The Potential of Antiseizure Drugs and Agents that Act on Novel Molecular Targets as Antiepileptogenic Treatments

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Abstract A major goal of contemporary epilepsy research is the identification of therapies to prevent the development of recurrent seizures in individuals at risk, including those with brain injuries, infections, or neoplasms; status epilepticus; cortical dysplasias; or genetic epilepsy susceptibility. In this review we consider the evidence largely from preclinical models for the antiepileptogenic activity of a diverse range of potential therapies, including some marketed antiseizure drugs, as well as agents that act by immune and inflammatory mechanisms; reduction of oxidative stress; activation of the mammalian target of rapamycin or peroxisome proliferator-activated receptors γ pathways; effects on factors related to thrombolysis, hematopoiesis, and angiogenesis; inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase; brain-derived neurotrophic factor signaling; and blockade of α2 adrenergic and cannabinoid receptors. Antiepileptogenesis refers to a therapy of which the beneficial action is to reduce seizure frequency or severity outlasting the treatment period. To date, clinical trials have failed to demonstrate that antiseizure drugs have such disease-modifying activity. However, studies in animal models with levetiracetam and ethosuximide are encouraging, and clinical trials with these agents are warranted. Other promising strategies are inhibition of interleukin 1β signaling by drugs such as VX-765; modulation of sphingosine 1-phosphate signaling by drugs such as fingolimod; activation of the mammalian target of rapamycin by drugs such as rapamycin; the hormone erythropoietin; and, paradoxically, drugs such as the α2 adrenergic receptor antagonist atipamezole and the CB1 cannabinoid antagonist SR141716A (rimonabant) with proexcitatory activity. These approaches could lead to a new paradigm in epilepsy drug therapy where treatment for a limited period prevents the occurrence of spontaneous seizures, thus avoiding lifelong commitment to symptomatic treatment.

Keywords Antiepileptogenic drug · Kindling model · Pilocarpine model · Anti-inflammatory · Sphingosine 1-phosphate receptor modulator · mTOR inhibitor

Introduction

Antiepileptic drugs, now commonly referred to as antiseizure drugs (ASDs), provide symptomatic benefit by preventing the occurrence of seizures in an individual at risk. ASD discovery and development has generated nearly 40 clinically effective agents since the introduction of potassium bromide by Sir Charles Locock in 1857 [1]. A major reason for this impressive productivity has been the availability of predictive animal screening models, particularly the maximal electroshock and pentylenetetrazol tests [2]. These models have led to the discovery of most presently used ASDs [3]. The current armamentarium of available ASDs effectively prevent seizures in many patients, but none has been shown to delay the onset or prevent the occurrence of epilepsy, and all have troubling side effects. Similarly, epilepsy treatment devices such as the vagal nerve stimulator and the responsive neurostimulator have not been demonstrated to confer disease modification. This leaves resective surgery as the only existing cure for epilepsy, and emphasizes a major unmet need for disease-modifying therapies that truly affect the underlying epilepsy [4]. Disease-modifying therapies may prevent or
delay the onset of spontaneous recurrent seizures in an individual at risk; they may reverse already established epilepsy; or they may prevent or ameliorate comorbidities, such as cognitive deficits that accompany some forms of epilepsy [5]. The key difference distinguishing disease-modifying therapies from symptomatic therapies is that the benefit conferred by disease-modifying therapies persists after drug treatment is withdrawn. For the purposes of this article, we mainly focus on treatments that prevent or delay the onset of epilepsy; such a treatment is referred to as “antiepileptogenic.” Antiepileptogenesis can also refer to conditions in which epilepsy develops despite treatment, but it is less severe, that is the seizure frequency is reduced, seizures are of shorter duration, or the seizure type is milder. Furthermore, antiepileptogenic treatment may also result in amelioration of comorbidities associated with epilepsy [6]. Evaluation of antiepileptogenic approaches often includes an assessment of their ability to protect against neural injury, although such injury is not a necessary part of epileptogenesis; in this review we may mention such results if they seem relevant.

It is believed that seizures occur when the excitability in certain brain circuits exceeds the restraints imposed by inhibitory mechanisms [7]. Although clinically used ASDs were all identified on the basis of animal models that are not biased with respect to mechanism, ASD mechanisms—to the extent they are understood—generally fall into two categories: negative modulation of excitatory mechanisms or positive modulation of inhibitory mechanisms [4, 8]. Agents that act in either of these ways effectively prevent seizures for many patients, but there is no reason to believe that targeting these same physiological mechanisms would lead to a beneficial modification in the underlying disease process [9]. In recent years, there has been an intense effort to understand the pathophysiological mechanisms underlying epileptogenesis, which has revealed new pathways and potential targets for future drug therapies intended to prevent or alter the course of the epileptogenic process [4]. Among the hypothetical mechanisms postulated to contribute to epileptogenesis are inflammation, neurodegeneration, blood–brain barrier (BBB) disruption, and acquired and genetically encoded changes in the functional activity or expression of ion channels or transporters. Attempts are being made to discover and develop novel agents that address these mechanisms and to repurpose drugs from other therapeutic areas with activities that could be beneficial when applied to epilepsy. The nature of potential antiepileptogenic agents may differ markedly from traditional small-molecule ASDs, and could include peptides, antibodies, nucleic acids, or even cellular or gene therapies. Challenges will be encountered in delivering these agents to the appropriate brain regions and in avoiding systemic side effects. Specialized carriers or delivery mechanisms may be required to address these issues (see, e.g., Rogawski [10]).

The first part of this article reviews the preclinical and clinical data obtained with current ASDs that have demonstrated antiepileptogenic properties in animal models. These include the older ASDs valproate and ethosuximide, and the newer agents topiramate and levetiracetam. The second part of the article considers novel mechanisms, targets, and agents. There is discussion of potential antiepileptogenic agents that modulate immune and inflammatory mechanisms; reduce oxidative stress; activate certain secondary messenger systems; influence thrombolysis, hematopoiesis, and angiogenesis targets; inhibit 3-methylglutaryl-coenzyme A (HMG-CoA) reductase; brain-derived neurotrophic factor (BDNF) signaling; block α2 adrenergic and cannabinoid receptors; and influence Cl⁻ homeostasis. When subjected to scrutiny, it is apparent that the support for certain of these mechanisms is sufficiently limited that it will be difficult to muster enthusiasm for attempts at clinical validation. In other cases, the base of evidence is more compelling: additional preclinical studies are of high priority, and clinical studies may soon be undertaken.

