Eslicarbazepine acetate (ESL) is a once daily antiepileptic drug (AED) approved by the European Medicines Agency (EMA), the Food and Drug Administration (FDA) and Health Canada as an adjunctive therapy in adults with partial-onset seizures (POS). In humans and in relevant animal laboratory species, ESL undergoes extensive first pass hydrolysis to its major active metabolite eslicarbazepine that represents ~95% of circulating active moieties. ESL and eslicarbazepine showed anticonvulsant activity in animal models. ESL may not only suppress seizure activity but may also inhibit the generation of a hyperexcitable network. Data reviewed here suggest that ESL and eslicarbazepine demonstrated the following in animal models: (1) the selectivity of interaction with the inactive state of the voltage-gated sodium channel (VGSC), (2) reduction in VGSC availability through enhancement of slow inactivation, instead of alteration of fast inactivation of VGSC, (3) the failure to cause a paradoxical upregulation of persistent Na\(^+\) current (\(I_{\text{NaP}}\)), and (4) the reduction in firing frequencies of excitatory neurons in dissociated hippocampal cells from patients with epilepsy who were pharmacoresistant to carbamazepine (CBZ). In addition, eslicarbazepine effectively inhibited high- and low-affinity hCa\(_{\text{V}3.2}\) inward currents with greater affinity than CBZ. These preclinical findings may suggest the potential for antiepileptogenic effects; furthermore, the lack of effect upon Kv7.2 outward currents may translate into a reduced potential for eslicarbazepine to facilitate repetitive firing.

**Abbreviations**

ADT, focal seizure threshold; AED, antiepileptic drug; CBZ, carbamazepine; CHO, Chinese hamster ovary; DGCs, dissociated dentate granule cells; EEG, electroencephalogram; ESL, eslicarbazepine acetate; \(I_{\text{NaP}}\), persistent Na\(^+\) current; \(I_{\text{NaT}}\), transient Na\(^+\) current; LCM, lacosamide; MES, maximal electroshock; OXC, oxcarbazepine; POS, partial-onset seizures; SE, status epilepticus; VGSC, voltage-gated sodium channel.

**Introduction**

Eslicarbazepine acetate (ESL) ((S)-10-acetoxy-10,11-dihydro-5H-dibenzo[b,f]azepine-5-carboxamide, with development code BIA 2-093) is a once daily antiepileptic drug (AED) that has been approved in 2009 by the European Medicines Agency and in 2013 by the Food and Drug Administration and Health Canada as an adjunctive therapy in adults' with partial-onset seizures (POS).

In humans, after oral administration, ESL undergoes extensive first pass hydrolysis to its major active metabolite eslicarbazepine that represents ~95% of circulating active moieties (Almeida and Soares-da-Silva 2007; Falcao et al. 2007; Almeida et al. 2008a,b, 2009; Maia et al. 2008; 2015).
Evidence from early preclinical studies suggested that ESL and eslicarbazepine may exert their anticonvulsant effects through blockade of voltage-gated sodium channels (VGSC) (Benes et al. 1999; Bonifacio et al. 2001; Cunha et al. 2002). Two of the concomitant AEDs most frequently used by the study population in the ESL phase III program (carbamazepine [CBZ] and lamotrigine) are also VGSC blockers (Walker et al. 2009). Suggestion has been made that combining AEDs with different mechanisms of action should be beneficial (Brodie and Yuen 1997). However, current knowledge on the mechanisms of action of various drugs is too limited to allow a rational application of this approach and, thus, AEDs are usually combined mainly on empirical grounds (Perucca 2009). Hence, the rationale was not to exclude CBZ or other VGSC blockers (except oxcarbazepine [OXC]), because it has some metabolic similarities with ESL [Almeida et al. 2008a; Maia et al. 2008]). From the adjunctive Phase III studies with ESL, ESL as an add-on treatment showed similar efficacy when added to subjects using CBZ as when added to subjects not using CBZ (Chung et al. 2014). The explanation for the efficacy of ESL as an adjunctive therapy in patients refractory to other VGSC blockers is certainly multifactorial, and includes pharmacokinetic/pharmacodynamic issues such as mechanisms of action that differ from VGSC blockade and the biopharmaceutical properties and pharmacokinetic profile of the ESL active entity, eslicarbazepine.

The present report assembles and reviews the available information on the pharmacodynamic properties of eslicarbazepine, namely its role as a VGSC blocker, and integrates it with information on other AEDs.

**Anticonvulsant Effects**

**General convulsant tests**

ESL and eslicarbazepine, the major active metabolite of ESL, were evaluated as anticonvulsants in the mouse maximal electroshock (MES), the 6-Hz psychomotor and Rotarod tests, and demonstrated distinctive anticonvulsant properties characterized by a wider (1.5- to 2.5-fold) protective index when compared to CBZ (Pires et al. 2011; Torrao et al. 2011). To assess anticonvulsant properties of ESL, eslicarbazepine and CBZ male NMRI mice were administered MES (50 mA, rectangular current: 0.6 msec pulse width, 0.4 sec duration, 50 Hz) via corneal electrodes connected to a constant current shock generator and the number of tonic convulsions recorded. ESL and CBZ demonstrated dose-dependent protection against MES-induced seizures with ED50 values (and 95% confidence intervals) of 23.0 (17.8, 28.3) mg/kg for ESL, 27.8 (18.9, 36.6) mg/kg for eslicarbazepine, and 13.5 (7.7, 19.2) mg/kg for CBZ. In the 6-Hz psychomotor seizure test, animals (n = 15) were administered a rectangular current (pulse: 0.2 msec, 6 Hz, 44 mA for 3 sec) via corneal electrodes connected to a constant current shock generator. The resulting seizures were scored 0 (absence of clonus), 1 (forelimb clonus, moderate), or 2 (bilateral forelimb clonus with rearing, high) during the first minute following current administration. ESL, eslicarbazepine, and CBZ exhibited a dose-dependent protection in forelimb seizure scores with ED50 values of 15.9 (7.7, 24.1) mg/kg for ESL, 12.1 (9.5, 14.8) mg/kg for eslicarbazepine, and 9.5 (3.3, 15.6) mg/kg for CBZ. The rotarod test was conducted in mice (n = 10 per group) trained to hold onto the rotarod apparatus until they maintain equilibrium up to 3 min while rotating at 15 rpm. The number of animals that drop off the rod was counted. CBZ and ESL were found to negatively affect motor coordination in a dose-dependent manner albeit with TD50 values for CBZ (110.2; 68.7, 289.0 mg/kg) that were much lower than for ESL (313.7; 274.0, 353.0 mg/kg) and eslicarbazepine (348.3; 296.1, 400.6 mg/kg). The corresponding protective indices (TD50/ED50) for CBZ (8.2 and 11.7, respectively, for the MES and 6 Hz tests) were markedly lower than those for ESL (13.8 and 19.7, respectively, for the MES and 6 Hz tests) and eslicarbazepine (12.5 and 28.7, respectively, of the MES and 6 Hz tests).

**Focal convulsant tests**

Another study determined the effect of ESL in the mouse amygdala kindling model of temporal lobe epilepsy. Male NMRI mice were stimulated once daily via an implanted depth electrode until 10 generalized seizures were elicited. ESL (100, 200, 300 mg/kg) was administered intraperitoneally 15 min before stimulation. Each ESL experiment was preceded by a vehicle control experiment in the same group of animals. ESL dose-dependently increased the focal seizure threshold (ADT), with effects statistically significant (P < 0.05) at 200 and 300 mg/kg. In 1 of the 10 animals receiving 200 mg/kg and in 7 of the 13 animals receiving 300 mg/kg, no seizure activity was observed until the maximum stimulation current of 1200 µA. In response to 200 and 300 mg/kg ESL, threshold increases reached >289% and >1319%, respectively. Seizure duration and after-discharge duration recorded at ADT were not significantly altered by ESL. ESL dose-dependently reduced seizure severity with a significant difference to the vehicle control experiment at 200 and 300 mg/kg. It
was concluded that ESL inhibited kindled seizures and protected against focal seizure activity in a kindling model. The effect of ESL on seizure severity suggests that it may interfere with seizure progression by inhibiting propagation of activity from the focus (Soerensen et al. 2011; Potschka et al. 2014).

