Is levetiracetam different from other antiepileptic drugs? Levetiracetam and its cellular mechanism of action in epilepsy revisited

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Abstract: Levetiracetam (LEV) is a new antiepileptic drug that is clinically effective in generalized and partial epilepsy syndromes as sole or add-on medication. Nevertheless, its underlying mechanism of action is poorly understood. It has a unique preclinical profile; unlike other antiepileptic drugs (AEDs), it modulates seizure-activity in animal models of chronic epilepsy with no effect in most animal models of acute seizures. Yet it is effective in acute *in-vitro* 'seizure' models. A possible explanation for these dichotomous findings is that LEV has different mechanisms of actions, whether given acutely or chronically and in 'epileptic' and control tissue. Here we review the general mechanism of action of AEDs, give an updated and critical overview about the experimental findings of LEV's cellular targets (in particular the synaptic vesicular protein SV2A) and ask whether LEV represents a new class of AED.

Keywords: levetiracetam, SV2A, antiepileptic drugs, synaptic transmission, epilepsy, ion channels

Introduction

Antiepileptic drug (AED) development has mainly taken place through trial and error. AEDs have been screened in animal models of seizures and epilepsy, often with an incomplete knowledge of their mechanism of action [Walker *et al.* 2004]. Indeed, the identification of drugs acting at putative 'antiepileptic' targets has rarely translated into successful AED therapies, because the drugs are often poorly tolerated or have poor efficacy. Moreover, AEDs that were designed to act at specific targets (e.g., gabapentin, lamotrigine) work via different mechanisms. Consequently, the underlying mechanism of action of an individual drug may only become apparent after its widespread clinical use. However, growing evidence suggests that many of the drugs that we use fall into one or more specific mechanistic groups – drugs that act at sodium channels, calcium channels or the GABAergic system [Walker and Fisher, 2004]. Other putative and potential targets include potassium channels, hyperpolarization-activated cation channels, and glutamate receptors. Here we briefly review the mechanism of action of

AEDs and ask whether levetiracetam (LEV) represents a new class of AED.

Main targets for AED

Sodium channels

Sodium channels provide the major target for a number of AEDs including phenytoin, carbamazepine, oxcarbazepine, and lamotrigine. Voltage-gated sodium channels are critical for action potential (AP) generation and propagation [Catterall, 2000a]. The sodium channel exists in three principal conformational states: at hyperpolarized potentials the channel is in the resting closed state; with depolarization the channel opens and permits the conduction of sodium ions; the channel then enters a nonconducting, inactivated state. This inactivation is removed (termed deinactivation) by hyperpolarization. In this manner, depolarization results in a transient inward sodium current that rapidly inactivates. In addition to these three states, there is also a slow inactivated state, which occurs with sustained or repeated depolarizations. This state

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is selectively enhanced by the new AED, lacosamide [Errington *et al.* 2008].

Phenytoin, lamotrigine, oxcarbazepine, and carbamazepine bind to and stabilize the inactivated state of the sodium channel [Kuo, 1998]. This has two effects: a greater proportion of channels are inactive at hyperpolarized membrane potentials, and second there is a delay in deinactivation. The effect on the excitability of neurons is 2-fold. The rate at which an axon can 'fire' is critically determined by the rate at which the sodium channels deinactivate. If this time is increased, then the 'refractory period' is prolonged, inhibiting sustained repetitive firing [McLean and Macdonald, 1983]. In addition, since these drugs bind to channels in their inactive state, then the greater the number of channels that have entered this state, the greater the drug binding. This results in a 'use dependent' phenomenon in which repetitive firing results in greater amounts of the drug bound and so greater inhibition. In addition, these drugs inhibit the persistent sodium current, which mediates long-lasting depolarizations [Lampl *et al.* 1998]. Other AEDs such as valproate, topiramate, and zonisamide may also have similar effects on sodium channels, but have been less well characterized.

Calcium channels

Calcium channels are also putative targets for AEDs, as they regulate not only neuronal excitability but also neurotransmitter release [Catterall, 2000b]. The voltage-gated calcium channels expressed in the brain can be subdivided into four main classes, L-, P/Q-, N-, and T-type channels. L-, P/Q-, and N-type channels are high-voltage activated (HVA) channels that require significant depolarization to open, while the T-type channel is a low-voltage activated (LVA) channel and is opened by relatively small depolarizations.

The L-type channels are mainly expressed postsynaptically. L-type channels are slowly inactivated thereby permitting sustained calcium entry following a depolarization. Calcium entering through L-type calcium channels may play a role in activity-dependent gene expression and synaptic plasticity. Some AEDs (such as carbamazepine) have been proposed to antagonise L-type calcium channels but the relevance of this to their antiepileptic effect is unclear [Ambrosio *et al.* 1999].

