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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 GABAergic activity

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[4, 5]. 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 stimuli usually takes a number of days or weeks and eventually reaches a plateau in which kindling stimuli evoke seizures and afterdischarges with reproducible be-

haviors and durations. 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 corneally 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 Ca^{2+} currents [62] but not low-voltage-activated calcium currents [61]. Although LEV has little direct effect on GABA-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 Ca^{2+} 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 Ca^{2+} channels [38].

Some of these AEDs may possess antiepileptogenic properties. For example, topiramate suppresses kindling devel-

Table 1. Mechanism of action and properties of levetiracetam (LEV) and other antiepileptic drugs (AEDs)

AED	Mechanism of action	Effects on kindling
LEV	Specifically reduces the N-type high-voltage-activated Ca^{2+} current Opposes the action of negative modulators of GABA and glycine receptors	Increases afterdischarge threshold Decreases seizure severity Reduces seizure spread Increases threshold for secondary generalized seizures Suppresses kindling development
Topiramate	Na^+ channel blocker, Enhances $GABA_A$ receptor currents Inhibits kainate and AMPA receptors Reduces the high voltage-activated Ca^{2+} current Inhibits type II and IV carbonic anhydrase	Suppresses kindling development Increases afterdischarge threshold Decreases seizure severity and duration Reduces seizure spread
Gabapentin	Increases GABA levels Reduces L-type Ca^{2+} currents	Increases afterdischarge threshold Decreases seizure severity Reduces seizure spread Increases threshold for secondary generalized seizures
Lamotrigine	Na^+ channel blocker Reduces Ca^{2+} conductances involved in transmitter release	Suppresses completed kindled seizures Blocks, has no effect, or facilitates kindling development Increases afterdischarge threshold Has no effect or decreases seizure severity and duration
Oxcarbazepine	Na^+ channel blocker Increases K^+ conductance Modulation of high voltage-activated Ca^{2+} channels Reduces Ca^{2+} conductances involved in transmitter release	Does not block (may facilitate) kindling development

opment [68]. 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 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

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

1. White HS, Johnson M, Wolf HH, Kupferberg HJ. The early identification of anti-seizure activity: role of the maximal electroshock and subcutaneous pentylenetetrazol seizure models. *Ital J Neurol Sci* 1995; 16: 73-7.

2. Dalby NO, Nielsen EB. Comparison of the preclinical anticonvulsant profiles of tiagabine, lamotrigine, gabapentin and vigabatrin. *Epilepsy Res* 1997; 28: 63-72.

3. Rho JM, Sankar R. The pharmacologic basis of antiepileptic drug action. *Epilepsia* 1999; 40: 1471-83.

4. White HS. Comparative anticonvulsant and mechanistic profile of the established and newer antiepileptic drugs. *Epilepsia* 1999; 40(Suppl 5): S2-10.

5. Czuczwar SJ, Patsalos PN. The new generation of GABA enhancers. Potential in the treatment of epilepsy. *CNS Drugs* 2001; 15: 339-50.

6. Tatum WO 4th, French JA, Faught E, *et al.* Post-marketing antiepileptic drug survey. Postmarketing experience with topiramate and cognition. *Epilepsia* 2001; 42: 1134-40.

7. Ketter TA, Post RM, Theodore WH. Positive and negative psychiatric effects of antiepileptic drugs in patients with seizure disorders. *Neurology* 1999; 53(5 Suppl 2): S53-67.

8. Drane DL, Meador KJ. Epilepsy, anticonvulsant drugs and cognition. *Baillieres Clin Neurol* 1996; 5: 877-85.

9. Matagne A, Klitgaard H. Validation of corneally kindled mice: a sensitive screening model for partial epilepsy in man. *Epilepsy Res* 1998; 31: 59-71.

10. Margineanu DG, Klitgaard H. Inhibition of neuronal hypersynchrony *in vitro* differentiates levetiracetam from classical antiepileptic drugs. *Pharmacol Res* 2000; 42: 281-5.

11. Temkin NR, Jarell AD, Anderson GD. Antiepileptogenic agents: how close are we? *Drugs* 2001; 61: 1045-55.

12. Cole AJ. Is epilepsy a progressive disease? The neurobiological consequences of epilepsy. *Epilepsia* 2000; 41(Suppl 2): S13-22.

