REVIEW ARTICLE

Molecular and cellular mechanisms of pharmacoresistance in epilepsy

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Epilepsy is a common and devastating neurological disorder. In many patients with epilepsy, seizures are well-controlled with currently available anti-epileptic drugs (AEDs), but a substantial (~30%) proportion of patients continue to have seizures despite carefully optimized drug treatment. Two concepts have been put forward to explain the development of pharmacoresistance. The transporter hypothesis contends that the expression or function of multidrug transporters in the brain is augmented, leading to impaired access of AEDs to CNS targets. The target hypothesis holds that epilepsy-related changes in the properties of the drug targets themselves may result in reduced drug sensitivity. Recent studies have started to dissect the molecular underpinnings of both transporter- and target-mediated mechanisms of pharmacoresistance in human and experimental epilepsy. An emerging understanding of these underlying molecular and cellular mechanisms is likely to provide important impetus for the development of new pharmacological treatment strategies.

Introduction to the clinical problem of pharmacoresistance in epilepsy

Epilepsy is a common and devastating neurological disorder. In many patients with epilepsy, seizures are well-controlled with currently available anti-epileptic drugs (AEDs). However, seizures persist in a considerable proportion of these patients. The exact fraction of epilepsy patients who are considered refractory varies in the literature, mostly because the criteria for classification as pharmacoresistant have varied. Nevertheless, a substantial proportion (~30%) of epilepsy patients do not respond to any of two to three first-line AEDs, despite administration in an optimally monitored regimen (Regesta and Tanganelli, 1999). The fraction of patients who are pharmacoresistant appears to correlate with certain features of the epileptic condition, such as a high seizure frequency or febrile seizures prior to treatment, early onset of seizures or the presence of certain types of structural brain lesions. In addition, pharmacoresistance occurs frequently in patients with partial seizures (Aicardi and

Shorvon, 1997; Regesta and Tanganelli, 1999 for a more complete discussion of clinical aspects of pharmacoresistance). Despite the obvious clinical relevance of uncontrolled seizures in a large fraction of epilepsy patients, the cellular basis of pharmacoresistance has so far remained elusive. The availability of tissue from epilepsy patients undergoing surgery for focal epilepsies, primarily temporal lobe epilepsy, has allowed to address some of the mechanisms underlying pharmacoresistance of focal epilepsies. The mechanisms underlying the development of resistance in certain forms of generalized epilepsies are still enigmatic.

Introduction to the cellular candidate mechanisms of pharmacoresistance

Which key mechanisms govern efficacy of CNS drugs? Firstly, in the presence of adequate, carefully monitored serum AED levels, drugs have to traverse the blood–brain barrier (BBB). Subsequently, CNS activity of AEDs is determined by a

multitude of factors, including physical properties, such as lipophilicity, that affect their distribution in different compartments within the CNS. Consequently, one scenario to explain pharmacoresistance could be that sufficient intraparenchymal AED concentrations are not attained, even in the presence of adequate AED serum levels. Such a phenomenon could arise via an enhanced function of multidrug transporters that control intraparenchymal AED concentrations (transporter hypothesis of pharmacoresistance, Kwan and Brodie, 2005).

Following permeation into the CNS parenchyma, drugs have to bind to one or more target molecules to exert their desired action. Thus, pharmacoresistance may also be caused by a modification of one or more drug target molecules (*see* Table 1). These modifications would then cause a reduced efficacy of a given AED at the target. This concept has been collectively termed the target hypothesis of pharmacoresistance (Fig. 1).

Modification in drug targets as basis for pharmacoresistance

The cellular mechanisms of AEDs have been examined to some extent in normal brain tissue, or ion channels and receptors in expression systems. These data are summarized qualitatively in Table 2. Many of these drug targets are altered on a molecular level in epilepsy. In the following sections, we will attempt to summarize briefly the known mechanisms of AEDs on ion channels. We will then focus on emerging experimental evidence supporting a loss of AED efficacy at selected targets, and discuss the possible molecular basis of these findings.

Changes in molecular drug targets for AEDs

Voltage-gated Na$^+$ channels

Voltage-gated Na$^+$ currents are ubiquitously expressed in excitable cells (Fig. 2A, Goldin, 1999; Goldin *et al.*, 2002), and appear to be targets for multiple first-line AEDs. Upon depolarization of the membrane, the channels activate and give rise to a fast 'transient' inward Na$^+$ current (I_{NaT}, Fig. 2B), responsible for the rising phase of the action potential, and—in some cells—a slowly-inactivating 'persistent' current (I_{NaP}, *see* Fig. 2C). Both current components represent major targets of several first-line AEDs including carbamazepine, phenytoin (PHT), lamotrigine and valproate (Ragsdale and Avoli, 1998; Catterall, 1999; Köhling, 2002, see also Table 2).

Most AEDs block Na$^+$ channels in their resting state (tonic block) at hyperpolarized membrane potentials (Ragsdale and Avoli, 1998), with a voltage-dependent enhancement of the block towards more depolarizing potentials. This voltage-dependent inhibition is associated with a shift of the steady-state inactivation curve in a hyperpolarized direction (Fig. 2D). Importantly, blocking effects are activity- or use-dependent, i.e. blocking effects are enhanced when neurons are repetitively depolarized at higher frequencies (Fig. 2E and F). This activity-dependence is expressed as a slowing of recovery from fast Na$^+$ channel inactivation (Ragsdale and Avoli, 1998; Catterall, 1999). It has been suggested that use-dependent blocking effects are important because they result in a preferential block of I_{NaT} during prolonged high-frequency neuronal activity, such as that occurring during seizures.

Several lines of evidence so far have indicated that reduced efficacy in inhibiting I_{NaT} may be a candidate mechanism of

Table 1 Changes in known AED targets or drug efflux transporters in experimental epilepsy models and human epileptic tissue

Target	Modification	Cell type	Human data (yes/no)
Voltage-gated sodium channels	Downregulation of accessory subunits	Dentate granule cells CA1 pyramidal neurons	Yes
	Altered alpha subunit expression, induction of neonatal isoforms	CA1 pyramidal neurons CA3 pyramidal neurons Dentate granule cells	Yes
Voltage-gated calcium channels	Increased expression of T-type channels	CA1 pyramidal neurons	No
Hyperpolarization-activated current (I_H)	Loss of dendritic I_H	Entorhinal cortex layer 3 neurons	No
GABA receptors	GABA$_A$ receptors: decrease of α_1 subunits increase of α_4 subunits	Dentate granule cells	Yes
P-Glycoprotein (MDR1)	Overexpression	Astrocytes Capillary endothelial cells Neurons	Yes
MRP1	Overexpression	Astrocytes Neurons	Yes
MRP2	Overexpression	Astrocytes Capillary endothelial cells	Yes
MVP (major vault protein)	Overexpression	Microglial cells	No
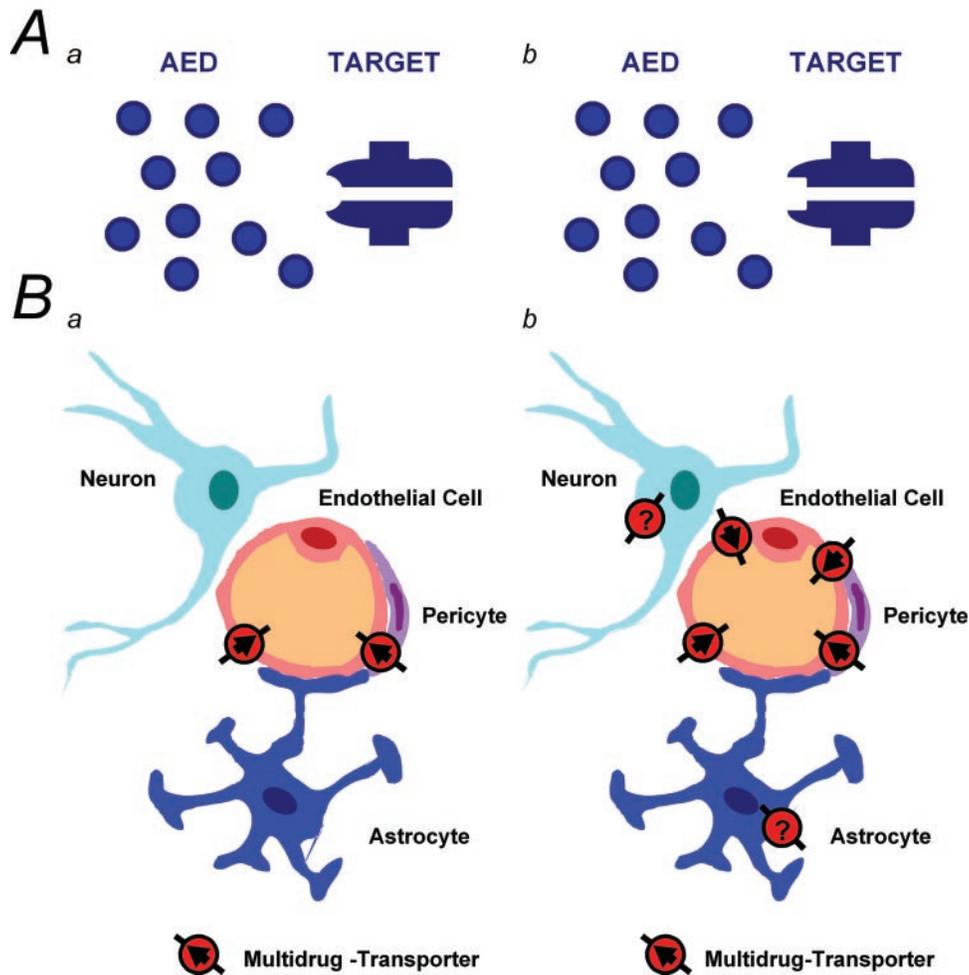
Fig. 1 In the pharmacoresistant patient the drug faces a modified target, with which it interacts less effectively (**A**, panel b). Putative candidate mechanisms resulting in target modifications are seizure-induced changes in transcription or alternative splicing of ion channel subunits, altered post-translational modification of the protein and/or phosphorylation by protein kinases. A second emerging concept to explain pharmacoresistance contends that increased expression or function of multidrug transporter proteins decreases availability of the AED at its target (**B**, panel b versus **B**, panel a). In the non-epileptic brain, drug transporter molecules are predominantly expressed in endothelial cells, and in some cases astrocytes (*see* text), and appear to regulate intraparenchymal AED concentrations by extruding certain AEDs over the blood–brain or blood–CSF barrier (indicated by arrows in **B**, panel a). An upregulation of various drug transporter molecules has been described in human epilepsy as well as in experimental epilepsy models (**B**, panel b). This upregulation may decrease the effective concentration of AEDs at their targets. In addition, in the setting of an epileptic brain, an ectopic expression of certain drug transporter genes has been observed in astrocytes and in neurons. It remains unresolved whether such transporters control access of AEDs to intracellular targets (indicated by the absence of an arrow in the neuron in **B**, panel b).

pharmacoresistance to some AEDs. Firstly, in CA1 neurons, the effects of carbamazepine on the steady-state inactivation properties of I_{NaT} were transiently reduced in the kindling model of epilepsy (Vreugdenhil and Wadman, 1999*b*). In contrast to these comparatively modest and transient effects, a complete and long-lasting loss of use-dependent blocking effects of carbamazepine was found in the pilocarpine model of epilepsy in hippocampal dentate granule cells, as well as in epilepsy patients with carbamazepine-resistant temporal lobe epilepsy (Remy *et al.*, 2003*a*). This dramatic loss of a major mechanism of action of carbamazepine did not extend to other AEDs known to affect I_{NaT}. Following pilocarpine-induced status epilepticus, the use-dependent effects of PHT were reduced, but not completely lost, while the effects

of lamotrigine were completely unchanged (Remy *et al.*, 2003*b*). Although the mechanisms of I_{NaT} inhibition induced by valproate are still controversial (Xie *et al.*, 2001); but see (Vreugdenhil *et al.*, 1998; Vreugdenhil and Wadman, 1999*b*), this substance exhibits potent voltage-dependent blocking effects in various preparations (Fohlmeister *et al.*, 1984; Zona and Avoli, 1990; Vreugdenhil and Wadman, 1999*b*; Köhling, 2002). Notably, in tissue obtained from pharmacoresistant patients and in experimental epilepsy no differences regarding valproic acid effects on I_{NaT} could be observed (Vreugdenhil *et al.*, 1998; Vreugdenhil and Wadman, 1999*b*; Remy *et al.*, 2003*b*). Collectively, these results suggest that epileptogenesis causes changes in the properties of I_{NaT} that may differ depending on the cell type examined