**ASDs with Potential Antiepileptogenic Properties**

Even though all currently used ASDs were discovered as a result of their ability to protect against seizures in rodent screening models, it has been of interest to assess whether these agents could potentially have antiepileptogenic properties. This has been facilitated by the availability of various animal models of epilepsy, including kindling, brain injury, and syndrome-specific models, as well as several rodent strains that exhibit reduced seizure threshold or spontaneous seizures [11, 12]. Although none of these models has been clinically validated as a tool for identifying agents that have antiepileptogenic activity in human epilepsy, they do permit proof-of-concept data to be generated to support clinical studies. There is some evidence that valproate [13] and levetiracetam [14, 15] may cause a delay in kindling acquisition that persists beyond the period of drug exposure, suggesting an antiepileptogenic action. In addition, long-term treatment with ethosuximide and levetiracetam has recently demonstrated a compelling ability to reduce the development of seizures in genetic animal models of absence epilepsy [16, 17]. The reduction in seizures persists after the treatment is withdrawn, suggesting a true antiepileptogenic effect. Post-traumatic epilepsy is considered the most feasible clinical situation for evaluating antiepileptogenic drug treatments. To date, phenytoin, phenobarbital, the combination of phenytoin and phenobarbital, carbamazepine, valproate, and magnesium have been rigorously studied in clinical trials for their ability to prevent the development of post-traumatic epilepsy [18]. None was found to have an antiepileptogenic effect. Comparable clinical trials have not been conducted with newer ASDs, although several, including topiramate and levetiracetam, exhibit
activity in certain animal antiepileptogenesis models [19], and, to date, human trials with levetiracetam suggest that additional study of this agent is warranted [20, 21].

Valproate

Valproate is one of the major older ASDs, possessing broad spectrum activity against diverse seizure types. While the precise way in which valproate protects against seizures is still obscure, the drug has a variety of actions, including effects on Na+ and T-type Ca²⁺ channels, and also on γ-aminobutyric acid (GABA) metabolism [8]. In addition, valproate is well recognized as an inhibitor of histone deacetylases [22] and may counteract aberrant neurogenesis [23], which might confer antiepileptogenic properties.

Studies have been conducted in the amygdala kindling model comparing the potential antiepileptogenic properties of valproate to carbamazepine and phenobarbital [13]. Valproate treatment prior to each kindling stimulation prevented the development of kindled behavioral seizures, which was interpreted as an antiepileptogenic action. However, valproate—at the doses used—also markedly reduced the duration of the afterdischarge (epileptiform discharge recorded by the stimulating electrode). Therefore, an alternative explanation of the result is that the drug treatment prevents the electrical stimulus from triggering the local seizure discharge that leads to epileptogenesis, but that it does not have a specific effect on the downstream events leading to epileptogenesis. The results with valproate contrast with NMDA, which can inhibit kindling development without attenuating the afterdischarge [24, 25]. Interestingly, in the study by Silver et al. [13], when kindling stimulation was continued in the absence of drug pretreatment, the afterdischarge duration was attenuated on the first stimulation without drug pretreatment when the drug was believed to have been completely cleared. In addition, in animals that had received high doses of valproate prior to kindling stimulations, kindling acquisition seemed delayed after drug treatment was stopped. Thus, this study raises the possibility that valproate could have a limited true antiepileptogenic action, apart from its ability to suppress the seizure discharge.

Further studies involving post-status epilepticus (SE) epileptogenesis models have found conflicting results with valproate. A first study in immature rats reported that valproate treatment following SE counteracted ensuing deficits in visuospatial learning, prevented histologic lesions, and there was an absence of spontaneous seizures [26]. However, the assessment of seizures was conducted at a time in which the animals were not completely drug free, raising questions as to whether the seizure suppression truly reflected an antiepileptogenic effect. In contrast, other studies have found that valproate treatment following SE studies in adult rats did not affect the later development of spontaneous seizures in drug-deprived animals, despite prevention of hippocampal damage [27, 28].

The lack of compelling evidence for an antiepileptogenic action of valproate in animals models in combination with a clinical study that failed to demonstrate a beneficial effect of valproate treatment on the development of epilepsy following traumatic brain injury (TBI) [18] reduces enthusiasm for valproate as a disease-modifying therapy. Also, valproate did not display any positive impact on cognitive function or psychopathology in the clinical study. However, issues related to choice of dose, timing of treatment initiation, and duration, as well as comorbid conditions in patients with TBI, such as drug and alcohol abuse and memory problems, render successful execution of clinical trials difficult in this population, so the interpretation of such studies is problematic [29]. Therefore, there is still uncertainty regarding the potential for valproate as an antiepileptogenic therapy.

Ethosuximide

Ethosuximide was introduced nearly 50 decades ago and continues to constitute a first-line treatment for the symptomatic treatment of absence seizures. Its primary mechanism relates to inhibition of T-type Ca²⁺ channels [30]. Recent experimental data obtained in genetic animal models now suggest that ethosuximide also may provide an antiepileptogenic therapy for absence epilepsy.

The first evidence that ethosuximide has such activity came from a study in WAG/Rij rats that exhibit spike-and-wave discharges (SWDs) during the course of their development [16]. Treatment was initiated 3 weeks after birth with ethosuximide in the drinking water and continued until 5 months of age. The exposure covered a period from before the onset of SWDs to a time in development when they are well established. The number, but not duration, of the SWDs was found to be markedly suppressed during 3 months of sequential recordings performed after termination of treatment, revealing an antiepileptogenic potential of ethosuximide. This observation has been reproduced in subsequent studies [17, 31] and extended to another genetic animal model for human absence epilepsy—the GAERS rat [32]. Furthermore, ethosuximide also seems to prevent comorbid anxiety [32] and depression [31, 33] in the genetic absence epilepsy models, based upon observations in the open field and the forced swim test, respectively.