13. Golarai G, Greenwood AC, Feeney DM, *et al.* Physiological and structural evidence for hippocampal involvement in persistent seizure susceptibility after traumatic brain injury. *J Neurosci* 2001; 21: 8523-37.

14. Romijn HJ, Voskuyl RA, Coenen AM. Hypoxic-ischemic encephalopathy sustained in early postnatal life may result in permanent epileptic activity and an altered cortical convulsive threshold in rat. *Epilepsy Res* 1994; 17: 31-42.

15. Yoshikawa T, Asano Y. Central nervous system complications in human herpesvirus-6 infection. *Brain Dev* 2000; 22: 307-14.

16. Garg RK. HIV infection and seizures. *Postgrad Med J* 1999; 75: 387-90.

17. Sloviter RS. Status epilepticus-induced neuronal injury and network reorganization. *Epilepsia* 1999; 40(Suppl 1): S34-9; discussion S40-1.

18. Santhakumar V, Ratzliff AD, Jeng J, *et al.* Long-term hyperexcitability in the hippocampus after experimental head trauma. *Ann Neurol* 2001; 50: 708-17.

19. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 1969; 25: 295-330.

20. DeLorenzo RJ, Garnett LK, Towne AR, *et al.* Comparison of status epilepticus with prolonged seizure episodes lasting from 10 to 29 minutes. *Epilepsia* 1999; 40: 164-9.

21. Covolan L, Mello LE. Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. *Epilepsy Res* 2000; 39: 133-52.

22. Turski WA, Cavalheiro EA, Schwarz M, *et al.* Limbic seizures produced by pilocarpine in rats: behavioural, electroencephalographic and neuropathological study. *Behav Brain Res* 1983; 9: 315-35.

23. Nadler JV. Minireview. Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sci* 1981; 29: 2031-42.

24. Nissinen J, Halonen T, Koivisto E, *et al.* A new model of chronic temporal lobe epilepsy induced by electrical stimulation of the amygdala in rat. *Epilepsy Res* 2001; 38: 177-205.

25. Albright PS, Burnham WM. Development of a new pharmacological seizure model: effects of anticonvulsants on cortical- and amygdala-kindled seizures in the rat. *Epilepsia* 1980; 21: 681-9.

26. McNamara JO. Kindling: an animal model of complex partial epilepsy. *Ann Neurol* 1984; 16(Suppl): S72-6.

27. Löscher, W, Jäckel R, Czuczwar SJ. Is amygdala kindling in rats a model for drug-resistant partial epilepsy? *Exp Neurol* 1986; 93: 211-26.

28. Sramka M, Sedlak P, Nadvornik P. Observation of kindling phenomenon in treatment of pain by stimulation in thalamus. In: Sweet WH. *Neurosurgical Treatment in Psychiatry, Pain, and Epilepsy*. Baltimore: University Park Press, 1977: 651-654.

29. Cavazos JE, Sutula TP. Progressive neuronal loss induced by kindling: a possible mechanism for mossy fiber synaptic reorganization and hippocampal sclerosis. *Brain Res* 1990; 527: 1-6.

30. Cavazos JE, Das I, Sutula TP. Neuronal loss induced in limbic pathways by kindling: evidence for induction of hippocampal sclerosis by repeated brief seizures. *J Neurosci* 1994; 14: 3106-21.

31. Cavazos JE, Golarai G, Sutula TP. Mossy fiber synaptic reorganization induced by kindling: time course of development, progression, and permanence. *J Neurosci* 1991; 11: 2795-803.

32. Sutula T, Lauersdorf S, Lynch M, *et al.* Deficits in radial arm maze performance in kindled rats: evidence for long-lasting memory dysfunction induced by repeated brief seizures. *J Neurosci* 1995; 15: 8295-301.

33. Gilbert TH, Hannesson DK, Corcoran ME. Hippocampal kindled seizures impair spatial cognition in the Morris water maze. *Epilepsy Res* 2000; 38: 115-25.

34. Hannesson DK, Howland J, Pollock M, *et al.* Dorsal hippocampal kindling produces a selective and enduring disruption of hippocampally mediated behavior. *J Neurosci* 2001; 21: 4443-50.

35. Löscher W, Honack D, Rundfeldt C. Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. *J Pharmacol Exp Ther* 1998; 284: 474-9.

36. Morimoto K, Sato H, Yamamoto Y, *et al.* Antiepileptic effects of tiagabine, a selective GABA uptake inhibitor, in the rat kindling model of temporal lobe epilepsy. *Epilepsia* 1997; 38: 966-74.

