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Mechanisms of Action of Antiseizure Drugs (Chapter 39)

Roger J Porter, University of Pennsylvania
Ashish Dhir, University of California, Davis
Robert L Macdonald, Vanderbilt University
Michael A Rogawski, University of California - Davis
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ROGER J. PORTER 1, ASHISH DHIR 2, ROBERT L. MACDONALD 3, AND MICHAEL A. ROGAWSKI 2*
1Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA, and Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA
2Department of Neurology, School of Medicine, University of California, Davis, Sacramento, CA, USA
3Departments of Neurology, Molecular Physiology and Biophysics, and Pharmacology, Vanderbilt University Medical Center, Nashville, TN, USA

INTRODUCTION

Most marketed antiseizure drugs were discovered by screening in animal models. Typically, only after considerable time has passed and the drug is in widespread clinical use is its mechanism elucidated. This chapter reviews the current understanding of how each of the marketed antiseizure drugs acts on brain mechanisms to protect against seizures.

There are many reasons why an understanding of the cellular mechanisms of action of these drugs is useful. An understanding of mechanism may serve as a guide to clinicians as to the seizure types and syndromes that a particular drug may or may not be useful to treat. For example, sodium channel-blocking antiseizure agents generally are useful to treat partial and generalized tonic–clonic seizures, but are not effective for absence epilepsy. However, empirical clinical validation is required as mechanism is not a foolproof predictor of clinical utility. For example, lamotrigine is believed to act through a sodium channel-blocking mechanism, but it does have activity against absence epilepsy. Another use of mechanistic knowledge is to determine rational combinations of drugs for polytherapy. It has been proposed that such combinations should include drugs acting by different “complementary” mechanisms (Perucca, 1995; Czuczwar and Borowicz, 2002). Some evidence from clinical trials has been obtained to support this notion (Sake et al., 2010). Finally, an understanding of mechanism may allow the identification of new molecules that act in a mechanistically similar fashion but have improved properties (Rogawski, 2008).

CLASSIFYING ANTISEIZURE DRUGS BY MECHANISM OF ACTION

In this chapter, we refer to drugs that provide symptomatic benefit (protect against seizures) as “antiseizure” drugs, although we recognize that the term “antiepileptic drug” is commonly used for this purpose. Antiseizure drugs prevent the occurrence of seizures and may also be used to stop ongoing seizure activity. These drugs act on the symptom of the epilepsies, the seizures themselves. There is no evidence that any marketed antiseizure drug has clinically relevant disease-modifying (antiepileptogenic) properties. Indeed, strategies for disease modification are likely to involve different brain mechanisms and different molecular targets than those applicable for antiseizure actions. However, in animal models in which epilepsy is triggered by seizure activity, antiseizure drugs may prevent the development of the epileptic state (Lösch, 2002a). The extent to which these experiments predict clinically relevant antiepileptogenic properties has been debated. Nevertheless, we know that some patients’ seizures stop after some years of antiseizure medications. Whether the drugs played a part in the cure or whether the natural history of the disease is really responsible is unknown.
(2) alterations in GABAergic inhibition via actions on GABA_A receptors or on GABA synthesis, reuptake, or degradation, (3) decreased synaptic excitation via actions on ionotropic glutamate receptors, and (4) modulation of neurotransmitter release via presynaptic mechanisms, with an action on glutamate release being most relevant. Figures 39.1 and 39.2 illustrate excitatory and inhibitory synapses, showing many of the known antiseizure drug targets.

**DRUGS DISCOVERED AND MARKETED BEFORE 1990**

**Phenobarbital**

Although bromides had been the mainstay of epilepsy therapy since the mid-19th century, the barbiturates came to the fore in 1912, when phenobarbital, which was being tested as a hypnotic, was serendipitously observed to reduce the frequency of seizures in patients with epilepsy. Phenobarbital has a diversity of pharmacological actions. Of particular importance is its ability to enhance GABA-mediated fast inhibitory synaptic transmission (Macdonald and Barker, 1978a). GABA is the major inhibitory neurotransmitter in the brain. GABA exerts fast synaptic inhibition via GABA_A receptors. In addition to localization at synapses, GABA_A receptors are also present perisynaptically and extrasynaptically; these receptors mediate nonsynaptic (tonic) inhibition (Sierra-Paredes and Sierra-Marcuno, 2007). Barbiturates have a powerful action on nonsynaptic δ-subunit-containing GABA_A receptors (Feng et al., 2004). The extent to which phenobarbital also enhances nonsynaptic GABA_A receptor isoforms is not known, but this action could potentially be significant.

