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Novel Targets for Developing Antiseizure and, Potentially, Antiepileptogenic Drugs

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Epilepsy is a chronic neurological disorder caused by abnormal changes in the functions of neuronal circuits and manifested by seizures. It affects patients of all age, substantially worsens the quality of life for the patients as well as their families, and imposes a huge economic burden on the healthcare system. Historically, efforts for discovering and developing antiseizure therapies have been focused on modulating the functions of receptors, transporters, and enzymes expressed by neurons. These drug development efforts have paid off, as we have over 25 antiseizure drugs available in the clinic. However, these drugs mainly provide symptomatic relief from seizures and often cause serious adverse effects. Importantly, almost one-third of patients with epilepsy do not have their seizures adequately controlled by available drugs. To address this problem, researchers are investigating cellular and molecular mechanisms fundamental to the optimal function of neuronal circuits. Evidence shows that disruptions in these mechanisms cause impairment in neuroglial interactions, uncontrolled inflammation, aberrant synaptogenesis, and neurodegeneration in genetic and acquired epilepsies. Many novel therapeutic targets have been identified to target these mechanisms for developing new antiseizure drugs. In addition, the field is exploring new drug targets which may impede the development of epilepsy. We have summarized some of these novel targets in this brief review.

Epilepsy is a devastating neurological disorder affecting nearly 65 million people worldwide. The etiology of epilepsy is heterogeneous in nature and a variety of factors—such as infection, stroke, traumatic brain injury, brain tumors, cerebral ischemia, and importantly, mutations in genes that are crucial for the development, migration, and function of neurons and glia—may cause seizures and lead to the development of epilepsy. Over 25 drugs are clinically available for the symptomatic treatment of the seizures that are the defining criteria of epilepsy. However, most of these drugs tend to optimize the balance between excitatory and inhibitory neurotransmission by modulating the functions of ion channels, receptors, enzymes, and transporters expressed by neurons (1). Importantly, around one-third of epilepsy patients are still pharmacoresistant, and many patients who respond to antiseizure drugs (ASDs) often present with significant adverse effects (2). In addition, people with epilepsy also suffer from co-morbidities such as anxiety, depression, and cognitive impairment. Many ASDs either do not address or, in some instances, aggravate these co-morbidities. Therefore, future ASD development efforts should 1) include novel mechanisms of action for ASDs, 2) evaluate potential therapeutic targets for disease modification, and 3) identify potential therapeutic interventions for the prevention of epilepsy. There are several promising targets for developing novel drugs that might prevent or impede epileptogenesis. Some of these novel targets are discussed in this brief review.

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Transforming Growth Factor β (TGFβ)

Sustained damage to the blood brain barrier (BBB) is associated with increased incidence of seizures (3). Animal studies show that the activation of the TGFβ signaling pathway, mainly in astrocytes by albumin extravasated into brain parenchyma following BBB injury, disrupts regulatory functions of astrocytes and causes hyperexcitability in cortical and limbic structures (4). Direct activation of the TGFβ signaling pathway by TGFβ1 also causes epileptiform activity in rats, whereas TGFβ receptor blockers ameliorate TGFβ1- or albumin-induced epileptiform activity (5). Mice overexpressing TGFβ1 specifically in astrocytes develop seizures (6). It was recently found that losartan, which inhibits TGFβ1 signaling, decreased the number of rats developing albumin-induced chronic spontaneous seizures and inhibited epileptogenesis in this vascular injury-induced model of epilepsy (7). It is evident from these animal studies that inhibition of the TGFβ signaling pathway might be an effective strategy to inhibit seizures and epilepto-

genesis, especially in conditions where the blood brain barrier is compromised (8). Importantly, losartan is an FDA-approved drug widely prescribed for the treatment of cardiovascular diseases and would be a good candidate for epilepsy prevention trials in patients.

Tyrosine Receptor Kinase B (TrkB)

TrkB is a receptor for brain-derived neurotrophic factor (BDNF). Targeted deletions of either BDNF or the TrkB gene, or the pharmacological inhibition of TrkB signaling, suppresses kindling-induced epileptogenesis in rodents (9–11). In contrast, mice overexpressing BDNF or TrkB develop severe chemoconvulsant-induced seizures and intrahippocampal administration of BDNF causes spontaneous behavioral seizures in rats (12–14). Seizures also induce the expression of TrkB in the hippocampus of rodents and uncontrolled BDNF-TrkB signaling may further exacerbate seizures (15). Given that activation of TrkB signaling is associated with the development of seizures and epilepsy, inhibitors of TrkB or its downstream signaling partners may be a promising antiseizure or antiepileptogenic strategy. However, global inhibition of TrkB might be counterproductive as TrkB signaling is also required for neuronal survival and growth (16). Mice treated with a novel inhibitor of TrkB, which specifically inhibits phospholipase-γ1 (PLCγ1)-mediated effects of TrkB following kainic acid-induced status epilepticus (SE), had a significantly reduced number of chronic seizures without compromising neuroprotective functions of TrkB (17). Clinical potential of TrkB-PLCγ1 inhibitors for the prevention of epilepsy should be validated in additional animal models of epilepsy and may eventually prove useful in the clinic.