Table 2 Summary of AED targets

	Voltage-dependent ion channels						Neurotransmitter systems	
	I_{NaT}	I_{NaP}	I_{Ca}	I_K	I_K (I_M)	I_H	GABA	Glu
Phenytoin	(Willow et al., 1985; Schwarz and Grigat, 1989; Ragsdale et al., 1991; Kuo, 1998; Remy et al., 2003b)	(Chao and Alzheimer, 1995; Segal and Douglas, 1997; Lampl et al., 1998)	High-threshold: (Stefani et al., 1997a, c; Schumacher et al., 1998) Low-threshold (Todorovic et al., 2000; Gomora et al., 2001)	(Nobile and Lagostena, 1998)				
Carbamazepine	(Willow et al., 1985; Schwarz and Grigat, 1989; Kuo, 1998; Reckziegel et al., 1999; Vreugdenhil and Wadman, 1999a; Remy et al., 2003a)		High-threshold (Schumacher et al., 1998)					
Oxcarbazepine	(McLean et al., 1994; Schmutz et al., 1994)		High-threshold (Schmutz et al., 1994; Stefani et al., 1995; 1997b)					
Lamotrigine	(Xie et al., 2001; Xie et al., 1995; Zona and Avoli, 1997; Remy et al., 2003b; Kuo, 1998)	(Spadoni et al., 2002)	High-threshold (Stefani et al., 1997c; Stefani et al., 1997a; Stefani et al., 1997b; Wang et al., 1996)	(Huang et al., 2004; Zona et al., 2002)		(Poolos et al., 2002)		
Valproic acid		(Taverna et al., 1998; but see Niespodziany et al., 2004)	High-threshold (Taverna et al., 1998) Low-threshold (Todorovic and Lingle, 1998)				(Löscher, 1989)	
Losigamone		(Gebhardt et al., 2001)						
Retigabine					(Main et al., 2000), (Tatulian et al., 2001; Yue and Yaari, 2004)			
Zonisamide	(Schauf, 1987)		Low-threshold (Suzuki et al., 1992)					

Felbamate
- (Taglialatela et al., 1996)
- High-threshold I_{Ca} (Stefani et al., 1997b)
- (Rho et al., 1997)
- (Subramaniam et al., 1995; White et al., 1995; Kuo et al., 2004)

Topiramate
- (Zona et al., 1997; Taverna et al., 1999; McLean et al., 2000); (Taverna et al., 1999)
- High-threshold I_{Ca} (Zhang et al., 2000)
- (Herrero et al., 2002)
- (White et al., 1997; Gordey et al., 2000)
- (Gibbs, III et al., 2000; Gryder and Rogawski, 2003)

Ethosuximide
- No effect: (McLean and Macdonald, 1986); (Leresche et al., 1998; Niespodziany et al., 2004)
- Low-threshold (Coulter et al., 1989; Todorovic and Lingle, 1998; Gomora et al., 2001), but see (Leresche et al., 1998)
- (Leresche et al., 1998)

Levetiracetam
- No effect: (Zona et al., 2001); No effect: (Niespodziany et al., 2004)
- High-threshold (Lukyanetz et al., 2002) Low-threshold, no effect: (Zona et al., 2001)
- (Madeja et al., 2003)

Gabapentin
- High-threshold (Alden and Garcia, 2001), but see (Schumacher et al., 1998)
- (Freiman et al., 2001)
- (Surges et al., 2003)

Pregabalin
- High-threshold (Ben Menachem, 2004), (McClelland et al., 2004)

Phenobarbital
- High-threshold (Ffrench-Mullen et al., 1993)
- (Twyman et al., 1989b)

Benzodiazepines
- (Study and Barker, 1981; Rogers et al., 1994; Eghbali et al., 1997; Rudolph et al., 1999)

Vigabatrin
- (Jolkkonen et al., 1992; Löscher and Horstermann, 1994; Wu et al., 2003)

Tiagabine
- (Fink-Jensen et al., 1992; Thompson and Gähwiler, 1992; Dalby, 2000)
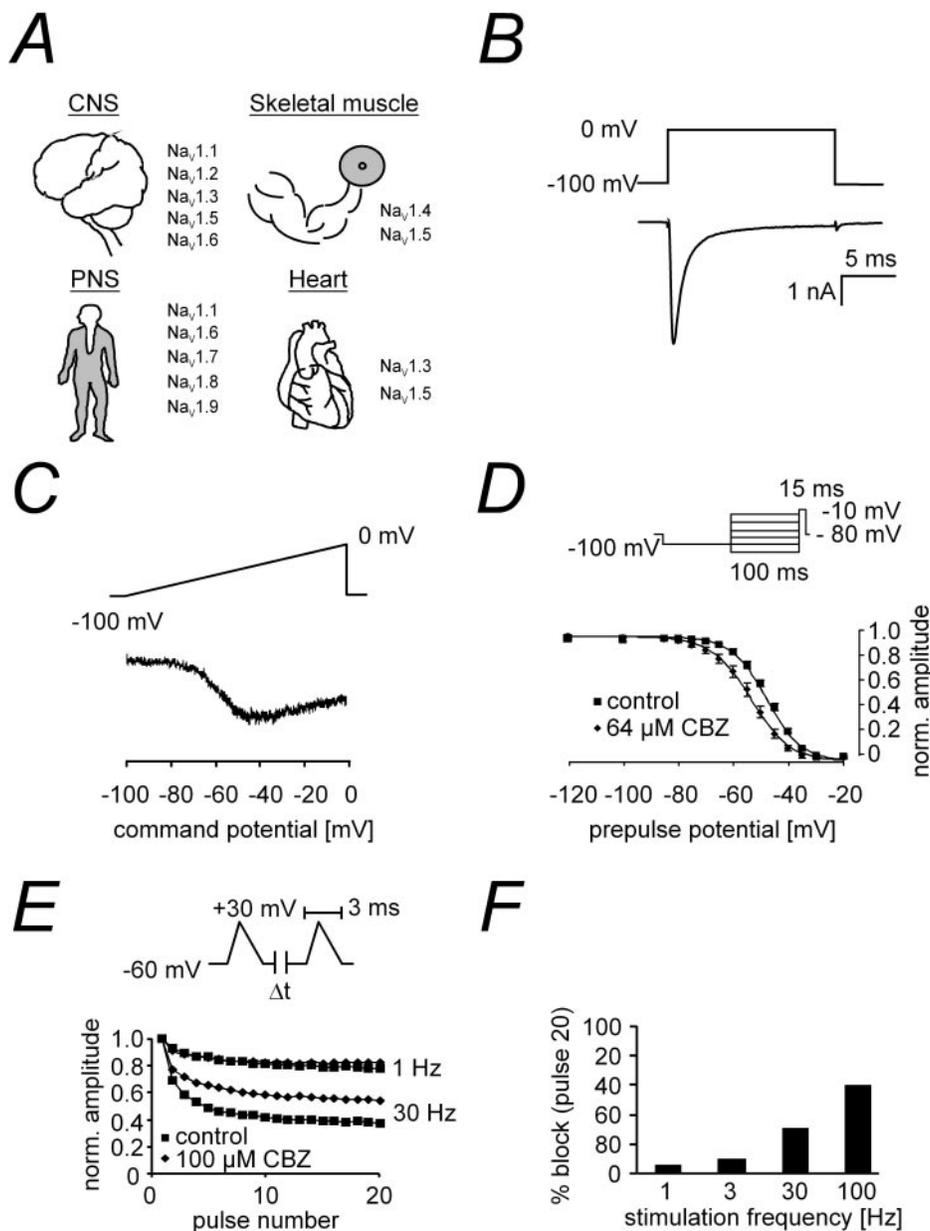
Fig. 2 Expression, functional properties and AED pharmacology of voltage-gated sodium channels. The Na$^+$ channel alpha subunits Na$_V$1.1–Na$_V$1.9 are widely distributed in excitable cells and exhibit a tissue-specific expression (**A**). Upon depolarization the channels activate and give rise to a rapidly inactivating 'transient' inward Na$^+$ current (I_{NaT}, **B**), responsible for the rising phase of the action potential. In addition, in some cells a slowly-inactivating 'persistent' current has been described (I_{NaP}, **C**). Slow voltage-ramp commands allow to describe the voltage-dependent properties of this current component in isolation. The persistent Na$^+$ current contributes to spike after-depolarizations in some neurons and plays a role in the generation and maintenance of subthreshold membrane oscillations. Na$^+$ channels are the main targets for a subset of AEDs including carbamazepine, PHT and lamotrigine. The drugs acting on I_{NaT} channels display two predominant mechanisms of action: 1. Voltage-dependent inhibition, associated with a shift of the steady-state inactivation curve in a hyperpolarized direction. This shift decreases the availability of the Na$^+$ channel during an action potential and, therefore, reduces the excitability of the cell (**D**). 2. Activity- or use-dependent blocking effects, i.e. blocking effects are enhanced when neurons are repetitively depolarized at higher frequencies (**E** and **F**). This results in a highly efficient block of I_{NaT} preferentially during prolonged high-frequency neuronal activity, such as that occurring during seizures.

(i.e. dentate granule cells versus CA1 neurons), and according to the epilepsy model studied. Changes in I_{NaT} may then dramatically affect sensitivity to some, but not all, AEDs.

Block of I_{NaP} may also be a crucially important mechanism of AED action. Pharmacological augmentation of I_{NaP} causes

an increased propensity of individual neurons to generate burst discharges (Su *et al.*, 2001), and several mutations that give rise to increased I_{NaP} cause epilepsy in mice or humans (Kearney *et al.*, 2001; Lossin *et al.*, 2002; Rhodes *et al.*, 2004; Spampanato *et al.*, 2004). I_{NaP} is efficiently

blocked by many AEDs, frequently at a concentration range lower than that observed for I_{NaT} (Köhling, 2002). In native neurons, I_{NaP} is potently inhibited by lamotrigine and PHT, as well as losigamone (Chao and Alzheimer, 1995; Lampl *et al.*, 1998; Gebhardt *et al.*, 2001; S. Remy and H. Beck, unpublished data). Despite the potential importance of I_{NaP} in the regulation of neuronal firing, altered AED effects on I_{NaP} following epileptogenesis have so far not been described.

What mechanisms can account for an altered sensitivity of Na$^+$ channels in epileptic tissue? One possibility may be that the subunit composition of these channels is altered, such that the expression of AED-insensitive subunits or subunit combinations is promoted. Indeed, numerous changes in Na$^+$ channel subunit expression have been observed in both human and experimental epilepsy (Bartolomei *et al.*, 1997; Aronica *et al.*, 2001; Whitaker *et al.*, 2001; Ellerkmann *et al.*, 2003). In this respect, the downregulation of accessory Na$^+$ channel β_1 and β_2 subunits following experimentally induced status epilepticus appears to be a consistent finding (Gastaldi *et al.*, 1998; Ellerkmann *et al.*, 2003). In addition to changes in mRNA levels, altered alternative splicing of pore-forming subunit mRNAs has also been observed (Gastaldi *et al.*, 1997; Aronica *et al.*, 2001). A recent, very interesting study underscores the potential importance of the β_1 subunit in the development of pharmacoresistance. In the paper by Lucas *et al.* (2005), the pharmacology of Na$^+$ channels containing a mutant β_1 subunit causing the epilepsy syndrome generalized epilepsy with febrile seizures plus was examined. Surprisingly, Na$^+$ channels containing mutant β_1 subunits displayed a dramatic and selective loss of use-dependent block by the AED PHT, that was very similar to the effects observed in chronic experimental epilepsy for PHT and carbamazepine (Remy *et al.*, 2003*a*, *b*). These results collectively suggest that changes in accessory subunits might be promising candidates for further investigation as a molecular correlate of the AED-insensitive sodium channel. They are also intriguing because they suggest that use-dependent effects on Na$^+$ channels require some form of interaction with β_1 subunits, whereas this may not be the case for tonic block of Na$^+$ channels.