The mechanism underlying the remarkable effects of ethosuximide remains to be determined. Knowing that ethosuximide is a relatively selective inhibitor of T-type Ca²⁺ channels it is intriguing to speculate that the effect on T-type Ca²⁺ channels could be responsible. Indeed, T-type Ca²⁺ channels seem to play a critical role in the epileptogenic process in the mouse pilocarpine SE model [34]. In this model, increased T-type Ca²⁺ current and intrinsic burst firing...
Topiramate

Topiramate is a newer, structurally novel ASD with multiple pharmacological actions that could contribute to its ability to protect against seizures [8]. These include modulatory effects on Na⁺ and Ca²⁺ channels, GABA_A receptors, and ionotropic glutamate receptors. This combination of actions has encouraged studies of the antiepileptogenic potential of topiramate.

An early study showed that topiramate could delay amygdala kindling in rats [35]. However, the protocol involved drug administration at the time of kindling stimulation and the afterdischarge was reduced, raising similar questions as in the case of valproate. More recent studies demonstrated an age-dependent ability to retard kindling acquisition in a rat rapid kindling model, with increasing efficacy as the age of the animals increased from 2 to 5 weeks [36, 37]. As with the prior study in adult rats, the translatability of the findings is uncertain.

Levetiracetam

Levetiracetam is another newer ASD which, like topiramate, possesses a novel chemical structure and has diverse pharmacological actions [51]. For example, the drug reduces high voltage-activated Ca²⁺ currents, inhibits intracellular Ca²⁺ release through the IP3 and ryanodine receptors associated with the endoplasmatic reticulum, reverses the inhibitory effects of zinc on both GABA_A and glycine receptor-mediated currents, and inhibits AMPA. However, the most likely target of relevance to the antiseizure actions of levetiracetam is synaptic vesicle protein 2A (SV2A), a ubiquitous presynaptic protein. SV2A-deficient mice show a marked reduction in the anticonvulsant activity of levetiracetam, confirming the role of SV2A in the seizure protection conferred by the drug [52]. Interestingly, SV2A expression has also been observed to be suppressed during the epileptogenic process in rat SE models and in hippocampal tissue from epilepsy patients. In addition, SV2A-deficient mice exhibit a proepileptic phenotype and accelerated epileptogenesis [53]. These various observations support a role for SV2A in the development of epilepsy, and raise the possibility that levetiracetam could have antiepileptogenic properties aside from its antiseizure activity.

In support of this possibility, numerous studies have demonstrated that levetiracetam counteracts the process of kindling when administrated immediately before kindling stimulation during the acquisition of kindling [14, 15, 54–57]. As for valproate (see above), levetiracetam was tested using an
experimental design consisting of a first phase in which drug treatment is administered before each kindling stimulation, and a second phase in which daily kindling stimulation is continued, but without drug pretreatment [14, 15]. In a first study, it was demonstrated that levetiracetam causes a dose-dependent inhibition of kindling development in rats, as assessed by behavioral seizure stage and also inhibits the increase in afterdischarge duration that occurs during kindling [14]. At a higher dose, but not at lower doses, the diminished afterdischarge duration persisted despite continued stimulation in the absence of drug pretreatment, indicating a persistent effect of the drug. However, the persistent kindling resistance did not extend to the behavioral seizure stage, which did increase with continued stimulation. These findings were reproduced in another study by a different group that showed levetiracetam to cause a persistent lack of increase in afterdischarge duration after cessation of treatment [15].

Studies in genetic animal models of epilepsy have consistently shown antiepileptogenic properties of levetiracetam, when administered before the developmental expression of seizure activity. This was first demonstrated in a strain of spontaneously epileptic rats from Kyoto University in which early long-term treatment with a therapeutically relevant dose of levetiracetam inhibited the developmental expression of tonic convulsions and SWDs associated with absence seizures [58]. Later studies in the WAG/Rij rats confirmed that early long-term treatment with a therapeutically relevant dose of levetiracetam inhibits the developmental expression of SWDs [17, 33].

In contrast to the results obtained in kindling and genetic models, studies in SE models have provided conflicting results with respect to levetiracetam’s antiepileptogenic properties. Studies in rats with SE induced by pilocarpine and electrical stimulation of the amygdala did not find chronic treatment with levetiracetam after SE to inhibit the later development of spontaneous seizures [28, 59]. In contrast, other studies in rats using paired perforant path stimulation, kainic acid, and pilocarpine for SE induction showed a significant ability of chronic treatment with levetiracetam after SE to reduce the number [60] and duration of subsequent spontaneous motor seizures [61]. In addition, the development of hippocampal hyperexcitability, as manifested by increased population spike amplitude in the dentate gyrus and reduced paired-pulse inhibition in the CA1 area, was inhibited [62]. With the exception of one study [59], levetiracetam has also exhibited neuroprotective properties in SE studies [28, 63] and an ability to suppress seizure-induced neurogenesis [62], whereas cognitive impairment induced by SE was not prevented [59, 63].

Experience from a limited number of human studies supports the antiepileptogenic potential of levetiracetam. An uncontrolled, retrospective analysis of patients undergoing craniotomy and treated postoperatively with either levetiracetam or phenytoin showed that fewer patients who received levetiracetam (26 %) developed epilepsy after 1 year than those treated with phenytoin (36 %), although the difference did not reach statistical significance [20]. A recent open-label, nonrandomized study in 126 adults and children assessing the safety, tolerability, and pharmacokinetics of levetiracetam treatment in TBI patients for 1 month, initiated within 8 h of the injury, also showed a nonsignificant trend toward fewer patients on levetiracetam treatment (11 %) developing epilepsy after 2 years than those who were untreated (20 %) [21]. An additional pilot study in 40 children aged 6–17 years in which half were treated with levetiracetam within 8 h of injury showed an overall low incidence of posttraumatic epilepsy [64]. Only one patient developed epilepsy, and this patient was in the treatment group. This study is inconclusive as to the effect of levetiracetam. Moreover, a benefit on seizure occurrence after TBI was not observed in a small randomized, single-blinded, comparative trial of levetiracetam versus phenytoin in patients with severe TBI [65].