Cytokines

Development of seizures and epilepsy in animal models and in humans following a variety of brain insults is correlated with neuroinflammation and increased expression of cytokines in brain (18). Cytokines can modulate neurotransmission by affecting the levels of excitatory and inhibitory neurotransmitters and their receptors, enzymes involved in the metabolism of neurotransmitters, and cellular signaling proteins (19). Interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα) are the most widely investigated cytokines for their roles in causing inflammation, ictogenesis, and epileptogenesis. Targeting these cytokines or their receptors and downstream signaling partners may provide a useful strategy for new antiseizure drugs.

IL-1β

Activation of the IL-1β signaling cascade following an epileptogenic brain insult causes neuronal hyperexcitability and long-term changes in the genes associated with aberrant neuronal sprouting, neurogenesis, inflammation, and glial dysfunction that reduce the threshold for chronic spontaneous seizures in animal models (20). Indeed, intrahippocampal injection of IL-1β prolonged the duration of kainic acid-induced seizures, whereas inhibition of IL-1β signaling by an IL-1R antagonist or by selective inhibition of IL-1β converting enzyme (ICE)/capsase-1 (which produces the biologically active form of IL-1β) decreased seizures in a number of animal models (21–23). Clinical studies also show increased expression of IL-1β, ICE, and IL-1β signal transduction molecules in glia and neurons in epileptic foci of patients with pharmacoresistant seizures and in neurological conditions associated with epilepsy, such as cortical malformation and Rasmussen's encephalitis (24, 25). A stage IIA clinical trial with VX-765 was completed in patients with epilepsy which found VX-765 safe and well tolerated; however, this caspase-1 inhibitor is not currently being developed for patients. Nevertheless, IL-1β remains an attractive molecular target for seizure suppression and potential disease modification.

TNFα

TNFα serves many regulatory functions in synaptic plasticity, neurotransmission and neuroglial interaction (26). Preclinical investigation suggests that there is a dichotomous role of TNFα in the pathophysiology of seizures and epilepsy. Studies using various mice strains deficient in TNFα or TNF receptors (TNFRs) show that TNFR1 and TNFR2 mediate pro- and anticonvulsant effects of TNFα, respectively, in animal models of epilepsy (27–29). Similarly, selective chronic activation of TNFR1 increased the numbers of kainic acid-induced seizures in rats (30). Anti-TNFα antibodies are widely prescribed for many peripheral inflammatory conditions, and several novel small molecules and biologics targeting central TNFα and TNFRs are under development that might be useful to treat seizures occurring as a consequence of neuroinflammation and to interfere in the development of epilepsy (31).

IL-6

Activation of IL-6 signaling may alter the expression of many receptor gated ion channel subunits through the JAK/STAT pathway (19). Increase in the levels of IL-6 can also influence synaptogenesis and tilt the balance of excitation and inhibition towards hyperexcitability (32). Animal studies investigating the role of IL-6 in seizure generation present diametric results. IL-6 decreased the latency and increased the duration of pentylenetetrazole-induced seizures in adult rats (33), whereas it increased the latency and decreased the duration of hyperthermia-induced seizures in developing rats (34). Mice overexpressing IL-6 in astrocytes have spontaneous seizures (35), while significantly fewer IL-$6^{-/-}$ mice infected with Theiler's virus develop acute seizures (28). In contrast, a significantly higher proportion of IL-$6^{-/-}$ mice exhibit kainic acid-induced seizures (36). Many variables related to animal model and experimental paradigm might have caused ambiguity in these results. Future efforts should be directed toward developing and evaluating the antiseizure effects of IL-6 modulators.