N- and P/Q-type channels are expressed at synaptic boutons where they mediate calcium entry necessary for neurotransmitter release. These channels rapidly inactivate, resulting in brief calcium transients. This calcium entry then triggers exocytosis of presynaptic vesicles. N- and P/Q-type calcium channels can be modulated by G-protein linked receptors such as GABA_B receptors. Inhibition of these channels would be expected to decrease neurotransmitter release. Gabapentin's and pregabalin's effect on HVA calcium channels is complex and novel; they both show strong and specific binding for the $\alpha 2\delta$ auxiliary calcium channel subunit and may modulate P/Q-type calcium channels [Dooley *et al.* 2007].

T-type calcium channels are activated at relatively hyperpolarized potentials. They open with small depolarization and then rapidly inactivate. They have been proposed to contribute to the generation of physiological rhythms within the thalamus, and have been implicated in the generation of spike-wave discharges associated with absence epilepsy [McCormick and Contreras, 2001]. There is evidence that ethosuximide mediates its effect through binding to and stabilizing the inactivated state of the T-type calcium channel [Gomora *et al.* 2001]. Other drugs such as zonisamide and valproate have also been suggested to act at this channel [Todorovic and Lingle, 1998; Suzuki *et al.* 1992].

GABAergic system

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the brain. It is formed and degraded in the GABA shunt. Glutamic acid decarboxylase (GAD) converts glutamate to GABA. Promotion of GABA synthesis has been proposed to contribute to the action of some AEDs including valproate [Löscher, 1989].

GABA is released into the synaptic space where it acts on two receptor types: ionotropic GABA_A and metabotropic GABA_B receptors (a third type, termed GABA_C receptors, is present predominantly in the retina) [Bormann, 2000]. Benzodiazepines act at specific GABA_A receptor subtypes [Mehta and Ticku, 1999], increasing the affinity of GABA_A receptors for GABA, and the probability of receptor opening. Topiramate also potentiates GABA_A receptor currents in a subunit specific manner [Simeone *et al.* 2006]. Barbiturates are less selective for GABA_A receptor subtypes, and prolong receptor

opening times. Drugs that act at GABA_B receptors have been less useful as AEDs, probably because GABA_B receptors have a complex function acting postsynaptically to decrease neuronal excitability but also presynaptically decreasing GABA release.

GABA is taken up by glial and neuronal GABA transporters, inhibition of which is another AED target (tiagabine) [Rekling *et al.* 1990]. Inside the cell, GABA is degraded by GABA transaminase to succinic semialdehyde, and inhibition of this enzyme by the AED vigabatrin increases GABAergic transmission [Gale and Iadarola, 1980].

Other targets

Potassium channels

Potassium channels form one of the most diverse groups of ion channels and have a critical role in determining neuronal excitability [Jan and Jan, 1997]. Persistent potassium currents play a crucial part in determining the resting membrane potential of neurons. Voltage-gated potassium channels can influence the resting membrane potential but also repolarize neurons following AP, thereby influencing neurotransmitter release. In addition, the rate of repolarization by potassium channels, affects the ability of a neuron to sustain rapid repetitive firing. Voltage-gated potassium channels in the brain can be subdivided into: channels that rapidly activate and inactivate (A-type channels), channels that open upon depolarization but do not significantly inactivate (delayed rectifier channels) and channels that close upon depolarization but are open at the resting potential (inward rectifying channels). There are other potassium channels that are similar in structure to the voltage-gated potassium channel, but are opened by intracellular calcium (calcium-activated potassium channels that mediate the afterhyperpolarization) or by cyclic nucleotides (mainly present in the retina where they mediate photoreceptor responses). There are also specific potassium channels that are inactivated by acetylcholine – termed M-type channels. Although, modulation of potassium channels would seem to be an ideal target for AEDs, most drugs have no or poorly characterized effects on potassium channels. However, phenytoin blocks the delayed rectifier potassium channels in neuroblastoma cells and retigabine, a putative AED, has as its main mode of action

potentiation of potassium M-type channels [Wuttke *et al.* 2005; Tatulian *et al.* 2001; Nobile and Lagostena, 1998].