Antiepileptogenic Effects

Bilateral corneal kindling

The effects of ESL, eslicarbazepine, and R-licarbazepine were evaluated on kindling epileptogenesis. In this study, NMRI female mice were kindled by bilateral corneal stimulation twice daily. The compounds ESL, eslicarbazepine, and R-licarbazepine were administered intraperitoneally 15 min before each kindling stimulation. Three dosages of ESL were tested (10, 30, and 100 mg/kg). The control group received injections of the corresponding vehicle solution. At dosages of 30 and 100 mg/kg ESL, the average number of stimulations to reach a fully kindled generalized seizure was increased by 217% and 280%, respectively. Administration of eslicarbazepine also had an inhibitory effect on the acquisition of kindling, whereas R-licarbazepine did not affect the number of stimulations necessary to induce a specific seizure stage, and did not exert any relevant effect on mean seizure severity during kindling progression. In conclusion, these data provide evidence of the anticonvulsant effect of ESL on POS in a kindling model. ESL may not merely suppress seizure activity but may also inhibit the generation of a hyperexcitable network (Pekcec et al. 2011; Potschka et al. 2014).

Hippocampal latrunculin A-induced chronic seizures

Latrunculin A microperfusion of the hippocampus induces acute epileptic seizures and long-term biochemical changes leading to spontaneous seizures. The effect of orally administered ESL on latrunculin A-induced acute and chronic seizures, and the changes in brain amino acid extracellular levels were described. Swiss mice hippocampus was continuously perfused with a latrunculin A solution (4 μmol/L, 1 μL/min, 7 h/day) with continuous electroencephalogram (EEG) and videotape recording for three consecutive days. Microdialysate samples were analyzed by HPLC and fluorescence detection of taurine, glycine, aspartate, glutamate, and gamma-aminobutyric acid (GABA). Thereafter, mice were continuously video monitored for 2 months to identify chronic spontaneous seizures or behavioral changes. Control EEG recordings (8 h) were performed in all animals at least once a week for a minimum of 1 month. Oral administration of ESL (100 mg/kg), previous to latrunculin A microperfusion, completely prevented acute latrunculin A-induced seizures as well as chronic seizures and all EEG chronic signs of paroxysmal activity. Hippocampal extracellular levels of taurine, glycine, and aspartate were significantly increased during latrunculin A microperfusion, while GABA and glutamate levels remained unchanged. ESL treatment reduced the increased extracellular taurine, glycine, and aspartate concentrations to basal levels and significantly reduced glutamate levels. Plasma and brain bioanalysis showed that ESL was completely metabolized within 1 h after administration to mainly eslicarbazepine, its major active metabolite. It is concluded that ESL treatment prevented acute latrunculin A-induced seizures as well as chronic seizures and all EEG chronic signs of paroxysmal activity, supporting a possible antiepileptogenic effect in mice (Sierra-Paredes et al. 2011, 2014).

Pilocarpine model of chronic epilepsy

Recently, we have also examined if ESL is also antiepileptogenic in the pilocarpine model of epilepsy, in which a single administration of the muscarinic agonist pilocarpine leads to the development of chronic epilepsy, and morphological and synaptic features similar to human temporal lobe epilepsy. CD-1 male mice were administered pilocarpine (300 mg/kg i.p.) to develop status epilepticus. Sham animals received injections of saline. After 1 week of recovery, epileptic mice were administered vehicle, 150 mg/kg or 300 mg/kg ESL p.o. once daily for 6 weeks. Nonepileptic sham mice received vehicle. Motor coordination (rotarod and wire test) and spontaneous locomotor behavior (open field test) were assessed 4 weeks after the end of ESL treatment. Cortical EEG telemetry monitoring was performed 8 weeks after the end of ESL treatment. Twelve weeks after the end of ESL, hippocampal slices were processed for Timm staining and mossy fiber sprouting were scored. EEG monitoring showed that transitory ESL treatment within the epileptogenic period caused a significant decrease in both the frequency (90% and 66% decrease for 150 and 300 mg/kg ESL versus vehicle, respectively) and duration (92% and 74% decrease for 150 and 300 mg/kg ESL as compared to vehicle, respectively) of epileptiform discharges. Conversely, 11% of the trains detected in vehicle-treated mice had a duration over 15 sec and are recognized to correspond to a pathologically epileptiform activity. In addition, vehicle-treated epileptic mice showed significant motor coordination impairment as compared to sham animals in the rotarod test. Treatment with 150 mg/kg ESL for 6 weeks resulted in significantly less coordination impairment as compared to vehicle-treated.
mice. Finally, transitory ESL treatment reduced the neuropathological hallmarks of chronic epilepsy. Timm staining of mossy fibers revealed significant mossy fiber sprouting into the inner dentate molecular layer of pilocarpine-injected animals compared with saline-injected controls. ESL treatment resulted in a significant decrease in mossy fiber sprouting. It was concluded that transitory ESL treatment attenuated both the functional and morphological sequelae of status epilepticus (SE), supporting a possible antiepileptogenic effect of ESL in the pilocarpine mouse model of chronic epilepsy (Pires et al. 2013; Döser et al. 2015).

**Pharmacodynamic/Pharmacokinetic Relationships**

In mouse, following the administration (p.o.) of 50 mg/kg ESL, a 97% protection against MES-induced seizures was observed (Pires et al. 2011). The levels of eslicarbazepine, the main active metabolite of ESL, in brain were 22 nmol/g total brain mass, which may be converted into an EC50 of 22 μmol/L (in total brain volume). Since eslicarbazepine has a strong preference to stick to tissue material in a ratio of about 50:1 and that such tissue constitutes only 20% of the total brain volume, it is expected that the effective concentration of eslicarbazepine in the organic fraction of the brain will be higher than that measured in the whole brain volume. The calculated LogP of eslicarbazepine is 1.72, according to the software package ADMET Predictor (SimulationsPlus, Inc., Lancaster, California, USA), which corresponds to an octanol:water partitioning coefficient of 52.5. In the brain, it is also expected that eslicarbazepine will associate preferentially with organic tissue (lipsids and proteins). After 72 h dehydration at 90°C, the water fraction in the mouse brain was found to be ~80% of the brain mass (or volume) and therefore, in an average mouse brain of 358 ± 8 mg (n = 12), water takes 281 ± 6 μL (n = 12), while the remaining 80 μL comprised cell structures, membranes, lipids, proteins, etc. As such, the concentration of eslicarbazepine in the organic fraction of the brain associated with effective 97% protection against MES-induced seizures was 102 μmol/L, which is 4.6 times higher than that measured directly in the total brain volume. However, eslicarbazepine does not show a strong affinity for binding (~30%) to nonspecific proteins and may be preferentially associated to membranes, which constitute a smaller fraction of the dry weight of the brain. Assuming that bilipid membranes constitute 10–12% of the total brain mass (Pratt et al. 1969; Mcllwain and Bachelard 1985; Banay-Schwartz et al. 1992), the corrected concentration of eslicarbazepine in the lipid fraction of the brain was ~183 μmol/L, which is 8.3 times higher than that measured directly in the total brain volume. The concentration of eslicarbazepine in plasma associated with a 97% protection against MES-induced seizures was found to be 32 μmol/L. In Table 1, the concentrations of eslicarbazepine in brain and in plasma in different epilepsy experimental models measured after oral or intraperitoneal administration are shown. Concentrations in brain tended to be higher than in plasma by a factor of 3.4–5.8, particularly when they represent $C_{max}$ concentrations (60 min after administration). This is an important aspect when in

### Table 1. Plasma and brain concentrations of eslicarbazepine after 15 or 60 min administration (i.p. or p.o.) of eslicarbazepine acetate (ESL) in the mouse maximal electroshock (MES) test, the mouse corneal kindling, the mouse amygdala kindling, or the mouse latrunculin A-induced chronic seizures.

| Ref. | Model                  | ESL dose mg/kg (route) | Time (min) | Plasma eslicarbazepine μmol/L | Brain (whole volume) Eslicarbazepine nmol/g | Brain (organic phase) Eslicarbazepine μmol/L | Brain (lipid fraction) Eslicarbazepine μmol/L |
|------|------------------------|------------------------|------------|------------------------------|---------------------------------------------|---------------------------------------------|-----------------------------------------------|
| Pires et al. (2011) | MES test               | 50 (p.o.)              | 60         | 31.5                         | 22.0                                        | 102                                         | 183                                           |
| Pekcec et al. (2011) | Corneal kindling        | 100 (i.p.)             | 60         | 168.1                        | 67.7                                        | 314                                         | 564                                           |
| Soerenesen et al. (2011) | Amygdala kindling      | 200 (i.p.)             | 15         | 236.2                        | 37.5                                        | 174                                         | 313                                           |
| Soorenensen et al. (2011) | Amygdala kindling      | 300 (i.p.)             | 15         | 282.8                        | 58.7                                        | 273                                         | 489                                           |
| Sierra-Paredes et al. (2014) | Latrunculin A-induced seizures | 100 (p.o.)      | 60         | 60.9                         | 36.4                                        | 169                                         | 303                                           |