37. Schmutz M, Klebs K, Baltzer V. Inhibition or enhancement of kindling evolution by antiepileptics. *J Neural Transm* 1988; 72: 245-57.

38. Schmutz M, Brugger F, Gentsch C, *et al.* Oxcarbazepine: preclinical anticonvulsant profile and putative mechanisms of action. *Epilepsia* 1994; 35(Suppl 5): S47-50.

39. O'Donnell RA, Miller AA. The effect of lamotrigine upon development of cortical kindled seizures in the rat. *Neuropharmacology* 1991; 30: 253-8.

40. Postma T, Krupp E, Li XL, *et al.* Lamotrigine treatment during amygdala-kindled seizure development fails to inhibit seizures and diminishes subsequent anticonvulsant efficacy. *Epilepsia* 2000; 41: 1514-21.

41. Michalakis M, Holsinger D, Ikeda-Douglas C, *et al.* Development of spontaneous seizures over extended electrical kindling. I. Electrographic, behavioral, and transfer kindling correlates. *Brain Res* 1998; 793: 197-211.

42. Nedivi E, Hevroni D, Naot D. Numerous candidate plasticity-related genes revealed by differential cDNA cloning. *Nature* 1993; 63: 718-22.

43. Sutula T, He XX, Cavazos J, *et al.* Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science* 1988; 239: 1147-50.

44. Crumrine RC, Bergstrand K, Cooper AT, *et al.* Lamotrigine protects hippocampal CA1 neurons from ischemic damage after cardiac arrest. *Stroke* 1997; 28: 2230-6; discussion 2237.

45. Lee SR, Kim SP, Kim JE. Protective effect of topiramate against hippocampal neuronal damage after global ischemia in the gerbils. *Neurosci Lett* 2000; 281: 183-6.

46. Edmonds HL Jr, Jiang YD, Zhang PY, *et al.* Topiramate as a neuroprotectant in a rat model of global ischemia-induced neurodegeneration. *Life Sci* 2001; 69: 2265-77.

47. Traystman RJ, Klaus JA, DeVries AC, *et al.* Anticonvulsant lamotrigine administered on reperfusion fails to improve experimental stroke outcomes. Stroke 2001; 32: 783-7.

48. Rataud J, Debarnot F, Mary V, *et al.* Comparative study of voltage-sensitive sodium channel blockers in focal ischaemia and electric convulsions in rodents. *Neurosci Lett* 1994; 172: 19-23.

49. Marciani MG, Santone G, Sancesario G, *et al.* Protective effect of clonazepam on ischemic brain damage induced by 10-minute bilateral carotid occlusion in Mongolian gerbils. *Funct Neurol* 1993; 8: 115-20.

50. Chen Xu W, Yi Y, Qiu L, *et al.* Neuroprotective activity of tiagabine in a focal embolic model of cerebral ischemia. *Brain Res* 2000; 874: 75-7.

51. Shuaib A, Murabit MA, Kanthan R, *et al.* The neuroprotective effects of gamma-vinyl GABA in transient global ischemia: a morphological study with early and delayed evaluations. *Neurosci Lett* 1996; 204: 1-4.

52. Löscher W, Honack D. Profile of ucb L059, a novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. *Eur J Pharmacol* 1993; 232: 147-58.

53. Klitgaard H, Matagne A, Gobert J, *et al.* Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy. *Eur J Pharmacol* 1998; 353: 191-206.

54. Gower AJ, Hirsch E, Boehrer A, *et al.* Effects of levetiracetam, a novel antiepileptic drug, on convulsant activity in two genetic rat models of epilepsy. *Epilepsy Res* 1995; 22: 207-13.

55. Klitgaard HV, Matagne AC, Vanneste – Goemaere J, *et al.* Effects of prolonged administration of levetiracetam on pilocarpine-induced epileptogenesis in rat. *Epilepsia* 2001; 42 (Suppl 7): 114-5.

56. Gower AJ, Noyer M, Verloes R, *et al.* ucb L059, a novel anti-convulsant drug: pharmacological profile in animals. *Eur J Pharmacol* 1992; 222: 193-203. (erratum *Eur J Pharmacol* 1993; 230: 389).

57. Lamberty Y, Margineanu DG, Klitgaard H. Absence of negative impact of levetiracetam on cognitive function and memory in normal and amygdala-kindled rats. *Epilepsy Behav* 2000; 1: 333-42.

