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Current understanding of the mechanism of action of the antiepileptic drug lacosamide

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Summary The antiepileptic drug lacosamide [(R)-2-acetamido-N-benzyl-3-methoxypropanamide], a chiral functionalized amino acid, was originally identified by virtue of activity in the mouse and rat maximal electroshock (MES) test. Attention was drawn to lacosamide because of its high oral potency and stereoselectivity. Lacosamide is also active in the 6Hz seizure model but inactive against clonic seizures in rodents induced by subcutaneous pentylenetetrazol, bicuculline and picrotoxin. It is also ineffective in genetic models of absence epilepsy. At doses greater than those required to confer protection in the MES test, lacosamide inhibits behavioral and electrographic seizures in hippocampal kindled rats. It also effectively terminates seizures in the rat perforant path stimulation status epilepticus model when administered early after the onset of seizures. Lacosamide does not exhibit antiepileptogenic effects in kindling or post-status epilepticus models. The profile of lacosamide in animal seizure and epilepsy models is similar to that of sodium channel blocking antiepileptic drugs, such as phenytoin and carbamazepine. However, unlike these agents, lacosamide does not affect sustained repetitive firing (SRF) on a time scale of hundreds of milliseconds or affect fast inactivation of voltage-gated sodium channels; however, it terminates SRF on a time scale of seconds by an apparent effect on sodium channel slow inactivation. Lacosamide shifts the slow inactivation curve to more hyperpolarized potentials and enhances the maximal fraction of channels that are in the slow inactivated state. Currently, lacosamide is the only known antiepileptic drug in clinical practice that exerts its anticonvulsant activity predominantly by selectively enhancing slow sodium channel inactivation.

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Introduction

The antiepileptic drug (AED) lacosamide was derived from synthetic chemistry efforts focusing on small molecules referred to as functionalized amino acids, which contain a N-benzyl-2-acetamidopropionamide core structure. In 1985, Harold Kohn and colleagues at the University of Houston identified N-acetyl-alanine-N-benzylamide as having protective activity in the mouse maximal electroshock (MES) test (Cortes et al., 1985), a widely used screening model for potential AEDs (Castel-Branco et al., 2009). Functionalized amino acids have substitutions on the C(2) carbon giving rise to a chiral center so that the molecules exist as (R) and (S) enantiomers. Subsequently, it was noted that anticonvulsant activity resides exclusively in the (R)-enantiomers whereas the (S)-enantiomers are virtually inactive (Choi et al., 1996; Kohn et al., 1988). The stereoselectivity was unusual for an AED and stimulated extensive exploration of derivatives to define the structural features conferring seizure protection. The project was enabled by the Anticonvulsant Screening Project (ASP) of the National Institute of Neurological and Communication Disorders and Stroke, a resource, directed at the time of lacosamide’s discovery by Harvey Kupferberg, that provides testing in animal models. Over 250 functionalized amino acids were subsequently synthesized and evaluated by the ASP. Within a series of 2-substituted N-benzyl-2-acetamidopropionamide analogs, the methyl ether derivative N-benzyl-2-acetamido-3-methoxypropionamide (originally designated as ADD 234037, SPA 927, harkoseride and erloksamide and currently known by the United States Adopted Name lacosamide; Fig. 1) exhibited particularly potent oral activity and a low propensity to cause motor toxicity in the rotorod test, conferring it with a wide therapeutic window (Choi et al., 1996). Activity in the MES test resided exclusively in the (R)-enantiomer (eudismic ratio > 22), as was generally the case with N-benzyl-2-acetamidoacetamide derivatives such as the lead compound N-acetyl-alanine-N-benzylamide.

Lacosamide (C₁₁H₁₈N₂O₃; MW 250.29) displays amphiphilic properties, which means that it is lipophilic enough to be orally bioavailable and penetrate the blood–brain barrier yet it is sparingly but sufficiently soluble in aqueous solution to permit the development of a 10 mg/ml parenteral formulation without adding a solubilizing agent (Hovinga, 2003). A 10 mg/ml oral solution (syrup) is also available.

Following initial identification, the properties of lacosamide were more extensively characterized in various animal models by the ASP. A summary of the results of these studies is provided in the next section. Pre-clinical studies using in vitro electrophysiological approaches revealed that lacosamide has a novel mechanism of action in that it selectively enhances the slow inactivation of voltage-gated sodium channels (VGSCs). Conventional sodium-channel blocking AEDs are believed to protect against seizures primarily by interacting with the fast inactivated state of VGSCs (Rogawski and Löscher, 2004). Lacosamide does not affect fast inactivation of VGSCs. While the precise mechanism by which lacosamide exerts its antiepileptic effect in humans remains to be fully elucidated, a review of our current understanding is presented in the following sections.

![Lacosamide and Functionalized Amino Acid](image)

**Figure 1** Chemical structure of lacosamide and a functionalized amino acid.
The efficacy and safety of lacosamide in the adjunctive treatment of focal seizures were demonstrated in three pivotal trials in a population of over 1200 patients (Ben-Menachem et al., 2007; Halasz et al., 2009; Chung et al., 2010). The long-term safety, as well as maintenance of efficacy for up to 8 years, were demonstrated in open-label extension trials (Rosenfeld et al., 2011; Rosenow et al., 2011; Husain et al., 2012). Since its approval in 2008 by both the European Medicines Agency and the US Food and Drug Administration, the drug has been widely used worldwide in the treatment of people with focal epilepsy. Its use however, is limited in some patients by side effects that include ataxia, dizziness, fatigue, and nausea (Sawh et al., 2013). Emerging evidence suggests that lacosamide may be effective in the treatment of status epilepticus (SE) and refractory seizure clusters in some patients and that it may have a reduced incidence of side effects compared with current standard of care treatments for these conditions (Höfler and Trinka, 2013; Legros et al., 2014; Kellinghaus et al., 2014).

Characterization in animal models

Animal seizure and epilepsy models have proven to be invaluable in the identification and characterization of AEDs. The MES test, developed in the 1940s by Swinyard and colleagues (Toman et al., 1946; Castel-Branco et al., 2009), remains one of the principal models used in the initial screening and characterization of novel compounds for activity as potential AEDs. Since the introduction of the MES test, a large number of other seizure and epilepsy models have become available for drug screening, including the subcutaneous pentyleneetrazol (sc MET) seizure test, introduced in 1944 by Everett and Richards (Everett and Richards, 1944; Goodman et al., 1953). An alternative electroshock test, the 6 Hz model, re-introduced in 2001 by Barton et al. (2001), displays a different pharmacological profile than either the MES or sc MET tests. In particular, typical sodium channel blocking AEDs fail to confer full protection in the model at non-toxic doses whereas drugs that act on the GABAergic inhibition are generally highly protective (Kaminski et al., 2004). According to the algorithm utilized by the ASP, once the activity of a novel compound is demonstrated using the aforementioned screening models, a series of differentiation tests is conducted to further characterize the spectrum of activity of the potential AED (Smith et al., 2007).