The shadowed area for brain concentrations reflects the assumptions that eslicarbazepine is distributed mainly in the organic phase of the brain (that represents 20% of whole brain volume) or the lipid fraction (that represents 12% of whole brain volume), which are based on the findings that the aqueous phase in the mouse brain represents 20% of total volume and that eslicarbazepine’ LogP of is 1.72, which corresponds to an organic:aqueous partitioning coefficient of 52.5.
vivo/in vitro relationships are addressed. Eslicarbazepine concentrations in plasma or even cerebrospinal fluid (because of its aqueous nature) should not be perceived as they reflect the concentrations attained in the brain neuronal biophase (mainly constituted by bilipidic membranes), since a lipophilic compound is expected to attain higher concentrations in brain than in aqueous media. At this stage it is also important to underscore that, although there are some conflicting reports in the literature on this issue (Zhang et al. 2011), eslicarbazepine has been shown not to be a substrate to reverse transport by P-glycoprotein (Fortuna et al. 2012), which is a factor determining the brain availability of drugs. Eslicarbazepine intestinal transport in the absorptive (mucosal to serosal, M–S) and excretive (serosal to mucosal, S–M or efflux) directions were found to be of the same magnitude and insensitive to the P-glycoprotein inhibitor verapamil, in contrast to that observed with CBZ-epoxide, OXC, and R-licarbazepine (Fortuna et al. 2012). To assess whether differences in brain penetration of eslicarbazepine and R-licarbazepine were related to susceptibility for efflux through P-glycoprotein or multidrug resistance protein (MRP), mice were pretreated with verapamil or probenecid. Verapamil and probenecid failed to affect the eslicarbazepine brain-to-plasma concentration ratio (Almeida et al. 2009; Bialer and Soares-da-Silva 2012). By contrast, verapamil, but not probenecid, markedly increased the R-licarbazepine brain-to-plasma ratio. This indicates that eslicarbazepine is not a substrate for either P-glycoprotein or MRP, whereas R-licarbazepine is a substrate for P-glycoprotein but not for MRP (Almeida et al. 2009; Bialer and Soares-da-Silva 2012).

**Ion Channel Pharmacology**

**Sodium currents**

ESL, on its own, preferentially blocks VGSC in rapidly firing neurons (Bonifacio et al. 2001), but the in vivo effects of ESL may be limited to its extensive conversion into eslicarbazepine. Mechanistically, however, it is important to underline that the affinity of eslicarbazepine for VGSC in the resting state is about 15- to fivefold lower than that of CBZ, OXC, and R-licarbazepine, a feature that may translate into an enhanced inhibitory selectivity of eslicarbazepine for rapidly firing “epileptic” neurons over those with normal activity (Hebeisen et al. 2011). This was observed in a series of in vitro pharmacology studies which were conducted to investigate the effects of eslicarbazepine, R-licarbazepine, OXC, and CBZ on the resting and inactivated states of endogenous VGSC from N1E-115 mouse neuroblastoma cells. It was concluded that eslicarbazepine demonstrated a greater selectivity for the inactive state of VGSC, which is the common feature of the rapidly firing neurons, over their resting state as compared to CBZ, OXC, and R-licarbazepine (Hebeisen et al. 2011).

An in vitro electrophysiology evaluation determined the effects of eslicarbazepine, the major active metabolite of ESL, R-licarbazepine, CBZ, OXC, and lacosamide (LCM) on the fast and slow inactivated states of VGSC. The whole-cell patch clamp technique was used to investigate the effects of eslicarbazepine, R-licarbazepine, CBZ, OXC, and LCM on sodium channels endogenously expressed in N1E-115 mouse neuroblastoma cells. The relative gene expressions as compared to NaV1.1 were as follows: NaV1.1: NaV1.2: NaV1.3: NaV1.6: NaV1.7 = 1.00: 1.31: 1.37: 2.17: 1.15. At a concentration of 250 μmol/L, the voltage dependence of the fast inactivation was not influenced by eslicarbazepine, whereas LCM, CBZ, and OXC shifted the V0.5 value by −4.8, −12.0, and −16.6 mV, respectively. Eslicarbazepine- and LCM-treated fast inactivated channels recovered similar to control conditions, whereas CBZ- and OXC-treated channels required longer pulses to recover. CBZ shifted the voltage dependence of the slow inactivation by −4.6 mV, whereas for eslicarbazepine and LCM the shift of the voltage dependence of the slow inactivation (V0.3) was −31.2 and −53.3 mV, respectively. For eslicarbazepine, LCM, CBZ, and OXC, the affinity to the slow inactivated state was 5.9, 10.4, 1.7, and 1.8 times higher than to the channels in the resting state, respectively. It was concluded that eslicarbazepine does not share with CBZ and OXC the ability to alter fast inactivation of VGSC. Both eslicarbazepine and LCM reduce VGSC availability through enhancement of slow inactivation, but LCM demonstrated higher interaction with VGSC in the resting state, with fast inactivation gating and shorter time to enter in the slow inactivated state (Aires et al. 2012; Bonifacio et al. 2012; Soares-da-Silva and Hebeisen 2012; Hebeisen et al. 2015).

Another in vitro electrophysiology evaluation study was aimed to compare the effects of eslicarbazepine, LCM, OXC, and CBZ on the fast and slow inactivated states of the human NaV1.3 sodium channels expressed in Chinese hamster ovary (CHO) CHO-K1 cells. The whole-cell patch clamp technique was used to investigate the effects of eslicarbazepine, LCM, OXC, and CBZ (all at 250 μmol/L), in conditions of fast and slow inactivation of sodium currents. In the fast inactivation protocol, the cells were depolarized to potentials between −120 and +10 mV for 500 msec (10 mV increments), followed by a 10 msec test pulse to −10 mV before stepping back to the holding potential of −80 mV (as shown below).

![Voltage Deposition of Fast and Slow Inactivation](image)

To evaluate the recovery from the fast inactivated state, a test pulse of 20 msec to −10 mV was applied. After
100 msec at the resting potential of −100 mV, the cell was depolarized for 500 msec to −10 mV. After a variable pulse to −100 mV, a second test pulse was applied. The variable pulse lasts for 1, 3, 10, 30, 100, 300, and 1000 msec (as shown below).

![Variable pulse](image)

To evaluate the entry into slow inactivated state, a test pulse of 20 msec to −10 mV was applied. After 1.5 sec at the resting potential of −90 mV, the cell was depolarized for 0, 10, 20, and 30 sec to −20 mV. After a 1.5 sec pulse to −90 mV, a second test pulse was applied (as shown below).

![Second test pulse](image)

In the slow inactivation protocol, the cells were depolarized to potentials between −120 and +50 mV for 5000 msec (10 mV increments), followed by a 1-sec pulse to −80 mV and the test pulse to −10 mV before stepping back to the holding potential of −80 mV (as shown below).

![Third test pulse](image)

To evaluate the recovery from the slow inactivated state, sodium channels were slow inactivated by a 10 sec pulse to −20 mV from a holding potential of −80 mV. To analyze the recovery from the slow inactivated state, test pulses (−10 mV, 50 msec) were applied every 1.5 sec for a duration of 75 sec (50 pulses) (as shown below).

![Fourth test pulse](image)

To determine IC$_{50}$s in the slow inactivated state, a test pulse of 20 msec to −10 mV was applied. After 1.5 sec at the resting potential of −80 mV, the cell was depolarized for 10 sec to −20 mV. After a 1.5 sec pulse to −90 mV, a second test pulse was applied in the presence of various concentrations of test drugs (as shown below).

![Fifth test pulse](image)

Steady state fast inactivation curves were shifted in the hyperpolarizing direction by OXC (−8.97 mV) and CBZ (−14.35 mV), but not by eslicarbazepine (−0.83 mV) and LCM (−1.90 mV) (Table 2). Eslicarbazepine- and LCM-treated fast inactivated channels recovered similar to control conditions (∆t = 3.3 msec), whereas OXC- and CBZ-treated channels required longer pulses to recover (∆t = 391.35 and 1291.47 msec, respectively) (Fig. 1). The time constant for entering the slow inactivated state of NaV1.3 channels was reduced by eslicarbazepine and LCM (from 20.29 sec to 14.02 sec and 10.91 sec, respectively), but was not affected by CBZ and OXC. OXC and CBZ shifted by −1.99 and −4.64 mV, respectively, the voltage dependence of the slow inactivation, whereas for eslicarbazepine and LCM the shift V$_{0.5}$ was −14.40 and −16.90 mV, respectively. For eslicarbazepine, LCM, OXC, and CBZ, the affinity to the slow inactivated state was 2.6, 4.8, 2.0, and 1.6 times higher than to the channels in the resting state, respectively. In conclusion, both eslicarbazepine and LCM preferentially enhanced the slow inactivation of human Nav1.3. Eslicarbazepine did not share with OXC and CBZ the ability to alter fast inactivation of human NaV1.3, but rather appeared to modify the kinetics and voltage dependence of slow inactivation states (Hebeisen and Soares-da-Silva 2013; Soares-da-Silva and Heibesen 2013).