HCN channels

HCN channels are permeable to both potassium and sodium and mediate a current termed the H-current. These channels are activated at hyperpolarised potentials and deactivated at depolarized potentials. H-currents depolarize neurons from the resting membrane potential and have an important role in potentiating and maintaining oscillations [Robinson and Siegelbaum, 2003]. They may play a part in terminating thalamic oscillations and the generation of spike-wave discharges of absence epilepsy. The H-current is also highly expressed in dendrites where it shunts excitatory inputs. Lamotrigine has been shown to enhance the H-current in dendrites [Poolos *et al.* 2002]. Likewise, gabapentin has been demonstrated to increase the H-current in pyramidal neurons [Surges *et al.* 2003]. This may have two potentially antiepileptic effects: in the hippocampus it would inhibit excitatory transmission to the soma, explaining the efficacy of lamotrigine and gabapentin in partial epilepsy. In the thalamus, it may inhibit or terminate spike-wave discharges and, therefore, could explain the efficacy of lamotrigine against absence seizures.

Glutamate and glutamate receptors

Glutamate is the major excitatory transmitter in the central nervous system and acts at distinct receptor types: N-methyl-D-aspartate (NMDA), nonNMDA [consisting of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) sensitive receptors] and metabotropic glutamate receptors. Inhibition of these receptors would seem to be an ideal target for AEDs, but such compounds have been associated with unacceptable side-effects. NMDA receptors influence memory, cognition, and learning and NMDA receptor antagonists have had unacceptable side effects in clinical use. Felbamate and remacemide, however, may modulate NMDA receptor-mediated transmission [Subramaniam *et al.* 1996; White *et al.* 1995].

Topiramate at high concentrations acts at AMPA/kainate receptors; whether this is responsible for its antiepileptic effect or dose-related side effects is unknown [Angehagen *et al.* 2004]. Low doses of phenobarbitone have been shown to block

AMPA receptors in the cerebral cortex [Sawada and Yamamoto, 1985], but the significance of this finding and its overall contribution towards the antiepileptic effects of phenobarbitone remains to be established. There are other drugs in clinical trials such as talampanel that are AMPA receptor antagonists.

Levetiracetam

LEV is a water soluble pyrrolidone derivative ((S)- α -ethyl-2-oxo-pyrrolidine acetamide), whose chemical structure differs from other AEDs. Since its approval for clinical use in 2002, LEV has become a widely used AED that is effective in partial and generalized epilepsy syndromes as sole or add-on medication [De Smedt *et al.* 2007]. Usual antiepileptic plasma concentrations range from trough levels between 35 and 100 μ M (5.95–17 μ g/ml) to peak levels between 90 and 250 μ M (15.3–42.5 μ g/ml) [Rigo *et al.* 2002; Patsalos, 2000]. Importantly, serum levels of LEV are very similar to corresponding LEV levels found in the brain tissue of individual patients [Rambeck *et al.* 2006]. Unlike other AEDs, LEV is probably not a substrate for multi-drug transporters [Potschka *et al.* 2004].

Except for rare instances of the treatment of acute seizures, AED therapy involves a regular daily, therefore chronic, intake of medication. Therefore, acute *in-vitro* and *in-vivo* experimental paradigms do not necessarily reflect the clinical use of an AED. Moreover, there are various epilepsy-associated modifications of brain physiology, and therefore models of acute seizures differ from models of chronic epilepsy. Intriguingly, LEV modulates seizure activity in animal models of chronic epilepsy (kindling models, pilocarpine model, genetic absence epilepsy rats from Strasbourg GAERS) with no effect in most models of acute seizures [Glien *et al.* 2002; Klitgaard *et al.* 1998; Löscher and Hönack, 1993]. This is consistent with the experimental observations that LEV only affects GABA_A receptors from epileptic tissue or under conditions that occur during epilepsy, whereas it has no effect on GABA_A receptors from controls [Palma *et al.* 2007; Rigo *et al.* 2002]. Taken together, these data suggest that LEV may preferentially work with chronic application or under chronic epilepsy-associated conditions. However, most of the experiments to investigate LEV's cellular mechanism of action have been performed by acute application, reporting its acute

cellular effects. A further consideration is that LEV is now being proposed as an acute treatment for seizures. An intravenous formulation is available [Ramael *et al.* 2006] that has already been shown to terminate status epilepticus after acute intravenous application [Knake *et al.* 2008]. Interestingly, LEV is effective in one model of acute epilepsy (6 Hz psychomotor seizure model) [Shannon *et al.* 2005; Barton *et al.* 2001] with the maximal effect occurring 1 h after injection [Barton *et al.* 2001]. These findings suggest that LEV can have a rapid-onset effect in some acute seizure models. One possible explanation for these dichotomous findings in animal models of acute and chronic epilepsy is that LEV may have different mechanisms of action whether given acutely or chronically and in epileptic and control tissue (see subsequently).