Interestingly, a recent retrospective analysis of seizure outcome implications of ASD use in patients who underwent temporal lobectomy demonstrated peripheral use of levetiracetam to predict a more favorable outcome [66]. While all ASDs were equally effective soon after surgery, at 5 years, patients taking levetiracetam exhibited a substantial “seizure freedom advantage” over other drugs, including carbamazepine, lamotrigine, oxcarbazepine, phenytoin, and topiramate. The authors speculated that this might reflect an antiepileptogenic action of levetiracetam under the assumption that the late recurrence of epilepsy reflects a new epileptogenic process following removal of the original epileptic focus. Overall, the available preclinical and clinical data indicate that continued evaluation of the antiepileptogenic potential of levetiracetam is worthwhile. More generally, it will be of interest to assess the role of pharmacological modulation of SV2A in antiepileptogenesis. In this context it is noteworthy that the selective, high-affinity SV2A ligand brivaracetam shows more pronounced antiepileptogenic effects than levetiracetam in the kindling model [57].

**Novel Antiepileptogenic Targets and Agents**

Although certain antiseizure drugs do have potential antiepileptogenic properties there is no reason to believe that an antiepileptogenic agent must protect against seizures or even that seizure suppression is a beneficial property for an antiepileptogenic therapeutic. Currently, there is an intense research effort focused on understanding the scientific basis of epileptogenesis. As a spin-off of this effort, a wide range of new molecular targets for antiepileptogenesis are being identified (see, e.g., Kobow et al. [67]). In many cases, these
targets are entirely different from those that have been defined through studies of the mechanism of action of antiepileptic drugs. To date, no novel antiepileptogenic drug has been identified as a result of screening against a unique antiepileptogenic target. Rather, studies have been conducted using previously known agents with central nervous system activity. In most cases, agents have been selected for study based on hypotheses regarding antiepileptogenic mechanisms. The strategy is to “repurpose” therapeutic agents designed for different purposes, for example to modulate immune function, reduce oxidative stress, or stimulate red cell production. The fact that many of these agents are already approved for use in other disease indications will facilitate evaluation of the novel strategies in human clinical studies. In the remainder of this article, we consider the evidence for the antiepileptogenic activity of various agents that are not traditional ASDs and that act on diverse putative antiepileptogenic mechanisms.

Immune and Inflammatory Mechanisms

Rapidly mounting evidence indicates a potential role of inflammation in the pathophysiology of epilepsy and epileptogenesis [68, 69]. A large number of inflammatory mechanisms have been implicated, but only relatively few have been experimentally investigated using pharmacological tools in animal epilepsy models. Marketed drugs and compounds in clinical development that possess specific anti-inflammatory actions are of particular interest in probing the therapeutic relevance of these mechanisms as they can be quickly translated into clinical development as epilepsy treatments.

Interleukin 1β Pathway

The interleukin 1β (IL1β) pathway is one of the best characterized inflammatory pathways in epilepsy. Seminal work performed by Dr. Annamaria Vezzani and her collaborators has provided compelling evidence for a pathophysiological role of IL1β in epilepsy [68]. In animal models, the levels of IL1β in the brain are elevated during epileptogenesis and also (to a lesser degree) during chronic epilepsy. Importantly, studies with brain tissue obtained at surgery for temporal lobe epilepsy (TLE) or focal cortical dysplasia have revealed that similar changes occur in human epilepsy. Furthermore, injection of IL1β into the brain leads to seizure exacerbation in rodents, while IL1β knockout mice are resistant to seizures. These observations have created a strong rationale for testing therapeutic modalities that inhibit IL1β synthesis in models of chronic epilepsy. For example, VX-765 is an orally active IL-converting enzyme/caspase-1 inhibitor that blocks IL1β secretion and produces a strong anti-inflammatory effect [70]. Subchronic treatment (4 days) with VX-765 before acute kainate injection reduces the duration and number of seizures during SE [71]. Moreover, treatment with VX-765 after epilepsy is established in the postkainate SE model, resulting in a reduction in the duration and number of spontaneous recurrent seizures [72]. A recent study extends this concept [73]. In these recent experiments, IL1β signaling was blocked by systemic administration of the recombinant IL1 receptor antagonist (anakinra) and VX-765. These compounds were administered either 60 mins after pilocarpine-induced SE or 180 mins after unrestrained electrically induced SE, and the treatment continued for 7 days. The drug combination significantly decreased IL1β expression and exerted a neuroprotective effect, although the onset of epilepsy and the frequency and duration of seizures were not significantly modified. While these particular studies did not indicate an antiepileptogenic action, VX-765 may have antiepileptogenic properties as it is able to delay kindling development [74]. The antiseizure mechanism of anti-inflammatory drugs that inhibit IL1β signaling seem quite different from most of the conventional ASDs acting on voltage-gated ion channels or inhibitory/excitatory mechanisms. However, IL1β may facilitate or exacerbate seizures via indirect modulation of NMDA receptors [75], and it will be of interest to further explore the disease-modifying potential of such treatments. Indeed, a recent trial with VX-765 in patients with treatment-resistant partial epilepsy did not meet the predefined endpoint, but a post-hoc analysis suggested that the treatment might produce a reduction in seizure frequency that is slow to develop, but which persists for at least 2 weeks after the drug is discontinued [76]. These encouraging, but preliminary, results represent the first evidence in humans that targeting immune mechanisms may have a role in epilepsy treatment.

Leukocyte Adhesion

Leukocytes and leukocyte adhesion mechanisms may play a role in seizure generation and in the pathology of epilepsy [77]. Experimental data from a mouse model of TLE indicate that seizures cause increased expression of vascular cell adhesion molecules, and enhance leukocyte rolling and arrest in brain vessels [78]. These effects could be mediated by specific leukocyte integrins, such as α4β1 integrin [very late antigen-4 (VLA-4)]. Remarkably, inhibition of leukocyte–vascular interactions with blocking antibodies markedly reduced seizures in this animal model, but, more importantly, treatment with blocking antibodies after SE prevented the development of epilepsy [78]. Interestingly, there is evidence that leukocyte infiltration into the brain is more abundant in individuals with epilepsy than in controls [78]. However, more recently, Zattoni et al. [79] have shown that in the kainate model leukocyte infiltration may actually be neuroprotective and could inhibit epileptogenesis. Thus, the role of immune cell trafficking during epileptogenesis is not fully elucidated, and may be dependent on experimental model or epilepsy...
syndrome. For example, the presence of T cells in human epilepsy tissue is not an unequivocal finding as it has only been established in focal cortical dysplasia type 2b, while a minor infiltration is observed in focal cortical dysplasia type 1, and virtually no infiltration is observed in TLE [80, 81]. Nevertheless, these observations suggest that inhibition of leukocyte migration into the brain could be a promising therapeutic strategy in epilepsy. This is particularly attractive as the VLA-4 blocking antibody natalizumab is already approved for the treatment of multiple sclerosis [82]. Small molecules blocking VLA-4 are also at different stages of clinical development [83] and could become useful tools to probe these mechanisms further in animal models of epilepsy.