The fundamental properties that enable sodium channels to carry out their physiological roles include rapid, voltage-dependent activation, which often opens the channel, and inactivation (Vilin and Ruben 2001). Inactivation closes the channel pore and prevents it from reopening until the cell is hyperpolarized. This makes the cell refractory to firing during a long depolarization (Goldin 2003; Eijkelkamp et al. 2012). There are at least two distinct kinetic classes of inactivation, termed fast and slow. Fast inactivation is important for action potential repolarization. Fast inactivation of Na$^+$ channels is thought to contribute to action potential termination and regulation of the refractory period. Fast inactivation in VGSC occurs by a “hinged lid” mechanism in which a cytoplasmic region (the inactivating particle) occludes the pore (Goldin 2003). Slow inactivation is a separate process that does not involve the inactivating particle and may result from a structural rearrangement of the pore (Vilin and Ruben 2001). Slow inactivation, on the other hand, may play a role in regulating excitability, such as by modulating burst discharges. In fact, this modulation appears to be complex since slow inactivation not only depends on resting potential but also on previous history of action potential firing. Slow inactivation occurs on a much slower time scale of seconds and is a more complex process involving amino acids lining the S6 segments. Mechanistically, fast inactivation has been shown to involve the short intracellular loop between domains III and IV; although additionally sites within IV S6 and within the S4-S5 loop of both domains III and IV have also been implicated. Slow inactivation is thought to contribute to overall membrane excitability by increasing action potential thresholds, thereby limiting action potential burst durations and importantly, limiting the propagation of action potentials within dendrites. Therefore, modulation of Na$^+$ channel availability through enhancement of slow inactivation by either endogenous or
pharmacological methods could have significant effects on membrane excitability, particularly during epileptic seizures. Amino acids located in the P loop form an outer ring of charges that were found to be associated with slow inactivation (Xiong et al. 2003). The data shown here indicate that CBZ does not significantly change any

Table 2. Midpoint of the inactivation curve ($V_{0.5}$) values obtained from fast and slow inactivation curves.

| Test item (250 μmol/L) | Fast inactivation | Slow inactivation |
|------------------------|-------------------|-------------------|
|                        | $V_{0.5}$ (mV)    | Individual shifts (mV) | $V_{0.5}$ (mV) | Individual shifts (mV) |
| Control (bath)         | $-39.85 \pm 2.84$ | $-0.83 \pm 1.04$ | $-12.21 \pm 2.59$ | $-14.40 \pm 2.88$ |
| Eslicarbazepine        | $-40.68 \pm 3.10$ |                     | $-26.61 \pm 2.74$ |                     |
| Control (bath)         | $-51.64 \pm 3.64$ | $-1.90 \pm 0.87$  | $-20.10 \pm 2.37$ | $-16.94 \pm 4.79$  |
| Lacosamide             | $-53.54 \pm 3.95$ |                     | $-37.04 \pm 2.72$ |                     |
| Control (bath)         | $-58.54 \pm 3.95$ | $-13.80 \pm 1.20^*$| $-16.92 \pm 0.98$ | $-4.64 \pm 2.26^*$ |
| Carbamazepine          | $-72.33 \pm 5.04$ |                     | $-21.56 \pm 3.23$ |                     |
| Control (bath)         | $-51.32 \pm 2.18$ | $-4.62 \pm 1.34$  | $-17.48 \pm 3.04$ | $-1.99 \pm 2.33^*$ |
| Oxcarbazepine          | $-55.95 \pm 2.91$ |                     | $-19.47 \pm 4.55$ |                     |

Values are mean ± SEM of $n = 3$–6. Significantly different from corresponding values for eslicarbazepine ($^*P < 0.05$).

Figure 1. Fast inactivation curves obtained in the presence of 250 μmol/L of (A) eslicarbazepine, (B) lacosamide, (C) carbamazepine, or (D)oxcarbazepine and (E) recovery from fast inactivated state in control conditions or in the presence of 250 μmol/L of eslicarbazepine, lacosamide, carbamazepine, or oxcarbazepine. Results are mean ± SEM ($n = 3$–5). The relative current amplitudes were normalized to the current amplitude of the longest recovery time (4262 msec). Results are mean ± SEM ($n = 3$–6).
characteristics of slow inactivation. Phenytoin, CBZ, and lamotrigine were demonstrated to block the pore of VGSCs by binding via aromatic-aromatic interaction to the side chain of a tyrosine and phenylalanine located in the IV S6 helix (Lipkind and Fozzard 2010). As eslicarbazepine and LCM may block the channel pore almost only during slow inactivation, it is likely that they may bind at a different position to the channel protein.

Additional in vitro pharmacology studies to investigate the effects of eslicarbazepine on rat Na\textsubscript{V}1.2, human Na\textsubscript{V}1.3, human Na\textsubscript{V}1.7, and human Na\textsubscript{V}1.8 sodium channels expressed in CHO cells were conducted. With the exception of CBZ, the potency of inhibition sodium currents through the rat Na\textsubscript{V}1.2 sodium channel was highly sensitive to the holding potential, increasing with depolarization, but the affinity of eslicarbazepine was ~two- to threefold lower than was highly sensitive to the holding potential, increasing with depolarization, but the affinity of eslicarbazepine was ~two- to threefold lower than that of OXC and CBZ in more depolarized conditions. CBZ was endowed with the potency to inhibit inward Na\textsubscript{V}1.2 sodium currents at ~80 and ~70 mV holding potentials. It was concluded that eslicarbazepine demonstrated a greater selectivity of the inactive state of Na\textsubscript{V}1.2 sodium channels, which is the common feature of the rapidly firing neurons, over their resting state as compared to CBZ and OXC (Pires et al. 2012). In the case of human Na\textsubscript{V}1.3 sodium channel expressed in CHO cells, the potency of inhibition was highly sensitive to the holding potential, increasing with depolarization, but the affinity of eslicarbazepine was ~threefold lower than CBZ and OXC in more depolarized conditions. All compounds demonstrated a much higher affinity for the inactivated state of the channel, but the affinity of eslicarbazepine and R-licarbazepine for VGSCs in the resting state was about twofold lower than that of CBZ and OXC. It was concluded that eslicarbazepine demonstrated a greater selectivity of the inactive state of Na\textsubscript{V}1.3 sodium channels, which is the common feature of the rapidly firing neurons, over their resting state as compared to CBZ and OXC (Wright et al. 2012). In the case of human Na\textsubscript{V}1.7 and Na\textsubscript{V}1.8 sodium channel expressed in CHO cells, at ~80 mV holding potential, eslicarbazepine and R-licarbazepine were equipotent in inhibiting inward sodium currents through Na\textsubscript{V}1.7 and Na\textsubscript{V}1.8 channels. However, the affinity of eslicarbazepine for the inactivated state of Na\textsubscript{V}1.7 and Na\textsubscript{V}1.8 channels was 10 to 2 times higher than that for R-licarbazepine. On the other hand, both compounds demonstrated a 10 to 5 times higher affinity for the inactivated state versus the resting state of the Na\textsubscript{V}1.7 channels, when compared to Na\textsubscript{V}1.8 channels. It was concluded that eslicarbazepine had a greater selectivity of the inactive state of Na\textsubscript{V}1.7 and Na\textsubscript{V}1.8 sodium channels, when compared with R-licarbazepine (Hebeisen et al. 2012).

