Antiepileptogenesis, neuroprotection, and disease modification in the treatment of epilepsy: focus on levetiracetam

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ABSTRACT – The search for antiepileptic drugs (AEDs) using drug screens that test for the ability to suppress paroxysmal events has primarily resulted in the discovery of AEDs that inhibit neuronal excitability. While profoundly reducing expression of epileptic seizures, current pharmacologic treatments have not been able to completely control seizures in all patients, and can impair normal neuronal excitation underlying cognition. A new approach to drug screening, including the process of epileptogenesis, may yield new classes of drugs that not only suppress seizures but also specifically act to protect against the neurobiological changes that contribute to the development of epilepsy. By preventing or reversing the neuronal circuit reorganizations that produce lowered seizure thresholds following brain insults such as head trauma or status epilepticus, these antiepileptogenic drugs could prevent, or reverse, progressive worsening of the epileptic process. It is likely that antiepileptogenic drugs will have mechanisms of action distinct from traditional AEDs, as the molecular mechanisms underlying epileptogenesis and ictogenesis probably differ. One new AED with potential antiepileptogenic properties is levetiracetam, which was discovered using non-conventional drug screens. It markedly suppresses kindling development at doses devoid of adverse effects, with persistent suppression of kindled seizures even after termination of treatment. Further design and implementation of antiepileptogenic drug screens are needed for the discovery of other novel disease-modifying agents.

KEY WORDS: epilepsy, AEDs, seizures, levetiracetam, kindling, epileptogenesis

Introduction

The discovery of new drugs with specific properties requires appropriate tests of drug function. Traditional screens for antiepileptic drugs (AEDs) examine anti-seizure properties; i.e., the ability to suppress expression of experimentally induced seizures in normal laboratory animals [1, 2]. For this reason, current drug treatment options for epilepsy predominantly combat ictogenesis, or the initiation of paroxysmal activity [3]. This has identified a number of classes of AEDs that primarily suppress neuronal excitability by blocking Na⁺ channels or enhancing inhibitory GABAAergic activity.
While these traditional AEDs have had a profound effect by reducing the expression of epileptic seizures, their function invariably elicits some impairment of the normal neuronal excitability underlying cognitive function [6-8]. Since ictogenesis and cognition are both mediated by neuronal excitability, it may not be possible to discover optimal non-impairing AEDs using traditional screens. This may be improved by performing drug screens in animal models of chronic epilepsy. Thus, by applying genetically modified or kindled animals [9] it may be possible to discover new AEDs that inhibit the neuronal hypersynchronization leading to an ictal event, without interfering with normal neuronal excitability [10]. An additional approach to the discovery of novel AEDs would be to examine processes of epileptogenesis in addition to ictogenesis [11]. Since the development of epilepsy is a multistep progressive process [12], there may be several mechanisms in addition to the neuronal excitability and hypersynchronization associated with the paroxysmal event that are susceptible to pharmacologic intervention. Thus, it appears possible to devise novel drug screens that may reveal new classes of AEDs with less compromising mechanisms of action.

Epileptogenesis refers to the multiphase process in which a normal brain undergoes alterations to support the generation of spontaneous seizures. It may be initiated by brain damage produced by events such as head trauma [13], stroke [14], infection [15, 16], or status epilepticus [17]. Following such an initial insult, a latency phase without seizures follows and may last for weeks to years. During these initial stages, progressive brain alterations result in lowered seizure thresholds which eventually cause spontaneous seizures [13, 18]. Once seizures occur, the epileptic disease state probably continues to progress, with each seizure having the potential to induce additional neuronal alterations that may further lower seizure thresholds [19].

In order to discover novel AEDs that combat these phases of epileptogenesis, new drug screening models must be employed. It is likely that such screens would identify drugs with mechanisms of action different from traditional AEDs, since the molecular mechanisms underlying epileptogenesis and ictogenesis are different. While anticonvulsants reduce the duration or frequency of seizures by suppressing neuronal excitation or excitability, real antiepileptogenic agents would act by blocking the initial epileptogenic process or by altering the epileptic disease state after the seizure onset [11]. Appropriate screens for antiepileptogenic drug action would be tests for the ability of drugs to reduce alterations in molecular, cellular, and network properties that occur during the epileptogenic process.