Cyclooxygenase-2

Cyclooxygenases are enzymes responsible for the formation of prostanoids, complex fatty acids that have diverse roles in inflammation. Selective inhibitors of cyclooxygenase-2 (COX-2) were developed to reduce the risk of peptic ulceration that occurs with nonselective cyclooxygenase inhibitors. There are several reports from experimental epilepsy models suggesting that COX-2 inhibitors may have potential disease-modifying properties; however, the results have not been consistent [84]. Jung et al. [85] reported that celecoxib has antiepileptic and neuroprotective effects in the lithium–pilocarpine model in rats. In this study, treatment with celecoxib was started 24 h after SE and continued during the seizure monitoring period. Celecoxib-treated animals showed a significantly reduced incidence of spontaneous recurrent behavioral seizures as detected in video recordings in the period 28–42 days after SE. In addition, the duration of seizures that occurred in the celecoxib-treated animals was reduced compared with those in vehicle-treated rats, and there was a neuroprotective effect in various hippocampal regions. In a later study, a different COX-2 inhibitor (SC58236) was investigated in the self-sustaining SE model [86]. Treatment with the COX-2 inhibitor was begun a few hours after the onset of SE and continued for 7 days. The animals were monitored by video-electroencephalography recording for 35 days. An additional experimental group was treated with SC58236 for 5 days after the development of spontaneous recurrent seizures. In contrast to the study of Jung et al. [85], treatment with SC58236 did not result in any significant effect on seizure incidence, duration, latency to the first spontaneous seizure, or seizure severity. In addition, no significant neuroprotection was observed in the SC58236-treated animals. Interestingly, the same group later reported that treatment with SC58236 before SE induction led to increased lethality within the first 2 weeks of SE, and 14-day treatment in chronic epileptic rats led to an increase in seizures in some animals [87]. In a third study, Polascheck et al. [88] used parecoxib, a prodrug of the potent and selective COX-2 inhibitor valdecoxib, in the rat pilocarpine model of TLE. In this study, the treatment was started 90 mins after SE and continued for 18 days. Despite thorough video-electroencephalography monitoring for 8 weeks, there was no evidence that parecoxib treatment led to a reduction in the incidence, frequency, or duration of spontaneous seizures or in the behavioral and cognitive alterations associated with epilepsy. The authors did find a mild effect of parecoxib treatment on seizure severity, and it appeared to reduce neuronal injury in the hippocampus.

Unfortunately, clinical use of selective COX-2 inhibitors has been associated with significantly increased risk of cardiovascular diseases [89, 90]; therefore, an alternative strategy selectively targeting of individual prostaglandin receptors has been propose. Indeed, recent data indicate that small molecule inhibitors of prostaglandin E2 receptor subtype EP2 are neuroprotective when administered after SE [91, 92]. Even though these compounds do not display clear-cut antiseizure or antiepileptogenic properties, at least when early seizures after SE are concerned, EP2 receptor antagonism could be an adjunctive therapeutic strategy for the pathological sequelae of SE or brain injury [91, 92].

Together, these studies suggest that despite strong induction of COX-2 during epileptic seizures pharmacological inhibition of this enzyme alone or selective inhibition of prostaglandin receptors may not be sufficient to provide a robust disease-modifying effect. However, COX-2 or prostaglandin E2 receptor inhibitors might provide benefit when used in conjunction with other anti-inflammatory treatments, such as IL1β inhibitors [93].

Sphingosine 1-Phosphate Receptors

Sphingosine 1-phosphate receptors belong to a large family of G protein-coupled receptors that are expressed by many cell types, including immune, glial, and neural cells. Fingolimod (FTY720), a sphingosine analog that acts as a sphingosine 1-phosphate receptor modulator, is an approved oral treatment for relapsing forms of multiple sclerosis. Fingolimod inhibits lymphocyte egress, selectively retaining lymphocytes within the lymph nodes, but, in addition, it acts on a number of neuronal and non-neuronal cells to exert neuroprotective and anti-inflammatory effects in the brain [94]. Although fingolimod has not been widely studied in epilepsy models, a recent report by Gao et al. [95] indicates that it may also have therapeutic potential in epilepsy. In this study, fingolimod was administered to rats in the lithium–pilocarpine model for 14 days beginning 24 h after SE. Interestingly, fingolimod produced a clear-cut neuroprotective effect, inhibiting pathological mossy fiber sprouting, decreasing activation of microglia and restoring abnormal expression of IL1β and tumor necrosis factor-α in the hippocampus. Furthermore, the incidence, duration, frequency, and severity of spontaneous
recurrent seizures was significantly decreased in fingolimod-treated animals [95]. These results are encouraging and suggest that further study is warranted.

Oxidative Stress Mechanisms

Oxidative stress, a biochemical state in which harmful reactive oxygen species are generated, has been hypothesized to occur in epilepsy and to be a cause of treatment refractoriness [96, 97]. Several antioxidant strategies have been proposed as a treatment approach. Here, we consider resveratrol, a stilbenoid found in the skin of red grapes and other fruits that demonstrates strong antioxidant and neuroprotective properties [98], and dimethyl fumerate, which exerts cytoprotective effects by induction of an antioxidant response [99].

Resveratrol

A plethora of largely preclinical studies has found broad therapeutic potential of resveratrol in the treatment of diverse conditions, including metabolic syndromes, cancer, and cardiovascular diseases. Some, but not all, recent experimental studies also suggest that resveratrol may have utility in epilepsy [100]. Wu et al. [101] examined the effects of resveratrol treatment on the occurrence of spontaneous seizures following SE induced by intrahippocampal kainate injection in rats. The incidence and rate of spontaneous seizures was markedly reduced in the resveratrol treatment group. The effect on seizures was associated with neuroprotection in the hippocampus, as well as a reduction in mossy fiber sprouting [101]. These promising results were not fully confirmed in a more recent study of younger kainate-treated rats [102], so that, at present, the potential of resveratrol for disease modification in epilepsy is uncertain.