Screening tests

Lacosamide was initially identified on the basis of its relatively potent activity in the MES test, comparable to that of the widely used AED phenytoin (Choi et al., 1996). The ED50 in mice when administered intraperitoneally is 4.5 mg/kg whereas the potency causing neurological toxicity (TD50) in the rotodot test is 27 mg/kg, so that the protective index (PI; TD50/ED50) is 6, which is comparable to that of other AEDs, such as phenoxy, where the value is 6.6 and superior to that of valproate, where the PI value is 1.7. Lacosamide also had high potency orally in MES test in rats (ED50, 3.9 mg) and failed to cause neurological impairment at doses as high as 500 mg/kg. In contrast, lacosamide was not active in the sc MET test in mice or rats, which is the case for AEDs that act primarily through an effect on voltage-gated sodium channels (VGSCs). In the intravenous PTZ test, which assesses the effect on seizure threshold and provides a means of assessing proconvulsant activity, lacosamide significantly increased the threshold for seizures (both first twitch and clonus) in mice at a dose equivalent to the MES ED50 (4.5 mg/kg) (Stöhr et al., 2007).

In the mouse 6 Hz model with stimulation at an intensity of 32 mA, lacosamide administered 30 min prior to application of current is protective with ED50 of 10 mg/kg (Shandra et al., 2013). As noted, other sodium channel blocking AEDs, including phenytoin, lamotrigine and carbamazepine, are only active in the 32 mA 6 Hz model at high, toxic doses (Barton et al., 2001; Shandra et al., 2013), thus differentiating lacosamide from these agents.

Differentiation tests

Lacosamide has also been demonstrated to confer protection against audiogenic (reflex) seizures in the Frings mouse (Beyreuther et al., 2007) and against NMDA-induced tonic convulsions and death, with partial protection against NMDA-induced clonic convulsions (Stöhr et al., 2007). Lacosamide is not active against bicuculline or picrotoxin-induced seizures (Stöhr et al., 2007). A summary of the anticonvulsant potencies in the models discussed so far is presented in Table 1.

Animal epilepsy models

In addition to the audiogenic seizure-susceptible mouse, e.g., the DBA/2J or Frings mouse, screening and differentiation of investigational AEDs are often conducted in healthy, non-epileptic animals in which seizures are induced by electrical or chemical stimulation. Potential AEDs can also be evaluated in epileptic animals. Epileptic animals may have a reduced seizure threshold, as in kindling models, or the animals may express recurrent spontaneous seizures, as occurs in human epilepsy. Epilepsy models exhibit greater face validity for various types of human epilepsy than do the seizure models used for initial screening, but they are more labor intensive. For example, rodent models of primary generalized epilepsy display an electrographic phenotype characterized by spike-wave discharges and a pharmacological profile that is consistent with the pharmacology of human absence; however, testing is based on evaluation of EEG and not behavior (van Luijtelaar et al., 2002). From this perspective, testing in epilepsy models is useful to more fully characterize the activity of a potential AED.

Kindling models, first described by Goddard in 1960s, are perhaps the oldest and best-studied epilepsy models. Kindling refers to the phenomenon whereby repetitive induction of focal seizures, typically via electrical stimulation, results in progressive and permanently heightened epileptic activity, both electrographic and behavioral, in response to stimuli (Morimoto et al., 2004). The rapid hippocampal kindling model described by Lothman and Williamson (1994) allows test compounds to be efficiently characterized and has therefore been adopted by the
ASP. After a recovery period of 1 week, fully kindled rats received lacosamide at various doses before seizure induction. According to protocol, results are presented as reductions in seizure score and the duration of afterdischarge (Table 3). A reduction from 5 (maximum) to 3 in the seizure score with no concomitant impact on afterdischarge duration indicates that the candidate AED may be effective against secondary generalized but not against focal seizures, whereas a greater decrease in seizure score with concomitant reduction in afterdischarge duration indicates potential effectiveness against focal seizures [White et al., 1998]. Treatment with lacosamide resulted in dose-dependent reductions in seizure score and afterdischarge duration relative to pre-drug control values (Table 2). The ED50 for reduction of the seizure score was 13.5 mg/kg (95% CI 9.11–17.8). Although lacosamide reduced both seizure score and afterdischarge duration indicating effects both on the local seizure discharge and on seizure spread (secondary generalization), the doses required were substantially greater than for the MES test.

The activity of lacosamide (25 mg/kg) was also compared with that of other AEDs administered at maximally effective doses (phenytoin 150 mg/kg, carbamazepine 50 mg/kg, valproic acid 250 mg/kg and ethosuximide 250 mg/kg) in kindled rats. Lacosamide was found to reduce the afterdischarge duration (>85%) to a greater extent than all other tested AEDs (Beyreuther et al., 2007).

**Genetic models of absence seizures**

Wistar Albino Glaxo from Rijswijk (WAG/Rij) rats and genetic absence epilepsy rats from Strasbourg (GAERS) are two well-established models of absence epilepsy (Marescaux et al., 1984; van Luijtelaar and Coenen, 1986). AEDs that suppress spike-wave discharges (SWDs) in these models, such as ethosuximide, trimethadione, sodium valproate and benzodiazepines, are effective against absence epilepsy in humans. In contrast, sodium channel-blocking AEDs including phenytoin and carbamazepine, but not lamotrigine, are known to increase SWDs in these genetic absence models (Peeters et al., 1988; Marescaux et al., 1992; van Rijn et al., 1994; Gurbanova et al., 2006; Liu et al., 2006) and in humans (Panayiotopoulos, 1999; Genton, 2000).

The impact of lacosamide on absence seizures has been tested in both models (Table 3). For experiments with both rat strains, electroencephalogram (EEG) activity was recorded in freely moving animals with implanted cortical electrodes (van Luijtelaar et al., manuscript in preparation). In WAG/Rij rats, lacosamide at doses of 3, 10 or 30 mg/kg produced a statistically significant increase in the number and duration of SWDs during the first hour following drug administration. Similarly in GAERS, lacosamide increased the duration and number of SWDs. However, in this latter model high doses were required (several-fold the dose conferring protection in the MES test; 3–6 × ED50). In light of these observations, lacosamide is unlikely to be clinically useful in the treatment of absence epilepsy.

### Status epilepticus

Models of SE may provide a means to assess the potential utility of AEDs in the treatment of human SE. One such model involves brief, intermittent stimulation of the hippocampal perforant path in rats for 30 min, which leads to the
Mechanism of action of lacosamide

Table 3  Anticonvulsant effects of lacosamide in animal models of chronic epilepsy.