Neuronal excitability is crucially determined by Na\textsuperscript{+}-mediated currents. The fast upstroke of the action potential is mediated by the transient Na\textsuperscript{+} current (I\textsubscript{NaT}) with its fast activation and inactivation. The inactivation, however, is not complete. A small fraction of the channel remains open even during prolonged depolarization. This persistent Na\textsuperscript{+} current (I\textsubscript{NaP}) activates in the subthreshold voltage range and thus it is thought to play a role as an important modulator of membrane excitability. In mice lacking β1 subunits, CBZ induced a hyperpolarizing shift in voltage-dependent activation, leading to a pronounced increase in I\textsubscript{NaP} at subthreshold voltages, while classical effects such as the use-dependent reduction in I\textsubscript{NaP} and effects on I\textsubscript{NaT} voltage dependence of inactivation remained unaltered. This paradoxical effect of CBZ on I\textsubscript{NaP} resulted in a complete loss of efficacy in reducing repetitive firing (Uebachs et al. 2010). In mice lacking β1 subunits, CBZ caused a paradoxical upmodulation of I\textsubscript{NaP} in the subthreshold range, leading to a failure in affecting neuronal firing (Uebachs et al. 2010). To determine the effects of eslicarbazepine on I\textsubscript{NaP} and the role of β subunits on the Na\textsuperscript{+} channel it was decided to use a mouse line lacking either the β1 or the β2 subunit, encoded by the Scn1b or Scn2b gene, respectively. Whole-cell patch clamp recordings were performed on CA1 neurons in hippocampal slices under control conditions and application of 300 μmol/L eslicarbazepine (Döser et al. 2013a, 2014). I\textsubscript{NaP} in acutely isolated CA1 neurons and repetitive firing in 300 μm hippocampal slices of mice were examined. Eslicarbazepine was found to significantly reduce maximal I\textsubscript{NaP} conductance and efficiently decrease the firing rate in wild-type mice. Eslicarbazepine did not cause a paradoxical upregulation of I\textsubscript{NaP} in Scn1b null mice. Consequently, the effects of eslicarbazepine on repetitive firing were maintained in these animals. These results indicate that eslicarbazepine exerted effects on I\textsubscript{NaP} similar to those known for CBZ (see Uebachs et al. 2010). However, in animals lacking the β1 Na\textsuperscript{+} channel subunit, these effects were maintained. Therefore, eslicarbazepine may potentially overcome a previously described putative mechanism of resistance to established Na\textsuperscript{+}-acting AEDs (Döser et al. 2013a, 2014).

Loss of use-dependent block of transient Na\textsuperscript{+} channels is a suggested key mechanism underlying pharmacoresistance to CBZ at the cellular level, both in human and experimental epilepsy. This study was aimed to determine the activity of eslicarbazepine in modulating transient Na\textsuperscript{+} channels in epileptic tissue from patients with therapy refractory seizures. Whole-cell patch clamp recordings were performed on dissociated granule cells from the human hippocampus obtained from therapy refractory
epileptic patients \( n = 26 \) under control conditions and after application of CBZ and eslicarbazepine. Consistent with previous published observations, only a slight but significant slowing of fast recovery was observed upon CBZ application (mean ± SEM, in msec): \( \tau = 11.0 ± 1.1 \) and 11.7 ± 1.7 before and during application of 100 \( \mu \)mol/L CBZ, respectively, \( n = 8, P = 0.05 \). A subsequent application of eslicarbazepine exerted a pronounced and significant additional slowing of fast recovery rates \( \tau = 21.8 ± 3.0 \) during application of 300 \( \mu \)mol/L eslicarbazepine. The effects of eslicarbazepine were concentration dependent: 57.9 ± 8.6%, 120.0 ± 23.5%, and 121.1 ± 21.7% increase following application of 30, 100, and 300 \( \mu \)mol/L eslicarbazepine, respectively. Accordingly, eslicarbazepine significantly inhibited neuronal firing in dissociated granule cells from human hippocampus (DGCs). In conclusion, these results suggest that eslicarbazepine exerted use-dependent effects in DGCs from patients with pharmacoresistant epilepsy resulting in reduced firing frequencies of excitatory neurons, and may potentially overcome a cellular resistance mechanism to conventional AEDs. This does not preclude additional effects of eslicarbazepine on other properties of sodium channels, that is, slow inactivation processes (Döser et al. 2013c, 2015).

A study in the pilocarpine model of temporal lobe epilepsy compared the effects of CBZ and eslicarbazepine on granule cells isolated from brain slices of control animals to those of animals who experienced SE following intraperitoneal administration of pilocarpine and who subsequently showed spontaneous seizures. Whole-cell patch clamp recordings were performed on dissociated dentate granule cells (DGCs) from the hippocampus obtained from control and pilocarpine-treated rats under control conditions and after application of either CBZ or eslicarbazepine. Eslicarbazepine reduced the maximal conductance of \( I_{NaT} \) in a concentration-dependent manner without effect on the voltage dependence of activation in DGCs from both control and SE-experienced rats. CBZ slowed the recovery from inactivation in granule cells from control, but not from those of SE-experienced rats. When applying 300 \( \mu \)mol/L eslicarbazepine to granule cells from control rats, it slowed the time course of recovery similar to CBZ. The effects of eslicarbazepine on the time course of recovery were more pronounced in DGCs from epileptic rats compared to control rats. Eslicarbazepine was equally effective in inhibiting firing of DGCs in control versus pilocarpine-treated rats. In contrast, the effects of CBZ on the maximal firing frequency were significantly reduced in chronic experimental epilepsy. In conclusion, eslicarbazepine exerted use-dependent effects resulting in reduced firing frequencies of excitatory neurons in a preclinical model of pharmacoresistant epilepsy and may potentially overcome a cellular resistance mechanism to conventional AEDs (Döser et al. 2013b, 2015).

### Calcium currents

Distinctive properties of eslicarbazepine over CBZ also include 10- to 60-fold higher potency for the blockade of low- and high-affinity hCa,3.2 inward currents (Brady et al. 2011), being devoid of effects upon hCa,2.1 inward currents, whereas CBZ inhibited hCa,2.1 calcium peak currents (Bonifacio et al. 2013). CaV2.1 calcium (P/Q-type) channels play a prominent role in initiating action potential-evoked neurotransmitter release at central nervous system synapses and impaired function of brain CaV2.1 may have a central role in the pathogenesis of certain cases of primary generalized epilepsy (Imbrici et al. 2004; Kullmann 2010; Pietrobon 2010). T-type calcium channels (CaV3.2) are critically important in controlling the excitability of the postsynaptic compartment of neurons, both in normal and epileptic neurons. Transcriptional induction of CaV3.2 is a critical step in epileptogenesis and neuronal vulnerability (Sanabria et al. 2001; Su et al. 2002; Becker et al. 2008). The whole-cell patch clamp technique was used to investigate the effects of eslicarbazepine and CBZ on hCa,2.1 and hCa,3.2 calcium channels stably expressed in CHO and HEK 293 cells, respectively. After formation of a Gigaohm seal between the patch electrodes and individual CaV,2.1 stably transfected CHO cells (pipette resistance range: 2.0–7.0 MΩ; seal resistance range: >1 GΩ) the cell membrane across the pipette tip were ruptured to assure electrical access to the cell interior (whole-cell patch configuration). In case the quality of the seal was poor, the process of seal formation was repeated with a different cell and a new pipette. As soon as a stable seal was established CaV,2.1 currents were measured upon depolarization of the cell membrane to +10 mV for 500 msec from a holding potential of −80 mV. This voltage protocol (as shown below) was run at least 20 times at intervals of 10 sec. If current density was judged to be too low for measurement, another cell was recorded.

![Voltage protocol](image.png)

Once control recordings were accomplished, cells were continuously perfused with a bath solution containing the test item. During wash-in of the test item, the voltage protocol indicated above was run continuously again at 10-sec intervals until the steady state level of block will be reached (Uchino et al. 2005). As soon as a stable seal was established calcium inward peak currents were measured upon depolarization of the cell membrane to −25 mV for 50 msec from a holding potential of −80 mV. This voltage protocol (as shown below) was run at intervals of 10 sec until stabilization of evoked CaV,3.2 calcium
GABA currents recorded in Ltk cells stably transfected with the test compounds on GABA receptors. Eslicarbazepine block of high affinity occurred with an IC50 of 0.43 and calcium peak currents, but CBZ affected hCav3.2 calcium currents (IC50 was reached. Complete cumulative dose–response analysis was accomplished per cell. Eslicarbazepine and CBZ were tested (0.3–1000 μmol/L; n = 3–5 cells) on hCa2,1 and hCa3,2 currents upon depolarization of the cell membrane to −10 or −25 mV for 500 msec or 50 msec from a holding potential of −80 mV, respectively. Ca2,1 and Ca3,2 blockers cobalt chloride, valproic acid, and mibefradil were used as reference (Fig. 2). Eslicarbazepine did not affect hCa2,1 calcium peak currents (IC50 = 7836.80 μmol/L), but CBZ inhibited hCa2,1 calcium peak currents (IC50 = 452.46 μmol/L). Eslicarbazepine potently inhibited hCa3,2 calcium peak currents, but CBZ affected hCav3.2 calcium peak currents at much higher concentrations (Fig. 2). A block of high affinity occurred with an IC50 of 0.43 and 27.10 μmol/L for eslicarbazepine and CBZ, respectively. A further block occurred at higher concentrations of the test agents, with an IC50 of 62.61 μmol/L for eslicarbazepine and 711.20 μmol/L for CBZ, respectively. These results demonstrated that eslicarbazepine effectively inhibits high- and low-affinity hCa3,2 inward currents with greater affinity than CBZ. These findings may have implications for the antiepileptic activity of ESL, namely on potential antiepilepticogenic effects (Becker et al. 2008).