It is at present unclear why use-dependent block by carbamazepine and PHT is lost or reduced, whereas use-dependent block by lamotrigine remains intact in experimental epilepsy. This is an intriguing question because it has been suggested that all three drugs bind to the same site on Na$^+$ channels in CA1 neurons based on coapplication experiments (Kuo *et al.*, 1998). The mutual exclusivity among the binding of drugs simultaneously applied to a channel may however result from either an allosteric mechanism or from direct competition of the drugs at a single binding site. Even though a single binding site appears to provide the most parsimonious explanation for these results, it is quite conceivable that allosteric interactions between different binding sites may also exist. This would provide a basis for the AED-selective loss of sensitivity observed in chronic experimental epilepsy.

Other types of voltage-gated channels

Other types of voltage-gated channels have also been screened as potential drug targets. In many cases, effects of AEDs on specific ion channel subunits or ion channels in native neurons or expression systems have been described (*see* Table 2).

Ca^{2+} channels can be subdivided into two groups: high-threshold Ca^{2+} currents, and a group of low-threshold currents (also termed T-type Ca^{2+} currents, Ertel *et al.*, 2000). A number of AEDs has been shown to inhibit high threshold Ca^{2+} channels in native neurons at high therapeutic concentrations (Stefani *et al.*, 1997*b*, 1998; Schumacher *et al.*, 1998; *see* Table 2). Additionally, the AED gabapentin has been shown to exhibit strong and specific binding to the accessory $\alpha_2\delta$ subunit (Gee *et al.*, 1996). It has been proposed that this effect underlies inhibition of presynaptically expressed high-threshold Ca^{2+} channels by gabapentin, which causes a reduction in neurotransmitter release (Fink *et al.*, 2000; *see* Table 2). Some AEDs potently inhibit low-threshold T-type Ca^{2+} channels, which are not expressed presynaptically (Yaari *et al.*, 1987; Coulter *et al.*, 1989; Gomora *et al.*, 2001; *see* Table 2). The effects of AEDs on the three T-type Ca^{2+} channel subunits, as well as in native neurons, are diverse (cf. Todorovic and Lingle, 1998; Todorovic *et al.*, 2000; Lacinova, 2004). T-type channels are critically important in controlling the excitability of the postsynaptic compartment of neurons (Huguenard, 1996), both in normal and epileptic neurons. For instance, aberrant bursting is seen in CA1 hippocampal neurons from epileptic animals (Sanabria *et al.*, 2001) that is mediated by increased expression of T-type Ca^{2+} channels (Su *et al.*, 2002). Additionally, T-type Ca^{2+} channels in thalamic neurons have been implicated in the generation of spike-wave discharges in absence epilepsy (Huguenard, 2002 and references therein). Consequently, inhibition of burst discharges in thalamic neurons is thought to contribute to the anti-epileptic effects of antiabsence AEDs. It is so far unknown if the sensitivity of either presynaptic or post-synaptic Ca^{2+} channels to AEDs changes during epileptogenesis. The same applies to other voltage-gated ion channels such as K$^+$ channels (*see* Table 2).

H-currents (I_H) are mixed cationic currents that are activated by hyperpolarization and deactivated following repolarization of the membrane. I_H has multiple functional roles; for instance, it mediates some forms of pacemaker activity in heart and brain, it regulates membrane resistance and dendritic integration and stabilizes the level of the resting potential (reviewed, Robinson and Siegelbaum, 2003). An interesting feature of I_H appears to be that the corresponding channels are predominantly located in dendrites, rather than the soma of neurons (Poolos *et al.*, 2002). Interestingly, dendritic H-currents are potently enhanced by the AEDs lamotrigine and gabapentin at clinically relevant concentrations (Poolos *et al.*, 2002; Surges *et al.*, 2003, *see* Table 2), resulting in I_H-mediated inhibitory effects on action potential firing by selectively reducing the excitability of the apical dendrites (Poolos *et al.*, 2002). Cell-type specific changes in I_H have

been described in models of epilepsy (Chen *et al.*, 2001), including a dramatic loss of dendritic I_H in entorhinal cortex neurons (Shah *et al.*, 2004). The importance of this change for pharmacoresistance to lamotrigine has not been directly addressed, but it is conceivable that a sufficiently large reduction of these channels could constitute a *de facto* loss of a major drug target for lamotrigine in this subregion.

Neurotransmitter systems: GABA

GABA is the predominant inhibitory neurotransmitter in the adult brain and plays a critical role in the regulation of excitability of neuronal networks (Mody and Pearce, 2004). GABA binding to ionotropic $GABA_A$ receptors causes opening of the receptor ionophore, which is permeable to Cl^- and—to a lesser extent—to HCO_3. In the presence of a normal adult transmembraneous Cl^- gradient, this results in expression of an inhibitory post-synaptic current that hyperpolarizes the post-synaptic neuronal membrane. Direct modulators of $GABA_A$ receptors include benzodiazepines and barbiturates. Benzodiazepines increase GABA affinity of the receptor complex and may augment their Cl^- conductance via allosteric modulation (Twyman *et al.*, 1989a; Rudolph *et al.*, 1999, 2001). Substances that interact with the GABA system in a more indirect way affect the handling and metabolism of synaptically released GABA. Vigabatrin (gamma-vinyl GABA) is a GABA analogue that inhibits one of the main enzymes controlling GABA concentrations in the brain, GABA transaminase. Consequently, application of vigabatrin causes large elevations in brain GABA levels. The AED tiagabine inhibits the high-affinity GABA transporter GAT1 that normally terminates synaptic action of GABA via rapid uptake. So far, available evidence indicates that neither the efficacy of GABA uptake, nor its sensitivity to tiagabine is altered in chronic experimental epilepsy (Frahm *et al.*, 2003).

Regarding $GABA_A$ receptor agonists, reduced activity of such substances has been described in a chronic model of epilepsy. In the pilocarpine model of epilepsy, $GABA_A$ receptors of dentate granule cells show a reduced sensitivity to drugs acting on the benzodiazepine receptor site 1. While augmentation of GABA-evoked currents by the broad-spectrum benzodiazepine clonazepam was slightly enhanced in epileptic animals, augmentation by the benzodiazepine site 1-selective agonist zolpidem was strongly decreased (Gibbs *et al.*, 1997; Brooks-Kayal *et al.*, 1998; Cohen *et al.*, 2003). In CA1 pyramidal cells, the effects of clonazepam were dramatically reduced in chronically epileptic animals (81% reduction relative to control, (Gibbs *et al.*, 1997). This suggests that the same might also apply to clinically employed benzodiazepines.

What is the molecular mechanism of this change in $GABA_A$ receptor pharmacosensitivity? An enormous diversity of $GABA_A$ receptors has been reported in the CNS, reflecting the fact that in each receptor at least three different subunits are present, which derive from one of eight structurally distinct and genetically distinct families (Costa, 1998;

Sperk *et al.*, 2004). Combined molecular and functional studies indicate that a transcriptionally mediated switch in the alpha subunit composition of $GABA_A$ receptors occurs in epileptic animals, in particular a decrease of α_1 subunits and an increase of α_4 subunits (Brooks-Kayal *et al.*, 1998). These findings correlate well with the observed changes in benzodiazepine receptor pharmacology.

Neurotransmitter systems: glutamate

Despite the undoubted importance of altered glutamate-mediated excitatory neurotransmission in chronic experimental (Mody and Heinemann, 1987; Martin *et al.*, 1992; Kohr *et al.*, 1993) and human epilepsy (Isokawa and Levesque, 1991), few substances acting on this system have been developed to clinical use so far. Felbamate exerts complex effects on the NMDA receptor (Kuo *et al.*, 2004), some of which may be mediated via the modulatory glycine binding site (White *et al.*, 1995). Some of the effects of felbamate have been shown to be affected by NMDA receptor subunit composition (Kleckner *et al.*, 1999; Harty and Rogawski, 2000). AEDs acting on AMPA receptors are also scarce, some drugs currently in clinical trials inhibit AMPA receptors (talampanel, *see* Chappell *et al.*, 2002). Likewise, topiramate has been shown to reduce excitatory synaptic transmission via an inhibition of AMPA receptors (Qian and Noebels, 2003). Altered cellular expression of glutamate receptors in epilepsy should be considered in future development of compounds acting on these receptors. Given the paucity of established AEDs acting on individual glutamate receptors, however, we will abstain from an in depth discussion of these changes here.

Role of changes in drug targets in the setting of a chronically epileptic brain

The specific changes in drug targets described above are an attractive concept to explain pharmacoresistance. It is important to realize, however, that not only changes in drug targets themselves, but also changes in other molecules that affect their function may have important consequences for AED efficacy. This idea is exemplified by recent findings regarding the role of GABA in epilepsy. GABA may on occasion act as an excitatory neurotransmitter in the immature brain. A depolarizing action of $GABA_A$ receptor activation arises because of an altered chloride homeostasis, resulting in a changed chloride gradient across the neuronal membrane. The altered chloride reversal potential then results in a net outward flux of Cl^- through the $GABA_A$ receptor ionophore, causing depolarization of the neuron (Mody and Pearce, 2004). Interestingly, in addition to the developing brain, depolarizing GABA responses appear to be a feature of some neurons in the epileptic brain (Cohen *et al.*, 2002; Wozny *et al.*, 2003). Augmenting such depolarizing GABA-mediated potentials by application of GABA agonists is likely to facilitate action potential generation to excitatory

input (Gulledge and Stuart, 2003), and thereby would increase neuronal excitability instead of decreasing it. Whether depolarizing GABA responses really play a role in pharmacoresistance to GABAmimetic drugs remains to be seen. These considerations do, however, illustrate the need to consider changes in drug targets within the more general setting of a chronically epileptic brain.

Molecular mechanisms underlying altered target sensitivity

So far, most of the mechanisms implicated in altered AED targets are changes in the transcription of ion channel subunits. Seizures appear to cause a highly coordinated change in transcription of certain groups of ion channel subunits, both in rat models of epilepsy (Brooks-Kayal *et al.*, 1998) and in human epilepsy patients (Brooks-Kayal *et al.*, 1999; Bender *et al.*, 2003). This seizure-induced transcriptional plasticity appears to be differentially regulated in different neuron types (cf. Bender *et al.*, 2003; Shah *et al.*, 2004). These transcriptional changes most probably affect both the density of ion channels in the neuronal membrane, as well as the subunit stochiometry of multisubunit channel complexes (Brooks-Kayal *et al.*, 1998). In addition to transcriptional mechanisms, seizure activity may also evoke multiple post-translational modifications of ion channel proteins, such as altered protein transport and targeting, phosphorylation or glycosylation (Bernard *et al.*, 2004). Indeed, increased phosphorylation of I_{Na} by protein kinase C has been shown to affect responsiveness to the AED topiramate in one study (Curia *et al.*, 2004). It is quite possible that other post-transcriptional modifications of ion channel proteins induced by seizures may profoundly affect their drug sensitivity. How seizures may modify the pharmacosensitivity of AED targets is summarized schematically in Fig. 3.

Relationship of molecular changes in AED sensitivity to pharmacoresistance observed *in vivo*

How is the loss of AED sensitivity on the level of an ion channel such as I_{NaT} related to pharmacoresistance observed in human epilepsy patients or intact animals? In epilepsy patients, properties of I_{NaT} seemed to differ when patients are separated into two groups, one resistant to carbamazepine and a smaller one responsive to carbamazepine. In the former, the use-dependent block of I_{NaT} proved to be abolished, similar to the findings in the pilocarpine model of epilepsy. In contrast, carbamazepine responsive patients showed potent use-dependent effects of carbamazepine on I_{NaT}. Thus, the sensitivity to carbamazepine on a cellular level appeared to correlate with the clinical responsiveness to the same drug. These results should be interpreted with caution on two levels. Firstly, the number of patients for which both clinical and *in vitro* data could be obtained is still limited, particularly when considering the group of pharmacoresponsive epilepsy

patients (Remy *et al.*, 2003*a*). Secondly, patients who are resistant to carbamazepine very frequently are resistant also to other AEDs (Kwan and Brodie, 2000). However, available data indicate that altered sensitivity of Na^+ channels may not be able to account for altered efficacy of other AEDs such as valproic acid or lamotrigine (Remy *et al.*, 2003*b*). This finding may indicate that resistance to AEDs in epilepsy patients is a complex phenomenon that possibly relies on multiple mechanisms. On a genetic level, association studies are beginning to yield candidate gene polymorphisms that may be associated with AED sensitivity (Tate *et al.*, 2005).