<table>
<thead>
<tr>
<th>Test</th>
<th>Lacosamide administration schedule</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kindling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampal kindling</td>
<td>7, 13, 19 or 25 mg/kg, i.p., 15 min before seizure induction in fully kindled animals</td>
<td>Significant reduction in afterdischarge duration compared with pre-treatment period at three highest doses (ED50 13.5 mg/kg; 95% CI 9.11–17.80)</td>
</tr>
<tr>
<td>Genetic models</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAERS</td>
<td>1.73, 5.2, 15.6 or 31.2 mg/kg, i.p. in animals displaying spontaneous SWDs</td>
<td>Transient and modest dose-dependent increase in the number and duration of SWDs, indicating lack of therapeutic effect on absence seizures</td>
</tr>
<tr>
<td>Wag/Rij</td>
<td>3, 10 or 30 mg/kg, i.p. in animals displaying spontaneous SWDs</td>
<td></td>
</tr>
</tbody>
</table>

All animals used in the tests were either Wistar or Sprague–Dawley rats. SWD, spike-wave discharges.

development of self-sustained status epilepticus (SSSE) that lasts approximately 16 h (Mazarati et al., 1998) (Table 4). Lacosamide was administered in this model either 10 or 40 min after the initiation of perforant path stimulation (Wasterlain et al., 2011). Doses as low as 10 mg/kg, i.p., administered at the early time point significantly reduced the number of post-treatment seizures. However, the drug was ineffective at the late treatment stage even at doses as high as 50 mg/kg. Lacosamide is therefore similar to many other AEDs, including benzodiazepines, phenytoin and fosphenytoin, which are effective early in the treatment of SE in this model but lose activity if seizures continue. A similar phenomenon is observed in clinical practice, where the treatment of SE becomes less successful with some AEDs as seizures continue.

The cobalt/homocysteine model is another model used to evaluate the therapeutic potential of drugs in SE. In this model, homocysteine thiolactone is administered to rats with cobalt-induced epileptogenic lesions, giving rise to secondarily generalized tonic–clonic seizures and subsequent convulsive SE (Walton and Treiman, 1988). Administration of lacosamide immediately after the onset of seizures in this model caused dose-dependent protection against generalized tonic–clonic seizures, with an ED50 of 45.4 mg/kg (Stöhr et al., 2007). While lacosamide was efficacious in this model, its potency was less than that seen in other models of seizures and epilepsy.

Antiepileptogenesis

Epileptogenesis is the process that leads to the development of epilepsy, which is defined as a disorder in which there are spontaneous recurrent seizures (SRS). Numerous types of brain insults such as trauma, tumors, neurodegenerative disease, cerebrovascular disease, status epilepticus and complex febrile seizures can lead to epileptogenesis (Lösch and Brandt, 2010). Epilepsy generally does not start immediately after the insult; indeed, the latency between the insult and the onset of epilepsy is highly variable and can range from months to years. At present, no drug treatment is known to have antiepileptogenic properties, that is to delay or eliminate the eventual development of SRS when administered during the latent period after an insult. It has been argued that an antiepileptogenic treatment would need to target the brain mechanisms involved in epileptogenesis. However, antiepileptogenesis clinical trials have generally utilized AEDs that were developed to protect against seizures and not to influence epileptogenesis. Therefore, it is not surprising that trials to date have failed. In order to identify antiepileptogenic therapies, drug screening would necessarily have to be conducted in models of epileptogenesis rather than in the seizure models that are used to identify anti-seizure agents.

Such antiepileptogenesis studies have been conducted with lacosamide in the amygdala kindling model and in

Table 4  Effects of lacosamide in status epilepticus models.

<table>
<thead>
<tr>
<th>Test</th>
<th>Lacosamide administration schedule</th>
<th>Results</th>
</tr>
</thead>
</table>
| Self-sustaining status epilepticus (electrical stimulation) | 3, 10, 30 or 50 mg/kg, i.p., 10 min (early) or 40 min (late) before initiation of perforant path stimulation | Early treatment
Significant, dose-dependent reduction in acute seizure activity with 10–50 mg/kg doses |
| Cobalt/homocysteine status epilepticus    | 10, 20, 40, 80 or 100 mg/kg, i.p., immediately after second generalized tonic-clonic seizure following homocysteine injection | Late treatment
Non-significant trend toward reduced seizure activity
Dose-dependent protection against generalized tonic-clonic seizures (ED50, 45.4 mg/kg) |
Table 5  Effects of lacosamide in antiepileptogenesis models.

<table>
<thead>
<tr>
<th>Test</th>
<th>Lacosamide administration schedule</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala kindling</td>
<td>3, 10 or 30 mg/kg i.p., for 5 days before kindling and during the 22–23 days of kindling</td>
<td>Apparent delay of kindling acquisition — significantly greater number of stimuli required with 10 and 30 mg/kg doses to achieve kindling criterion (stage 5 seizure); however, no effect of drug treatment parameters after washout; spontaneous seizures in some animals treated with 30 mg/kg dose during kindling</td>
</tr>
</tbody>
</table>
| Self-sustaining status epilepticus (electrical stimulation) | 3, 10, 30 or 50 mg/kg, i.p., 10 (early) or 40 (late) minutes before initiation of perforant path stimulation | Early treatment: Significant, dose-dependent reduction in acute seizure activity at 10–50 mg/kg doses and in number of SRS following 6-week waiting period at 50 mg/kg dose  
Late treatment: Non-significant trend toward reduced seizure activity. Significant reduction in number of animals that developed SRS at 30–50 mg/kg doses, but no significant reduction in seizure severity or frequency in rats that developed SRS |
| Electrical stimulation of basolateral amygdala | Low or high doses orally and via minipump (initially 20 and 60 mg/kg/day) for 23 days after status epilepticus induction | No antiepileptogenic effect, but dose-dependent neuroprotection observed                                                                 |

SRS = spontaneous recurrent seizures.

the post-status epilepticus model (Table 5). In the kindling model, Wistar rats received daily doses of lacosamide (3, 10, or 30 mg/kg/day) or saline over a period of 22–23 days during the kindling process (Brandt et al., 2006). The animals were not kindled or treated over the weekends. Administration of 3 mg/kg lacosamide had no impact on kindling acquisition, i.e., all animals reached the kindling criterion (a generalized stage 5 seizure) at the same rate as controls; however, treatment with lacosamide at 10 or 30 mg/kg resulted in a reduction in the rate and extent of kindling acquisition. While control animals developed fully kindled stage 5 seizures after an average of 8.6 ± 0.7 stimulations, those treated with 10 mg/kg lacosamide did so after an average of 16.5 ± 1.4 stimulations, corresponding to an increase of >90%. Inhibition in the acquisition of kindling was also observed with the 30 mg/kg dose, but this dose also was also paradoxically associated with the occurrence of spontaneous seizures in some animals. The authors therefore concluded that lacosamide has a dose-dependent ability to exert proconvulsant effects in epileptic animals. However, as discussed above, a proconvulsant effect was not observed in normal animals in the PTZ threshold test. Nonetheless, there is evidence that nearly all AEDs can aggravate epilepsy, particularly at high, supratherapeutic doses (Gayatri and Livingston, 2006) and lacosamide may be no different in this respect (Cuzzola et al., 2010). Following termination of lacosamide treatment, animals were stimulated after a 2.5 month washout period to determine if lacosamide treatment exerted an enduring antiepileptogenic or disease-modifying effect. There was no difference between lacosamide-treated animals and controls in terms of kindled seizure severity or afterdischarge duration. This indicates that lacosamide does not cause an enduring antiepileptogenic effect. It is likely that the apparent antiepileptogenic efficacy observed during concomitant stimulation and lacosamide treatment is due to lacosamide-induced suppression of seizure expression. Despite the suppression of seizure expression, kindling epileptogenesis continues unabated.