GABA_A receptors are formed by the assembly of 19 subunit subtypes (α1–α6, β1–β3, γ1–γ3, δ, ε, π, θ, and ρ1–ρ3) into a pentamer; the most common subunit composition is two α subunits, two β subunits and a γ subunit (Olsen and Sieghart, 2008). Pentameric GABA_A receptors serve as chloride ion channels, responsible for synaptic and extrasynaptic inhibition. The current carried by these channels can be modulated by a number of antiepileptic drugs, including barbiturates and benzodiazepines (Fig. 39.3). Although both barbiturates and

**Fig. 39.1.** Diverse molecular targets for antiseizure drugs at excitatory glutamatergic synapses. Seizure protection can be conferred by effects on voltage-gated sodium channels (Na_v), M-type voltage-gated potassium channels (K_v7), and voltage-gated calcium channels (Ca_v) located in presynaptic terminals. Additional presynaptic targets include the synaptic vesicle protein SV2A, G-protein-coupled metabotropic glutamate receptors (mGluR), and excitatory amino acid transporters (EAAT). These presynaptic targets may act to diminish glutamate release. Postsynaptic targets include ionotropic glutamate receptors of the NMDA, AMPA, and kainate types and also voltage-gated channels and mGluRs. Astrocytes also contain glutamate receptors and transporters that may be linked to glutamate uptake and release, and these cells may therefore influence the excitability of neurons.
Fig. 39.2. Diverse molecular targets for antiseizure drugs at inhibitory GABAergic synapses. Seizure protection can be conferred by effects on synaptic or extrasynaptic GABA_A receptors or on GABA transaminase (GABA-T) or GABA transporters (GAT). Additional elements of the synapse are illustrated; abbreviations are as in the caption for Fig. 39.1. Astrocytes contain elements that can influence the dynamics of GABA and therefore influence the excitability of the postsynaptic neuron. In particular, inwardly rectifying potassium channels (K_{ir}) regulate extracellular potassium levels.

Fig. 39.3. (A) Schematic illustration of the membrane topology of a GABA_A receptor subunit showing the location of binding sites for GABA and benzodiazepines in the extracellular N-terminal domain and the suspected region of barbiturate binding in transmembrane domains. (B) Pentameric structure of a typical GABA_A receptor complex. Each subunit has the topology represented in (A). The GABA and benzodiazepine recognition sites are formed from adjacent subunits. Other components of the GABAergic synapse are shown including glutamic acid decarboxylase (GAD), the enzyme that catalyzes the decarboxylation of glutamate to GABA; GABA-T, the enzyme that catalyzes the catabolism of GABA to succinic semialdehyde (SSA); GABA-containing synaptic vesicles (circles containing GABA molecules) and the GAT-1 GABA transporter (cylinders). Vigabatrin irreversibly inhibits GABA-T whereas tiagabine competitively inhibits GAT-1.
benzodiazepines have been observed to enhance GABAergic inhibition by interacting directly with the GABA<sub>A</sub> receptors (Macdonald, 1989), the mechanisms of action are different. Barbiturates increase the burst duration of GABA receptor currents while benzodiazepines augment the frequency of channel opening.

Structurally, each subunit of the GABA<sub>A</sub> receptor consists of a large (200 amino acid) extracellular N-terminal domain that contains binding sites for GABA and benzodiazepines, an extracellular loop (M3–M4 loop), a large cytoplasmic loop (M3–M4 loop), and four transmembrane domains (M1–M4) of about 20 amino acids in length. GABA binds at the interface of α and β subunits, whereas benzodiazepines bind at a homologous site at the interface of α and γ subunits. The extracellular N-terminus region contains three sites for N-linked glycosylation and the two cysteine residues, which form a β-loop, and may function in binding GABA. The M2 domain, which is composed of several hydrophilic residues, is thought to line the channel pore. The extremely variable M3–M4 loop region contains consensus sequences for phosphorylation by protein kinase A and the two cysteine residues, which form a β-loop, and may function in binding GABA. The M2 domain contains binding sites for GABA and benzodiazepines (both in length and in composition) intracellular loop between M3 and M4 of some subunits contains consensus sequences for phosphorylation by protein kinase A and the tyrosine protein kinase. This domain is important for receptor trafficking and surface clustering, through interactions with cytoplasmic proteins. The specific way in which binding of barbiturates modulates the activity of GABA<sub>A</sub> receptors has not yet been fully defined. However, the second and third transmembrane domains of the β-subunit appear to be critical for binding (Amin, 1999; Mercado and Czajkowski, 2008; Olsen and Li, 2011).

Barbiturates enhance activation of GABA<sub>A</sub> receptors by GABA and, at higher concentrations, they can directly activate GABA<sub>A</sub> receptors, in the absence of GABA (Rho et al., 1996). GABA may act as a partial agonist at extrasynaptic δ-subunit-containing GABA<sub>A</sub> receptor isoforms that mediate tonic inhibition, which may render them particularly sensitive to allosteric modulation by barbiturates (Feng et al., 2004). Interestingly, chronic treatment with barbiturates causes an increase in GABA<sub>A</sub> receptor δ subunit mRNA; withdrawal results in downregulation (Lin and Wang, 1996). Thus, the relative activity of barbiturates on synaptic and extrasynaptic GABA<sub>A</sub> receptors may vary with drug exposure.

Besides its GABAergic actions, phenobarbital blocks voltage-activated calcium channels current (ffrench-Mullen et al., 1993) and non-NMDA (AMPA/kainate) receptors (Frandsen et al., 1990; Ko et al., 1997). These actions may contribute to the anticonvulsant action and also to side-effects.