Potassium currents

Activation of voltage-gated potassium channels ( Kv7/M) during the initial stages of an action potential discharge suppresses later action potentials. Inhibition of channel activity strongly enhanced repetitive firing (Brown andPassmore 2009). Kv7.2 knockout mice have a reduced electroconvulsive threshold and increased sensitivity to convulsing agents (Peters et al. 2005). The whole-cell patch clamp technique was used to investigate the effects of eslicarbazepine and CBZ on hKv7.2 potassium channels stably expressed in CHO cells (Soares-da-Silva et al. 2011). Cells were transfected with the human KCNQ2 cDNA using lipofectamine according to Invitrogen’s standard protocol and 24–48 h later used for electrophysiological experiments. A stable cell line was obtained after G418 selection. Kv7.2 outward currents were measured at the end of a depolarizing pulse of +20 mV or for 500 msec from a holding potential of −80 mV. This voltage protocol was run at intervals of 10 sec until stabilization of evoked potassium currents. Once control recordings were accomplished and after steady state, cells were perfused with bath solution containing eslicarbazepine, CBZ, or the reference compound XE991, a known Kv7-blocker. Vehicle was 0.2% DMSO. Eslicarbazepine had no inhibitory effect on Kv7.2 currents, while CBZ reduced the current amplitudes in a concentration-dependent manner with an IC50 value of 571.8 μmol/L (Fig. 4). The IC50 value determined for XE991 was 1.3 μmol/L. The obtained data demonstrated that eslicarbazepine differed from CBZ by the lack of inhibitory effects upon Kv7.2 outward currents. While preliminary, these results may translate into a reduced potential for eslicarbazepine to facilitate repetitive firing, which is apparently not the case with CBZ.

NMDA/AMPA currents

Altered glutamate-mediated excitatory neurotransmission may play a role in chronic experimental and human
epilepsy (Isokawa and Levesque 1991; Martin et al. 1992). The whole-cell patch clamp technique was used to study the effect of eslicarbazepine and CBZ on the submaximal NMDA-receptor-mediated and AMPA-receptor-mediated currents recorded from, respectively, HEK cells expressing the GRIN1/GRIN2B subunits and CHO cells expressing

Figure 2. Inhibition concentration–response curves for the blockade of (A) hCa.2.1 and (B) hCa.3.2 currents by eslicarbazepine and carbamazepine, and representative current traces of the effect of test compounds on (A) hCa.2.1 and (B) hCa.3.2 calcium channels. Data represent mean ± SEM (n=3–9).
the human GRIA1 receptor subtype (Bulling et al. 2011). HEK cells were stably transfected with human GRIN1 receptor (variant NR1-3) and transiently transfected with human GRIN2B receptor cDNAs and CHO cells were transiently transfected with human GRIA1 cDNA. Eslicarbazepine and CBZ inhibited NMDA receptor-mediated currents in a concentration-dependent manner with IC$_{50}$ values of 1232 and 214 µmol/L, respectively (Fig. 5A). The IC$_{50}$ value of 7 µmol/L obtained for D-AP5 (d-(-)-2-amino-5-phosphonopentanoic), a negative allosteric NMDA receptor modulator, is consistent with its known pharmacological profile. Regarding AMPA currents, neither eslicarbazepine nor CBZ significantly changed AMPA currents. NBQX (2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide), a known negative allosteric AMPA modulator, reduced the currents with an IC$_{50}$ of 19 µmol/L. Eslicarbazepine was less potent than CBZ in inhibiting NMDA receptor currents and none of the compounds affected AMPA currents (Bulling et al. 2011).

Table 3. Effects of eslicarbazepine, carbamazepine, and bicuculline on GABA inward current stimulation measured in Ltk cells stably transfected with α1β2γ2, α2β2γ2, α3β2γ2, or α5β2γ2 GABA receptors.

| Compound   | µmol/L | α1β2γ2 | Mean ± SEM | n  | α2β2γ2 | Mean ± SEM | n  | α3β2γ2 | Mean ± SEM | n  | α5β2γ2 | Mean ± SEM | n  |
|------------|--------|--------|-----------|----|--------|-----------|----|--------|-----------|----|--------|-----------|----|
| Vehicle    | 96.2 ± 3.7 | 97.6 ± 3.6 | 100.3 ± 5.9 | 6  | 95.3 ± 4.6 | 6  | 95.3 ± 4.6 | 6  |
| Eslicarbazepine | 50      | 103.3 ± 2.0 | 91.6 ± 2.7 | 5  | 95.6 ± 4.8 | 5  | 95.2 ± 2.2 | 5  |
|            | 100     | 95.9 ± 1.0 | 97.0 ± 2.7 | 5  | 97.9 ± 4.2 | 5  | 93.9 ± 0.6 | 5  |
|            | 250     | 97.8 ± 1.1 | 88.6 ± 3.2** | 5  | 96.4 ± 2.2 | 5  | 93.5 ± 1.8 | 5  |
|            | 500     | 99.4 ± 0.8 | 89.8 ± 4.0 | 5  | 91.6 ± 5.6 | 5  | 89.7 ± 3.4 | 5  |
| Carbamazepine | 50      | 104.3 ± 5.0 | 97.9 ± 3.3 | 5  | 93.3 ± 4.1 | 6  | 103.8 ± 1.5 | 6  |
|            | 100     | 117.4 ± 4.9 | 111.4 ± 2.7 | 5  | 112.8 ± 5.4 | 6  | 103.8 ± 4.5 | 6  |
|            | 250     | 115.7 ± 5.3 | 109.0 ± 7.6 | 5  | 131.1 ± 9.4 | 6  | 93.6 ± 4.3 | 6  |
|            | 500     | 132.4 ± 5.3* | 103.6 ± 7.6 | 5  | 120.3 ± 11.8 | 6  | 80.7 ± 5.9 | 6  |
|            | 1000    | 150.3 ± 15.5* | 104.2 ± 2.7 | 5  | 166.7 ± 21.0* | 6  | 63.0 ± 4.5* | 6  |
| Bicuculline | 0.1     | 70.5 ± 3.2** | 62.7 ± 2.6* | 5  | 66.1 ± 3.1 | 5  | 60.2 ± 3.5* | 5  |
|            | 1       | 24.3 ± 1.7* | 16.4 ± 2.2* | 5  | 25.8 ± 5.1* | 5  | 10.3 ± 1.8* | 5  |
|            | 3       | 8.2 ± 1.0* | 2.2 ± 1.1* | 5  | 10.5 ± 1.4* | 5  | 5.9 ± 2.5* | 5  |
|            | 30      | 0.4 ± 0.5* | 1.9 ± 1.4* | 5  | 3.1 ± 1.4* | 5  | 1.7 ± 0.7* | 5  |

Vehicle was 0.4% DMSO.
Significantly different from vehicle (*P < 0.01; **P < 0.05).

Figure 3. Representative current traces at α1β2γ2 GABA receptors.
Glycine serves as an important mediator of synaptic inhibition, and can evoke inhibition of the neuronal activity by activating GlyR\textsubscript{a3} glycine receptor. Altered glycine-mediated neurotransmission may play a role in chronic experimental and human epilepsy (Wang et al. 2006; Eichler et al. 2008). The whole-cell patch clamp technique was used to investigate effects of eslicarbazepine and CBZ on GlyR\textsubscript{a3} receptor-mediated inward currents in CHO cells stably expressing the \(\alpha3\) glycine receptor. Eslicarbazepine and CBZ were tested at concentrations of 50, 100, 250, 500, and 1000 \(\mu\text{mol}/\text{L}\) \((n = 5 \text{ cells})\) together with glycine (150 \(\mu\text{mol}/\text{L}\)) to elicit submaximal currents. Eslicarbazepine and CBZ inhibited GlyR\textsubscript{a3} receptor-mediated inward currents in a concentration-dependent manner, with IC\textsubscript{50} values of 1186 and 441 \(\mu\text{mol}/\text{L}\), respectively (Fig. 5B). Strychnine, a glycine channel blocker, reduced the GlyR\textsubscript{a3} inward currents (Fig. 5B) with an IC\textsubscript{50} value of 52 nmol/L, which is consistent with its known pharmacological profile. The reduced potency of eslicarbazepine in inhibiting glycine GlyR\textsubscript{a3} receptor-mediated inward currents, when