Action of LEV on voltage-gated ion channels and regulation of intracellular ions

Neuronal excitability and firing behavior are crucially shaped by voltage-gated ion channels. Acute application of LEV at a relatively low concentration (10 μ M) did not alter neuronal properties such as membrane potential, input resistance, AP amplitude, AP duration, or fast and slow afterhyperpolarization of CA3 pyramidal cells [Birnstiel *et al.* 1997]. However, at higher concentrations (similar to those used in clinical practice) there is substantial experimental evidence that acutely applied LEV (and prolonged application for up to 1 h) modulates cellular targets that are important for neuronal excitability and synaptic transmission (cf. Tables 1 and 2).

Voltage-gated ion channels

HVA Ca²⁺ currents in different cell preparations (acutely isolated striatal, neocortical, and hippocampal CA1 neurons, CA1 pyramidal neurons in slices) were inhibited by an average of 18–40% when LEV was acutely applied at different concentrations (1–300 μ M) [Costa *et al.* 2006; Pisani *et al.* 2004; Lukyanetz *et al.* 2002; Niespodziany *et al.* 2001]. Pharmacological separation of different HVA Ca²⁺ channel subtypes revealed that mainly N-type, and to a lesser extent P/Q-type calcium channels were affected [Costa *et al.* 2006; Pisani *et al.* 2004; Lukyanetz *et al.* 2002]. Changes in steady-state activation or inactivation properties were not observed [Lukyanetz *et al.* 2002]. In contrast, LEV did not modulate amplitudes, steady-state activation/inactivation properties or kinetics of T-type Ca²⁺ currents

Table 1. LEV effects on voltage-gated ion currents.

	Effect	Concentration	Application time	Tissue	Reference
Na⁺ currents					
Fast Na ⁺ currents	No effect	10 μ M to 1 mM	Acute up to 10 min	Cultured neocortical neurons	Zona <i>et al.</i> 2001
	No effect	100 μ M	Acute	Acutely isolated CA1 neurons	Madeja <i>et al.</i> 2003
	No effect	1–500 μ M	Probably acute	Acutely isolated striatal neurons	Costa <i>et al.</i> 2006
Persistent Na ⁺ currents	No effect	10–100 μ M	Acute for up to 1 h	CA1 neurons in slices	Niespodziany <i>et al.</i> 2004
Ca²⁺ currents					
HVA	Inhibition by ~30%	32 μ M	Acute up to 30 min	CA1 pyramidal neurons in slices	Niespodziany <i>et al.</i> 2001
	Inhibition by ~18% via N-type Ca ²⁺ channel blockade	200 μ M	Acute	Acutely isolated hippocampal CA1 neurons	Lukyanetz <i>et al.</i> 2002
	Inhibition by 35%, mainly via blockade of N-type and to a lower extent of P/Q-type Ca ²⁺ channels	100 μ M	Acute	Acutely isolated neocortical neurons	Pisani <i>et al.</i> 2004
	Inhibition by 40% (30% N-type, 10% P-type)	Half-maximal inhibition at 22 μ M	Probably acute	Acutely isolated striatal neurons	Costa <i>et al.</i> 2006
LVA	No effect on T-type Ca ²⁺ currents	32–100 μ M	Acute for up to 40 min	CA1 pyramidal neurons in slices	Zona <i>et al.</i> 2001
K⁺ currents					
Delayed rectifier K ⁺ current	Inhibition by ~30%	100 μ M	Acute	Acutely isolated CA1 neurons	Madeja <i>et al.</i> 2003
A-type K ⁺ current	No effect	100 μ M	Acute	Acutely isolated CA1 neurons	Madeja <i>et al.</i> 2003

[Zona *et al.* 2001]. A reduction of N-type and P/Q-type calcium currents can lead to a decrease in presynaptic Ca²⁺-dependent processes involved in neurotransmitter release. These channels have been implicated in both ictogenesis and also epileptogenesis.

Delayed rectifier K⁺ currents were decreased by about 30% when LEV was acutely applied to CA1 neurons, whereas A-type K⁺ currents were unaffected [Madeja *et al.* 2003]. The reduction of delayed rectifier K⁺ currents led to a reduction in repetitive AP generation and a slight prolongation of AP duration. The relevance of this to its antiepileptic effect remains unknown, but may decrease neuronal firing.