**Primidone**

Primidone is a phenobarbital analog first identified as having protective activity against electrically and chemically induced convulsions in animals in 1949 (Handley and Stewart, 1952). In animals, it exhibited a large separation between the dose conferring seizure protection and the hypnotic dose, and therefore was believed to be of low toxicity. Primidone is now well recognized to serve as a prodrug for phenobarbital and phenylethylmalonamide (PEMA), both of which have antiseizure activity in their own right, but the parent compound may have activity independent of the active metabolites. However, in one animal study, the antiseizure action of primidone was independent of plasma and brain levels of primidone but correlated with the appearance of phenobarbital and PEMA (Baumel et al., 1973), suggesting that the contribution of the parent is minor. Phenobarbital is the major active metabolite of primidone whereas PEMA is a minor metabolite (Gruber et al., 1962). Moreover, PEMA is 16 times less potent than phenobarbital against electroshock and pentylenetetrazol-induced convulsions in mice; further, PEMA may contribute to the neurotoxicological effects of primidone (Bourgeois et al., 1983). Therefore, the anticonvulsant activity of primidone is dominated by phenobarbital, which, as noted above, primarily acts via modulation of GABA<sub>A</sub> receptors. Primidone itself does not act on GABA<sub>A</sub> receptors (Harrison and Simmonds, 1983). At concentrations that exceed the usual human therapeutic levels, primidone has been found to limit the sustained high-frequency repetitive firing of action potentials, indicating an action on voltage-gated sodium channels (see below; Macdonald, 1988). The extent to which this action contributes to primidone’s clinical activity is uncertain.

**Phenytoin**

Phenytoin is useful for the treatment of partial seizures and generalized tonic–clonic seizures but not primary generalized seizures such as absence seizures or myoclonic seizures (Rogawski and Löscher, 2004). In animal models, phenytoin is protective against tonic seizures in the maximal electroshock test, but does not protect against pentylenetetrazol-induced clonic seizures. Phenytoin is also active against seizures induced by blockers of voltage-gated potassium channels (4-aminopyridine and dendrotoxin), which enhance the release of glutamate and other neurotransmitters (Coleman et al., 1992; Yamaguchi and Rogawski, 1992).

Although phenytoin has many pharmacological actions, the action most relevant to its antiseizure activity is the inhibition of high-frequency repetitive firing of voltage-gated sodium channels (McLean and Macdonald, 1983). Some of the other actions include blockade of voltage-gated calcium channels (Tuttle and Richelson, 1979; McLean and Macdonald, 1983) and ionotropic glutamate receptors (Wamil and McLean, 1993), which occur at supratherapeutic concentrations.
The first clue to the mechanism underlying the antiseizure activity of phenytoin came from a study by Toman (1949), in which the drug was found to inhibit the rebound spike that appears a few milliseconds after the first spike induced by supramaximal stimulation of frog sciatic nerve. Thus, phenytoin specifically inhibited the repetitive firing of action potentials at high frequency. The ability of phenytoin to inhibit high-frequency trains of action potentials was later demonstrated by McLean and Macdonald (1983). This action was believed to be due to an interaction of phenytoin with voltage-gated sodium channels (Rogawski and Löscher, 1993). These channels can transition into three conformational states: a closed resting state, an open activated state, and an inactivated state. Phenytoin has low affinity for resting sodium channels at hyperpolarized membrane potentials. However, when neurons are depolarized and the channels transition into the open and inactivated states, greater binding and block occur. The inhibitory potency is strongly use dependent, so that block accumulates with prolonged or repetitive activation. This is largely due to preferential binding to the inactivated conformation. An important feature of the blocking of sodium channels by phenytoin is its slow onset. The timecourse of fast sodium currents is therefore not altered in the presence of the drug and action potentials evoked by synaptic depolarizations of ordinary duration are not blocked.

Mutational analysis has revealed that sodium channel-blocking antiseizure drugs bind to a common recognition site on sodium channels. These channels consist of a 260-kDa pore-forming α subunit composed of four homologous domains each with six transmembrane segments. S5 and S6 segments are believed to form the lining of the sodium channel pore. Specific phenylalanine (F1764) and tyrosine (Y1771) residues in the S6 segment of domain IV are crucial for use-dependent block by the antiseizure drugs; in addition, adjacent residues spread across ~160° of the circumference of the IVS6 segment that flanks F1764 also contribute to binding. When the sodium channel is inactivated, the structure of the channel pore is altered, making drug binding with these residues more favorable. Channel gating is believed to be associated with rotation of the segment containing F1764, Y1771, and the surrounding amino acids so that these residues are brought into the pore, thereby facilitating drug binding. The pore-lining residues leucine 1465 and isoleucine 1469 in S6 of domain III also form a portion of the high-affinity binding site for sodium channel-blocking antiseizure agents.