**Glycine currents**

Glycine serves as an important mediator of synaptic inhibition, and can evoke inhibition of the neuronal activity by activating GlyR\textsubscript{a3} glycine receptor. Altered glycine-mediated neurotransmission may play a role in chronic experimental and human epilepsy (Wang et al. 2006; Eichler et al. 2008). The whole-cell patch clamp technique was used to investigate effects of eslicarbazepine and CBZ on glycine channels in CHO cells stably expressing the \(\alpha3\) glycine receptor. Eslicarbazepine and CBZ were tested at concentrations of 50, 100, 250, 500, and 1000 \(\mu\text{mol}/\text{L}\) \((n = 5 \text{ cells})\) together with glycine (150 \(\mu\text{mol}/\text{L}\)) to elicit submaximal currents. Eslicarbazepine and CBZ inhibited glycine GlyR\textsubscript{a3} receptor-mediated inward currents in a concentration-dependent manner, with IC\textsubscript{50} values of 1186 and 441 \(\mu\text{mol}/\text{L}\), respectively (Fig. 5B). Strychnine, a glycine channel blocker, reduced the GlyR\textsubscript{a3} inward currents (Fig. 5B) with an IC\textsubscript{50} value of 52 nmol/L, which is consistent with its known pharmacological profile. The reduced potency of eslicarbazepine in inhibiting glycine GlyR\textsubscript{a3} receptor-mediated inward currents, when

**Figure 4.** Representative traces for \(K\textsubscript{7.2}\) currents in the absence and the presence of DMSO, eslicarbazepine, carbamazepine, and XE991 and inhibition concentration–response curves of relative \(K\textsubscript{7.2}\) current amplitudes by eslicarbazepine, carbamazepine, and XE991 in CHO transfected cells. Data represent mean ± SEM \((n = 5 \text{ cells})\).
compared with CBZ, may translate into a better safety profile for ESL (Wright et al. 2011).

Receptors, Enzymes, and Transporters

The interaction of eslicarbazepine, the main active metabolite of ESL, with potential biological targets was also evaluated (Loureiro et al. 2012). Displacement of binding of specific ligands or substrates for 95 human G-protein coupled and ligand gated receptors, enzymes, and transporters was tested in the presence of 400 μmol/L eslicarbazepine (Table 4). In the presence of 400 μmol/L eslicarbazepine, no relevant interactions were observed with receptors (acetylcholine, adenosine, adrenergic, angiotensin II, cannabinoid, cholecystokinin, dopamine, glutamate, glycine, histamine, muscarinic, nicotinic, purinergic, serotonin, and sigma) or transporters (adenosine, dopamine, norepinephrine, and serotonin). Glutamate transporters (glutamate, GABA, and glycine) were also tested, and no relevant interactions were observed with these targets.

Table 4. Summary of the in vitro selectivity profile of eslicarbazepine (400 μmol/L) against diverse biological targets.

| Biological target | % inhibition |
|-------------------|--------------|
| Adenosine transporter (h) | 10.13 |
| Adenosine, A1 (h) | 32.34 |
| Adenosine, A2 | 0.0 |
| Adenosine, A2A (h) | 6.81 |
| Adrenergic, alpha2A (h) | 15.62 |
| Cannabinoid, CB1 (h) | 20.44 |
| Dopamine transporter (h) | 2.85 |
| Dopamine, D1 (h) | 6.85 |
| Dopamine, D2 (h) | 0.0 |
| Dopamine, D3 | 0.0 |
| Dopamine, D4 (h) | 0.0 |
| Dopamine, D5 (h) | 0.0 |
| GABA A, agonist site | 0.0 |
| GABA A, BDZ, alpha 1 site | 25.92 |
| GABA A, BDZ, alpha 2 site | 23.75 |
| GABA A, BDZ, alpha 3 site | 0.0 |
| GABA-B | 11.72 |
| Glutamate, AMPA site | 0.0 |
| Glutamate, chloride-dependent site | 9.36 |
| Glutamate, kainate site | 1.56 |
| Glutamate, MK-801 site | 15.45 |
| Glutamate, NMDA agonist site | 0.0 |
| Glutamate, NMDA, phenylcyclidine site | 0.0 |
| Glutamate, mGlur1 | 1.82 |
| Glutamate, mGlur5 | 14.42 |
| Glutamate, NMDA, glycine (strychnine-insensitive) | 13.21 |
| Glysine, strychnine-sensitive | 25.95 |
| Histamine, H1 | 23.23 |
| Histamine, H2 | 0.0 |
| Imidazoline, I2 [central] | 17.45 |
| Melatonin, nonselective | 17.43 |
| Muscarinic, M1 (h) | 0.0 |
| Muscarinic, M4 (h) | 6.08 |
| Muscarinic, M5 (h) | 15.98 |
| Nicotinic, neuronal [a-BnTx insensitive] (h) | 23.46 |
| Norepinephrine transporter (h) | 9.33 |
| Opioid, delta 2 (h) | 7.91 |
| Opioid, kappa (h) | 5.39 |
| Opioid, Mu (h) | 0.0 |
| Purinergic, P2Y | 0.0 |
| Serotonin transporter (h) | 0.0 |
| Serotonin, 5HT1, nonselective | 11.43 |
| Serotonin, 5HT1A (h) | 0.4 |
| Serotonin, 5HT1B | 2.1 |
| Serotonin, 5HT1D (h) | 16.40 |
| Serotonin, 5HT2A (h) | 19.22 |
| Serotonin, 5HT2C | 3.67 |
| Serotonin, 5HT3 | 10.41 |
| Serotonin, 5HT4 | 7.38 |
| Serotonin, 5HT5A (h) | 11.88 |
| Serotonin, 5HT6 (h) | 5.86 |
| Serotonin, 5HT7 (h) | 0.0 |
| Sigma 1 | 28.43 |
| Sigma 2 | 6.11 |

(Continued)
GABA, glutamate, histamine, melatonin, neurokinin, neuropeptide Y, neurotensin, opioid, purine, serotonin, somatostatin, and TRH, enzymes (acetylcholine esterase, acetylcholine transferase, carbonic acid, decarboxylase, COX-2, MAO-A, and MAO-B, caspasas [1–10]; kinases [CK1d, Fyn, GSK3b, MAPK1, MAPK3, PKA, PKC, PKC, ROCK2, SGK1]; and nitric oxide synthase), and transporters (dopamine, noradrenaline, serotonin, and GABA). In this study, eslicarbazepine was devoid of effects upon major G-protein-coupled and ligand-gated receptors, enzymes, and transporters (Louereo et al. 2012). In what concerns displacement of GABA ligands by eslicarbazepine, the findings concerning alpha 2 and 5 interaction that result in a 23–25% decrease in radioligand binding by eslicarbazepine did not correlate with the lack of effect of eslicarbazepine upon chloride currents through α1β2γ2 and α5β2γ2 GABA receptors, as shown in Table 3.

Discussion

In an attempt to further investigate the mechanism of action of ESL and its metabolites and evaluate the effects in animal models of epilepsy, a number of primary pharmacology studies were conducted. The overall conclusions are the following. ESL and eslicarbazepine – the major active metabolite of ESL – demonstrated distinctive anticonvulsant properties, characterized by a wider (1.5- to 2.5-fold) protective index, when compared to CBZ. ESL may also interfere with seizure progression by inhibiting propagation of activity from the focus. ESL may also inhibit the generation of a hyperexcitable network and therefore provide an antiepileptogenic effect, as evidenced in kindling-induced epileptogenesis. ESL treatment prevented acute latrunculin A-induced seizures as well as chronic seizures and all EEG chronic signs of paroxysmal activity, supporting a possible antiepileptogenic effect in mice. The major active metabolite of ESL, eslicarbazepine, demonstrated a greater selectivity of the inactive state of VGSC, which is the common feature of the rapidly firing neurons, over their resting state as compared to CBZ, OXC, and R-licarbazepine. Eslicarbazepine did not share with CBZ and OXC the ability to alter fast inactivation of VGSC, but reduced VGSC availability through enhancement of slow inactivation. Eslicarbazepine did not cause a paradoxical upregulation of $I_{NaP}$, which contrasts with that described for CBZ, and may potentially overcome a previously described putative mechanism of resistance to established Na$^+$-acting AEDs. Eslicarbazepine exerted use-dependent effects resulting in reduced firing frequencies of excitatory neurons, in DGCs from patients with pharmacoresistant epilepsy and potentially overcomes a cellular resistance mechanism to conventional AEDs. Eslicarbazepine was equally effective in inhibiting firing of DGCs in control versus pilocarpine-treated rats. In contrast, the effects of CBZ on the maximal firing frequency were significantly reduced in chronic experimental epilepsy.