The correlation of target pharmacosensitivity and sensitivity to AEDs *in vivo* in experimental models of epilepsy is quite unclear. The pilocarpine model of epilepsy has been frequently used to study changes in pharmacosensitivity of drug targets. Leite and Cavalheiro (1995) have provided some evidence that high doses of common AEDs such as carbamazepine, PHT and valproate reduce the spontaneous seizure frequency in these animals. This is in apparent contradiction to the finding that Na^+ channels in the same model are resistant to carbamazepine. It is possible that this may be due to the high doses of AEDs used, or alternatively to differences in the rat strains and/or pilocarpine protocols used. Furthermore, it is likely that some groups responsive and resistant to AED may exist in chronic epilepsy models (Löscher *et al.*, 1998; Nissinen and Pitkänen, 2000). Ideally, these questions could, therefore, be resolved by first examining pharmacosensitivity in intact animals, and subsequently comparing these *in vivo* results to the cellular effects of AEDs in the same individuals. So far, this important approach has only been implemented in few experiments (Jeub *et al.*, 2002). For these experiments, kindled rats were used that could be separated into two groups based on their responsiveness to PHT (Löscher *et al.*, 1998). When rats responsive to PHT were compared to a group of rats that were not, no difference in PHT sensitivity of I_{NaT} emerged. It should be noted, however, that PHT effects on the recovery from inactivation and use-dependent block, where the most dramatic effects were seen in the pilocarpine model of epilepsy, were not examined in this study (Jeub *et al.*, 2002). Nevertheless, animal models in which groups with differential pharmacosensitivity can be defined represent a promising avenue to study mechanisms of pharmacoresistance (Nissinen and Pitkänen, 2000). It remains to be seen how far such animal models mirror mechanisms of pharmacoresistance in human epilepsy patients.

Modification in multidrug transporters as basis for pharmacoresistance
Overview of multidrug transporter molecules expressed in the brain

The second main emerging concept to explain pharmacoresistance contends that increased expression or function of multidrug transporter proteins decreases the effective concentration of AEDs at their targets (*see* Fig. 1B). Intense
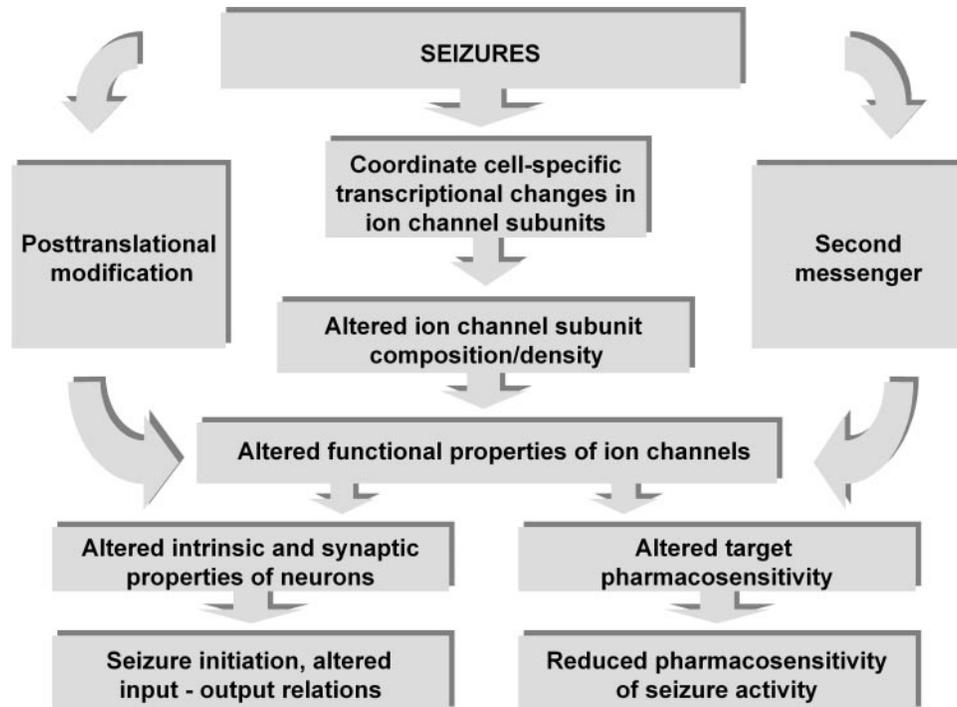
Fig. 3 Epileptic seizures trigger an activity-dependent sequence of events resulting in alterations in neuronal firing and pharmacosensitivity. Known changes triggered by seizures include coordinate changes in ion channel transcription, altered post-translational processing of ion channel proteins, or altered modification of channels by second-messenger systems. Many of these changes result in defined pharmacological and functional changes in ion channels. These changes may underlie altered responses to AEDs as well as altered excitability. It should be noted that this model depicts acquired activity-dependent changes caused by seizures. Reduced responses to AEDs may also be a pre-existing condition caused by genetic polymorphisms that affect specific AED targets (see discussion in text).

interest has been focused on understanding the molecular basis of multidrug transport in the brain in recent years, primarily because of their potential importance in mediating resistance to anticancer drugs. This effort has led to the discovery of several genes encoding transmembrane proteins that function as drug efflux pumps. These genes are highly conserved and the vast majority belongs to the superfamily of adenosine triphosphate-binding cassette (ABC) proteins. A large number of human genes belonging to this superfamily have been identified, which have been systematically classified into seven subfamilies [ABCA, ABCB, ABCC, ABCD, ABCE ABCF and ABCG (Dean *et al.*, 2001)]. Most of these genes encode ATP-driven pumps that are able to transport a wide range of substrates.

Studies addressing the role of multidrug transporters in the development of pharmacoresistant epilepsy have hitherto been focused mainly on a subset of these transporters. *MDR1* (belonging to the ABCB subfamily, systematic nomenclature ABCB1) encodes P-glycoprotein (PGP, Silverman *et al.*, 1991; Ueda *et al.*, 1993), which transports a wide range of lipophilic substances across cell membranes. A further family of genes [multidrug-resistance associated proteins or MRPs, systematic nomenclature ABCC subfamily (Borst *et al.*, 2000)] transports a range of substances partially overlapping with those transported by PGP. Most of the proteins encoded by these genes (i.e. MRP1 to 6 and PGP) are expressed in endothelial cells of the blood–brain or blood–CSF barrier

(Schinkel *et al.*, 1996; Huai-Yun *et al.*, 1998; Rao *et al.*, 1999; Zhang *et al.*, 1999). In addition, MRP1 and one of the two rodent isoforms of PGP are present in astrocytes (Pardridge *et al.*, 1997; Regina *et al.*, 1998; Golden and Pardridge, 1999; Decleves *et al.*, 2000). The functional role of this expression is currently a matter of debate (Golden and Pardridge, 2000).

Altered expression of multidrug transporters in human and experimental epilepsy, and consequences for intraparenchymal AED concentrations

Several lines of evidence indicate a role of multidrug transporters in the development of resistance to AEDs, which are set out in more detail as follows. Firstly, drug transporters transport some AEDs in isolated cell systems (Batrakova *et al.*, 1999; Marchi *et al.*, 2004). Secondly, drug transporters appear to regulate intraparenchymal drug concentrations *in vivo* in many cases. Mice or rats lacking certain drug transporters display increased accumulation of AEDs (Schinkel *et al.*, 1996, 1997; Rizzi *et al.*, 2002; Potschka *et al.*, 2003*b*, but see Sills *et al.*, 2002; *see* Table 3). It should be noted that interpretation of data from such animal models is complicated by two issues: firstly, there may be a compensatory regulation of other drug transporter molecules and, secondly, the wide

Table 3 Drug efflux transporters and their anticonvulsant drug substrates (adapted from Loescher and Potschka, 2005)

Drug efflux transporter	Substrate
PGP (MDR1, ABCB1)	Phenytoin (Tishler *et al.*, 1995; Schinkel *et al.*, 1996; Potschka and Löscher, 2001; Rizzi *et al.*, 2002) Carbamazepine (Potschka *et al.*, 2001) Phenobarbital (Potschka *et al.*, 2002) Lamotrigine (Potschka *et al.*, 2002) Felbamate (Potschka *et al.*, 2002) Topiramate (Sills *et al.*, 2002)
MRP2 (ABCC2)	Phenytoin (Potschka *et al.*, 2003*b*)

expression of drug transporters in other tissues may result in complex pharmacokinetic effects of deleting drug transporter genes. These issues do not apply when drug transporters are inhibited pharmacologically *in vivo*. Indeed, pharmacological inhibition of drug transporters alters brain distribution of some AEDs (Potschka and Löscher, 2001; Potschka *et al.*, 2001, 2003*b*; Löscher and Potschka, 2002, *see* Table 3). It should be stated, however, that the drug transporter inhibitors employed thus far are not very specific, and that studies using more specific novel inhibitors are currently being undertaken.

Interestingly, the results in the literature with regard to one of the most frequently employed AEDs, carbamazepine, are not uniform. This drug is not transported in PGP-containing cell systems, and its brain concentration remains unaltered in mice lacking PGP (Owen *et al.*, 2001), or animals lacking MRP2 (Potschka *et al.*, 2003*a*). On the other hand, pharmacological (Potschka *et al.*, 2001) or genetic (Rizzi *et al.*, 2002) inhibition of PGP does appear to be associated with a change in intraparenchymal carbamazepine concentration under some conditions. The reasons for this discrepancy are currently under scrutiny. That not all AEDs may be transported equally by drug transporters has also been suggested in the case of some benzodiazepines, which appear not to be transported by MDR1 (Schinkel *et al.*, 1996). As a further possibility, carbamazepine has been shown to itself inhibit the activity of human P-glycoprotein, albeit at high concentrations which may not be clinically relevant (Weiss *et al.*, 2003).

A large body of evidence suggests that different drug transporter molecules are indeed upregulated in human epilepsy, as well as in experimental models of epilepsy. For instance, increased MDR1 expression on the mRNA and/or protein level occurs in patients with different forms of epilepsy (Tishler *et al.*, 1995; Lazarowski *et al.*, 1999; Sisodiya *et al.*, 1999, 2002; Aronica *et al.*, 2003; Volk and Löscher, 2005) and after chemically-induced status epilepticus or audiogenic seizures (Zhang *et al.*, 1999; Kwan *et al.*, 2002; Rizzi *et al.*, 2002). Similar findings have been obtained for MRP1 [(Sisodiya *et al.*, 2001, 2002), MRP2 (Dombrowski *et al.*, 2001)] and major vault protein (Van Vliet *et al.*, 2004). These studies have also shown that expression of drug

transporter genes in epileptic foci is observed in cell types that do not usually express them. For instance, PGP and MRP1 appear to be strongly upregulated on the protein level in astrocytes, especially surrounding blood vessels (Sisodiya *et al.*, 2002). Likewise, there may be upregulation of drug transporter expression in dysplastic neurons in focal cortical dysplasia (Sisodiya *et al.*, 2001), as well as in hippocampal neurons in temporal lobe epilepsy (Marchi *et al.*, 2004; Volk *et al.*, 2004). It is yet unclear how this ectopic expression might contribute to altered pharmacosensitivity. It is entirely possible, however, that expression of multidrug transporters in neuronal membranes inhibits access of AEDs to intracellular sites of action.

Relationship of molecular changes in AED sensitivity to pharmacoresistance observed *in vivo*

If multidrug transporters play a significant role in pharmacoresistance, then upregulation of transporters on the molecular or functional level should correlate with the clinically observed responsiveness to AEDs. Indeed, increased drug transporter expression appeared to be correlated with less efficient seizure control in one study (Tishler *et al.*, 1995). This correlation is also found in an animal model of resistance to AEDs. Rats resistant to phenobarbital showed a dramatic overexpression of PGP in limbic brain regions compared to rats responsive to phenobarbital (Volk and Löscher, 2005). Similar findings have been obtained in an elegant study using MRP2-deficient rats. In this study, MRP2-deficient kindled rats have higher PHT brain levels than wild-type rats, and are more susceptible to PHT treatment (Potschka *et al.*, 2003*b*). These findings are interesting because they constitute the first controlled experiment in which deficiency in a specific drug transporter is associated with differential susceptibility to AED treatment.