Phenytoin, besides blocking the sodium channel during its peak current phases, also blocks the persistent late sodium current, which is probably also mediated by voltage-gated sodium channels. The persistent current may critically regulate the firing properties of some neurons and could also play an important role in epileptic activity; blocking the persistent current rather than the peak current may be of particular importance in seizure control (Segal and Douglas, 1997).

Succinimides

The oxazolidinedione trimethadione was originally identified as a specific treatment for absence epilepsy by Lennox (1945). Sales of trimethadione were discontinued in the USA in 1996, although the drug remains available in Europe. The succinimides were the second class of antiabsence drugs to be identified and they have continued to be successful in this one clinical niche. By far the most widely used is ethosuximide, which is not useful in the treatment of other seizure types but is effective and relatively nontoxic for absence seizures. Other succinimides include phensuximide and methosuximide.

Absence seizures are characterized by brief episodes of impaired consciousness accompanied by generalized 3-Hz spike-and-wave discharges recorded in the electroencephalogram; these discharges are generated from complex interactions between the thalamus and the cerebral cortex. The spontaneous pacemaker oscillatory activity of thalamocortical circuitry involves low-threshold T-type Ca²⁺ currents in the thalamus (Suzuki and Rogawski, 1989). Ethosuximide reduces T-type Ca²⁺ currents in thalamic neurons (Coulter et al., 1989a), with higher affinity for inactivated channels (Gomora et al., 2001). The effect occurs at therapeutic concentrations (0.25–0.75 mM), but is not of large magnitude. Nevertheless, the degree of block is likely sufficient to exert an antiabsence action. The effect is shared by other succinimides with antiabsence actions and also by dimethadione, the active metabolite of trimethadione (Coulter et al., 1989b, c, 1990). Three different types of T-type calcium channels are found in neurons: Ca₃.1, Ca₃.2, and Ca₃.3. Ethosuximide is more active on the Ca₃.2 isoform and less active on the Ca₃.1 isoform (Lacinová, 2004). α-Methyl-α-phenylsuccinimide, an active metabolite of methsuximide, is moderately active on all three subtypes.

Contradictory findings have been reported. For example, even millimolar concentrations of ethosuximide failed to affect calcium currents in acutely isolated hippocampus CA3 and neocortical neurons (Sayer et al., 1993; Avery and Johnston, 1996). Similarly, Lerescue et al. (1998) found no evidence for ethosuximide action on calcium currents in rat and cat thalamic neurons. However, in a study with cloned human T-type calcium channels, ethosuximide, methsuximide, and α-methyl-α-phenylsuccinimide were all active (Gomora et al., 2001). Thus, despite the conflicting results, the evidence supports the view that ethosuximide does have an effect
on T-type calcium channels, although the drug is not a potent blocker. In addition to the effect on T-type calcium channels, Leresche et al. (1998) found that ethosuximide (0.75–1 mM) decreased persistent sodium current, as does phenytoin, but, in contrast to phenytoin, had no effect on fast inactivating sodium current (McLean and Macdonald, 1986a). In addition, these investigators observed an effect on calcium-activated potassium current. Recently, ethosuximide has been found to inhibit inwardly rectifying potassium channels (Kobayashi et al., 2009). Cronelli and Leresche (2002) concluded that the actions on sodium and potassium channels contribute to the overall therapeutic effect of ethosuximide.

**Carbamazepine and oxcarbazepine**

Like phenytoin, carbamazepine is effective in the treatment of partial seizures and generalized tonic–clonic seizures. It also has a similar profile of activity in animal seizure models. Carbamazepine is metabolized to an active metabolite, carbamazepine epoxide, which is stable and which contributes to the antiseizure activity.

Carbamazepine blocks voltage-activated sodium channels in a similar fashion to phenytoin (Willow et al., 1985; McLean and Macdonald, 1986b). However, in contrast to phenytoin, carbamazepine produced less pronounced frequency-dependent block. Thus, although phenytoin and carbamazepine have qualitatively similar effects on sodium channels, their actions are quantitatively somewhat different. This could account for differences in efficacy in different patients. Pharmacokinetic–pharmacodynamic correlation studies support the concept that the sodium channel effects of the drug account for its antiseizure effects. Concentrations of 20–25 μM are achieved in rat brain after *in vivo* administration of therapeutic doses of carbamazepine; 40–45% blockade of sodium current occurs at this concentration.

Besides blocking sodium currents, carbamazepine has other pharmacological actions, but the relevance of these actions to its therapeutic activity is uncertain. For example, carbamazepine blocks calcium channels (particularly those of the L-type), but this is often observed only at concentrations in excess of therapeutic levels (Kito et al., 1994; Schumacher et al., 1998). In one study, carbamazepine, at therapeutically relevant concentrations (10–20 μM), was found to activate a voltage-dependent potassium current in rat neocortical neurons in culture (Zona et al., 1990). Such an action on potassium currents is expected to oppose or diminish hyperexcitability and could potentially be relevant to seizure protection.