Unlike some classical AEDs, acute and prolonged application (for up to 1 h) of LEV at different concentrations (ranging from 1 μ M–1 mM) in different cell preparations (acutely isolated striatal and hippocampal CA1 neurons, cultured neocortical neurons) had no effect on either

the amplitudes of fast Na⁺ currents [Costa *et al.* 2006; Madeja *et al.* 2003; Zona *et al.* 2001] or on steady-state activation and inactivation properties or current kinetics [Zona *et al.* 2001]. Likewise, persistent Na⁺ currents were not affected [Niespodziany *et al.* 2004]. However, activity-dependent inhibition (use-dependence) of Na⁺ currents has, to our knowledge, not been investigated, but given the above findings it is unlikely that LEV would have an effect.

Intraneuronal Ca²⁺ stores and Cl[−]/HCO₃[−] exchanger

Intriguingly, other cellular targets for LEV that are involved in the regulation of neuronal excitability have recently been identified, including intraneuronal calcium stores. These play an important role in the regulation of neuronal excitability, neurotransmission and synaptic plasticity as well as disease-related processes such as epileptogenesis and seizure-like activity [Bardo *et al.* 2006; Pal *et al.* 2001]. In most neurons Ca²⁺-release from these stores is mainly

Table 2. LEV effects on ligand-gated ion currents and other targets.

	Effect	Concentration	Application time	Tissue	Reference
AMPA receptors	Inhibition by 10–25%	200 μ M	Acute	Cultured cortical neurons	Carunchio <i>et al.</i> 2007
GABA_A receptors	Complete reversal of zinc-induced inhibition	30 μ M	Acute	Cultured hippocampal neurons	Rigo <i>et al.</i> 2002
	Alleviation of run-down upon repetitive activation	0.5–100 μ M	Incubation for 3 h	Membrane preparations transplanted into frog oocytes	Palma <i>et al.</i> 2007
Glycine receptors	Complete reversal of zinc-induced inhibition	Half-maximal effect at 0.04 μ M	Acute	Cultured hippocampal neurons	Rigo <i>et al.</i> 2002
Ca²⁺ stores					
Ryanodine-regulated Ca ²⁺ release	Inhibition by ~50%	32 μ M	After 5 min	Cultured hippocampal neurons	Angehagen <i>et al.</i> 2003
IP3-regulated Ca ²⁺ release	Inhibition by 25–50%	10 μ M	After 5 min	PC12 cells (rat pheochromocytoma cells)	Cataldi <i>et al.</i> 2005
Cl⁻/HCO₃⁻ exchanger	Inhibition	10–50 μ M	Up to 20 min	CA3 neurons in slices	Leniger <i>et al.</i> 2004

regulated by inositol (1,4,5)-triphosphate (IP3) and ryanodine receptors with varying relative contributions of each. Activation of ryanodine receptors by caffeine in mixed hippocampal cell cultures led to a transient increase of intracellular Ca²⁺ as well as to spontaneous bursts in hippocampal slices. These Ca²⁺ transients were reduced by almost 50% after 5 min incubation with LEV and occurrence of spontaneous bursts in slices was delayed by LEV [Angehagen *et al.* 2003]. Furthermore, Gq-protein coupled IP3-dependent Ca²⁺ release in rat PC12 pheochromocytoma cells was inhibited by about 25–50% after 5 min incubation with LEV [Cataldi *et al.* 2005]. Thus, inhibition of Ca²⁺ release from intraneuronal Ca²⁺ stores induced by LEV may contribute to its antiepileptic effects.

The function of many proteins is pH dependent. Changes in intraneuronal pH modulate neuronal activity and intraneuronal acidification reduces seizure-like activity in *in-vitro* preparations [Bonnet *et al.* 2000]. In hippocampal slices acutely applied LEV for up to 20 min acidified the internal pH of CA3 neurons [Leniger *et al.* 2004]. Moreover, LEV administration decreased the frequency of spontaneous AP and bursts induced by 4-aminopyridine. Both effects were reversible upon washout and the latter could be reversed by application of a membrane-permeable base. Further analysis revealed that the acidification in the presence of LEV was probably linked to the blockade of the Na⁺-dependent

Cl⁻/HCO₃⁻ exchanger. This exchanger participates in the extrusion of intracellular acid, thus a LEV-induced blockade was proposed to acidify the intracellular milieu, thereby reducing seizure-like activity [Leniger *et al.* 2004].

Action of LEV on synaptic transmission

AEDs can act at pre- or post-synaptic sites to modulate synaptic transmission. Evoked presynaptic release of neurotransmitters is triggered by an AP-induced calcium influx via P/Q-, N-, and R-type calcium channels. We have already described the effect of LEV on presynaptic calcium channels. In the following paragraphs we will further discuss LEV action on synaptic transmission including its interaction with presynaptic vesicular proteins and modulation of post-synaptic ligand-gated receptors.