In the SSSE model described above, the impact of early and late administration of lacosamide on the occurrence of SRS was evaluated. In addition to suppressing seizure activity as discussed previously, early administration (10 min after initiation of perforant path stimulation) of lacosamide reduced the number of SRS and the cumulative seizure time in a dose-dependent fashion; statistically significant effects were obtained in the 30 and 50 mg/kg lacosamide treatment groups (Wasterlain et al., 2011). Significant reductions in spike frequency were also observed following treatment with lacosamide at doses of 10, 30 or 50 mg/kg. Fewer animals that received late treatment (40 min after stimulation) with high-dose lacosamide (30–50 mg/kg) developed SRS, but the treatment did not significantly reduce seizure severity or frequency in rats that developed SRS. Overall, this study demonstrates that epileptogenesis can be suppressed when SE is effectively treated. However, the study was not designed to evaluate whether lacosamide has antiepileptogenic properties apart from its ability to effectively modify the duration of SE; i.e., insult modification, which is the insult that provokes epilepsy development.

A more recent study was specifically designed to determine if lacosamide has antiepileptogenic and disease-modifying effects in status epilepticus-induced epileptogenesis (Licko et al., 2013). The investigators used a model of
SSSE induced by brief, intermittent stimulation of the basolateral amygdala; SE was limited to 4 h by administration of diazepam. Lacosamide was then administered at 10 mg/kg (low dose) and 30 mg/kg (high dose) for 24 days following the episode of SE. The development of spontaneous seizures was assessed by continuous video/EEG-monitoring over a 3-week period. Treatment with lacosamide at either dose during the period following SE failed to affect the development of spontaneous seizures during the monitoring period. However, drug treatment did produce a dose-dependent neuroprotective effect manifest as a significant reduction in neuronal cell loss in the hippocampal CA1 region and piriform cortex. Lacosamide also attenuated the impact of SE on the rate of hippocampal neurogenesis and the aberrant extension of granule cell basal dendrites into the hilus instead of the molecular layer. Results of this study fail to provide support for an antiepileptogenic action of lacosamide, although the drug does appear to reduce SE-associated neuropathology. The results reinforce the conclusion from many other studies that neuronal injury and cell loss is not the basis of epileptogenesis.

Lacosamide does not affect normal synaptic transmission

Fast synaptic transmission in the central nervous system is mediated by excitatory and inhibitory ligand gated ion channels, including AMPA, NMDA and GABA<sub>A</sub> receptors. Cellular electrophysiological studies have demonstrated that lacosamide does not interact with these receptors at therapeutically relevant concentrations (Errington et al., 2006). For example, extracellular recordings of fast excitatory post synaptic potentials in the CA1 region of the hippocampus, evoked by Schaffer collateral stimulation, were unaffected by concentrations of lacosamide in the range of 1—1000 μM (Lees et al., 2006). These studies also suggest that lacosamide does not directly affect the synaptic release machinery. Therapeutic plasma concentrations of lacosamide are typically in the range 20—40 μM (Cawello & Bonn, 2012; Greenaway et al., 2011), and the brain to plasma ratio is 0.553 (Koo et al., 2011). Therefore, brain concentrations far in excess of those expected to be obtained during treatment with lacosamide do not perturb physiological synaptic transmission.

Lacosamide effects on in vitro epileptiform activity

Seizure-like discharges can be induced in brain slices by exposure to proconvulsant agents such as 4-aminopyridine (4-AP). In contrast to its lack of effect on non-epileptic activity, lacosamide suppresses epileptiform activity in brain slices (Lees et al., 2006). For example, in visual cortex slices, lacosamide reduced the duration and maximum firing frequency of epileptiform discharges induced by 4-AP with EC<sub>50</sub> of 41 and 71 μM respectively, and completely abolished epileptiform discharges at 320 μM. The anti-epileptiform activity of lacosamide exhibited absolute stereoselectivity as the (S)-enantiomer at concentrations of 100—320 μM did not produce any significant effect on the duration or frequency of spontaneous epileptiform discharges (Lees et al., 2006).

The induction of high frequency repetitive firing in vitro preparation is often used to simulate neural bursting activity, comparable to what occurs in a paroxysmal depolarization shift or an ictal epileptiform discharge. AEDs thought to act by blocking VGSCs — e.g., phenytoin, carbamazepine and lamotrigine — terminate action potential trains induced by current injection within hundredths of milliseconds. Lacosamide has no effect on repetitive firing on this time scale. However, lacosamide strongly inhibits repetitive firing on a time scale of seconds (Fig. 2). This indicates that the kinetic properties of lacosamide action on the mechanisms of action potential firing are markedly different from that of other VGSC-blocking AEDs or that lacosamide inhibits action potential firing by a different mechanism. As discussed in the next section, there is strong evidence that the latter interpretation is correct. Indeed, the delay in the onset of lacosamide’s suppression of neuronal excitability is compatible with the fact that the drug must interact with the slow inactivated state of VGSCs (discussed in detail below) in order to exert its pharmacological actions on the channel. In a model of post traumatic epileptogenesis, chronic treatment with lacosamide (100 mg/kg, 7 days) did not prevent the occurrence of epileptiform activities but significantly reduced the mean amplitude of the events (Wilson et al., 2012).

![Figure 2](image-url)
Toward an understanding of the mechanism of action

New AEDs are most often identified by screening in animal models before their molecular targets and mechanism of action are known (Rogawski, 2006). Identification of the molecular mode of action can be a major challenge and it may take years after market authorization before an understanding is achieved of how the new AED protects against seizures. This was certainly the case with lacosamide. It is noteworthy that the success of phenotypic screening in the identification of new AEDs is not uncharacteristic of the situation in drug discovery overall. Even though in recent decades target-based screening has become the predominant method of drug discovery, most recently discovered first-in-class drugs with new molecular mechanisms of action were identified in phenotypic screens (Swinney and Anthony, 2011). Indeed, most marketed AEDs were discovered in phenotypic screens and even when the target is well established, such as in the case of VGSCs, target based screening has so far not been a successful approach for AED discovery.

Since the first cloning of a VGSC nearly three decades ago (Noda et al., 1984) there has been remarkable progress in defining the structural features responsible for the different gating modes of the channel (Payandeh et al., 2012). Nevertheless, it is not yet possible to design a modulatory drug that is able to subtly modulate VGSCs in a way that inhibits seizure behavior without affecting the function of the channel during normal brain function. Consequently, all structurally novel sodium channel blocking AEDs were identified through screening in animal models rather than through in vitro studies with VGSCs. Once a drug is identified in animal models and demonstrates a spectrum of activity suggesting an interaction with VGSCs it is then possible to confirm the interaction through electrophysiological studies with native and recombinant VGSCs.