Oxcarbazepine, the 10-keto analog of carbamazepine, has a similar clinical utility and a similar spectrum of activity in animal models to carbamazepine (Kalis and Huff, 2001). It is actually a prodrug for its mono-hydroxylated derivative (10,11-dihydro-10-hydroxy-carbamazepine; licarbazepine; MHD), to which it is rapidly converted and which provides the bulk of the antiseizure activity. Unlike carbamazepine, oxcarbazepine is not converted to a stable epoxide metabolite. Some have proposed that the epoxide could contribute to carbamazepine toxicity (Reinikainen et al., 1987), but the evidence that oxcarbazepine is safer than carbamazepine is scant. Oxcarbazepine, like phenytoin and carbamazepine, blocks the sustained high-frequency repetitive firing of sodium-dependent action potentials (Wamil et al., 1994; Schmutz et al., 2007). Oxcarbazepine can also inhibit calcium currents (Stefani et al., 2005; Schmutz et al., 2007), but, with the exception of ethosuximide, there is little evidence that effects on calcium channels play a substantial role in the antiseizure activity of any clinical agents.

**Valproate**

Valproate, a branched fatty acid, is available throughout the world in different formulations, including the sodium salt, the magnesium salt, the free acid, and the coordination compound divalproex sodium. It is active in a broad range of animal seizure models but generally requires high doses in the range of 150–400 mg/kg (Rogawski and Porter, 1990). Clinically, valproate has among the broadest range of activity of any antiseizure agent and still is considered among the first choice of drugs for the treatment of primary generalized epilepsies.

Although valproate has a variety of pharmacological actions, the contributions of these actions to clinical activity remain uncertain (Löschler, 2002b). For example, valproate (6–200 μM) has been reported to inhibit sustained high-frequency repetitive firing of sodium-dependent action potentials in a use- and voltage-dependent fashion in spinal cord and cortical neurons, similar to phenytoin and carbamazepine (McLean and Macdonald, 1986a). At higher concentrations (200 μM–2.4 mM), valproate inhibits fast sodium currents in hippocampal and neocortical neuronal preparations (Van Dongen et al., 1986). *Trans*-2-en-sodium valproate, a major metabolite of valproic acid (resulting from mitochondrial β-oxidation of the parent), also inhibits sustained action potential firing, but probably not at concentrations achieved in patients receiving valproate (Wamil et al., 1997). Valproate has also been reported to block persistent sodium current at lower concentrations (5–50 μM) (Taverna et al., 1998); such an action...
has been proposed as a key factor in the anticonvulsant activity of phenytoin (see above). In addition to its effect on sodium channels, valproic acid, at an EC50 value of 330 μM, produces a small (17%) block of T-type calcium channels (Todorovic and Lingle, 1998). These various actions of valproate on voltage-dependent ion channels could potentially contribute to the antiseizure activity of the drug but do not explain the broad spectrum of activity seen in animal models and in patients with diverse seizure types and syndromes.

A second class of actions that could contribute to valproate’s clinical activity is effects on GABA-mediated neurotransmission. The drug is protective against seizures induced by chemoconvulsants acting on the GABA system, such as methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), a benzodiazepine receptor inverse agonist (Petersen, 1983), and the drug is also effective in the 6-Hz model, an alternative electroshock model that is sensitive to agents that act through GABA systems (Barton et al., 2001; Kaminski et al., 2004a). Indeed, valproate administration increases the concentration of GABA in the whole brain (Godin et al., 1969; Johannessen, 2000) and also increases GABA turnover (Loscher, 1989). However, only very high concentrations of valproate (>300 μM) stimulated GABA release in cultured neurons (Gram et al., 1988). Similarly, there is little evidence that valproate enhances postsynaptic GABA responses (McLean and Macdonald, 1986a). Therefore, whether and how valproate modulates GABergic processes is not clear.

**Benzodiazepines**

Benzodiazepines have proven invaluable for the acute treatment of status epilepticus, but their role in chronic antiseizure therapy is limited both by their sedative nature and by tolerance that occurs in many patients. In addition, discontinuation of benzodiazepines may lead to withdrawal symptoms. Approximately 35 benzodiazepines are available for the treatment of various central nervous system disorders including epilepsy. Of these, six are typically prescribed for epilepsy: diazepam, clonazepam, clorazepate, and midazolam (Riss et al., 2008).

All of the pharmacological actions of benzodiazepines result from their actions on GABA_A receptors. Benzodiazepines have a distinct binding site on the receptor and they modulate GABA_A receptor function in an allosteric manner (Macdonald and Barker, 1978b; Campo-Soria et al., 2006). The γ subunit of the GABA_A receptor, when coexpressed with α1, α2, α3, or α5 receptor subtypes, is essential for benzodiazepine activity (Riss et al., 2008). Once bound, benzodiazepines produce a conformational change in the GABA_A receptor such that the neurotransmitter GABA has a much higher affinity for the GABA_A receptor. This results in an increase in the frequency of channel opening, which contrasts with the action of barbiturates that alter the burst duration (Rogers et al., 1994).