Even though, collectively, these findings appear to support a role for multidrug transporters in pharmacoresistant epilepsy, there are a number of conceptual questions that remain enigmatic. Firstly, epileptic seizures are known to result in a disruption of the BBB, which would be expected to result in better access of AEDs to brain parenchyma despite the upregulation of multidrug transporters. Secondly, patients are in many cases treated with AEDs until CNS side effects develop. This seems to indicate that relevant CNS concentrations of AEDs are reached despite transporter upregulation, yet, these patients are resistant to treatment. This apparent discrepancy could potentially arise via local upregulation of drug transporters that only affects AED concentrations at the epileptic focus.

The genetic basis of pharmacoresistance

How can the wide spectrum of pharmacoresistance observed in human epilepsy patients and some animal models be explained? A number of recent studies have suggested that

sequence variants in drug transporter or ion channel genes affect either function or expression of the corresponding proteins. In the case of drug transporters, a number of functionally relevant polymorphisms have been identified (Kerb *et al.*, 2001*a*, *b*). Furthermore, a polymorphism (C3435T) has been identified in exon 25 of the gene encoding MDR1 that is associated with increased expression of the protein (CC-genotype). Based on these findings, Siddiqui *et al.* (2003) conducted a population-based association study testing the hypothesis that the C3435T polymorphism is associated with resistance to AED treatment. They found that patients with drug-resistant epilepsy were more likely to have the CC-genotype than the TT-genotype [OR 2.66, 95% CI 1.32–5.38, *P* = 0.006 (Siddiqui *et al.*, 2003)]. As suggested by the authors of this study, the C3435T polymorphism by itself is very unlikely to confer a biologically relevant effect. Since this variant is localized in an extensive block of linkage disequilibrium spanning the gene, the as yet unidentified causal variant is supposed be in linkage disequilibrium with the C-allele of the C3435T polymorphism. It should be noted that the results of Siddiqui *et al.* (2003) have not been confirmed in a subsequent study by Tan *et al.* (2004). To further address how polymorphisms can contribute to drug resistance, two major obstacles will have to be overcome. Firstly, it will be necessary to address experimentally whether polymorphisms found in association studies have biologically relevant effects. Secondly, it will be necessary to significantly increase the size of carefully matched patient cohorts to increase reproducibility of such results (Soranzo *et al.*, 2004; Cavalleri *et al.*, 2005). Finally, it will be interesting to extend current studies to include polymorphisms in other multidrug transporters. In this respect, the development of single nucleotide polymorphism tagging for classes of genes important in resistance is a very important step that may enable screening of large numbers of patients (Ahmadi *et al.*, 2005).

It is important to note that gene polymorphisms relevant for pharmacoresistance may occur both in promoter regions as well as in introns and exons. Gene polymorphisms within the coding regions of such genes would result in a difference in ion channel or transporter proteins that precedes the onset of epilepsy. Polymorphisms in promoter regions, which affect the transcription of such genes, may affect activity-dependent transcriptional regulation of these genes by seizures. This provides a potential mechanism for the acquisition of a pharmacoresistant phenotype during epileptogenesis in pharmacoresistant—as opposed to pharmacoresponsive—patients.

Interplay between target and transporter-mediated mechanisms

Collectively, the experimental results described in the above sections indicate that functionally relevant alterations in both AED targets and AED transporters exist. Clearly, these mechanisms are not mutually exclusive. It is entirely possible

that decreased permeation of AEDs into brain tissue, in synergy with changes in targets for these drugs, mediate pharmacoresistance. This does not exclude that—for some AEDs—predominant mechanisms underlying pharmacoresistance to these drugs can be identified.

Carbamazepine, for instance, appears not to be a substrate of some multidrug transporters (Owen *et al.*, 2001); rather, sodium channels display a potent loss of sensitivity to carbamazepine (Remy *et al.*, 2003*a*). These and other results imply that expression of multidrug transporters at the BBB is not the main factor in the development of resistance, and imply a target mechanism for this drug. For other AEDs, this may be different. For instance, intraparenchymal PHT concentration is potently regulated by multidrug transporters (Potschka and Löscher, 2001; Rizzi *et al.*, 2002; Potschka *et al.*, 2003*b*), and epileptic animals lacking a multidrug transporter protein (MRP2) are more sensitive to PHT treatment than wild-type animals (Potschka *et al.*, 2003*b*). In contrast, target mechanisms seem to be less important for PHT compared to carbamazepine (Jeub *et al.*, 2002; Remy *et al.*, 2003*b*). Although the evidence supporting this view is far from conclusive, it is tempting to speculate that predominant resistance mechanisms may exist for specific AEDs.

Future directions of research

What are the key pieces of evidence that we should consider to be prerequisites in order to state that drug transporters and/or altered targets play a role in the development of resistance to a given AED? We believe that the following sets of experimental results should be available:

(i) Evidence that the multidrug transporter regulates intraparenchymal concentrations of the drug: this should include work with specific drug transporter inhibitors, and mice lacking specific drug transporter subtypes, as well as a combination of pharmacological and genetic inhibition of transporter function.

(ii) Evidence that multidrug transporter expression and/or transporter function is upregulated in human and experimental epilepsy. Regarding drug targets, evidence should be available that drug targets are less sensitive to a given AED in chronic epilepsy. In both cases, functional and molecular changes should correlate with AED sensitivity of seizures in experimental animals or epilepsy patients.

(iii) Evidence that genetic or pharmacological manipulation of drug transporters/drug targets affects sensitivity of spontaneous seizures to AEDs *in vivo* in chronic models of epilepsy.

In addition, the following data on human epilepsy patients should be obtained.

(iv) Association of polymorphisms in drug transporter/drug target genes with clinical pharmacoresistance. This could also include association of polymorphisms with the drug
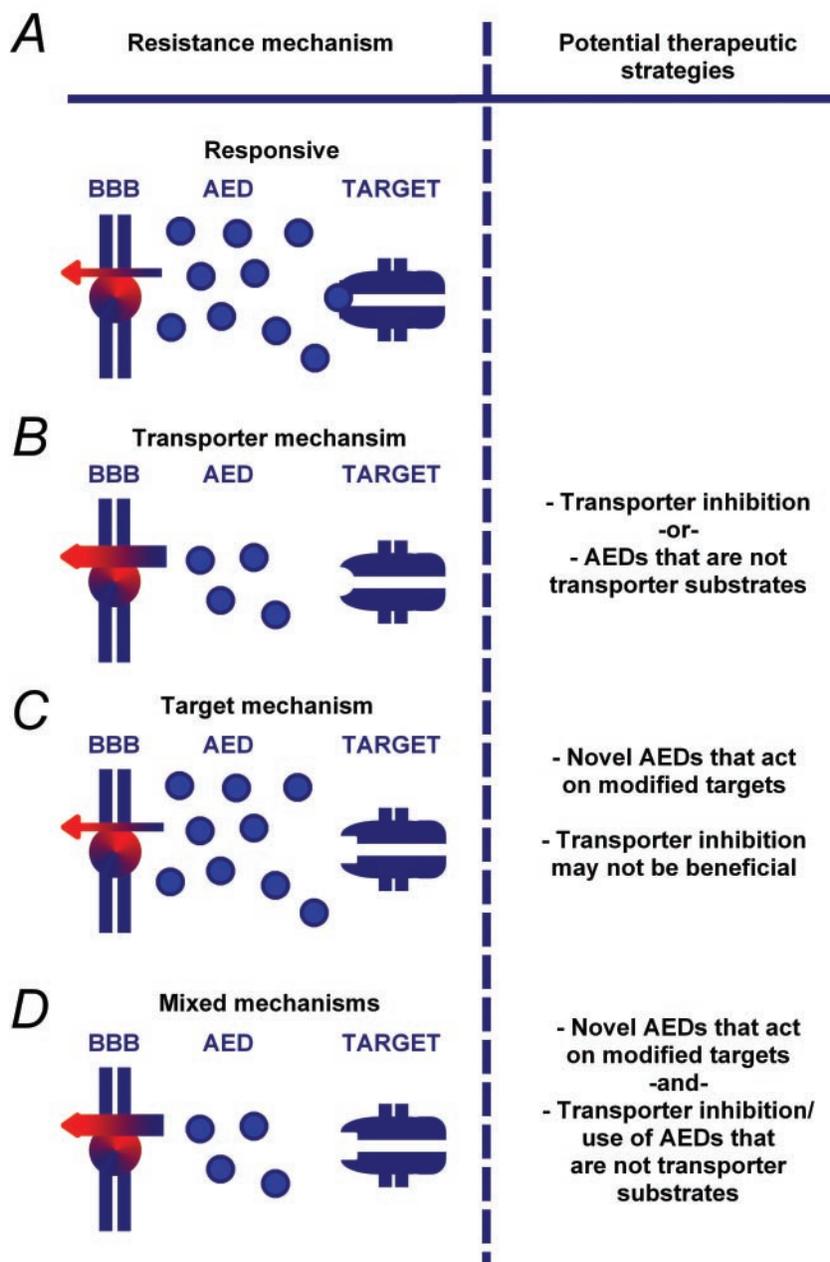
Fig. 4 Potential implications for treatment of pharmacoresistant epilepsy when taking into account different mechanisms underlying pharmacoresistance. Responsive patients (**A**): In patients responsive to a given AED, the AED gains sufficient access to a target. In addition, the AED has sufficient pharmacological effects on the target. Resistant patients, predominant transporter mechanism of resistance (**B**): In a scenario of increased drug transport without any changes in the AED target, the use of AEDs that are not transporter substrates and may additionally exhibit transporter-inhibiting properties would be advantageous in obtaining therapeutic drug levels at the site of action. Alternatively, the use of transporter inhibitors may increase the intraparenchymal drug concentration to therapeutic levels and may help to overcome the reduced efficacy of the drug. Resistant patients, predominant target mechanism of resistance (**C**): If resistance for a given AED is due to a change in its target, increasing the intraparenchymal AED concentration by comedication with transporter inhibitors would not be expected to be beneficial. In this case, the development of drugs that specifically act on the modified target would be the more appropriate approach. Resistant patients, mixed resistance mechanism (**D**): If both transporter- and target-mediated mechanisms of resistance apply, both strategies outlined in panel B and C would have to be combined.

transporter function or drug target pharmacology measured *in vitro* in tissue obtained from epilepsy surgery.

(v) Demonstration that drug transporter polymorphisms have functional effects resulting in a decreased intraparenchymal AED concentration (i.e. effects on either expression or function of drug transporter molecules). Likewise, polymorphisms in drug target genes should have demonstrable effects on expression or pharmacology of these targets.

Potential future clinical implications

Once we have obtained a detailed picture of the mechanisms underlying the development of pharmacoresistance to individual AEDs, this knowledge may become increasingly important both in drug development, as well as clinically. First, detailed information on drug target changes can be used to inform the development of new drugs for the treatment of epilepsy. Currently, identification of AEDs is performed mostly in acute seizure models in normal experimental animals. These animals do not show any of the chronic changes in ion channels or receptors discussed here, and may not represent the best models to develop novel compounds useful in human epilepsy. Targeting novel drugs to 'epileptic' ion channels based on information from chronic models of epilepsy or even tissue from epileptic patients represents a promising avenue for rational drug development in the future.

Information on specific resistance mechanisms might also be used to guide potential treatment with drug transporter inhibitors in conjunction with AEDs. The simplest scenario in which such substances might be used would be as comedication with an AED that is ineffective predominantly due to transporter-mediated mechanisms (depicted schematically in Fig. 4B). On the other hand, comedication with transporter inhibitors in a patient in whom resistance to an AED is predominantly target mediated (*see* Fig. 4C) would not be expected to be beneficial. In this case, the development of drugs that specifically act on the modified target would be the more appropriate approach. Ideally, these compounds would not be substrates of drug transporter, but could also be coadministered with transporter inhibitors. For some AEDs, both resistance mechanisms may be relevant and synergistic (Fig. 4D); in this case, strategies to overcome resistance would have to combine the approaches outlined in Fig. 4B and C. A further clinical issue that may gain more and more importance in the coming years is the identification of predictors that identify clinically resistant or responsive patient populations. If clear genetic polymorphisms in either transporter or ion channel genes can be identified that reliably predict the occurrence and the probable mechanism of drug resistance (Soranzo *et al.*, 2004; Tan *et al.*, 2004; Ahmadi *et al.*, 2005), these data would obviously strongly influence initial therapy, and perhaps increase the chances of its success.