In an attempt to understand how lacosamide produces its anticonvulsant activity in vivo, and to identify its underlying mechanism of action, a systematic approach was used, which first consisted of testing lacosamide for its ability to compete for binding with a large panel of known drug receptor targets (Errington et al., 2006). More recent studies using [3H]lacosamide with high specific activity also failed to detect specific, high affinity binding in rat brain homogenates (Wolff et al., 2012). These negative results in binding studies are similar to those seen with other sodium-channel blocking AEDs, where the interaction with VGSCs is of low affinity, not detected by radioligand binding and, difficult to characterize with standard receptor binding approaches.

Lacosamide was tested for functional activity on a broad range of central nervous system ion channels targets expressed in neurons using electrophysiological recording. At concentrations of 100–300 μM the drug did not produce tonic inhibition of voltage-gated calcium channels (L, N-, P/Q, T-type channels), nor did it affect the voltage dependence of activation or fast inactivation of these channels (Errington et al., 2008; Wang et al., 2011). Many AEDs inhibit voltage-gated calcium channels, including phenytoin, carbamazepine, oxcarbazepine, lamotrigine (Schumacher et al., 1998; Stefani et al., 1997a,b; Salvati et al., 1999; Lingamaneni and Hemmings, 2003), zonisamide (Matar et al., 2009) and ethosuximide (Crunelli and Leresche, 2002) (Table 6). The lack of effect on calcium channels distinguishes lacosamide from these diverse AEDs.

K,7 (KCNQ) voltage-gated potassium channels have been demonstrated to be a target for the recently approved AED ezogabine (retigabine), an opener of these channels (Rundfeldt, 1997). Although lacosamide was shown not to affect the activity of A-type and delayed rectifier potassium channels in hippocampal neurons (Errington et al., 2008), it has not yet been studied on the diverse families of voltage-gated potassium channels (Table 6). Nevertheless, the spectrum of activity in animal models (see above) is distinct from that of ezogabine, suggesting that K,7 potassium channels are not likely to be a target of lacosamide.

**Lacosamide enhances slow inactivation of voltage-gated sodium channels**

VGSCs play a fundamental role in the generation of action potentials during normal brain function and also during the high frequency firing characteristic of epileptic discharges. The channels cycle between three biophysical states: the resting or closed state, the open state (triggered by cell membrane depolarization) and the inactivated state (representing a non-conductive state from which channels cannot open) (Fig. 3). The availability of VGSCs is regulated at the molecular level by the fraction of channels in the inactivated state. VGSC inactivation consists of two processes: fast inactivation, occurring on a millisecond timescale, and slow inactivation, occurring within seconds to minutes. Fast inactivation contributes to action potential termination and regulation of the refractory period, while slow inactivation is proposed to contribute to overall membrane excitability by increasing action potential threshold. Modulation of VGSC availability through enhancement of either fast or slow inactivation could have significant effects on membrane excitability (Nau and Wang, 2004), particularly during epileptic seizures (Rogawski and Löscher, 2004). Indeed, several human mutations associated with central nervous system disorders have been identified in genes encoding VGSCs and are reported to modify the fast and slow inactivation processes of the channels (Vilin and Ruben, 2001; Catterall et al., 2008; Lossin et al., 2012).

The fast and slow inactivation processes of VGSCs are mediated by different structural features of the channels. Fast inactivation is mediated by an intracellular peptide loop located between domains III–IV (Vassiliev et al., 1988), while it is thought that slow inactivation may involve rearrangement of the inner pore structure (Ulbricht, 2005; Vilin and Ruben, 2001; Payandeh et al., 2012). To study VGSC slow inactivation, specific voltage protocols must be applied to cells and generally long depolarization steps are used (>5s) to drive the channel into the slow inactivated state (Niespodziany et al., 2013).

Several studies, using a broad range of drug concentrations, have confirmed the lack of effect of lacosamide on fast inactivation of VGSCs (Errington et al., 2008; Sheeets et al., 2008; Wang et al., 2011; Hebeisen et al., 2015). These studies utilized native VGSCs expressed by neuroblastoma cells and recombinant VGSCs expressed in heterologous cell
expression systems. Classic VGSC blocking AEDs, including phenytoin, carbamazepine and lamotrigine, have been shown to exert their anti-seizure activity predominantly via fast inactivation of VGSCs (Rogawski and Löscher, 2004). These drugs bind with greater affinity to VGSCs when they are in the inactivated state than when they are open or closed (Kuo, 1998). The result of this preferential binding is stabilization of the inactivated state. In the presence of therapeutically relevant concentrations of these AEDs, the voltage-dependency of inactivation is shifted to more hyperpolarized potentials (Mantegazza et al., 2010). Several newer AEDs such as zonisamide and rufinamide may also act, at least in part, through effects on VGSC fast inactivation (Kuo and Lu, 1997; Biton, 2007; Arroyo, 2007).

Conventional VGSC-blocking AEDs produce little tonic block of VGSCs at therapeutically relevant concentrations. In contrast, lacosamide produces a tonic block of VGSCs and this effect is maintained after the channels have recovered from the fast inactivated state (Errington et al., 2008), suggesting that lacosamide acts on different biophysical properties of VGSCs. More detailed investigations showed that lacosamide is able to enhance the entry of VGSC into the slow inactivated state (Errington et al., 2008) (Fig. 3). Importantly, lacosamide increases the maximal fraction of VGSCs in the slow inactivated state, thereby reducing the overall availability of the channels. Facilitation of slow inactivation exhibits the same stereoselectivity as does the anticonvulsant activity: the (S)-enantiomer is inactive (Niespodziany et al., 2013). A more recent study comparing actions of AEDs on VGSC slow inactivation, confirmed that lacosamide acts differently from other AEDs and potently shifts the voltage dependence of slow inactivation to more hyperpolarized potentials (Niespodziany et al., 2013) (Fig. 4). The shift of the slow inactivation curve has an important physiological impact; when the neuronal membrane potential is near the resting potential, lacosamide produces a reduction in the number of available channels, thereby reducing neuronal excitability. The effects of lacosamide on slow inactivation have been confirmed in studies using different neuronal cell lines and with various recombinant VGSC isoforms, including Nav1.3, Nav1.7, and also tetrodotoxin-resistant VGSCs (Nav1.8) in dorsal root ganglion neurons (Sheets et al., 2008; Wang et al., 2011; Wilson et al., 2012; Hebeisen et al., 2015). In recombinant VGSCs, lacosamide reportedly prolongs recovery from the slow inactivated state (Sheets et al., 2008); such an effect on recovery was not detected in experiments using native VGSCs expressed in neuroblastoma cells (Errington et al., 2008). Despite the wealth of evidence supporting the specific action of lacosamide on slow inactivation of VGSCs, simulation of various modes of block by kinetic modeling have indicated that voltage-clamp protocols used