**DRUGS DISCOVERED AND MARKETED AFTER 1990**

**Ezogabine (Retigabine)**

Ezogabine (USAN; international nonproprietary name: retigabine) is approved in the United States and the European Union as an adjunctive therapy in adults with partial-onset seizures. The drug has broad-spectrum activity in animal seizure models, conferring protection in the maximal electroshock and pentylenetetrazol tests as well as many other models (Large et al., 2012). Ezogabine is particularly active against kindled seizures (Rogawski, 2006), and also confers protection in models such as the lamotrigine-resistant amygdala kindled rat and the 6 Hz model in which sodium channel blocking antiseizure agents are inactive (Large et al., 2012). In vitro brain slice preparations, ezogabine inhibits epileptiform activity at concentrations as low as 1–2 μM.

Ezogabine acts as a positive modulator of KCNQ2-KCNQ5 (Kv7.2–Kv7.5) potassium channels that mediate an M-type, non-inactivating voltage-dependent potassium current in neurons. The drug therefore has a new and unique target of action not shared by other marketed antiseizure agents. KCNQ channels are localized to axons, including the axon initial segment, where they control action potential threshold (Shah et al., 2011). In addition, they are expressed in neuronal somata where they regulate synaptic integration. M-current is modestly activated at resting potential and the current amplitude increases as neurons are depolarized. The current causes an inhibitory influence on excitability that counters repetitive firing and epileptiform activity. The kinetics of M-current is such that it selectively attenuates high frequency or burst firing without affecting single action potentials, thus accounting for the ability of ezogabine to suppress epileptic activity without impairing normalongoing activity (Gunthorpe et al., 2012).

Ezogabine causes a hyperpolarizing shift in the activation of KCNQ channels such that more M-current is generated near resting potential. In addition, the drug causes a change in the kinetics of single KCNQ channels to favor channel opening, thus increasing the macroscopic M-current; ezogabine does not alter the single-channel conductance of individual KCNQ channels (Tatulian and Brown, 2003). Many KCNQ channels in brain are believed to be KCNQ2/KCNQ3 heteromers, which are among the most sensitive isoforms
(EC\textsubscript{50}, 1.6 \mu M; Gunthorpe et al., 2012). At doses that were efficacious in clinical trials, free plasma concentrations of ezogabine are about 0.4–0.8 \mu M; brain concentrations are estimated to be similar. Therefore, therapeutic concentrations are sufficient to affect KCNQ channels. Additional evidence supporting the involvement of KCNQ channels comes from studies showing that the KCNQ blocker XE-991 can reverse the antiseizure activity of retigabine and that mice with a genetic defect in KCNQ2 show reduced sensitivity to the anticonvulsant activity of ezogabine (Large et al., 2012).

The binding site for ezogabine in KCNQ2/KCNQ3 heteromers is in a pocket formed by the S5 membrane segment of one subunit and the S6 membrane segment of the neighboring subunit (Wuttke et al., 2005; Lange et al., 2009). Channel opening may expose the pocket, permitting binding of ezogabine, which stabilizes the open channel conformation.

Ezogabine also can potentiate the activity of GABA\textsubscript{A} receptors and affect other ion channels, but the concentrations required are greater than those believed to be obtained at therapeutic doses (Large et al., 2012).

**Felbamate**

Felbamate is a dicarbamate that is closely related to the sedative mebrobamate (Burdette and Sackellares, 1994). Felbamate’s antiseizure activity was discovered by the National Institute of Neurological Disorders and Stroke Anticonvulsant Screening Program, and the drug was shown in clinical trials to be effective in the treatment of partial seizures. Felbamate is also useful in Lennox–Gastaut syndrome (Jensen, 2007). Substantial idiosyncratic toxicities mandate limited use of this drug.

Felbamate has a broad spectrum of activity in animal models. It is effective in the maximal electroshock model, and against pentylenetetrazol and other chemoconvulsant-induced seizures in mice and rats (McCabe et al., 1993). It also protects against seizures induced by intracerebroventricular NMDA and quisqualic acid (White et al., 2005). There is evidence that the drug has a unique dual action on excitatory (NMDA receptor) and inhibitory (GABA\textsubscript{A} receptor) mechanisms, such that actions on both mechanisms contribute to seizure protection. Thus, at therapeutic concentrations (0.1–3 mM) felbamate inhibits NMDA receptor currents and at the same time enhances GABA\textsubscript{A} receptor currents (Rho et al., 1994). A variety of studies have confirmed the effects of felbamate on NMDA receptor responses (Taylor et al., 1995; White et al., 1995); felbamate is the only antiseizure agent for which there is substantial evidence supporting the view that effects on NMDA receptors could contribute to clinical efficacy.

