, resulting in transcriptional downregulation of Kir4.1 and GLT-1 (68) and generation of an epileptic focus. Interestingly, albumin-induced epileptiform activity in hippocampal slices can be reduced with a TGF-β receptor kinase inhibitor (67). Although these studies are in agreement with the hypothesis that blood-brain barrier dysfunction and activated astrocytes are epileptogenic, it remains unknown whether inhibiting TGF-β receptor signaling is a viable strategy to modify disease or prevent epileptogenesis in model systems.

Tumor Necrosis Factor-α Receptors

The proinflammatory cytokine TNFα is a member of the TNF superfamily of ligands, which are involved in inflammatory reactions as well as cell death and proliferation (124, 125). TNFα is synthesized as a transmembrane protein (tmTNFα) that can be subsequently cleaved by the matrix metalloprotease TNFα converting enzyme (TACE or ADAM17), thereby generating soluble TNFα ligand (sTNFα) (126). Both tmTNFα and sTNFα are biologically active molecules that signal through two receptors, TNF receptor 1 (TNFR1 or p53) and TNF receptor 2 (TNFR2 or

p75). Similar to tmTNFα, both membrane TNFR1 and TNFR2 can be proteolytically cleaved by TACE to create biologically active, soluble receptors. Soluble TNF receptors act as decoys that attenuate the biological effects of TNFα by competing with membrane-bound receptors (127). Thus, TNFα signaling is mediated by numerous factors including expression level, TACE activity, and receptor availability.

Intrahippocampal injection of recombinant TNFα prior to intrahippocampal kainate reduced the number and duration of acute seizures, and this was phenotyped in transgenic animals over-expressing TNFα. Conversely, *Tfnr2* knockout mice exhibited more intense kainate-induced seizures than their control counterparts, suggesting that TNFα can also act as an anticonvulsant (128). However, other studies have shown that high astrocytic expression of human TNFα, which preferentially activates TNFR1, can be proconvulsive (129, 130), pointing to a context dependence of the disease-modifying potential of TNFα. These results suggest that a specific TNFR1 inhibitor or TNFR2 agonist could be useful in epilepsy. Indeed, a specific TNFR2 agonist appears to be neuroprotective for dopaminergic neurons exposed to oxidative stress (131), and a selective TNFR1 antagonist attenuates arterial inflammation in an ischemia model (132).

Restrictive element 1 silencing transcription factor (REST): a DNA-binding protein complex that represses many genes expressed in the nervous system by recruiting nuclear enzymes that modify DNA/histone complexes

Restrictive Element 1 Silencing Transcription Factor and Its Effector Enzymes

Post-translational modifications of histones associated with nuclear DNA, including acetylation, methylation, phosphorylation, and ribosylation, have a profound impact on the transcription of genes as they alter DNA/histone interactions, rendering the nucleoprotein complex accessible to proteins that control transcription (133). The ability of histone modifications to regulate access of transcription factors to DNA contributes profoundly to regulation of gene expression. The transcription factor REST (restrictive element 1 silencing transcription factor) mediates transcriptional repression through the recruitment of histone deacetylases, demethylases, and methyltransferases. REST binds to a 17–33-base-pair DNA sequence known as repressor element-1 (RE1) or neuron restrictive silencing element (NRSE). In the human genome, approximately 1,800 genes contain RE1 sites within their regulatory regions (134), and many of these genes are involved in neuronal excitability (135). Given the impact of post-translational modification of histones on transcriptional control and the large number of genes with RE1 sites, it is reasonable to speculate that REST might mediate an important role in diseases wherein pathological alterations are accompanied by widespread gene expression changes.

Notably, elevated levels of REST are encountered after prolonged seizure activity (136, 137). Moreover, some ion channel genes, including hyperpolarization-activated cyclic nucleotide-regulated cation channel (HCN1), which typically reduces excitability in neurons, contain RE1 sites. Mice deficient in HCN1 in forebrain neurons display more seizures and higher mortality in both kindling and pilocarpine models, suggesting that decreased HCN1 may contribute to epileptogenesis (138). McClelland and colleagues (139) have recently shown that REST binding to the RE1 site of HCN1 is enhanced 2 days after kainic acid–induced SE. Intraventricular administration of antisense oligonucleotides targeted to the HCN1-RE1 genomic region inhibited REST/RE1 binding and prevented downregulation of HCN1. Repeated antisense oligonucleotide therapy led to fewer spontaneous seizures in the 14 days following SE (139). However, researchers have yet to test whether this reduced number of seizures persists after oligonucleotide therapy is discontinued.

CONCLUSIONS AND FUTURE DIRECTIONS

The search for epilepsy prevention therapies has been ongoing for several decades without success. Although anticonvulsants can prevent the acute convulsions that often accompany epileptogenic

trigger events, they have clearly been ineffective in preventing the subsequent development of epilepsy itself. Thus, the biology driving the conversion of a normal brain to a brain with epilepsy must be different than the biology driving seizures in the epileptic brain. Investigators have therefore largely shifted their focus to potential disease-modifying or antiepileptogenic strategies such as anti-inflammation, neuroprotection, and regulation of neuronal plasticity to identify molecular targets for preventing the development or progression of epilepsy. Promising preclinical data strongly support the further development of inhibitors of ICE, adenosine kinase, TrkB, and mTOR (perhaps only for tuberous sclerosis). Inhibitors of REST effector enzymes, JAK/STAT, TNFR1, and the EP2 receptor for PGE$_2$ also show potential for disease modification or epilepsy prevention. In our opinion, adequate control of epileptogenesis will likely require combining drugs that target synergistic pathways. Nonetheless, as is the case for all chronic neurologic disorders, a major challenge will be to interrupt the pathologic mechanism(s) without disrupting pathways involved in daily life.

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