<table>
<thead>
<tr>
<th>Voltage-gated sodium channels</th>
<th>Lacosamide</th>
<th>Phenytoin</th>
<th>Carbamazepine</th>
<th>Lamotrigine</th>
<th>Zonisamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast inactivation</td>
<td>No(^b)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Slow inactivation</td>
<td>Yes</td>
<td>Yes(^a)</td>
<td>No effect</td>
<td>Yes(^a)</td>
<td>No effect</td>
</tr>
<tr>
<td>Total inactivation</td>
<td>Not</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Persistent Na Current</td>
<td>Inhibition</td>
<td>Inhibition</td>
<td>Inhibition</td>
<td>Inhibition</td>
<td>?</td>
</tr>
<tr>
<td>Na(_x) isoform selectivity</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Use dependence</td>
<td>Weak</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td>Voltage-gated calcium channels</td>
<td>No effect on N-, P/Q-, L-type</td>
<td>Inhibition of high voltage activated channels</td>
<td>Inhibition of high voltage activated channels</td>
<td>Inhibition of L-type</td>
<td>Inhibition of L, T-type</td>
</tr>
<tr>
<td>Voltage-gated potassium channels</td>
<td>No effects on delayed rectifier and A-type</td>
<td>Inhibition of delayed rectifier, A-type, hERG</td>
<td>No effects, potentiation, inhibition reported</td>
<td>Potentiation of 4-AP sensitive</td>
<td>Activation of BK, inhibition of A-type</td>
</tr>
<tr>
<td>References</td>
<td>1–4, 14, 22, 28</td>
<td>2, 5, 11, 12, 17, 19, 23, 24, 25, 30</td>
<td>1, 4, 5, 6, 12, 16, 17, 26, 29</td>
<td>1, 2, 7, 8, 12, 13, 17–19, 27</td>
<td>2, 6, 9, 10, 15, 20, 21</td>
</tr>
</tbody>
</table>

\(^a\) Effects on slow inactivation curves are different than those of lacosamide (Niespodziany et al., 2013).
\(^b\) A mechanism involving slow binding to the fast inactivated state have also been suggested (Jo and Bean, 2013).

1: Errington et al. (2008); 2: Niespodziany et al. (2013); 3: Uebachs et al. (2012); 4: Sheets et al. (2008); 5: Schumacher et al. (1998); 6: Zhu et al. (2002); 7: Wegerer et al. (1997); 8: Wang et al. (1998); 9: Huang et al. (2007); 10: Suzuki et al. (1992); 11: Lenkowski et al. (2007); 12: Kuo (1998); 13: Liu et al. (2003); 14: Wang and Khanna (2011); 15: Schauf (1987); 16: Sun et al. (2007); 17: Lang et al. (1993); 18: Kuo and Lu (1997); 19: Stafstrom (2007); 20: Kito et al. (1996); 21: Biton (2007); 22: Wang et al. (2011); 23: Nobile and Lagostena (1998); 24: Pisciotto and Prestipino (2002); 25: Danielsson et al. (2003); 26: Ambrósio et al. (2002); 27: Zona et al. (2002); 28: Errington et al. (2006); 29: Chen et al. (2013); 30: Hebeisen et al. (2015).
A

Activation

C

Recovery from inactivation

Fast inactivation

Slow inactivation

B

RMP (-70 mV)

C/I

RMP

Recovery from inactivation

O

Threshold for AP firing

I

AP firing

Fast inactivation

Slow inactivation

Figure 3  Inactivation of voltage-gated sodium channels and role in neuronal firing (A) VGSCs cycle between three major states: the closed state (C), open state (O) and the inactivated state (I). VGSCs may enter different inactivation states depending on the cell membrane potential and the metabolic state of the channel. Fast inactivation (FI) occurs on a millisecond timescale and involves a rapid closure of the pore from the intracellular side (ball and chain mechanism). Slow inactivation (SI) occurs over a timescale of seconds to minutes and involves yet undefined pore structures. The recovery from inactivation also involves a rapid or slow process depending on the inactivation state of the channel. (B) VGSCs play a key role in shaping action potentials (AP). At the neuronal resting membrane potential (RMP) of approximately −65 to −70 mV, VGSC are present in mixed states including closed and inactivated channels (C/I). Only channels in the closed state will contribute to the generation of an action potential. The opening of VGSC generates a large and rapid membrane depolarization which then also triggers the fast inactivation process. The repolarization phase of the action potential is mediated by the combination of voltage-gated potassium channel activation and VGSC inactivation. VGSCs rapidly recover from fast inactivation (less than 10 ms) and during a prolonged interspike interval, channels may revert to the closed state and participate in a new action potential. (C) The generation of action potentials is a physiological process that mediates electrical signal propagation and synaptic transmission. Under conditions where APs are fired at a low frequency, VGSCs undergo fast inactivation at each AP and rapidly revert to the closed state (see Fast inactivation illustration, green traces). The fraction of VGSC in the slow inactivated state is not greatly affected by single APs and during normal physiological firing conditions. However, under pathophysiological conditions where neuronal cells are in a depolarized state and generate high frequency firing, VGSC readily enter the slow inactivated state during sustained AP firing (see slow inactivation illustration, green trace). The entry of VGSCs in the slow inactivated state is enhanced by lacosamide and leads to a reduction or block of the AP firing process (see Fig. 2). Fast inactivated channels are targeted by classic VGSC blocking AEDs which increase the fraction of inactivated channels leading to a block of repetitive AP firing.

to demonstrate drug preference for the slow inactivated state may not be reliable (Karoly et al., 2010; Jo and Bean, 2013). Interactions with both the fast and slow inactivated state of Nav1.5 channels have been reported (Wang and Wang, 2014), and in one study, while lacosamide did not affect the short term availability of fast inactivated channels, it was shown to bind to the fast inactivated channel, but with very slow kinetics (Jo and Bean, 2013).

Some other AEDs, which are believed to act mainly on fast inactivation have been reported to also influence slow inactivation (Table 6). For example, while carbamazepine does influence slow inactivation of tetrodotoxin (TTX)-sensitive VGSCs (Errington et al., 2008; Sheets et al., 2008), it was reported to decrease the availability of slow inactivated TTX-resistant (Nav1.8-like) VGSCs in dorsal root ganglion neurons (Cardenas et al., 2006; Sheets et al., 2008). Phenytoin has also been reported to modulate the slow inactivation of VGSCs (Quandt, 1988; Niespodziany et al., 2013) but more detailed studies indicated that the drug binds to the fast inactivated state with slow binding and unbinding kinetics rather than to the slow inactivated state (Kuo, 1998; Kuo and Lu, 1997; Kuo and Bean, 1994). Eslicarbazepine, a major metabolite of eslicarbazepine acetate and oxcarbazepine, has recently been reported to mediate its effects specifically by interacting with the slow inactivated state of VGSCs (Bonifácio et al., 2012). The effects of eslicarbazepine on VGSC slow inactivation are observed at very high concentrations (IC_{50} approximately 559 \mu M) (Hebeisen et al., 2015). Since the maximal levels of eslicarbazepine reached in clinical use are substantially lower (C_{max} of 96 \mu M
Mechanism of action of lacosamide