Studies of NMDA receptors expressed in mammalian cells and Xenopus oocytes have indicated that felbamate has differential activity on different NMDA receptor isoforms (Kleckner et al., 1999; Harty and Rogawski, 2000). It is most potent at NMDA receptors composed of GluN1/GluN2B (NR1/NR2B) subunits (IC\textsubscript{50} 0.93 mM), of intermediate potency on GluN1/GluN2C (NR1/NR2C) (2.02 mM), and only weakly active on GluN1/GluN2A (NR1/NR2A) (8.56 mM). Recently, felbamate was reported to inhibit presynaptic NMDA receptors in rat entorhinal cortex and prevent glutamate release (Yang et al., 2007). Kuo et al. (2004) have proposed that felbamate effectively modifies NMDA channel gating and significantly inhibits the late sustained current, but not the early peak current. This property might confer a selectivity of felbamate to block seizures, since the prolonged pathological activation that occurs during seizures would be suppressed by felbamate more strongly than normal (rapid) synaptically generated responses.

Felbamate also produces a barbiturate-like modulation of GABA\textsubscript{A} receptors (Rho et al., 1997). The drug is active on GABA\textsubscript{A} receptors consisting of \( \alpha_1\beta_2\gamma_2\), \( \alpha_1\beta_3\gamma_2\), \( \alpha_2\beta_2\gamma_2\), and \( \alpha_2\beta_3\gamma_2\) subunits but not on other subunit combinations (Simeone et al., 2006a). The threshold concentration for felbamate modulation of GABA\textsubscript{A} receptor currents is in the range of 0.1–1 mM, and the IC\textsubscript{50} value for allosteric inhibition of \( \gamma\text{H} \)-butylbicycloorthobenzoate binding to GABA\textsubscript{A} receptors in rat brain slices is 250 \mu M (Kume et al., 1996).

In addition to its actions on excitatory and inhibitory synaptic function, felbamate may also affect voltage-gated sodium channels. The drug blocks both sustained repetitive firing in mouse spinal cord neurons (White et al., 2005) and voltage-dependent sodium currents (Pisani et al., 1995). Felbamate has also been reported to inhibit dihydropyridine-sensitive calcium channels in central neurons (Stefani et al., 1996), but this action is unlikely to contribute to seizure protection.

**Gabapentin and pregabalin**

Gabapentin is a low-potency antiseizure drug whose structure closely resembles GABA; indeed, gabapentin was developed as a lipophilic, blood–brain barrier permeable form of GABA. The drug, however, does not interact with GABA systems (Bryans and Wustrow, 1999; Rogawski and Bazil, 2008). Pregabalin has a similar mechanism of action to gabapentin but it has improved pharmacokinetic properties. Both gabapentin and pregabalin (collectively referred to as “gabapentinoids”) are active in the maximal electroshock seizure test and also have variable activity against tonic seizures in chemoconvulsant models.
Gabapentin and pregabalin act by binding to \( \alpha_2\delta \) proteins, which are believed to be accessory subunits of voltage-dependent calcium channels (Gee et al., 1996). \( \alpha_2\delta \) are highly glycosylated proteins of molecular mass \( \sim 150 \text{ kDa} \) (997–1150 amino acid residues) that exist in four homologous forms; only types 1 and 2 bind gabapentin and pregabalin with high affinity (Hendrich et al., 2008). Both drugs bind to an extracellular epitope present on the \( \alpha_2 \)-1 and \( \alpha_2 \)-2 subunits. This binding appears to be responsible for the anticonvulsant, anxiolytic, and analgesic activity of these two drugs. Each of the \( \alpha_2\delta \) subunits are products of a single gene that is posttranslationally cleaved into \( \alpha_2 \) and \( \delta \) peptides, but the peptides are covalently linked by a disulfide bridge. Neither peptide alone binds gabapentin; both peptides are required, but the disulfide linkage itself is not essential (Wang et al., 1999). The \( \delta \) subunit portion of \( \alpha_2\delta \) subunits serves as an anchor for the \( \alpha_2 \)-1 subunit, which is largely extracellular. Interestingly, deletion of \( \alpha_2\delta \)-1 or \( \alpha_2\delta \)-2 in mice is associated with absence epilepsy or enhanced seizure susceptibility (Snell, 1955; Ivanov et al., 2004).

The precise way in which binding of gabapentin and pregabalin to \( \alpha_2\delta \)-1 or \( \alpha_2\delta \)-2 proteins confers seizure protection is not well understood (Rogawski and Bazil, 2008). Some studies have indicated that the drugs inhibit calcium channel currents (Stefani et al., 1998; van Hooft et al., 2002) whereas others have failed to demonstrate such an action (Brown and Randall, 2005). Regardless of whether the drugs inhibit calcium channel function, they do seem to block the release of various neurotransmitters, including glutamate; this may account for the antiseizure activity (Dooley et al., 2007).

Although the acute effects gabapentin are unlikely to be mediated by effects on calcium currents, chronic exposure to the drug does lead to inhibition of calcium currents (Hendrich et al., 2008). This occurs as a result of a reduction in the total number of \( \alpha_2\delta \) and \( \alpha_1 \) subunits at the cell surface because of an intracellular effect of gabapentin to disrupt the trafficking of \( \alpha_2\delta \) subunits (Tran-Van-Minh and Dolphin, 2010). Specifically, gabapentin prevents the Rab11-dependent recycling of \( \alpha_2\delta \), inhibiting its movement from post-Golgi compartments to the plasma membrane. In contrast, internalization of \( \alpha_2\delta \) is unaffected by gabapentin. The action on calcium channel trafficking could be relevant to the antiseizure activity of gabapentinoids in long-term therapy. However, it is difficult to appreciate how this effect could account for the rapid action of gabapentinoids on seizures in animal models.