Complement proteins

The complement system is suspected to contribute to seizures and epileptogenesis in patients with mesial temporal lobe epilepsy (37), Rasmussen's encephalitis (38), and tuberous sclerosis complex (39). Activation of complement system occurs in animal models of limbic epilepsy (37). Complement 5a receptor 1 (C5aR1) was found to be upregulated in the kainic acid and pilocarpine models of epilepsy, and the C5aR1 antagonist, PMX53, reduced electrographic spontaneous seizures in the kainic acid model, and decreased the severity and mortality associated with pilocarpine-induced SE (40). PMX53 also had anticonvulsant effects in the 6 Hz and corneal kindling models

of acute seizures (40). Several anticomplement therapies for a number of inflammatory conditions are currently under clinical development and might be beneficial in the treatment of seizures (41).

Cyclooxygenase-2 (COX-2) and Prostaglandins (PGs)
COX-2 is an inducible rate-limiting enzyme in the synthesis of prostanoids that include various prostaglandins (PGs) and thromboxane A_2 (TXA_2). The expression level of various prostanoids and their receptors contributes to the regulation of inflammation and vascular responses under pathophysiological conditions (42). COX-2 expression is rapidly increased in the brain following seizures in both animal models and humans (43). Overexpression of COX-2 in neurons increases the severity of kainic acid-induced seizures in mice (44), whereas COX-2 knockout mice have a reduced incidence of hippocampal seizures following repetitive electrical stimulation of the perforant path (45). Importantly, COX-2 inhibitors such as celecoxib and aspirin decrease pilocarpine-induced chronic spontaneous seizures and neuronal damage in rats when treated after the period of SE (46, 47). Given the regulation of vascular diameter by prostanoids, especially by PGI_2 and TXA_2, long-term inhibition of COX-2 may cause cardiovascular adverse effects (42). Also, animal studies have demonstrated that PGE_2 signaling via EP1 and EP2 receptors are crucially involved in causing neuroinflammation and cell death following seizures. Therefore, specific inhibitors of PGE_2 signaling via EP1 and EP2 could be a promising strategy for the treatment of epilepsy (48).

microRNA (miRNA)
Gene expression profiling studies have found changes in the expression levels of a number of miRNAs in animal models of epilepy and in resected brain tissues from patients with epilepsy. Importantly, mice deficient in the expression of miR-128 developed fatal epilepsy, whereas overexpression of miR-128 suppressed kainic acid-induced seizures (49). Pretreatment of mice with ant-134 (miR-134 antagomirs), which silences miR-134, reduced severity of kainic acid-induced seizures and imparted long-term neuroprotection (50). In addition, ant-134 also decreased the occurrence of chronic spontaneous seizures after kainate-induced SE, which suggests that targeting miRNAs may provide disease-modifying anti-epileptogenic effects (50). Although the mechanisms by which miRNAs may contribute to the development of hyperexcitable neural networks are largely unknown, the evidence so far suggests that miRNAs regulate the expression of glutamate receptors and the morphology of dendritic spines (51). Some miRNAs may also modulate neuroimmune interactions by inducing changes in inflammatory molecules (51). Indeed, recent work in the intra-amygdala mouse model of TLE demonstrated that short-term treatment with an miR-146a mimic that inhibits IL-1R1/TLR4 intracellular signaling could dramatically reduce the seizure frequency observed in mice several weeks after treatment. This suggests that a transient block of IL-R1 and TLR4 signaling by a miRNA mimic could be disease modifying (52), thus demonstrating the utility of miRNA targets of inflammatory signals. Given the importance of miRNAs in controlling gene expression of numerous proteins contributing to epileptogenesis, they may prove to be important therapeutic targets.

Galanin
Galanin is one of several neuropeptides that has demonstrated antiseizure activity (53). Activation of galanin receptor subtypes 1 and 2 (GalR1 and GalR2) confers antiseizure effects, whereas the role of subtype 3 (GalR3) in brain hyperexcitability and seizure activity has not been fully elucidated (53). Thus far, the potential for a novel galanin therapy has been explored by use of systemically administered, receptor subtype-preferring analogs (54). However, as systemic administration of GalR1-preferring analogs produces hyperglycemia resulting from receptor-mediated insulin inhibition in the pancreas (55, 56), GalR2 has emerged as the more likely candidate for galanin-based therapy. The lead GalR2-preferring analog, NAX 810-2, is metabolically stable and reduces seizures following sytemic and i.v. administration in the mouse 6 Hz and corneal kindling models (57, 58). Further, this lead analog is active using the mouse 6 Hz 44 mA stimulus intensity, which suggests that this novel analog may be useful in the treatment of pharmacoresistant epilepsy.