Synaptic vesicle binding site for LEV

Lynch *et al.* (2004) showed that LEV specifically binds to the synaptic vesicle protein SV2A, whereas it does not bind to its two isoforms SV2B or SV2C. Moreover it was demonstrated that SV2A binding affinity of different LEV derivatives positively correlated with their antiepileptic potency in different animal models of epilepsy [Kaminski *et al.* 2008; Lynch 2004]. These findings strongly suggest that LEV binding to SV2A is involved in its antiepileptic effect. Indeed, SV2A knock-out (KO) mice strains display a severe seizure

phenotype after the first postnatal week [Crowder *et al.* 1999; Janz *et al.* 1999] indicating that SV2A may normally regulate signaling cascades involved in seizure generation. Unfortunately no data on the actual CSF levels of different LEV derivatives were provided in the above correlation studies. Thus, it is still possible that the potency of different LEV derivatives in seizure-prevention was related to different CSF levels rather than to different binding affinities to SV2A. It is also unclear how these LEV derivatives act on voltage- or ligand-gated ion channels involved in synaptic transmission.

SV2 is a major vesicular protein that contains 12 putative transmembrane regions and resembles membrane transporters. [Janz *et al.* 1999]. Unfortunately to date, there are no available reports on the specific binding site for LEV on the SV2A molecule. Also the functions of SV2 proteins in synaptic transmission are still debated. Electrophysiological recordings in cultured autaptic hippocampal neurons (which form synapses with themselves) from single and double SV2A/SV2B KO mice suggest several, somewhat disparate, possible mechanisms by which SV2 may regulate vesicular exocytosis. At high extracellular calcium concentrations (i.e., when the initial release probability is high) neurons from the SV2A/SV2B double KO exhibited no change in the initial probability of release but revealed a sustained increase in the AP-evoked synaptic transmission during trains of AP [Janz *et al.* 1999]. Importantly the increase could be partially reversed by loading presynaptic terminals with slow calcium buffer EGTA. These results prompted two alternative hypotheses: (i) the function of SV2s is to regulate presynaptic calcium levels during repetitive activity or (ii) SV2s function as targets for residual calcium in regulating vesicular exocytosis. In contrast, when synaptic transmission was assessed at physiological extracellular calcium concentrations, loss of SV2A, and SV2B led to reduced initial release probability but had no effect on steady-state responses during the trains of AP [Custer, 2006]. These results suggest that SV2A may regulate priming of docked synaptic vesicles, a process that makes them ready for exocytosis, and thus selectively enhancing low frequency neurotransmission.

Overall, although SV2A was identified as a specific binding site for LEV, a direct demonstration of SV2A mediating the antiepileptic effect of

LEV via a change in synaptic transmission is still lacking.

Synaptic transmission in-vivo

In-vivo studies are an important tool to judge the overall efficacy of AEDs in native environment of preserved neuronal circuitry. However, very often it is not possible to precisely control the AED concentrations during an *in-vivo* experiment. This in turn makes difficult dissecting specific AED actions on different signaling pathways. The effects of acute and chronic LEV application on neurochemical parameters in the mouse brain were examined after single or repeated intraperitoneal LEV injections [Sills *et al.* 1997]. No effect of LEV on whole brain preparations was detected either on the overall GABA and glutamate concentrations or on the overall activities of GABA transaminase and GAD. In contrast, when studied *in-vivo* on a regional basis, acute LEV application had different effects within different brain regions with the most pronounced (and opposite) effects on GABA turnover in the cortex and striatum [Löscher *et al.* 1996]. The implications for this finding are uncertain but suggest that LEV may operate through different mechanisms in different brain areas.

Acute systemic application of LEV had no effect on evoked population spikes in the hippocampal CA3 region recorded *in-vivo*, but prevented the increase of the population-spikes induced by local application of bicuculline [Margineau and Wülfert, 1995]. Since the paired-pulse ratio in the presence or absence of bicuculline was not altered by LEV, LEV probably has little effect at presynaptic sites when applied acutely. Furthermore, acute LEV prevented the bicuculline-induced increase in population-spikes probably independent of a GABAergic pathway [Margineau and Wülfert, 1995]. In addition, in *in-vivo* experiments, acute systemic application of antiepileptic LEV doses also failed to alter evoked field potentials and GABAergic (GABA_A and GABA_B receptor) mechanisms in the hippocampal dentate gyrus [Margineau and Klitgaard, 2003].