Nrf2 Pathway

Nuclear erythroid-2-related factor 2 (Nrf2) is a redox-sensitive, basic leucine zipper transcription factor. Under physiological conditions, Nrf2 is sequestered in the cytoplasm, tethered to the regulatory protein Keap1. Oxidative stress or other disturbances in cell homeostasis lead to nuclear translocation of Nrf2, where it binds to the antioxidant response element in promoters of target genes. Overall, Nrf2 coordinates the expression of numerous genes encoding detoxification, antioxidant, and anti-inflammatory mediators, and calcium homeostasis and signaling proteins resulting in an orchestrated protective response. Nrf2 has been described as the "master regulator" of the antioxidant response, and it represents a molecular target with broad therapeutic potential [103]. Indeed, Nrf2 exerts a protective role in a number of neurodegenerative disease models [104]. Interestingly, Nrf2 has also been identified to be at the center of a network of over 250 coexpressed genes that were specifically altered in the hippocampi of animals with kainate-induced seizures [105]. Furthermore, Nrf2-deficient animals are more vulnerable to kainate-induced seizures [106]. Recently, Mazzuferi et al. [107] confirmed that Nrf2 is activated in hippocampal tissue obtained from patients and mice with TLE. They also reported that overexpression of Nrf2 by viral gene transfer in mice with TLE led to seizure reduction, as well as neuroprotective and anti-inflammatory effects. Together, these recent results provide a strong rationale for the investigation of small molecule activators the Nrf2 pathway to treat epilepsy. Several natural products and synthetic molecules that activate the Nrf2 pathway are under development or already approved for clinical use [108]. For example, dimethyl fumarate (BG-12) has recently been licensed for the treatment of relapsing–remitting multiple sclerosis [109]. It remains to be determined whether this compound, or other Nrf2 activators, will indeed provide therapeutic efficacy in epilepsy.

Signaling Pathways

The Mammalian Target of Rapamycin Pathway

The mammalian target of rapamycin (mTOR) is a well-conserved serine/threonine kinase of the phosphatidylinositol kinase-related kinase family [110]. In brain, it plays a role in neurite growth, synaptic plasticity and cell survival by regulating metabolism and protein synthesis [110, 111]. The mTOR signaling pathway is critical to tuberous sclerosis (TS), a genetic multisystem disorder characterized by hamartomas in several organs, including the brain. Patients with TS usually have seizures. The disease occurs as a result of mutations in the TSC1 and TSC2 tumor suppressor genes, which act as negative regulators of the mTOR complex. A mutation in TSC1 or TSC2 results in hyperactivation of mTOR and its downstream genes, causing tumor formation [112, 113]. Recent studies have indicated that mTOR plays a role in the epileptogenic process in TS inasmuch as early treatment with the mTOR inhibitor rapamycin can prevent the development of epilepsy in mice with conditional inactivation of the Tsc1 gene [114]. A retrospective clinical study confirmed the potential utility of rapamycin for the treatment of seizures in children with TSC; whether this action is disease-modifying remains to be determined [115]. Interestingly, mTOR dysregulation has been demonstrated in a variety of other types of epilepsy, including epilepsies associated with brain injury, SE, genetic mutations, brain tumors, and focal cortical dysplasias [116]. Indeed, mTOR inhibitors may have antiseizure and antiepileptogenic actions in various types of acquired epilepsy [114, 117, 118]. The efficacy of rapamycin in TLE models is inconsistent, and may be dependent on study design, model or species choice [119–121]. Consequently,
mTOR inhibition may not be a universal antiepileptogenic strategy.

**Peroxisome Proliferator-Activated Receptors**

The peroxisome proliferator-activated receptors (PPARs) constitute a group of three nuclear receptor isoforms: PPARγ, PPARα, and PPARδ. They operate as ligand-regulated transcription factors, which heterodimerize with retinoid X receptor and upon agonist binding interact with various cofactors to initiate gene transcription. Fatty acids are the natural ligands for PPARs, which thereby serve as lipid sensors and regulators of lipid metabolism. Consequently, synthetic PPAR ligands have been developed for the treatment of dyslipidemias and diabetes. However, emerging research indicates broader potential of these ligands as therapies for atherosclerosis, inflammation, cancer, demyelination, and several neurological diseases [122].

Many PPARγ agonists have been tested in various models of epilepsy and it has generally been found that these agents protect against seizures. For example, pioglitazone delayed the development of seizures in genetically epileptic EL mice, which was associated with overall reduction in several neuroinflammation biomarkers [123]. Fenofibrate displayed anticonvulsant effects in the lithium–pilocarpine SE model and against pentylenetetrazol-induced seizures [124]. Another PPARγ agonist, rosiglitazone, consistently displayed significant neuroprotective effects and attenuated inflammatory responses after induction of SE [125–127]. Importantly, intracerebroventricular rosiglitazone administered prior to the administration of lithium–pilocarpine also decreased the number of spontaneous recurrent seizures in the chronic phase beginning 2 weeks after SE, although it did not affect the severity of SE in the acute phase; some of its protective effects were indirectly mediated by TrkB signaling [128]. PPARγ agonists seem to have promise as anticonvulsant agents and there is a suggestion that they might be disease-modifying. Given the availability of diverse PPAR agonists that are approved for use in other clinical indications, further investigation of this class of agents is warranted in epilepsy.