The induction of status epilepticus (SE) and kindling represent two animal paradigms in the preclinical evaluation of AEDs. SE is defined as long-duration seizures, typically lasting for more than 30 min [20]. Experimentally, SE can be induced by acute systemic exposure to epileptogenic agents, including drugs that block GABAergic inhibition or facilitate glutamatergic or cholinergic excitatory transmission. The glutamate receptor agonist kainic acid [21] and the cholinergic agonist pilocarpine [22] are commonly used in SE models. SE can also be induced by electrical stimulation [1]. Anti-seizure properties of potential AEDs can be tested by measuring the ability of drugs to suppress SE initiation, duration, or seizure intensity following administration of convulsant agents.

Experimentally induced SE can also be used as a model for epileptogenesis, since SE induces neuronal alterations similar to those seen in epileptic patients. Further, the long-duration seizures characteristic of SE produce neuronal damage similar to Ammon’s horn sclerosis observed in patients with temporal lobe epilepsy [23, 24]. SE induces cell loss in specific neuronal populations in multiple brain regions, including the hippocampus, amygdala, and entorhinal cortex [22, 23]. The damage induced by kainic acid-induced SE is produced by the evoked seizure activity and not by direct activation of glutamate receptors by kainic acid [23]. There are two phases of cell death following SE. Acute necrotic cell loss occurs during the prolonged seizure event, while other cells undergo delayed cell death hours or days following seizure termination. Surviving brain cells undergo morphological alterations including axonal sprouting and altered density of dendritic spines. In addition, SE causes widespread changes in gene expression, the extracellular matrix, and neurogenesis. SE also causes alteration in non-neuronal brain cells, such as changes in number and morphology of astrocytes and microglia. Functionally, SE produces long-lasting deficits in cognition, behavior, and memory. Critical to the use of SE as a model of epileptogenesis is that spontaneous seizures develop after a latency following SE. SE can be used to test for antiepileptogenic properties of potential AEDs by administering the AED following SE and examining the effect on neuronal pathology and expression of spontaneous seizures.

A second animal model commonly used for evaluating anti-seizure properties of AEDs is focal, electrical kindling. In the kindling model, repeated exposure to an initial sub-convulsive stimulus eventually evokes seizures [19, 25, 26]. Initially, electrical kindling stimuli only elicit short-duration afterdischarges produced by a synchronous neuronal discharge near the site of stimulation. Each additional kindling stimulation induces longer afterdischarges which incorporate larger brain regions, with the limbic system quickly becoming involved. Behavioral seizures accompany the afterdischarges and become more complex and longer with repeated stimuli. This progressive increasing sensitivity to a previously subconvulsant stimulus usually takes a number of days or weeks and eventually reaches a plateau in which kindling stimuli evoke seizures and afterdischarges with reproducible be-
The kindling-induced reduction in seizure threshold is permanent. The seizures evoked by focal, electrical kindling stimuli in the temporal lobe involve limbic circuits and are analogous to human complex partial seizures with secondary generalization [26, 27]. Pharmacologic convulsants or electrical stimuli can induce kindling [26]. Kindling can be used as a screen for anti-seizure effects since kindled seizures are inducible and have durations and electrographic and behavioral manifestations that are easily characterized. After animals have been fully kindled, potential AEDs can be administered and the effects on behavioral and electrographic seizures measured.

The progressive nature of kindling, in which repeated seizures cause a reduction in seizure thresholds over time, may share features with the epileptogenic process in humans. It is possible that the long delay between trauma and seizure expression in posttraumatic epilepsy may reflect a slow kindling process [26]. This idea is supported by the development of generalized seizures in a patient receiving electrical stimulation of the thalamus [28]. Evidence against kindling as a mechanism underlying epilepsy in man relates to the observation that although primates can be kindled, they are much more resistant to kindling stimuli than are rodents [26].

An association between alterations of neuronal circuits and increased seizure susceptibility has also been found in kindling. Even relatively brief kindled seizures, lasting seconds to minutes, have been shown to produce limited neuronal alterations similar to those seen following SE. Kindled seizures induce progressive, but limited, cell loss in limbic regions including the dentate gyrus, hippocampus and entorhinal cortex [29, 30], and sprouting of axonal collaterals in the dentate gyrus [31]. Kindling also induces behavioral alterations and causes long-term deficits in cognitive function [32-34]. In contrast to SE, however, kindling rarely results in the development of spontaneous seizures.