Synaptic transmission in-vitro

As stated in the previous paragraph, there is no electrophysiological evidence for a direct effect of acutely applied LEV on the pre- or postsynaptic site of naïve GABAergic synapses. Further, there is no direct LEV effect on naïve GABA_A receptor mediated inhibitory currents in

neuronal cell cultures, or in hippocampal and hypothalamic slices [Poulain and Margineanu, 2002; Rigo *et al.* 2002; Birnstiel *et al.* 1997]. Intriguingly, however, there have been two studies describing an effect of LEV on GABA_A receptors that have undergone modifications associated with epilepsy. First, in hippocampi from epileptic brains GABA_A receptor function can be impaired via allosteric inhibition by zinc, thereby reducing the overall inhibitory effect of GABA [Coulter 2000]. This zinc-induced inhibition of GABA_A receptors was fully reversed by acute application of LEV in cultured hippocampal neurons, whereas GABA_A-induced currents in controls were unchanged [Rigo *et al.*, 2002]. Second, Palma *et al.* (2007) prepared neuronal membranes from human tissue removed during brain surgery. They then micro-transplanted the membrane preparations into frog oocytes and investigated evoked GABA_A receptor currents. The GABA_A receptor currents from epileptic hippocampi were substantially impaired upon repeated GABA application without changes in decay time. This run-down was substantially reduced by pre-incubation with LEV for 3 h, suggesting stabilization of GABA_A receptor currents during repeated activation. The stabilizing effect was also present with testing on GABA_A receptor currents in neocortical pyramidal neurons in slices from temporal lobe epilepsy patients. This particular effect probably involved a protein kinase C dependent pathway, needed more than 1 h to develop and slowly diminished upon withdrawal of LEV.

LEV may also reduce glutamatergic transmission. Whereas acutely applied LEV at a low concentration (10 μ M) had no appreciable effect on evoked excitatory postsynaptic potentials (EPSPs) in hippocampal slices [Birnstiel *et al.* 1997], acutely applied LEV at concentrations within the usual clinical range (100–300 μ M) decreased evoked EPSPs by about 20% in striatal slices [Costa *et al.* 2006]. This effect was probably not due to an inhibition of presynaptic N- and P/Q-type Ca²⁺ channels, since the paired-pulse ratio (an indicator of a presynaptic site of action) was unaltered. Interestingly, LEV acutely applied to cultured cortical neurons reversibly reduced AMPA-receptor currents by ~25% [Carunchio *et al.* 2007]. However, Yang *et al.* (2007) did not observe any change in evoked field potentials in the CA1 region of hippocampal slices during acute LEV application. In contrast, 3 h pre-incubation of the slices with 100 μ M LEV resulted in a relative decrease in the amplitude of

late field potentials during an 80 Hz train. Thus, LEV exhibited a use-dependent decrease in neurotransmitter release. The same group also showed that, using the FM dye technique, the synaptic exocytosis rate at 1 Hz stimulation was diminished after pre-incubation with LEV, suggesting a presynaptic site of action. Unfortunately, these imaging experiments did not distinguish between LEV's action on glutamatergic or GABAergic terminals, and thus the effect of this mechanism on network behavior remains unclear.

Action of LEV on in-vitro models of epilepsy

So far, we have reviewed the cellular targets of LEV that were mostly identified *in-vitro* under 'physiological' conditions. One way to judge the anticipated antiepileptic efficacy of a drug is to apply it to *in-vitro* models of acute seizures. Surprisingly, in contrast to most *in-vivo* animal models of acute seizures (apart from the 6 Hz psychomotor seizure model, Barton *et al.* 2001), LEV had significant effects in *in-vitro* seizure models. Spontaneous NMDA-induced epileptiform bursts in hippocampal slices were substantially reduced during acute application of 10 μ M LEV (with a maximum effect 20–30 min after drug application) [Birnstiel *et al.* 1997]. Similarly acute bath application of LEV at concentrations between 10–500 μ M led to consistent reduction of seizure-like activity induced by bicuculline in both human neocortical slices from epileptic patients and in rat hippocampal slices [Gorji *et al.* 2002; Birnstiel *et al.* 1997]. The underlying mechanism is likely unrelated to a direct effect of LEV on GABA_A receptors and remains unclear. LEV has also been tested in a different model in which reduction of extracellular magnesium, application of bicuculline and 4-aminopyridine led to spontaneous seizure-like activity characterized by paroxysmal depolarization shifts in pyramidal neurons of neocortical brain slices [Pisani *et al.* 2004]. In this model, acute application of LEV for 10–20 min reversibly decreased the duration of the paroxysmal depolarization shift as well as the relative increase in calcium with a half-maximal inhibition at concentrations between 140 and 180 μ M LEV. In hippocampal slices, bath application of caffeine-induced spontaneous bursts whose occurrence was considerably delayed after 5 min incubation with LEV [Angehagen *et al.* 2003], suggesting an inhibitory effect of LEV on Ca²⁺ release from intracellular stores. Moreover, LEV administration decreased the frequency of spontaneous