58. Noyer M, Gillard M, Matagne A, *et al.* The novel antiepileptic drug levetiracetam (ucb L059) appears to act via a specific binding site in CNS membranes. *Eur J Pharmacol* 1995; 286: 137-46.

59. Birnstiel S, Wülfert E, Beck SG. Levetiracetam (ucb LO59) affects *in vitro* models of epilepsy in CA3 pyramidal neurons without altering normal synaptic transmission. *Naunyn Schmiedebergs Arch Pharmacol* 1997; 356: 611-8.

60. Rigo JM, Hans G, Nguyen L, *et al.* The anti-epilectic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal GABA- and glycine-gated current. *Br J Pharmacol* 2002; 136: 659-72.

61. Zona C, Niespodziany I, Marchetti C, *et al.* Levetiracetam does not modulate neuronal voltage-gated Na^+ and T-type Ca^{2+}currents. *Seizure* 2001; 10: 279-86.

62. Niespodziany I, Klitgaard H, Margineanu D-G. Levetiracetam: inhibits the high-voltage activated Ca^{2+} current in pyramidal neurons of rat hippocampal slices. *Neurosci Lett* 2001; 306: 5-8.

63. Bischoff U, Schlobohm I. Levetiracetam had no effect on voltage-gated potassium currents in cultured mouse hippocampal neurons. *Epilepsia* 2002; 43(Suppl 8): 88.

64. Klitgaard H, Matagne A, Grimee R, Vanneste-Goemaere J, Margineanu DG. Electrophysiological, neurochemical and regional effects of levetiracetam in the rat pilocarpine model of temporal epilepsy. *Seizure* 2003;12: 92-100.

65. White HS, Brown SD, Woodhead JH, *et al.* Topiramate enhances GABA-mediated chloride flux and GABA-evoked chloride currents in murine brain neurons and increases seizure threshold. *Epilepsy Res* 1997; 28: 167-79.

66. Ng GY, Bertrand S, Sullivan R, *et al.* Gamma-aminobutyric acid type B receptors with specific heterodimer composition and postsynaptic actions in hippocampal neurons are targets of anticonvulsant gabapentin action. *Mol Pharmacol* 2001; 59: 144-52.

67. Cheung H, Kamp D, Harris E. An *in vitro* investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. *Epilepsy Res* 1992; 13: 107-12.

68. Amano K, Hamada K, Yagi K, *et al.* Antiepileptic effects of topiramate on amygdaloid kindling in rats. *Epilepsy Res* 1998; 31: 123-8.

69. Niebauer M, Gruenthal M. Topiramate reduces neuronal injury after experimental status epilepticus. *Brain Res* 1999; 837: 263-9.

70. Stratton SC, Large CH, Cox B, *et al.* Effects of lamotrigine and levetiracetam on seizure development in a rat amygdala kindling model. *Epilepsy Res* 2003; 53: 95-106.

71. Hanon, E, Klitgaard H. Neuroprotective properties of the novel antiepileptic drug levetiracetam in the rat middle cerebral artery occlusion model of focal cerebral ischemia. *Seizure* 2001; 10: 287-93.

72. Jehle T, Lagreze WA, Blauth E, *et al.* Gabapentin-lactam (8-aza-spiro [5,4] decan-9-on; GBP-L) inhibits oxygen glucose deprivation-induced [3H] glutamate release and is a neuroprotective agent in a model of acute retinal ischemia. *Naunyn Schmiedebergs Arch Pharmacol* 2000; 362: 74-81.

73. Shuaib A, Mahmood RH, Wishart T, *et al.* Neuroprotective effects of lamotrigine in global ischemia in gerbils. A histological, *in vivo* microdialysis and behavioral study. *Brain Res* 1995; 702: 199-206.

74. Wiard RP, Dickerson MC, Beek O, *et al.* Neuroprotective properties of the novel antiepileptic lamotrigine in a gerbil model of global cerebral ischemia. *Stroke* 1995; 26: 466-72.

75. Smith SE, Meldrum BS. Cerebroprotective effect of lamotrigine after focal ischemia in rats. *Stroke* 1995; 26: 117-21.

76. Halonen T, Nissinen J, Pitkanen A. Effect of lamotrigine treatment on status epilepticus-induced neuronal damage and memory impairment in rat. *Epilepsy Res* 2001; 46: 205-23.