Therefore, kindling stands as a model to investigate the effects of potential antiepileptogenic compounds on the reorganization of neuronal circuits which have similarities to those that occur after SE and lead to the development of spontaneous seizures [11, 35]. Drugs with antiepileptogenic properties may inhibit the development of kindling. Some antiepileptogenic drugs might function to block spread of the synchronous neuronal discharge underlying seizure activity or prevent the formation of secondary foci. This model is confounded by the fact that during kindling development, it is the kindled seizures that induce the neuronal alterations underlying lowered afterdischarge thresholds. Therefore, drugs with anti-seizure effects might inhibit kindling development simply by preventing, or shortening, the expression of seizures, not by inhibiting the effects of seizures. In this sense, anti-seizure compounds might have disease-modifying effects by shortening seizure duration. However, this problem may be solved by continued evaluation of kindling inhibition after cessation of treatment with an AED. It has consistently been reported that AEDs which enhance GABAergic transmission delay development of kindling [36]. In contrast, most AEDs that block Na+ channels do not delay the development of kindling [37-40].

The main problem with kindling as a model of epileptogenesis is that kindled seizures must be induced. Since the emergence of spontaneous seizures following kindling is rare, it may be questioned if kindling produces a true epileptic state [41]. It is possible that the neuronal alterations produced by kindling, including cell loss and aberrant axonal sprouting, are relatively mild and may not be sufficient to mediate epileptogenesis [29-31]. Furthermore, it may be argued that the neuronal damage in the kindling model is the result of, and not the cause of, seizures.

Animal models for testing neuroprotective effects of AEDs

A wide range of brain insults, including SE, head trauma, and stroke, produce a pattern of brain damage. Different initial events may induce a similar sequence of events, including acute neuronal necrosis, followed by delayed glutamate release and excitotoxicity, which commonly results in the death of specific neuronal populations. Long-term alterations, evoked by activity-induced gene expression [42] or compensatory responses to cell damage and death, appears to produce changes in neuronal circuits [17]. It is likely that at least a subset of these alterations underlie the reduced seizure thresholds and expression of spontaneous seizures that define the epileptogenic disease state. For example, altered neuronal circuitry from axonal sprouting and aberrant excitatory synapse formation may produce hyperexcitable recurrent circuits [43]. Altered glial cell function observed following SE may disrupt extracellular K+ buffering contributing to neuronal hyperexcitability. The multistep process of epileptogenesis provides a number of sites for potential pharmacologic intervention. Drug screens may be designed to specifically target the discovery of agents that inhibit the initial damage produced by brain insults. Alternatively, antiepileptogenic drug screens may seek compounds that block excitotoxic cell death or other secondary damage. Other agents may prevent or reverse the compensatory alterations in neuronal circuits that contribute to lowered seizure thresholds.

Ischemia models

Screens for antiepileptogenic drugs may identify compounds that protect against altered neuronal circuits and...
neuronal damage. SE models can be used to test drugs for effects against SE-induced neuronal death, morphological alterations, altered excitability, and seizure expression. Temporary global ischemia in rodents produced by arterial occlusion or cardiac arrest is used as a model of stroke. Neuronal pathology following global ischemia has many similarities to damage following SE [44-47]. Both can lead to expression of spontaneous seizures [14]. The ability of drugs to block the ischemia-induced neuronal damage or the emergence of neurological deficits and seizures in SE models can be considered as a screen for antiepileptogenic drug properties. In that respect, tests of traditional AEDs in ischemia models has found that Na⁺ channel blockers (carbamazepine, phenytoin, lamotrigine) [48] and GABAergic transmission enhancers (clonazepam, tiagabine, topiramate, vigabatrin) [46, 49-51] reduce ischemic damage.

In addition to attenuating the initial alterations in neuronal circuits and brain damage preceding the first spontaneous seizures, antiepileptogenic drugs also might function after the epileptic state has been established to change the underlying disease state. Antiepileptogenic agents may alter neuronal circuits, making them less seizure-prone, and neuroprotective agents may reduce further seizure-induced damage. It remains to be determined to what extent these two approaches may alleviate the consequences of the epileptogenic process in man.