Lacosamide enhances slow inactivation of voltage-gated sodium channels. (A) The steady state slow inactivation curves of VGSCs expressed in N1E-115 cells have been generated using the manual patch clamp method. The cell membrane potential is indicated on the abscissa (mV) and the fraction of available channels (I/I_{max}) is plotted on the ordinate. Under control conditions (open circles) VGSCs start to enter the slow inactivated state from a potential of approximately −60 mV and reach a maximum level of slow inactivated channels at 0 mV of approximately 25–37%. Lacosamide produces a large leftward shift of the inactivation curve (ΔV_{50}, approximately −33 mV, horizontal arrow) without significantly changing the maximum level of slow inactivated channels at 0 mV. However, the large shift of the inactivation curve produces a significant increase in slow inactivated channels at the neuronal resting potential of approximately −70 mV, where lacosamide increases the fraction of slow inactivated channels by 5-fold (from approximately 4–20%, vertical arrow). Adapted from Niespodziany et al. (2013) with permission. (B) Lacosamide differentiates from other VGSC AEDs by producing a large and specific shift (ΔV_{50}, approximately −33 mV) in steady state slow inactivation. Other AEDs tested in parallel did not affect the voltage dependence of slow inactivation (ΔV_{50} not significant from control) although lamotrigine and rufinamide produced a slight opposite shift of the slow inactivation curve in these experiments. Two-tailed t-test are indicated with *P < 0.05, **P < 0.005, or ***P < 0.0005. Adapted from Niespodziany et al. (2013) with permission. (C) Recording of VGSC currents (I_{Na}) in N1E-115 cells using fast or slow inactivation voltage protocols. Lacosamide (300 μM) does not affect the peak I_{Na} current when channels are entering the fast inactivated state (upper panel) but significantly reduces I_{Na} when channels are entering the slow inactivated state (lower panel). A time matched control group (Control), only perfused with vehicle, was measured in parallel. Currents were recorded before (black) or after 3 min perfusion (red) with control or lacosamide. (Wolff, Unpublished results).

and 30 μM in plasma and CSF, respectively) (Nunes et al., 2013), it seems unlikely that the therapeutic actions of eslicarbazepine are related to effects on slow inactivation.

Lacosamide does not affect the activation properties of VGSCs (Wang et al., 2011; Errington et al., 2008) and does not modulate the resting or closed state of the channel (Sheets et al., 2008). The relative selectivity of lacosamide for the inactivated versus resting state was 40–50 fold higher than for carbamazepine (Sheets et al., 2008), indicating that lacosamide is a highly specific state dependent inhibitor of VGSCs. A more extensive study, including the testing of lacosamide against all of the central nervous system-specific VGSC subtypes, would help to define whether it has a preferred selectivity for a specific VGSC isofrom.

Another feature of the VGSC-blocking action of AEDs is that the block occurs in a use-dependent manner (Table 6). This mechanism is related to the repeated activation of the channel producing additional or tighter binding of the drug to its receptor and hence an increased inhibition of the channel (Rogawski and Löscher, 2004). This action may allow such AEDs to selectively inhibit high frequency pathological discharges without affecting normal ongoing activity that occurs at lower frequency. Lacosamide produces a very low level of use-dependent inhibition (approximately 10%), which is observed only after prolonged stimulation of the channel by trains of depolarising pulses (Errington et al., 2008; Wang et al., 2011). Use-dependent inhibition is not likely to significantly contribute to lacosamide’s mode of action.

Although lacosamide has a distinct mechanistic impact on VGSCs from that of traditional VGSC blockers such as carbamazepine, combination therapy with traditional agents still results in the well recognized pharmacodynamic interaction between two VGSCs leading to increased CNS-related side effects (French and Faught, 2009). Clinical trials and post marketing studies revealed that patients receiving lacosamide with a concomitant VGSC blocker were more likely to discontinue due to poor tolerability compared with patients receiving a concomitant non-VGSC blocker (Sake et al., 2010; Novy et al., 2011). In the real life setting, without fixed dosing, high doses of lacosamide beyond the maximum approved daily dose of 400 mg were reported to be well tolerated and efficacious in patients with uncontrolled epilepsy, if the dose of the concomitant VGSC blocker was down titrated (Edwards et al., 2012).

Persistent sodium current

In addition to the fast and slow inactivation properties of VGSCs, many excitatory cells express VGSCs that possess a non-inactivating component, called the persistent sodium current (I_{nap}) (Kiss, 2008). I_{nap} represents only a small fraction of the sodium currents generated by VGSCs (approximately 5%) and is regulated by e.g. accessory protein
interactions (Aman et al., 2009) and the metabolic state of the channel. It has been observed that $I_{\text{NaP}}$ is increased after status epilepticus in animals (Agrawal et al., 2003; Chen et al., 2011) and in hippocampal CA1 neurons from patients with drug-resistant epilepsy (Vreugdenhil et al., 2004). The increase in $I_{\text{NaP}}$ has been proposed as an epileptogenic mechanism inasmuch as $I_{\text{NaP}}$ may cause maintained depolarization of the neurons and facilitate the generation and maintenance of epileptiform activity. Inhibition of $I_{\text{NaP}}$ by several AEDs has been proposed as a mechanism whereby these drugs could protect against seizures with minimal effects on normal brain function (Chao and Alzheimer, 1995; Spadoni et al., 2002; Taverna et al., 1998; Rogawski and Löscher, 2004; Sun et al., 2007) (Table 6). Recent investigations demonstrate that lacosamide is able to reduce $I_{\text{NaP}}$ by about 60% in hippocampal neurons, without modifying the activation properties of $I_{\text{NaP}}$ (Uebachs et al., 2012). In addition to the effects on slow inactivation, modulation of $I_{\text{NaP}}$ by lacosamide may contribute to therapeutic activity in epilepsy, especially under conditions where $I_{\text{NaP}}$ is increased. The effects of AEDs on $I_{\text{NaP}}$ are strongly influenced by changes of the physiological conditions. For example, carbamazepine inhibits $I_{\text{NaP}}$ under most conditions it paradoxically increases $I_{\text{NaP}}$ at membrane potentials close to the neuronal resting potential (Uebachs et al., 2010). This paradoxical effect of carbamazepine was observed in mice lacking the VGSC accessory subunit $\beta_1$ (encoded by Scn1B) and which translated in a complete loss in the ability of carbamazepine to reduce repetitive action potential firing in $\beta_1$ deficient mice (Uebachs et al., 2012). The effects of lacosamide on $I_{\text{NaP}}$ were maintained in the absence of the $\beta_1$ subunit (Uebachs et al., 2012) indicating that activity of lacosamide is less sensitive to changes in subunit composition. Mutations in the $\beta_1$ subunit have been associated with genetic epilepsy with febrile seizures plus (GEFS+) and possibly other epilepsy types in humans (Scheffer et al., 2007). The fact that the activity of lacosamide is resistant to changes in $\beta_1$ could potentially confer the drug with therapeutic activity in certain forms of epilepsy, such as those with mutations in $\beta_1$, where carbamazepine and possibly other VGSC blockers are ineffective. This theoretical possibility requires clinical verification.