**Erythropoietin**

Erythropoietin (EPO), a glycoprotein hormone that controls erythropoiesis in the bone marrow, has other actions that have been widely recognized, including effects in the central nervous system [136]. In particular, there is evidence that exogenous EPO administration can protect against seizures [137, 138]. Moreover, EPO receptors are expressed by hippocampal neurons, and their abundance is increased following lithium–pilocarpine SE [139, 140]. This may occur because SE increases transcript levels of hypoxia-inducible factor-1α, the regulatory subunit of hypoxia-inducible factor-1, which regulates the gene for EPO and vascular endothelial growth factor (VEGF; see below). In a recent study of rats subjected to lithium–pilocarpine SE, EPO administration started immediately after SE cessation and continued for 7 days led to prevention of BBB leakage, neuronal death, and microglia activation, and inhibited the ectopic granule cell generation that occurs with epileptogenesis [140]. More importantly, EPO treatment reduced the risk for the development of spontaneous recurrent seizures and led to a reduction in the seizure duration. An obvious concern with the use of EPO is that there could be a dangerous increase in red cell production. However, the dosing regimen used in this study does not elevate the hematocrit beyond safe levels. A separate study found similar neuroprotective effects of EPO in a rat model of SE [141]. Interestingly, Eid et al. [142] demonstrated an increased density of EPO receptors in sclerotic hippocampi resected from patients with treatment-resistant TLE. It will be of interest to determine the mechanism whereby EPO protects against epileptogenesis, and clinical evaluation may be warranted.
VEGF

Profound cerebrovascular changes are observed in patients with focal epilepsies and in rodent models, which appear to be an important factor for the development and maintenance of the disease pathology [143]. In rodent models of TLE, BBB impairment occurs early after SE and is typically followed by a progressive increase in vascularization. However, in humans and rodents, ongoing angiogenesis and BBB disruption are still evident in the epileptic focus during the chronic phase of the disease, probably as a result of ongoing recurrent seizures [144]. VEGF is a key signal promoting angiogenesis, but it also alters BBB permeability and enhances monocyte infiltration into brain parenchyma. These and other actions of VEGF directly or indirectly affect neuronal excitability, brain plasticity, and the biology of several neurological diseases, including epilepsy [145]. Numerous reports confirm that there is a rapid increase in VEGF expression and signaling shortly after SE induction, and this enhanced VEGF activity persists in the chronic phase of focal epilepsies [145–148]. If VEGF is involved in the maintenance of the epileptic state, anti-VEGF therapies such as bevacizumab and ranibizumab that are marketed for several cancer indications and for ophthalmic diseases could be beneficial in therapy of epilepsy or epileptogenesis. It is possible that therapeutic antibodies such as bevacizumab are able to penetrate the BBB, although whether and under what conditions this occurs is uncertain [149]. Unfortunately, VEGF plays an important neuroprotective role so that approaches aiming at diminution of VEGF signaling could be problematic [145, 150]. VEGF-R2, a receptor tyrosine kinase, mediates most of the cellular responses to VEGF, including its neuroprotective actions. Targeting VEGF-R2 could, theoretically, be an alternative antiepileptogenic strategy [151]. However, in line with the role of VEGF in neuroprotection, blocking VEGF-R2 was found to increase hippocampal neuronal loss in a model of TBI [152]. Moreover, VEGF-R2 overexpression in mice exerted an antiseizure effect, but failed to affect kindling epileptogenesis [153]. At present, there is insufficient information to conclude that VEGF or its receptors are appropriate targets for an antiepileptogenic therapy.

Other Molecular Targets

Inhibition of HMG-CoA Reductase

Statins are a class of drugs used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver [154]. In addition, this class of compound exerts a plethora of other actions, including modulation of inflammatory responses and improvement of endothelial functions [155]. Therefore, statins may have broad therapeutic utility, including for the treatment neurological diseases. Interestingly, there has been a flurry of reports providing evidence that statins could be useful in the treatment of epilepsy. For example, treatment with atorvastatin and simvastatin produced clear-cut neuroprotective and anti-inflammatory effects in the kainate model of TLE [156, 157], while lovastatin inhibited aberrant mossy fiber sprouting in the pilocarpine model [158]. In contrast, atorvastatin had no significant effect on brain inflammation and neuronal death in the self-sustaining SE model [159]. Moreover, in none of these cases did treatment with statins affect the frequency of spontaneous recurrent seizures. Interestingly, however, treatment with lovastatin corrected excess hippocampal protein synthesis and prevented epileptogenesis in Fmr1 mice, a model of fragile X syndrome in which the Fmr1 gene is deleted [160]. It is noteworthy that the genetic defect in these mice differs from that in the human disorder, where there is a CGG trinucleotide repeat expansion. These animals exhibit audiogenic seizures, but not other seizure types, and they do not exhibit a reduced seizure threshold to chemoconvulsants; the significance of the audiogenic seizures that occur in these animals for human fragile X syndrome is uncertain.

In a recent epidemiological study, a large patient cohort with cardiovascular diseases, treated with statins, was compared with patients not on these medications. Patients taking a statin were less likely to be hospitalized for epilepsy, suggesting that statins might play a role in the prevention or treatment of epilepsy in populations at high risk of seizures [161]. Although the results are intriguing, this observational study requires confirmation with prospective trials.

Neurotrophic Factors

Emerging evidence suggests that activation of BDNF receptor TrkB promotes epileptogenesis caused by SE. Indeed, animal models of TLE are associated with a strong increase in BDNF expression and enhanced activation of TrkB, while infusion of BDNF and transgenie overexpression of BDNF or TrkB increase seizure susceptibility or severity. Consequently, conditional knockout of TrkB completely abolishes epileptogenesis in the kindling model [162]. Combined augmentation of fibroblast growth factor-2 (FGF-2) and BDNF increased neurogenesis, reduced neuronal loss, and, most importantly, reduced the occurrence of spontaneous seizures in a model of TLE [163]. Interestingly, antiepileptogenic effects of FGF-2 and BDNF might involve inhibition of neuroinflammatory mechanisms [164]. These exiting observations have been confirmed recently and extended to another post-SE model of epileptogenesis in which transient inhibition of TrkB achieved by a chemical–genetic approach prevented the onset of recurrent seizures, ameliorated anxiety-like behaviors, and limited loss of hippocampal neurons [165]. These recent findings have more firmly established FGF-2 and TrkB signaling as an attractive target for developing preventive treatments for human epilepsies.
Potential Antiepileptogenic Treatments