It should be underscored that OXC on its own provides limited exposure when administered systemically, because it is rapidly metabolized to eslicarbazepine and R-licarbazepine in 4:1 ratio. However, small amounts of nonmetabolized OXC persist in the body (~5% of the parent dose) and, therefore, its pharmacodynamic effect may be of relevance, as the potency of OXC is greater than eslicarbazepine for the resting state of the VGSC. Following OXC administration (600 mg BID), $C_{max}$ plasma and CSF...
Cmax of nonmetabolized OXC attained levels were 8.44 and 1.82 μmol/L, respectively (Nunes et al. 2013). In the same study, while using 1200 mg QD ESL, Cmax plasma and CSF Cmax of nonmetabolized OXC levels were 1.00 and 0.38 μmol/L, respectively (Nunes et al. 2013).

Other distinctive features between eslicarbazepine and CBZ with regard to their antiseizure effects relate to differences toward GABA_A, potassium, and calcium channels. Eslicarbazepine, in contrast to CBZ, is devoid of effects upon submaximal GABA currents, which may translate into a reduced potential to aggravate absence seizures. Eslicarbazepine differed from CBZ by the lack of inhibitory effects upon Kv7.2 outward currents, which may translate into a reduced potential for eslicarbazepine to facilitate repetitive firing. Eslicarbazepine, when compared to CBZ, is endowed with a 10- to 60-fold higher potency for the blockade of low- and high-affinity hCa2.3 inward currents, which may translate into an enhanced potential to elicit antiepileptogenic effects. Eslicarbazepine, in contrast to CBZ, is devoid of effects upon hCa2.1 inward currents, the impairment of which may have a central role in the pathogenesis of certain cases of primary generalized epilepsy.

In the in vitro studies described above on the interaction with VGSCs, the concentration of eslicarbazepine (250–300 μmol/L) is approximately five times greater than the concentrations obtained in plasma (and ~10–20 times greater than concentrations obtained in CSF) during human use of oral ESL at doses of 1200 mg QD (peak plasma concentrations of 90 μmol/L). The concentrations of OXC used are 5- to 20-fold greater than the concentration of OXC obtained in plasma during human clinical use of oral OXC. In contrast, LCM concentrations during clinical use in plasma are in the 100 μmol/L range. However, the concentrations used in this study may be justified for the following reasons. Since eslicarbazepine has a strong preference to stick to tissue material in a ratio of about 50:1 and that such tissue constitutes only 20% of the total brain volume, it is expected that the effective concentration of eslicarbazepine in the organic fraction of the brain are higher than that measured in the whole brain volume. Eslicarbazepine does not show a strong affinity for binding (~30%) to nonspecific proteins (Bialer and Soares-da-Silva 2012) and may preferentially be associated to membranes, which constitute a smaller fraction (10% to 12%) of the dry weight of the brain (Pratt et al. 1969; McIlwain and Bachelard 1985; Banay-Schwartz et al. 1992). The corrected EC50 values in the organic fraction (102 μmol/L) or the lipid fraction (183 μmol/L) of the brain are within the range of the IC50 values obtained in patch clamp experiments measuring the effects of eslicarbazepine upon VGSC, particularly under depolarizing conditions at less negative holding potentials (Hebeisen et al. 2011, 2012; Pires et al. 2012; Wright et al. 2012). The corresponding mouse plasma levels are within the range to those observed in healthy volunteers taking ESL at therapeutic doses (Elger et al. 2013) or patients with POS (Perucca et al. 2011).

Taking into consideration that if eslicarbazepine protein binding corresponds to 30% (Bialer and Soares-da-Silva 2012), then the systemic peak plasma concentrations of 90 μmol/L eslicarbazepine after oral administration of ESL at 1200 mg once daily (Perucca et al. 2011) falls to 63 μmol/L eslicarbazepine-free drug, which is twice the CSF Cmax eslicarbazepine levels (30 μmol/L) previously described while using 1200 mg QD oral ESL (Nunes et al. 2013). Assuming that eslicarbazepine therapeutic level in the CNS tissue is in equilibrium to that in the CSF, which is most probably not the case because of the high lipid:water partition coefficient (50:1) described above, then the in vitro experiments performed with 300 μmol/L were 10 times higher the putative therapeutic level. The study establishing that LCM promotes the slow inactivation of VGSC was conducted at concentrations ranging from 32 to 320 μmol/L, the most relevant results obtained at 100 μmol/L LCM (Errington et al. 2008). In human clinical use, oral administration of LCM at 600 mg twice daily results in approximate peak plasma concentrations of (14.5 μg/mL or 58 μmol/L) (Ben-Menachem et al. 2007). Since LCM protein binding corresponds to ~15% (Doty et al. 2007), then the systemic peak plasma concentrations of 58 μmol/L LCM falls to 49 μmol/L LCM-free drug, which is 2 times lower the putative therapeutic level used by Errington et al. (2008). Recently, however, the plasma LCM protein binding was challenged to be as high as 90%, with LCM-free drug levels in the range of 0.1–6.1 μmol/L, in 98 patients that were prescribed the daily LCM dose was 242 ± 99 mg (range 50–550 mg) (Greenaway et al. 2011). Therefore, the LCM-free drug would be 1000 to 16 times lower the putative therapeutic level used by Errington et al. (2008).

A rational approach to AED polytherapy for patients that do not achieve seizure control with a single AED is a matter of considerable debate. Theoretically, AED combinations with the same mechanism of action may be less optimal than targeting different mechanisms of action (French and Faught 2009; St Louis 2009; Brodie and Sills 2011). However, this may be difficult to implement, since many AEDs exert multiple mechanisms (Rogawski and Loscher 2004; White et al. 2007; Stafstrom 2010) and there is lack of clear evidence to support this hypothesis. Another unresolved issue is whether AEDs with different mechanisms of action are more likely to interact synergistically than AEDs with similar or differing mechanisms (Kwan and Brodie 2006; Stafstrom 2010). Combinations of AEDs with similar mechanisms of action may cause
exaggerated additive/supra-additive adverse effects; combinations of AEDs that target VGSC are regarded as offering only additive improvements in efficacy and frequently supra-additive enhancement of neurotoxicity (Kwan and Brodie 2006; Brodie and Sills 2011). While most VGSC blockers used in the treatment of epileptic seizures interfere with the fast inactivation pathway, eslicarbazepine and LCM selectively influence slow inactivation, which fits well the view that they can be usefully combined with VGSC blockers that act on fast inactivation. In the phase III clinical program, the efficacy of adjunctive oral ESL, which results in exposure to largely eslicarbazepine, in reducing the frequency of POS in adults receiving CBZ or another AED was similar (Elger et al. 2009; Chung et al. 2014; Ben-Menachem et al. 2010; Halasz et al. 2010; Hufnagel et al. 2013). The fact that adjunctive ESL was efficacious in patients uncontrolled with CBZ may be explained by the findings previously described in this article that eslicarbazepine, the major active metabolite of ESL, retained activity at the VGSC of DGCS of patients with CBZ-resistant epilepsy, and differed from CBZ in demonstrating activity in VGSC β-subunit deficient models.

Overall, the ESL nonclinical data, a summary of which is provided in Table 5, further underscore the overall conclusions supporting the use of ESL in the adjunctive treatment of adults with POS.

**Conclusion**

The data gathered here demonstrate that ESL and eslicarbazepine have distinctive pharmacological properties, namely (1) anticonvulsant properties characterized by a wider (1.5- to 2.5-fold) protective index, (2) possible antiepileptogenic effects in mouse models of chronic epilepsy, (3) selectivity of the inactive state of VGSC, (4) potentially reduce VGSC availability through enhancement of slow inactivation, instead of altering fast inactivation of VGSC as promoted by CBZ, (5) the failure to cause a paradoxical upregulation of \( I_{NaP} \), which contrasts with that described for CBZ, and (6) the reduction in firing frequencies of excitatory neurons in CBZ pharmacoresistant human epilepsy, which potentially overcomes a cellular resistance mechanism, (7) ability to effectively inhibit high- and low-affinity hCaV3.2 inward currents with greater affinity than CBZ, which may have implications for antiepileptic activity of ESL, and (8) the lack of effect upon Kv7.2 outward currents, which may translate into a low potential for eslicarbazepine to facilitate repetitive firing.

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Patrício Soares-da-Silva, Nuno Pires, Maria João Bonifácio, Ana I. Loureiro, Nuno Palma, and Lyndon C. Wright are or were employees of BIAL – Portela & Cª S.A. at the time of the studies.

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