References

Ahmadi KR, Weale ME, Xue ZY, Soranzo N, Yarnall DP, Briley JD, et al. A single-nucleotide polymorphism tagging set for human drug metabolism and transport. Nat Genet 2005; 37: 84–9.

Aicardi J, Shorvon SD. Intractable epilepsy. In: Engel J, Pedley TA, editors. Epilepsy: a comprehensive textbook. Philadelphia: Lippincott-Raven; 1997. p. 1325–31.

Alden KJ, Garcia J. Differential effect of gabapentin on neuronal and muscle calcium currents. J Pharmacol Exp Ther 2001; 297: 727–35.

Aronica E, Yankaya B, Troost D, van Vliet EA, Lopes da Silva FH, Gorter JA. Induction of neonatal sodium channel II and III alpha-isoform mRNAs in neurons and microglia after status epilepticus in the rat hippocampus. Eur J Neurosci 2001; 13: 1261–6.

Aronica E, Gorter JA, Jansen GH, van Veelen CW, van Rijen PC, Leenstra S, et al. Expression and cellular distribution of multidrug transporter proteins in two major causes of medically intractable epilepsy: focal cortical dysplasia and glioneuronal tumors. Neuroscience 2003; 118: 417–29.

Bartolomei F, Gastaldi M, Massacrier A, Planells R, Nicolas S, Cau P. Changes in the mRNAs encoding subtypes I, II and III sodium channel alpha subunits following kainate-induced seizures in rat brain. J Neurocytol 1997; 26: 667–78.

Batrakova EV, Li S, Miller DW, Kabanov AV. Pluronic P85 increases permeability of a broad spectrum of drugs in polarized BBMEC and Caco-2 cell monolayers. Pharm Res 1999; 16: 1366–72.

Ben Menachem E. Pregabalin pharmacology and its relevance to clinical practice. Epilepsia 2004; 45 Suppl 6: 13–8.

Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, et al. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. J Neurosci 2003; 23: 6826–36.

Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. Science 2004; 305: 532–5.

Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 2000; 92: 1295–302.

Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Coulter DA. Selective changes in single cell GABA(A) receptor subunit expression and function in temporal lobe epilepsy. Nat Med 1998; 4: 1166–72.

Brooks-Kayal AR, Shumate MD, Jin H, Lin DD, Rikhter TY, Holloway KL, et al. Human neuronal gamma-aminobutyric acid(A) receptors: coordinated subunit mRNA expression and functional correlates in individual dentate granule cells. J Neurosci 1999; 19: 8312–8.

Catterall WA. Molecular properties of brain sodium channels: an important target for anticonvulsant drugs. Adv Neurol 1999; 79: 441–56.

Cavalleri GL, Lynch JM, Depondt C, Burley MW, Wood NW, Sisodiya SM, et al. Failure to replicate previously reported genetic associations with sporadic temporal lobe epilepsy: where to from here? Brain 2005; 128: 1832–40.

Chao TI, Alzheimer C. Effects of phenytoin on the persistent Na$^+$ current of mammalian CNS neurones. NeuroReport 1995; 6: 1778–80.

Chappell AS, Sander JW, Brodie MJ, Chadwick D, Lledo A, Zhang D, et al. A crossover, add-on trial of talampanel in patients with refractory partial seizures. Neurology 2002; 58: 1680–2.

Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. Nat Med 2001; 7: 331–7.

Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 2002; 298: 1418–21.

Cohen AS, Lin DD, Quirk GL, Coulter DA. Dentate granule cell GABA(A) receptors in epileptic hippocampus: enhanced synaptic efficacy and altered pharmacology. Eur J Neurosci 2003; 17: 1607–16.

Costa E. From GABAA receptor diversity emerges a unified vision of GABAergic inhibition. Annu Rev Pharmacol Toxicol 1998; 38: 321–50.

Coulter DA, Huguenard JR, Prince DA. Characterization of ethosuximide reduction of low-threshold calcium current in thalamic neurons. Ann Neurol 1989; 25: 582–93.

Curia G, Aracri P, Sancini G, Mantegazza M, Avanzini G, Franceschetti S. Protein-kinase C-dependent phosphorylation inhibits the effect of the antiepileptic drug topiramate on the persistent fraction of sodium currents. Neuroscience 2004; 127: 63–8.

Dalby NO. GABA-level increasing and anticonvulsant effects of three different GABA uptake inhibitors. Neuropharmacology 2000; 39: 2399–407.

Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001; 11: 1156–66.

Decleves X, Regina A, Laplanche JL, Roux F, Boval B, Launay JM, et al. Functional expression of P-glycoprotein and multidrug resistance-associated protein (Mrp1) in primary cultures of rat astrocytes. J Neurosci Res 2000; 60: 594–601.

Dombrowski SM, Desai SY, Marroni M, Cucullo L, Goodrich K, Bingaman W, et al. Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. Epilepsia 2001; 42: 1501–6.

Eghbali M, Curmi JP, Birnir B, Gage PW. Hippocampal GABA(A) channel conductance increased by diazepam. Nature 1997; 388: 71–5.

Ellerkmann RK, Remy S, Chen J, Sochivko D, Elger CE, Urban BW, et al. Molecular and functional changes in voltage-dependent Na$^+$ channels following pilocarpine-induced status epilepticus in rat dentate granule cells. Neuroscience 2003; 119: 323–33.

Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, et al. Nomenclature of voltage-gated calcium channels. Neuron 2000; 25: 533–5.

Ffrench-Mullen JM, Barker JL, Rogawski MA. Calcium current block by (−)-pentobarbital, phenobarbital, and CHEB but not (+)-pentobarbital in acutely isolated hippocampal CA1 neurons: comparison with effects on GABA-activated Cl$^-$ current. J Neurosci 1993; 13: 3211–21.

Fink K, Meder W, Dooley DJ, Göthert M. Inhibition of neuronal Ca^{2+} influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. Br J Pharmacol 2000; 130: 900–6.

Fink-Jensen A, Suzdak PD, Swedberg MD, Judge ME, Hansen L, Nielsen PG. The gamma-aminobutyric acid (GABA) uptake inhibitor, tiagabine, increases extracellular brain levels of GABA in awake rats. Eur J Pharmacol 1992; 220: 197–201.

Fohlmeister JF, Adelman WJ Jr, Brennan JJ. Excitable channel currents and gating times in the presence of anticonvulsants ethosuximide and valproate. J Pharmacol Exp Ther 1984; 230: 75–81.

Frahm C, Stief F, Zuschratter W, Draguhn A. Unaltered control of extracellular GABA-concentration through GAT-1 in the hippocampus of rats after pilocarpine-induced status epilepticus. Epilepsy Res 2003; 52: 243–52.

Freiman TM, Kukolja J, Heinemeyer J, Eckhardt K, Aranda H, Rominger A, et al. Modulation of K+-evoked [3H]-noradrenaline release from rat and human brain slices by gabapentin: involvement of K$_{ATP}$ channels. Naunyn Schmiedebergs Arch Pharmacol 2001; 363: 537–42.

Gastaldi M, Bartolomei F, Massacrier A, Planells R, Robaglia-Schlupp A, Cau P. Increase in mRNAs encoding neonatal II and III sodium channel alpha-isoforms during kainate-induced seizures in adult rat hippocampus. Brain Res Mol Brain Res 1997; 44: 179–90.

Gastaldi M, Robaglia-Schlupp A, Massacrier A, Planells R, Cau P. mRNA coding for voltage-gated sodium channel beta2 subunit in rat central nervous system: cellular distribution and changes following kainate-induced seizures. Neurosci Lett 1998; 249: 53–6.

Gebhardt C, Breustedt JM, Noldner M, Chatterjee SS, Heinemann U. The antiepileptic drug losigamone decreases the persistent Na$^+$ current in rat hippocampal neurons. Brain Res 2001; 920: 27–31.

Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the $\alpha_2\delta$ subunit of a calcium channel. J Biol Chem 1996; 271: 5768–76.

Gibbs JW III, Shumate M, Coulter D. Differential epilepsy-associated alerations in postsynaptic GABA$_A$ receptor function in dentate granule and CA1 neurons. J Neurophysiol 1997; 77: 1924–38.

Gibbs JW III, Sombati S, DeLorenzo RJ, Coulter DA. Cellular actions of topiramate: blockade of kainate-evoked inward currents in cultured hippocampal neurons. Epilepsia 2000; 41 Suppl 1: S10–S16.

Golden PL, Pardridge WM. P-Glycoprotein on astrocyte foot processes of unfixed isolated human brain capillaries. Brain Res 1999; 819: 143–6.

Golden PL, Pardridge WM. Brain microvascular P-glycoprotein and a revised model of multidrug resistance in brain. Cell Mol Neurobiol 2000; 20: 165–81.

Goldin AL. Diversity of mammalian voltage-gated sodium channels. Ann N Y Acad Sci 1999; 868: 38–50.

Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, et al. Nomenclature of voltage-gated sodium channels. Neuron 2002; 25: 365–8.

Gomora JC, Daud AN, Weiergraber M, Perez-Reyes E. Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. Mol Pharmacol 2001; 60: 1121–32.

Gordey M, DeLorey TM, Olsen RW. Differential sensitivity of recombinant GABA(A) receptors expressed in Xenopus oocytes to modulation by topiramate. Epilepsia 2000; 41 Suppl 1: S25–S29.

Gryder DS, Rogawski MA. Selective antagonism of GluR5 kainate-receptor-mediated synaptic currents by topiramate in rat basolateral amygdala neurons. J Neurosci 2003; 23: 7069–74.

Gulledge AT, Stuart GJ. Excitatory actions of GABA in the cortex. Neuron 2003; 37: 299–309.

Harty TP, Rogawski MA. Felbamate block of recombinant N-methyl-D-aspartate receptors: selectivity for the NR2B subunit. Epilepsy Res 2000; 39: 47–55.

Herrero AI, Del Olmo N, Gonzalez-Escalada JR, Solis JM. Two new actions of topiramate: inhibition of depolarizing GABA(A)-mediated responses and activation of a potassium conductance. Neuropharmacology 2002; 42: 210–20.

Huai-Yun H, Secrest DT, Mark KS, Carney D, Brandquist C, Elmquist WF, et al. Expression of multidrug resistance-associated protein (MRP) in brain microvessel endothelial cells. Biochem Biophys Res Commun 1998; 243: 816–20.

Huang CW, Huang CC, Liu YC, Wu SN. Inhibitory effect of lamotrigine on A-type potassium current in hippocampal neuron-derived H19-7 cells. Epilepsia 2004; 45: 729–36.

Huguenard JR. Low-threshold calcium currents in central nervous system neurons. Annu Rev Physiol 1996; 58: 329–48.

Huguenard JR. Block of T-type calcium channels is an important action of succinimide antiabsence drugs. Epilepsy Curr 2002; 2: 49–52.

Isokawa M, Levesque MF. Increased NMDA responses and dendritic degeneration in human epileptic hippocampal neurons in slices. Neurosci Lett 1991; 132: 212–6.

Jeub M, Beck H, Siep E, Ruschenschmidt C, Speckmann EJ, Ebert U, et al. Effect of phenytoin on sodium and calcium currents in hippocampal CA1 neurons of phenytoin-resistant kindled rats. Neuropharmacology 2002; 42: 107–16.

Jolkkonen J, Mazurkiewicz M, Lahtinen H, Riekkinen P. Acute effects of gamma-vinyl GABA on the GABAergic system in rats as studied by microdialysis. Eur J Pharmacol 1992; 229: 269–72.

Kearney JA, Plummer NW, Smith MR, Kapur J, Cummins TR, Waxman SG, et al. A gain-of-function mutation in the sodium channel gene Scn2a results in seizures and behavioral abnormalities. Neuroscience 2001; 102: 307–17.

Kerb R, Aynacioglu AS, Brockmoller J, Schlagenhaufer R, Bauer S, Szekeres T, et al. The predictive value of MDR1, CYP2C9, and CYP2C19 polymorphisms for phenytoin plasma levels. Pharmacogenomics J 2001a; 1: 204–10.

Kerb R, Hoffmeyer S, Brinkmann U. ABC drug transporters: hereditary polymorphisms and pharmacological impact in MDR1, MRP1 and MRP2. Pharmacogenomics 2001b; 2: 51–64.