APs and bursts induced by 4-aminopyridine [Leniger *et al.* 2004] that could be linked to a LEV-induced intracellular acidification. Surprisingly and unlike other AEDs with high extracellular K^+ and low Ca^{2+} (both of which increase neuronal excitability), 32 μ M LEV (application for up to 80 min) selectively impaired the number of evoked epileptiform and hypersynchronous field potentials in CA3 area of hippocampal slices without altering the number of APs recorded intracellularly [Niespodziany *et al.* 2003]. These results suggest an uncoupling of interneuronal synchrony; however, its underlying mechanism remains to be elucidated.

Taken together, most of the observed 'antiepileptiform' effects of LEV *in-vitro* can be explained by the sum of the known mechanisms of action, although it is still difficult to dissect out which are most important. This raises the question of why there is an 'antiepileptic' LEV effect in *in-vitro*, but not in most of the *in-vivo* models of acute seizures. This may be due to obtaining a sufficiently high and persistent LEV concentration in brain slices or in cell cultures as compared to *in-vivo* models with acute systemic LEV application. In the latter, there may be complex concentration profiles in different brain areas with an insufficient total increase or local increase in the seizure generating areas of LEV. Alternatively, specific models of acute seizures *in-vivo* models may select for AEDs with distinct mechanisms of actions (i.e., generation and propagation of ictal activity in a specific model may be critically dependent on particular pathways and cellular targets). If this is true, why is LEV effective in chronic models of epilepsy? Epileptic tissues undergo various alterations in voltage- and ligand-gated ion channels [Walker *et al.* 2007]. As already described beforehand, e.g., GABA_A receptors from 'epileptic' tissue or under conditions occurring in chronic epilepsy (e.g., zinc-induced allosteric inhibition) were modulated by LEV. Thus, 'epileptic' tissue could be more sensitive to LEV than 'nonepileptic' tissue and so require lower LEV concentrations. At the same time, drug resistance and tolerance, that is a reduction in response to a drug after repeated applications, can occur during chronic treatment or long-standing epilepsy that might be related to target-specific compensatory mechanisms or disease-specific modifications. Indeed, development of tolerance to LEV was observed in some animal models of epilepsy [van Vliet *et al.* 2008; Löscher and Hönack, 2000]. However,

development of drug tolerance and resistance in animal models probably depends on the specific epilepsy models used and can be highly selective for specific AEDs (e.g., LEV is effective in phenytoin-resistant amygdala-kindled rats [Löscher *et al.* 2000]).

Summary and conclusions

Unlike other AEDs, LEV exerts antiepileptic effects predominantly in animal models of chronic epilepsy with no effect in most common animal models of acute epilepsy. This is consistent with the observation that LEV only affected GABA_A receptors from 'epileptic' tissue or under conditions that occur during epilepsy, whereas naïve GABA_A receptors were not modulated. However, LEV is effective in *in-vitro* models of acute epilepsy and also in one animal model of acute epilepsy. Moreover, an increasing number of naïve cellular targets for LEV have been identified to date whose modulation has potential antiepileptic effects. From these studies, it emerges that LEV may have preferential sites and mechanisms of action, depending on the speed of administration and the condition of the tissue. Acutely applied LEV in clinically relevant concentrations may preferentially modulate neuronal activity by inhibition of intracellular Ca^{2+} increase (via blockade of N- and P/Q-type Ca^{2+} channels and Ca^{2+} release from intraneuronal stores), delayed rectifier K^+ currents, Cl^-/HCO_3^- exchanger and AMPA-receptors (cf. Tables 1 and 2). In contrast and unlike other AEDs, chronically applied LEV may predominantly act via binding to SV2A, probably leading to decreased transmitter release, which, together with the modulation of ion channels and other targets, potentially prevents epileptic activity. In tissue of epileptic patients, the disease-associated modifications may make neuronal circuits more sensitive to LEV and new targets may emerge, such as altered GABA_A receptors, thereby strengthening inhibitory neurotransmission.

In conclusion, although LEV shares some targets (such as delayed rectifier channels and N- and P/Q-type calcium channels) with other AEDs, LEV exhibits unique mechanisms of action and a novel and unique binding site.

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Conflict of interest statement

None declared.

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