**Preclinical findings with levetiracetam (LEV)**

The novel AED LEV has interesting properties that may suggest both anti-seizure and antiepileptogenic properties. LEV differs from most AEDs in that it has no anti-seizure effect in the acute maximal chemoconvulsive or electroshock seizure tests [52, 53], but it markedly suppresses seizures in kindled and genetically epileptic animals [52-54]. The ability of LEV to delay the development of kindling [35] suggests that it has the potential to interfere with circuitry modifications underlying the progressive development of lowered seizure threshold. Of particular interest is the finding that, unlike any other currently available AED, LEV treatment results in a persistent suppression of afterdischarge duration in kindled brain, even after the termination of treatment. Further support for an antiepileptogenic potential of LEV derives from recent observations showing that LEV attenuates both hippocampal cell death and enhancement in hippocampal excitability following a pilocarpine-induced SE [55].

**Safety of LEV in animal models**

One of the promising features of LEV is a highly favorable safety profile in animal models. LEV elicits only mild sedation at doses more than 50 to 100 times higher than the anti-seizure dose [53]. LEV demonstrates low toxicity in rats and mice in an Irwin-type observation test, the rotarod test, and open-field exploration [52, 53, 56]. Thus, LEV induces only mild behavioral alterations in normal and amygdala kindled rats at anti-seizure doses [52, 53]. In coronally kindled mice, LEV had a high safety margin between rotarod impairment and seizure suppression [53]. Furthermore, at doses which produced seizure suppression, LEV did not alter cognitive performance of normal and amygdala kindled rats in the Morris water maze test [57]. Furthermore, at clinically relevant doses, LEV also did not affect induction of long-term potentiation in rat hippocampal slices, a model of memory [57].

**LEV mechanisms of action**

Although LEV’s mechanism of action is still not fully elucidated, it appears to differ from that of other known AEDs. LEV has a specific membrane binding site within the brain [58], but it does not directly affect glutamate — or GABA — receptor mediated synaptic transmission at therapeutically relevant concentrations [59, 60]. Furthermore, LEV does not alter Na⁺ channel current properties [61]. LEV produces a limited reduction in high-voltage-activated Ca²⁺ currents [62] but not low-voltage-activated calcium currents [61]. Although LEV has little direct effect on GABAA-receptor mediated currents, it opposes the action of negative modulators of GABA and glycine receptors [60]. Conflicting reports exist as to LEV’s ability to induce a modest inhibition of the delayed rectifier K⁺ current [63]. LEV’s antiepileptic action appears mediated through selective inhibition of neuronal burst firing and blocking synchronized firing of populations of neurons [10, 64]. Indeed, the ability of LEV to selectively suppress synchronized and burst firing interferes with spike propagation from the hippocampus to cortex [64] and may underlie both its unique anti-seizure and antiepileptogenic effects.

**Comparison of LEV to other AEDs**

LEV’s mechanism of action appears to be distinct from the other new AEDs (table 1), including topiramate, gabapentin, lamotrigine, and oxcarbazepine, which appear to directly affect neuronal excitability. Topiramate is principally a Na⁺ channel blocker that may also enhance GABA_A-receptor currents [65]. The mechanism of action of gabapentin is unclear but relates to reduction in L-type Ca²⁺ currents and increases in GABA levels [66]. Lamotrigine is also principally a Na⁺ channel blocker [67]. Oxcarbazepine is a Na⁺ channel blocker that also increases K⁺ conductance and modulates high-voltage-activated Ca²⁺ channels [38]. Some of these AEDs may possess antiepileptogenic properties. For example, topiramate suppresses kindling develop-
It acts primarily by blocking the spread of seizures. When administered after SE, topiramate attenuates seizure-induced hippocampal cell death [69]. Oxcarbazepine, however, prolongs afterdischarge duration during kindling induction and increases the rate of kindling development [37]. Lamotrigine has been reported to increase, decrease [70], or have no effect on kindling development [39, 40]. It is interesting that the AEDs that are Na+ channel blockers have primarily anti-seizure properties, while AEDs that modulate GABAergic transmission also appear to possess antiepileptogenic properties. Indeed, most AEDs that enhance GABAergic transmission have neuroprotective effects against SE-induced neuronal damage.