Kleckner NW, Glazewski JC, Chen CC, Moscrip TD. Subtype-selective antagonism of N-methyl-D-aspartate receptors by felbamate: insights into the mechanism of action. J Pharmacol Exp Ther 1999; 289: 886–94.

Köhling R. Voltage-gated sodium channels in epilepsy. Epilepsia 2002; 43: 1278–95.

Kohr G, De Koninck Y, Mody I. Properties of NMDA receptor channels in neurons acutely isolated from epileptic (kindled) rats. J Neurosci 1993; 13: 3612–27.

Kuo CC. A common anticonvulsant binding site for phenytoin, carbamazepine, and lamotrigine in neuronal Na$^+$ channels. Mol Pharmacol 1998; 54: 712–21.

Kuo CC, Lin BJ, Chang HR, Hsieh CP. Use-dependent inhibition of the N-methyl-D-aspartate currents by felbamate: a gating modifier with selective binding to the desensitized channels. Mol Pharmacol 2004; 65: 370–80.

Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med 2000; 342: 314–9.

Kwan P, Brodie MJ. Potential role of drug transporters in the pathogenesis of medically intractable epilepsy. Epilepsia 2005; 46: 224–35.

Kwan P, Sills GJ, Butler E, Gant TW, Meldrum BS, Brodie MJ. Regional expression of multidrug resistance genes in genetically epilepsy-prone rat brain after a single audiogenic seizure. Epilepsia 2002; 43: 1318–23.

Lacinova L. Pharmacology of recombinant low-voltage activated calcium channels. Curr Drug Targets CNS Neurol Disord 2004; 3: 105–11.

Lampl I, Schwindt P, Crill W. Reduction of cortical pyramidal neuron excitability by the action of phenytoin on persistent Na^+ current. J Pharmacol Exp Ther 1998; 284: 228–37.

Lazarowski A, Sevlever G, Taratuto A, Massaro M, Rabinowicz A. Tuberous sclerosis associated with MDR1 gene expression and drug-resistant epilepsy. Pediatr Neurol 1999; 21: 731–4.

Leite JP, Cavalheiro EA. Effects of conventional antiepileptic drugs in a model of spontaneous recurrent seizures in rats. Epilepsy Res 1995; 20: 93–104.

Leresche N, Parri HR, Erdemli G, Guyon A, Turner JP, Williams SR, et al. On the action of the anti-absence drug ethosuximide in the rat and cat thalamus. J Neurosci 1998; 18: 4842–53.

Löscher W. Valproate enhances GABA turnover in the substantia nigra. Brain Res 1989; 501: 198–203.

Löscher W, Horstermann D. Differential effects of vigabatrin, gamma-acetylenic GABA, aminooxyacetic acid, and valproate on levels of various amino acids in rat brain regions and plasma. Naunyn Schmiedebergs Arch Pharmacol 1994; 349: 270–8.

Löscher W, Potschka H. Role of multidrug transporters in pharmacoresistance to antiepileptic drugs. J Pharmacol Exp Ther 2002; 301: 7–14.

Löscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci 2005; 6: 591–602.

Löscher W, Cramer S, Ebert U. Selection of phenytoin responders and nonresponders in male and female amygdala-kindled Sprague-Dawley rats. Epilepsia 1998; 39: 1138–47.

Lossin C, Wang DW, Rhodes TH, Vanoye CG, George AL Jr. Molecular basis of an inherited epilepsy. Neuron 2002; 34: 877–84.

Lucas PT, Meadows LS, Nicholls J, Ragsdale DS. An epilepsy mutation in the beta1 subunit of the voltage-gated sodium channel results in reduced channel sensitivity to phenytoin. Epilepsy Res 2005; 64: 77–84.

Lukyanetz EA, Shkryl VM, Kostyuk PG. Selective blockade of N-type calcium channels by levetiracetam. Epilepsia 2002; 43: 9–18.

Madeja M, Margineanu DG, Gorji A, Siep E, Boerrigter P, Klitgaard H, et al. Reduction of voltage-operated potassium currents by levetiracetam: a novel antiepileptic mechanism of action? Neuropharmacology 2003; 45: 661–71.

Main MJ, Cryan JE, Dupere JR, Cox B, Clare JJ, Burbidge SA. Modulation of KCNQ2/3 potassium channels by the novel anticonvulsant retigabine. Mol Pharmacol 2000; 58: 253–62.

Marchi N, Hallene KL, Kight KM, Cucullo L, Moddel G, Bingaman W, et al. Significance of MDR1 and multiple drug resistance in refractory human epileptic brain. BMC Med 2004; 2: 37.

Martin D, McNamara JO, Nadler JV. Kindling enhances sensitivity of CA3 hippocampal pyramidal cells to NMDA. J Neurosci 1992; 12: 1928–35.

McClelland D, Evans RM, Barkworth L, Martin DJ, Scott RH. A study comparing the actions of gabapentin and pregabalin on the electrophysiological properties of cultured DRG neurones from neonatal rats. BMC Pharmacol 2004; 4: 14.

McLean MJ, Macdonald RL. Sodium valproate, but not ethosuximide, produces use- and voltage- dependent limitation of high frequency repetitive firing of action potentials of mouse central neurons in cell culture. J Pharmacol Exp Ther 1986; 237: 1001–11.

McLean MJ, Schmutz M, Wamil AW, Olpe HR, Portet C, Feldmann KF. Oxcarbazepine: mechanisms of action. Epilepsia 1994; 35 Suppl 3: S5–S9.

McLean MJ, Bukhari AA, Wamil AW. Effects of topiramate on sodium-dependent action-potential firing by mouse spinal cord neurons in cell culture. Epilepsia 2000; 41 Suppl 1: S21–S24.

Mody I, Heinemann U. NMDA receptors of dentate gyrus granule cells participate in synaptic transmission following kindling. Nature 1987; 326: 701–4.

Mody I, Pearce RA. Diversity of inhibitory neurotransmission through GABA(A) receptors. Trends Neurosci 2004; 27: 569–75.

Niespodziany I, Klitgaard H, Margineanu DG. Is the persistent sodium current a specific target of anti-absence drugs? NeuroReport 2004; 15: 1049–52.

Nissinen JPT, Pitkänen A. An animal model with spontaneous seizures: a new tool for testing the effects of antiepileptic compounds. Epilepsia 2000; 41: 136.

Nobile M, Lagostena L. A discriminant block among K^+ channel types by phenytoin in neuroblastoma cells. Br J Pharmacol 1998; 124: 1698–702.

Owen A, Pirmohamed M, Tettey JN, Morgan P, Chadwick D, Park BK. Carbamazepine is not a substrate for P-glycoprotein. Br J Clin Pharmacol 2001; 51: 345–9.

Pardridge WM, Golden PL, Kang YS, Bickel U. Brain microvascular and astrocyte localization of P-glycoprotein. J Neurochem 1997; 68: 1278–85.

Poolos NP, Migliore M, Johnston D. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. Nat Neurosci 2002; 5: 767–74.

Potschka H, Löscher W. In-vivo evidence for P-glycoprotein-mediated transport of phenytoin at the blood-brain barrier of rats. Epilepsia 2001; 42: 1231–40.

Potschka H, Fedrowitz M, Löscher W. P-glycoprotein and multidrug resistance-associated protein are involved in the regulation of extracellular levels of the major antiepileptic drug carbamazepine in the brain. Neuroreport 2001; 12: 3557–60.

Potschka H, Fedrowitz M, Löscher W. P-Glycoprotein-mediated efflux of phenobarbital, lamotrigine, and felbamate at the blood-brain barrier: evidence from microdialysis experiments in rats. Neurosci Lett 2002; 327: 173–6.

Potschka H, Fedrowitz M, Löscher W. Brain access and anticonvulsant efficacy of carbamazepine, lamotrigine, and felbamate in ABCC2/MRP2-deficient TR- rats. Epilepsia 2003a; 44: 1479–86.

Potschka H, Fedrowitz M, Löscher W. Multidrug resistance protein MRP2 contributes to blood-brain barrier function and restricts antiepileptic drug activity. J Pharmacol Exp Ther 2003b; 306: 124–31.

Qian J, Noebels JL. Topiramate alters excitatory synaptic transmission in mouse hippocampus. Epilepsy Res 2003; 55: 225–33.

Ragsdale DS, Avoli M. Sodium channels as molecular targets for antiepileptic drugs. Brain Res Brain Res Rev 1998; 26: 16–28.

Ragsdale DS, Scheuer T, Catterall WA. Frequency and voltage-dependent inhibition of type IIA Na^+ channels, expressed in a mammalian cell line, by local anesthetic, antiarrhythmic, and anticonvulsant drugs. Mol Pharmacol 1991; 40: 756–65.

Rao VV, Dahlheimer JL, Bardgett ME, Snyder AZ, Finch RA, Sartorelli AC, et al. Choroid plexus epithelial expression of MDR1 P-glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. Proc Natl Acad Sci USA 1999; 96: 3900–5.

Reckziegel G, Beck H, Schramm J, Urban BW, Elger CE. Carbamazepine effects on Na^+ currents in human dentate granule cells from epileptogenic tissue. Epilepsia 1999; 40: 401–7.

Regesta G, Tanganelli P. Clinical aspects and biological bases of drug-resistant epilepsies. Epilepsy Res 1999; 34: 109–22.

Regina A, Koman A, Piciotti M, El Hafny B, Center MS, Bergmann R, et al. Mrp1 multidrug resistance-associated protein and P-glycoprotein expression in rat brain microvessel endothelial cells. J Neurochem 1998; 71: 705–15.

Remy S, Gabriel S, Urban BW, Dietrich D, Lehmann TN, Elger CE, et al. A novel mechanism underlying drug-resistance in chronic epilepsy. Ann Neurol 2003a; 53: 469–79.

Remy S, Urban BW, Elger CE, Beck H. Anticonvulsant pharmacology of voltage-gated Na^+ channels in hippocampal neurons of control and chronically epileptic rats. Eur J Neurosci 2003b; 17: 2648–58.

Rho JM, Donevan SD, Rogawski MA. Barbiturate-like actions of the propanediol dicarbamates felbamate and meprobamate. J Pharmacol Exp Ther 1997; 280: 1383–91.

Rhodes TH, Lossin C, Vanoye CG, Wang DW, George AL Jr. Noninactivating voltage-gated sodium channels in severe myoclonic epilepsy of infancy. Proc Natl Acad Sci USA 2004; 101: 11147–52.

Rizzi M, Caccia S, Guiso G, Richichi C, Corter JA, Aronica E, et al. Limbic seizures induce P-glycoprotein in rodent brain: functional implications for pharmacoresistance. J Neurosci 2002; 22: 5833–9.

Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 2003; 65: 453–80.

Rogers CJ, Twyman RE, Macdonald RL. Benzodiazepine and β-carboline regulation of single GABA$_A$ receptor channels of mouse spinal neurones in culture. J Physiol (Lond) 1994; 475: 69–82.

Rudolph U, Crestani F, Benke D, Brunig I, Benson JA, Fritschy JM, et al. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature 1999; 401: 796–800.

Rudolph U, Crestani F, Mohler H. GABA(A) receptor subtypes: dissecting their pharmacological functions. Trends Pharmacol Sci 2001; 22: 188–94.

Sanabria ERG, Su H, Yaari Y. Initiation of network bursts by Ca^{2+}-dependent intrinsic bursting in the rat pilocarpine model of temporal lobe epilepsy. J Physiol 2001; 205–16.

Schauf CL. Zonisamide enhances slow sodium inactivation in Myxicola. Brain Res 1987; 413: 185–8.

Schinkel AH, Wagenaar E, Mol CA, Van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 1996; 97: 2517–24.

Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smitt JJ, et al. Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci USA 1997; 94: 4028–33.

Schmutz M, Brugger F, Gentsch C, McLean MJ, Olpe HR. Oxcarbazepine: preclinical anticonvulsant profile and putative mechanisms of action. Epilepsia 1994; 35 Suppl 5: S47–S50.

Schumacher TB, Beck H, Steinhauser C, Schramm J, Elger CE. Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal granule cells from patients with temporal lobe epilepsy. Epilepsia 1998; 39: 355–63.