Cannabinoids
The use of marijuana (*Cannabis sativa*) and marijuana-derived compounds (phytocannabinoids) to treat various neurological conditions has been described previously (59). More recently, largely due to the success of cannabidiol (CBD) in preclinical and clinical settings, CBD has emerged as an attractive ASD candidate for the treatment of various forms of epilepsy. CBD protects against seizures in a variety of preclinical seizure models including electrically and chemically induced acute seizures and SE (59). Currently, CBD has orphan drug designation for Dravet Syndrome, infantile spasms, Lennox-Gastaut syndrome, and tuberous sclerosis complex. The lead candidate, Epidiolex (GW Pharmaceuticals, Carlsbad, CA), a liquid formulation of purified CBD, was tested in a Phase III clinical trial in children with Dravet Syndrome and found to result in a 22.8% reduction in median frequency of convulsive seizures per month compared to placebo treatment (60). In a similar manner, the phytocannabinoid cannabidivarin (CBDV) is also being investigated (clinical and preclinical development) by GW Pharmaceuticals as a potential therapy for pharmacoresistant epilepsy (58). As interest in these molecules increases, it is anticipated that additional studies evaluating efficacy will continue.

Metabotropic Glutamate Receptors
Activation of metabotropic glutamate receptors, and in particular subtypes 2 and 3 ($mGlu_2$, $mGlu_3$), can affect excitatory synaptic transmission (61) and agonists of these receptors can block or reduce seizures in a variety of seizure models (62, 63). Further, as these receptors possess both orthosteric and allosteric modulator sites, positive allosteric modulators (PAMs) of $mGlu_2$ have emerged as a potential therapy for a variety of neurologic conditions, including epilepsy. Moreover, as $mGlu_2$ receptors are expressed pre- and perisynaptically (64), they are uniquely positioned to respond to excess glutamate levels that may occur during seizures. $mGlu_2$ PAMs (e.g., JNJ-42153605 and JNJ-40411813) have been evaluated both alone and in combination with the ASD levetiracetam (LEV; 65). Both of these compounds are efficacious in the mouse 6 Hz model at both the 32 mA and the more pharmacoresistant 44 mA stimulus

intensities (65). Further, in contrast to LEV, which is dramatically less potent at the 44 mA stimulus intensity (at least 18-fold vs 32 mA), $mGlu_2$ PAMs are effective in this model (44 mA) at doses only 1.5- to 2-fold greater than efficacious doses using the 32 mA stimulus intensity (65). This suggests that these compounds present a potentially novel therapy for pharmacoresistant epilepsy. When combined with LEV, $mGlu_2$ PAMs show a potentially synergistic effect (65), suggesting a combination therapy strategy that may be effective for pharmacoresistant seizures.

Conclusion

Currently available ASDs generally target receptor gated or voltage gated ion channels and are beneficial to a large number of epilepsy patients. Despite this advancement, significant unmet medical need exits for pharmacoresistant cases of epilepsy and epilepsy-associated comorbidites. As far as antiseizure/antiepileptic targets are concerned, it appears that the low-hanging fruits have already been plucked and future efforts should focus on exploring molecular mechanisms fundamental in regulating synatpic and network activity. As the basic mechanisms of the epilepsies are understood, especially with respect to the genetic epilepsies, novel molecular targets will be identified and provide unique opportunities to address the problems of refractory epilepsy and epileptogenesis.

Emerging evidence strongly suggests that CNS inflammation is a common occurrence in animals and patients with epilepsy, and there may be a causative link between uncontrolled inflammation and epilepsy. Testing clinical safety and efficacy of anti-inflammtory agents in epilepsy should be prioritized. VX-765, which is the only known anti-inflammatory molecule underwent early stage clinical trials in chronic epilepsy, was safe and well tolerated, and therefore, warrants larger efficacy trials. Since inflammation occurs in many CNS diseases, anti-inflammatory agents may have a wider application and that may incentivize pharmaceutical companies to develop novel anti-inflammatory therapies. Cannabinoid-based therapies have also generated well-deserved interest among epileptologists due to remarkable improvement in managing intractable epilepsy and quality of life in some patients. The results of a recent clinical trial have shown that cannabidiol is efficacious in controlling seizures in pediatric patients with Dravet's syndrome, suggesting that more trials in other epilepsies is warranted (60). Future studies should also test the combination of molecules acting on novel targets described here with the existing ASDs, as they may impart synergistic effects given their unique mechanisms of action and provide higher safety and efficacy for the treatment of epilepsy. Basic research has contributed enormously to the identification of novel targets in different types of epilepsy and we anticipate that future work will continue to provide hope to the patient with epilepsy and their families and caregivers.

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