α2 Adrenergic Receptor Blockade

Adrenergic receptors are a family of G protein-coupled receptors that serve as the targets of the neurotransmitter norepinephrine. Several decades of research have demonstrated that pharmacological activation or blockade of the various adrenergic receptor types can influence seizure susceptibility. Specifically, stimulation of α2 adrenergic receptors is generally anticonvulsant, while blockade of these receptors is proconvulsant [166]. Therefore, the observation of Pitkänen et al. [167] that inhibition of α2 adrenergic receptors may have disease-modifying and neuroprotective effects in a model of TLE is intriguing. In this study, chronic treatment with atipamezole, a selective α2 adrenergic receptor antagonist, was started 1 week after the induction of SE by electrical stimulation of the amygdala. Although there were no differences in the proportion of animals that developed epilepsy, the atipamezole-treated rats displayed lower seizure frequency, and the seizure frequency did not increase over time as in the control animals. Furthermore, atipamezole-treated animals had less damage in the hilar region of the hippocampus and less pronounced mossy fiber sprouting, although this did not correlate with the severity of the epilepsy. Inasmuch as atipamezole, like other α2 adrenergic receptor blockers, has proconvulsant activity [168], this study clearly rebuts the conventional view that assumes antiseizure treatments will lead to antiepileptogenesis. In fact, just the opposite might be true. At present, it is not known whether the effect of atipamezole seen in this study will extend to other epilepsy models, whether antiepileptogenesis is a general property of proconvulsant agents (repeated treatment with certain proconvulsant agents leads to kindling, so caution is warranted), and whether it would be feasible to use a proconvulsant agent clinically. Nevertheless, this study has been influential in changing thinking about the appropriate strategy to pursue in the search for disease-modifying agents in epilepsy [6].

Cannabinoid Receptors

Cannabinoid receptor antagonists provide another example of agents that enhance brain excitability, but which may paradoxically confer antiepileptogenesis. Many studies have demonstrated that cannabinoid agonists are acutely anticonvulsant, whereas cannabinoid antagonists, including the selective CB1 antagonist SR141716A (rimonabant), are acutely proconvulsant [169]. Surprisingly, however, SR141716A treatment prevents the development of increased seizure susceptibility when administered during or shortly after a brain insult in models of prolonged febrile seizures [170] or TBI [171]. The observation that the cannabinoid system can regulate epileptogenesis indicates that caution is warranted when contemplating the use of agents that interact with cannabinoid signaling in epilepsy therapy.

Na⁺ K⁺ 2Cl⁻ Cotransporter

In immature neurons in the developing brain, the balance between Na⁺ K⁺ 2Cl⁻ cotransporter (NKCC1) and K⁺Cl⁻ cotransporter activity is such that the Cl⁻ equilibrium potential is more positive than in adult neurons so that the neurotransmitter GABA causes depolarization. If GABA is depolarizing, seizures may occur, which could lead to epileptogenesis [172, 173]. Depolarizing GABA may also be relevant to epileptogenesis in the adult brain. Various epileptogenic brain insults downregulate K⁺Cl⁻ cotransporter and upregulate NKCC1 causing a recapitulation of the state of hyperexcitability in the immature brain. Bumetanide, an inhibitor of NKCC1, prevents the outward flow of Cl⁻, opposing the shift in Cl⁻ equilibrium potential and the depolarizing action of GABA, which leads to an anticonvulsant action [174, 175]. By treating rats chronically with bumetanide in the pilocarpine-induced SE model of TLE, Brandt et al. [176] examined the hypothesis that depolarizing GABA is a factor in epileptogenesis. No significant effect on development of spontaneous seizures was obtained. This negative result was later confirmed in the presence of a metabolic inhibitor that increases bumetanide brain exposure [177]. A small observational clinical case series indicates that bumetanide might confer seizure protection in patients with treatment-resistant TLE [172]. Thus, agents that block NKCC1 might provide symptomatic benefit under some circumstances, but there is no evidence that they would be antiepileptogenic [173].

Conclusion

Evidence from preclinical studies indicates that certain ASDs, including levetiracetam, ethosuximide, and possibly valproate, may produce effects on seizure susceptibility that outlast the treatment period, suggesting that these agents might have disease-modifying activity in epilepsy. To date, no ASD has been demonstrated to have antiepileptogenic activity in a rigorous clinical trial. However, the most promising drugs, notably levetiracetam and ethosuximide, have not been adequately studied. Whether they can provide clinically useful disease-modifying effects is uncertain, but some attempts are being made to find out [21, 64]. Although the emphasis to date in antiepileptogenesis clinical trials has almost exclusively been on antiseizure drugs, it is apparent that an antiepileptogenic agent need not have antiseizure activity, and the evidence from studies of atipamezole and SR141716A indicates that even drugs with proconvulsant activity can have antiepileptogenic properties.

At present, there are many theories of epileptogenesis, but none are firmly established. However, the diverse theories of epileptogenesis currently under investigation suggest many potential treatment approaches. There are a remarkable
number of treatments that were developed for other disease indications and are now in clinical use, which, the theories suggest, could be antiepileptogenic. These treatments could be repurposed for epilepsy therapy. Clinical studies can be expected with many of these agents.

It is worth noting that epilepsy encompasses many distinct conditions and it seems unlikely that a single agent will be universally antiepileptogenic in all epilepsies. More likely, disease-modifying treatments will be specific to the epilepsy type. An important consideration is the timing of the treatment. Early treatment before epilepsy is established is most likely to be successful. For many epilepsy types, this will require predictive biomarkers, which are not available at present [178]. Also, new clinical trial designs are urgently required [5]. The recent clinical trial of VX-765, which was originally developed as a treatment for autoimmune conditions and represents the first nonconventional therapy to be studied in humans, leads credence to the repurposing strategy. However, the study was structured as a conventional epilepsy drug trial and failed to meet its predefined endpoint. It was only when the investigators considered the novel biological actions of VX-765 as an antiinflammatory agent and reanalyzed the data to recognize the possibility that benefit might develop slowly, but persist beyond the course of treatment, that it was possible to discern a suggestion of efficacy. Clearly, the design of clinical trials of potential antiepileptogenic agents will need to be founded on a deep understanding of the underlying biological mechanisms, as well as comprehensive knowledge of the pharmacology of the therapeutic agent. Despite the not inconsequential hurdles, the base of relevant scientific information is expanding rapidly and there is cause for cautious optimism. It is not difficult to imagine that in the not too distant future antiepileptogenic therapies will emerge, ushering in a new era in which physicians administer drug treatment for a limited period to prevent the occurrence of epilepsy instead of committing patients to an often lifelong course of one or more symptomatic treatments.

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References


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