Schwarz JR, Grigat G. Phenytoin and carbamazepine: potential- and frequency-dependent block of Na currents in mammalian myelinated nerve fibers. Epilepsia 1989; 30: 286–94.

Segal MM, Douglas AF. Late sodium channel openings underlying epileptiform activity are preferentially diminished by the anticonvulsant phenytoin. J Neurophysiol 1997; 77: 3021–34.

Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. Neuron 2004; 44: 495–508.

Siddiqui A, Kerb R, Weale ME, Brinkmann U, Smith A, Goldstein DB, et al. Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. N Engl J Med 2003; 348: 1442–8.

Sills GJ, Kwan P, Butler E, de Lange EC, van den Berg DJ, Brodie MJ. P-glycoprotein-mediated efflux of antiepileptic drugs: preliminary studies in mdr1a knockout mice. Epilepsy Behav 2002; 3: 427–32.

Silverman JA, Raunio H, Gant TW, Thorgeirsson SS. Cloning and characterization of a member of the rat multidrug resistance (mdr) gene family. Gene 1991; 106: 229–36.

Sisodiya SM, Heffernan J, Squier MV. Over-expression of P-glycoprotein in malformations of cortical development. Neuroreport 1999; 10: 3437–41.

Sisodiya SM, Lin WR, Squier MV, Thom M. Multidrug-resistance protein 1 in focal cortical dysplasia. Lancet 2001; 357: 42–3.

Sisodiya SM, Lin WR, Harding BN, Squier MV, Thom M. Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. Brain 2002; 125: 22–31.

Soranzo N, Cavalleri GL, Weale ME, Wood NW, Despondt C, Marguerie R, et al. Identifying candidate causal variants responsible for altered activity of the ABCB1 multidrug resistance gene. Genome Res 2004; 14: 1333–44.

Spadoni F, Hainsworth AH, Mercuri NB, Caputi L, Martella G, Lavaroni F, et al. Lamotrigine derivatives and riluzole inhibit INa,P in cortical neurons. Neuroreport 2002; 13: 1167–70.

Spampanato J, Kearney JA, de Haan G, McEwen DP, Escayg A, Aradi I, et al. A novel epilepsy mutation in the sodium channel SCN1A identifies a

cytoplasmic domain for beta subunit interaction. J Neurosci 2004; 24: 10022–34.

Sperk G, Furtinger S, Schwarzer C, Pirker S. GABA and its receptors in epilepsy. Adv Exp Med Biol 2004; 548: 92–103.

Stefani A, Pisani A, De Murtas M, Mercuri NB, Marciani MG, Calabresi P. Action of GP 47779, the active metabolite of oxcarbazepine, on the corticostriatal system. II. Modulation of high-voltage-activated calcium currents. Epilepsia 1995; 36: 997–1002.

Stefani A, Spadoni F, Bernardi G. Differential inhibition by riluzole, lamotrigine, and phenytoin of sodium and calcium currents in cortical neurons: implications for neuroprotective strategies. Exp Neurol 1997a; 147: 115–22.

Stefani A, Spadoni F, Bernardi G. Voltage-activated calcium channels: targets of antiepileptic drug therapy? Epilepsia 1997b; 38: 959–65.

Stefani A, Spadoni F, Bernardi G. Gabapentin inhibits calcium currents in isolated rat brain neurons. Neuropharmacology 1998; 37: 83–91.

Study RE, Barker JL. Diazepam and (−)-pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responses in cultured central neurons. Proc Natl Acad Sci USA 1981; 78: 7180–4.

Su H, Alroy G, Kirson ED, Yaari Y. Extracellular calcium modulates persistent sodium current-dependent intrinsic bursting in rat hippocampal neurons. J Neurosci 2001; 21: 4173–82.

Su H, Sochivko D, Becker A, Chen J, Jiang Y Yaari Y, et al. Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus. J Neurosci 2002; 22: 3645–55.

Subramaniam S, Rho JM, Penix L, Donevan SD, Fielding RP, Rogawski MA. Felbamate block of the N-methyl-D-aspartate receptor. J Pharmacol Exp Ther 1995; 273: 878–86.

Surges R, Freiman TM, Feuerstein TJ. Gabapentin increases the hyperpolarization-activated cation current Ih in rat CA1 pyramidal cells. Epilepsia 2003; 44: 150–6.

Suzuki S, Kawakami K, Nishimura S, Watanabe Y, Yagi K, Seino M, et al. Zonisamide blocks T-type calcium channel in cultured neurons of rat cerebral cortex. Epilepsy Res 1992; 12: 21–7.

Taglialatela M, Ongini E, Brown AM, Di Renzo G, Annunziato L. Felbamate inhibits cloned voltage-dependent Na$^+$ channels from human and rat brain. Eur J Pharmacol 1996; 316: 373–7.

Tan NC, Heron SE, Scheffer IE, Pelekanos JT, McMahon JM, Vears DF, et al. Failure to confirm association of a polymorphism in ABCB1 with multidrug-resistant epilepsy. Neurology 2004; 63: 1090–2.

Tate SK, Depondt C, Sisodiya SM, Cavalleri GL, Schorge S, Soranzo N, et al. Genetic predictors of the maximum doses patients receive during clinical use of the anti-epileptic drugs carbamazepine and phenytoin. Proc Natl Acad Sci USA 2005; 102: 5507–12.

Tatulian L, Delmas P, Abogadie FC, Brown DA. Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. J Neurosci 2001; 21: 5535–45.

Taverna S, Mantegazza M, Franceschetti S, Avanzini G. Valproate selectively reduces the persistent fraction of Na$^+$ current in neocortical neurons. Epilepsy Res 1998; 32: 304–8.

Taverna S, Sancini G, Mantegazza M, Franceschetti S, Avanzini G. Inhibition of transient and persistent Na$^+$ current fractions by the new anticonvulsant topiramate. J Pharmacol Exp Ther 1999; 288: 960–8.

Thompson SM, Gähwiler BH. Effects of the GABA uptake inhibitor tiagabine on inhibitory synaptic potentials in rat hippocampal slice cultures. J Neurophysiol 1992; 67: 1698–701.

Tishler DM, Weinberg KI, Hinton DR, Barbaro N, Annett GM, Raffel C. MDR1 gene expression in brain of patients with medically intractable epilepsy. Epilepsia 1995; 36: 1–6.

Todorovic SM, Lingle CJ. Pharmacological properties of T-type Ca^{2+} current in adult rat sensory neurons: effects of anticonvulsant and anesthetic agents. J Neurophysiol 1998; 79: 240–52.

Todorovic SM, Perez-Reyes E, Lingle CJ. Anticonvulsants but not general anesthetics have differential blocking effects on different T-type current variants. Mol Pharmacol 2000; 58: 98–108.

Twyman RE, Rogers CJ, Macdonald RL. Differential regulation of gamma-aminobutyric acid receptor channels by diazepam and phenobarbital. Ann Neurol 1989a; 25: 213–20.

Twyman RE, Rogers CJ, Macdonald RL. Pentobarbital and picrotoxin have reciprocal actions on single GABA$_A$ receptor channels. Neurosci Lett 1989b; 96: 89–95.

Ueda K, Shimabuku AM, Konishi H, Fujii Y, Takebe S, Nishi K, et al. Functional expression of human P-glycoprotein in Schizosaccharomyces pombe. FEBS Lett 1993; 330: 279–82.

Van Vliet EA, Aronica E, Redeker S, Gorter JA. Expression and cellular distribution of major vault protein: a putative marker for pharmacoresistance in a rat model for temporal lobe epilepsy. Epilepsia 2004; 45: 1506–16.

Volk HA, Löscher W. Multidrug resistance in epilepsy: rats with drug-resistant seizures exhibit enhanced brain expression of P-glycoprotein compared with rats with drug-responsive seizures. Brain 2005; 128: 1358–68.

Volk HA, Burkhardt K, Potschka H, Chen J, Becker A, Löscher W. Neuronal expression of the drug efflux transporter P-glycoprotein in the rat hippocampus after limbic seizures. Neuroscience 2004; 123: 751–9.

Vreugdenhil M, Van Veelen CWM, Van Rijen PC, Da Silva FHL, Wadman WJ. Effect of valproic acid on sodium currents in cortical neurons from patients with pharmaco-resistant temporal lobe epilepsy. Epilepsy Res 1998; 32: 309–20.

Vreugdenhil M, Wadman WJ. Modulation of sodium currents in rat CA1 neurons by carbamazepine and valproate after kindling epileptogenesis. Epilepsia 1999; 40: 1512–22.

Wang SJ, Huang CC, Hsu KS, Tsai JJ, Gean PW. Inhibition of N-type calcium currents by lamotrigine in rat amygdalar neurones. Neuroreport 1996; 7: 3037–40.

Weiss J, Kerpen CJ, Lindenmaier H, Dormann SM, Haefeli WE. Interaction of antiepileptic drugs with human P-glycoprotein in vitro. J Pharmacol Exp Ther 2003; 307: 262–7.

Whitaker WR, Faull RL, Dragunow M, Mee EW, Emson PC, Clare JJ. Changes in the mRNAs encoding voltage-gated sodium channel types II and III in human epileptic hippocampus. Neuroscience 2001; 106: 275–85.

White HS, Brown SD, Woodhead JH, Skeen GA, Wolf HH. Topiramate enhances GABA-mediated chloride flux and GABA-evoked chloride currents in murine brain neurons and increases seizure threshold. Epilepsy Res 1997; 28: 167–79.

White HS, Harmsworth WL, Sofia RD, Wolf HH. Felbamate modulates the strychnine-insensitive glycine receptor. Epilepsy Res 1995; 20: 41–8.

Willow M, Gonoi T, Catterall WA. Voltage clamp analysis of the inhibitory actions of diphenylhydantoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. Mol Pharmacol 1985; 27: 549–58.

Wozny C, Kivi A, Lehmann TN, Dehnicke C, Heinemann U, Behr J. Comment on 'On the origin of interictal activity in human temporal lobe epilepsy in vitro'. Science 2003; 301: 463.

Wu Y, Wang W, Richerson GB. Vigabatrin induces tonic inhibition via GABA transporter reversal without increasing vesicular GABA release. J Neurophysiol 2003; 89: 2021–34.

Xie X, Dale TJ, John VH, Cater HL, Peakman TC, Clare JJ. Electrophysiological and pharmacological properties of the human brain type IIA Na$^+$ channel expressed in a stable mammalian cell line. Pflugers Arch 2001; 441: 425–33.

Xie X, Lancaster B, Peakman T, Garthwaite J. Interaction of the antiepileptic drug lamotrigine with recombinant rat brain type IIA Na+ channels and with native Na$^+$ channels in rat hippocampal neurones. Pflugers Arch 1995; 430: 437–46.

Yaari Y, Hamon B, Lux HD. Development of two types of calcium channels in cultured mammalian hippocampal neurons. Science 1987; 235: 680–2.

Yue C, Yaari Y. KCNQ/M channels control spike after depolarization and burst generation in hippocampal neurons. J Neurosci 2004; 24: 4614–24.

Zhang L, Ong WY, Lee T. Induction of P-glycoprotein expression in astrocytes following intracerebroventricular kainate injections. Exp Brain Res 1999; 126: 509–16.

Zhang X, Velumian AA, Jones OT, Carlen PL. Modulation of high-voltage-activated calcium channels in dentate granule cells by topiramate. Epilepsia 2000; 41 Suppl 1: S52–60.

Zona C, Avoli M. Effects induced by the antiepileptic drug valproic acid upon the ionic currents recorded in rat neocortical neurons in cell culture. Exp Brain Res 1990; 81: 313–17.

Zona C, Avoli M. Lamotrigine reduces voltage-gated sodium currents in rat central neurons in culture. Epilepsia 1997; 38: 522–5.

Zona C, Ciotti MT, Avoli M. Topiramate attenuates voltage-gated sodium currents in rat cerebellar granule cells. Neurosci Lett 1997; 231: 123–6.

Zona C, Niespodziany I, Marchetti C, Klitgaard H, Bernardi G, Margineanu DG. Levetiracetam does not modulate neuronal voltage-gated Na+ and T-type Ca2+ currents. Seizure 2001; 10: 279–86.

Zona C, Tancredi V, Longone P, D'Arcangelo G, D'Antuono M, Manfredi M, et al. Neocortical potassium currents are enhanced by the antiepileptic drug lamotrigine. Epilepsia 2002; 43: 685–90.