LEV pretreatment significantly reduced the infarct volume induced by transient cerebral artery occlusion [71]. Topiramate post-treatment has also been reported to protect against global ischemia-induced hippocampal cell death and motor impairment [45, 46] and to reduce the severity of seizures induced by ischemic insults [46]. Topiramate post-treatment has also reduced the hippocampal damage when administered 140 min after the onset of SE induced by unilateral hippocampal stimulation [69].

Gabapentin has been shown to reduce glutamate release in hippocampal models of ischemia but not in vivo [72]. Lamotrigine post-treatment has been shown to be neuroprotective both in focal and global ischemia models in rats and gerbils [44, 48, 73-75]. Furthermore, lamotrigine administration before or after electrical stimulation-induced SE protected against cell death in the hippocampus and piriform cortex, but did not alter subsequent memory impairments [76].

**Conclusions**

Traditional epilepsy treatment has focused on seizure suppression using anti-seizure drugs. With the understanding that epilepsy arises as a progressive change in neural circuits and frequently manifests as neuronal damage, it may be more appropriate to complement this treatment with antiepileptogenic and neuroprotective drugs. The molecular basis of epileptogenesis and ictogenesis have a very different neurobiologic basis and may therefore be addressed by different classes of drugs or drug actions. Thus, novel antiepileptogenic compounds may be found by using screens specifically designed to test for neuroprotection or the ability to alter the reorganization of neuronal circuits underlying the development of lowered seizure threshold. Such new drugs would be important as prophylactic antiepileptogenic drugs following head trauma, stroke, cerebral infection, and SE to prevent the potential development of spontaneous seizures. Importantly, continual antiepileptogenic and neuroprotective drug administration may be required, since molecular, cellular, and network reorganization continues after the diagnosis of epilepsy, particularly in patients who are not seizure-free. Reducing the ongoing circuitry reorganization in this difficult-to-treat subpopulation of patients may result in

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<td>LEV</td>
<td>Specifically reduces the N-type high-voltage-activated Ca2+ current</td>
<td>Increases afterdischarge threshold</td>
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<td></td>
<td>Opposes the action of negative modulators of GABA and glycine receptors</td>
<td>Decreases seizure severity</td>
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<td>Reduces seizure spread</td>
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<td>Topiramate</td>
<td>Na+ channel blocker, Enhances GABA&lt;sub&gt;A&lt;/sub&gt; receptor currents</td>
<td>Increases threshold for secondary generalized seizures</td>
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<td></td>
<td>Inhibits kainate and AMPA receptors</td>
<td>Suppresses kindling development</td>
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<td>Reduces the high voltage-activated Ca&lt;sup&gt;2+&lt;/sup&gt; current</td>
<td>Increases afterdischarge threshold</td>
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<td>Inhibits type II and IV carbonic anhydrase</td>
<td>Decreases seizure severity and duration</td>
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<td>Gabapentin</td>
<td>Increases GABA levels</td>
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<td>Suppresses completed kindled seizures</td>
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<td>Lamotrigine</td>
<td>Na+ channel blocker</td>
<td>Does not block (may facilitate) kindling development</td>
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<td>Reduces Ca&lt;sup&gt;2+&lt;/sup&gt; conductances involved in transmitter release</td>
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<td>Oxcarbazepine</td>
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<td>Modulation of high voltage-activated Ca&lt;sup&gt;2+&lt;/sup&gt; channels</td>
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less severe epilepsy expression. Neuroprotection may constitute a critical component of epileptogenesis alleviation, of neuronal loss after brain-damaging insults, and alleviation of the continuous remodeling of neuronal circuits in established epilepsies.

The novel AED LEV may be the first of a new class of drugs which meet these needs. Whether the permanent shortening of afterdischarge duration by LEV treatment during kindling development is associated with antiepileptogenesis in models in which the spontaneous seizure development is triggered by brain damage remains an intriguing hypothesis [35]. Furthermore, whether this reflects a significant disease-modifying effect (i.e., seizures will be shorter) remains to be confirmed in spontaneous seizure models. LEV also supports the notion that drugs which do not act directly to suppress neuronal excitability may have more favorable safety profiles. It is likely that the application of drug screens specifically testing for antiepileptogenesis may yield additional promising AEDs.

References