Effects on other molecular targets

Inhibition of carbonic anhydrases

Carbonic anhydrases are zinc-containing enzymes that play a critical role in the regulation of cellular pH and HCO$_3^-$ levels. Carbonic anhydrase inhibition is believed to account for the anticonvulsant activity of acetazolamide (Rogawski and Porter, 1990). The AEDs topiramate and zonisamide have activity as carbonic anhydrase inhibitors, which may contribute to side effects (Meldrum and Rogawski, 2007) but is not believed to be a factor in clinical efficacy (Shank et al., 1994). A recent study demonstrated that lacosamide potently inhibits human carbonic anhydrase isoforms with $K_i$ values between 300 and 5000 nM (Temperini et al., 2010). The $K_i$ for inhibition of the physiologically important type II isoform is 331 nM. In contrast, the $K_i$ of topiramate for the same isoform is 5 nM. As noted above, therapeutic blood concentrations of lacosamide are in the range 20–40 µM. Even accounting for a brain to plasma ratio of 0.553 (Koo et al., 2011), brain concentrations associated with therapeutic doses are expected to far exceed the $K_i$ for all carbonic anhydrase isoforms, suggesting that effects on the enzymes could contribute to therapeutic activity. However, tolerance ordinarily develops to the anticonvulsant activity of carbonic anhydrase inhibitors so that inhibition is not useful as a long-term epilepsy treatment strategy (Rogawski and Porter, 1990). Moreover, lacosamide does not exhibit side effects typical of other carbonic anhydrase inhibitors, including metabolic effects, raising doubt as to whether the inhibition observed in in vitro systems is clinically relevant.

Is collapsin response mediator protein 2 a target?

Collapsin response mediator proteins (CRMPs) are a family of five intracellular phosphoproteins implicated in neurotrophic signaling and neuronal outgrowth. Among the five members of the CRMP family, CRMP-2 is the most abundantly expressed in adult brain (Charrier et al., 2003). Changes in expression levels of CRMPs have been observed in patients with temporal lobe epilepsy (Čech et al., 2004; Wang et al., 2010a). Initial experiments identified an interaction between lacosamide and CRMP-2 using a proteomic fish-hook approach, suggesting that lacosamide binds to CRMP-2 with an apparent dissociation constant of approximately 5 µM (Beyreuther et al., 2007). Park and colleagues (2011) showed that lacosamide affinity baits bind stereoselectively to CRMP-2 in mouse brain membranes but this interaction could only be weakly competed with high millimolar concentrations of lacosamide, suggesting that the interaction is of weak affinity. More recent radioligand binding studies failed to detect specific [3H]lacosamide binding to isolated or membrane bound CRMP-2 expressed in mammalian cells or bacteria (Wolff et al., 2012).

In silico docking studies, where lacosamide was virtually docked to the CRMP-2 structure or biochemical experiments where lacosamide affinity bait analogs were attached to brain tissue suggested an interaction of lacosamide with the CRMP-2 protein (Wang et al., 2010a,b). One study found that CRMP-2 expression in neuronal model CAD (catecholamine A differentiated) cells modulates the effects of lacosamide on VGSC slow inactivation by reducing the number of available channels in the slow inactivated state (Dustrude et al., 2013). However, another recent study, where CRMP-2 was expressed in hippocampal neurons, showed the opposite effects, in that CRMP-2 expression increased the fraction of available channels in the presence of lacosamide (Wilson et al., 2012). The precise mechanistic link that may underlie these observations is not clear and the idea that CRMP-2 is able to modify the binding site for lacosamide on VGSCs remains speculative. Interestingly, over-expression of CRMP-2 affects the cell surface cycling of voltage-gated calcium and sodium channels (Chi et al., 2009; Brittain et al., 2011; Dustrude et al., 2013). However, lacosamide does not modulate channel cycling under such conditions (Wang et al., 2011) further suggesting that lacosamide does not directly interact with CRMP-2. Overall, there is no evidence that lacosamide directly binds to CRMP-2 but the possibility
of an indirect functional interaction has not been ruled out.

14-3-3 Protein

The 14-3-3 protein has also been identified as a novel potential binding partner for lacosamide using affinity bait analogs (Park et al., 2011). 14-3-3 proteins are cellular signaling molecules and alterations of different 14-3-3 isoforms have been reported in patients with temporal lobe epilepsy (Schindler et al., 2006). However, additional studies are required to clearly establish a molecular and functional link between 14-3-3 and lacosamide.

Conclusions

Lacosamide was introduced into clinical practice in 2008. In the relatively short time that the drug has been available, it has gained increasing acceptance, largely because of its efficacy, acceptable safety and tolerability profile and favorable pharmacokinetic properties, including a relatively long half-life allowing for twice-daily administration, good bioavailability, and low potential for clinically relevant drug–drug interactions (Stephen et al., 2011; Biton, 2012; Krauss et al., 2012). The drug is a member of a class of anticonvulsant compounds that Kohn refers to as functionalized amino acids (Choi et al., 1996), which originally gained attention because of their stereoselectivity, a property that is unusual for AEDs and suggested the existence of a specific protein target. Among the many functionalized amino acid analogs that were evaluated for activity in screening models, lacosamide was of particular interest because of its high oral potency in the mouse and rat MES test. Studies in a broad range of animal models have indicated that lacosamide has a spectrum of activity similar to that of the VGSC-blocking AEDs phenytoin, carbamazepine and lamotrigine. However, lacosamide differentiates itself by its apparent ability to selectively enhance the rate of entry of VGSCs into a slow inactivated state, distinct from the fast inactivated state stabilized by conventional VGSC-blocking AEDs. Lacosamide is the only clinically used AED that is known to alter VGSC slow inactivation without affecting fast inactivation. It thus acts by a differentiated molecular mechanism even though the molecular target through which it acts is shared by other AEDs. Given the shared molecular target, it is not surprising that lacosamide has a spectrum of activity in animal models similar to conventional VGSC-blocking AEDs. However, given the selective action of lacosamide on VGSCs slow inactivation, it is presumably able to terminate the prolonged, high frequency firing that occurs during seizure discharges, while having little effect on briefer runs of high frequency firing, including those that are associated with normal brain function. As a result, lacosamide may be especially able to discriminate between normal and pathological activity. AEDs that affect VGSC fast inactivation are expected to have less ability to discriminate in this way since they terminate much briefer trains of action potential firing and are therefore more likely to interfere with normal activity. Lacosamide’s block of persistent sodium current (INap) may also be a factor in its ability to protect against pathological activity without affecting normal brain function. These speculations require confirmation by quantitative approaches.

Disclosures

M.A.R. has served as a consultant for UCB and H.S.W. has served on the UCB Speaker’s Bureau; neither was compensated for the preparation of this article. C.W., A.M. and A.T. are employees of UCB.

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