**Lacosamide**

Lacosamide is a functionalized amino acid originally identified because it had stereoselective protective activity in the mouse maximal electroshock seizure test (ED\(_{50} \) 4.5 mg/kg) (Choi et al., 1996; Doty et al., 2007). Activity resides exclusively in the \( R \)-enantiomer; \( S \)-lacosamide is inactive. The stereoselectivity stimulated interest in lacosamide’s development. Lacosamide is inactive in the pentylentetrazol test but is highly potent against sound-induced seizures in Frings mice (ED\(_{50} \) 0.63 mg/kg) and is also active in the 6-Hz mouse electroshock model (32 mA) (ED\(_{50} \) 10 mg/kg) (Stohr et al., 2005). Conventional sodium channel-blocking antiseizure drugs are inactive in the 6-Hz model. Therefore, lacosamide has a unique spectrum of activity. Lacosamide is also active against hippocampal and amygdala kindling and in the self-sustaining status epilepticus models (LeTiran et al., 2001; Stohr et al., 2005; Brandt et al., 2006). Despite its unique spectrum of activity, the antiseizure activity of lacosamide is believed to relate to its effects on voltage-gated sodium channels.

Although lacosamide seems to act through sodium channels, the specific way in which the drug interacts with these channels is unique and distinct from that of other sodium channel-blocking antiseizure agents. In particular, lacosamide (32–100 \( \mu \)M) enhances slow sodium channel inactivation (Errington et al., 2008; Sheets et al., 2008; Wang et al., 2011). In contrast to conventional sodium channel inactivation, which occurs within the timeframe of the action potential, slow inactivation is produced during prolonged depolarizations (such as might occur during epileptic phenomena) over tens of seconds (60–90 seconds), or may accumulate over a series of more brief depolarizations. Slow inactivation is difficult to study and little is known about the way it occurs. Lacosamide shifts the slow inactivation voltage curve to more hyperpolarized potentials and accelerates the entry of the sodium channels into the slow inactivated state. By promoting slow inactivation, lacosamide would limit high-frequency neuronal firing in a similar way to drugs that enhance fast inactivation except that the effect would occur over a longer timescale, which might confer lacosamide with less effects on normal ongoing neuronal activity and theoretically therefore less propensity for side-effects. As noted below, there is limited evidence that zonisamide may also promote the slow inactivation of voltage-gated sodium channels.

**Lamotrigine**

Lamotrigine is approved for the treatment of partial seizures and primary generalized tonic–clonic seizures. In addition, the drug is effective in the treatment of certain generalized seizure types such as absence seizures and seizures occurring in the Lennox–Gastaut syndrome. Lamotrigine acts on voltage-gated sodium channels in a similar fashion to phenytoin and carbamazepine.
(Lees and Leach, 1993). Since lamotrigine is clinically useful in absence seizures and other types of primary generalized seizures, it is unlikely that blockade of sodium channels is its sole antiseizure mechanism (Coulter, 1997).

In preclinical studies, lamotrigine confers protection against maximal electroshock seizures and is effective in various kindling models (Leach et al., 1986; Rogawski and Porter, 1990; Cheung et al., 1992). Lamotrigine may act by inhibiting glutamate release from nerve terminals (Leach et al., 1991). For example, it inhibits veratridine-evoked release of glutamate and aspartate in rat cortical neurons (ED$_{50}$ 21 µM) (Miller et al., 1986). This likely occurs by blocking the repetitive firing of action potentials through effects on voltage-gated sodium channels (Lang and Wang, 1991; Wang et al., 2001). Lamotrigine may preferentially inhibit glutamate release relative to GABA release, as has been observed for other sodium channel agents (Prakriya and Mennerick, 2000). This observation may be critical to the understanding of how such drugs reduce brain excitability and seizures. Whether lamotrigine is a more selective glutamate release inhibitor than other antiseizure agents is uncertain.

In addition to its inhibitory action on sodium channels, lamotrigine may also modify voltage-gated calcium currents (Grunze et al., 1998). In one study, lamotrigine blocked N-type calcium current in rat amygdala neurons (Wang et al., 1996). In another study, lamotrigine, at a therapeutic concentration of 10 µM, inhibited R-type ($\alpha_{1R}$) calcium currents by approximately 30%; it did not, however, affect $\alpha_{1T}$ T-type calcium currents and was a weak inhibitor of $\alpha_{1G}$ T-type calcium currents (Hainsworth et al., 2003). Further, lamotrigine, at the higher concentration of 300 µM, inhibited calcium-sensing nonselective cation channels in hippocampal neurons (Xiong et al., 2001). These actions of lamotrigine on calcium channels may possibly account for its broader clinical spectrum of activity than other sodium channel-blocking antiseizure agents.

**Levetiracetam**

Levetiracetam is a piracetam derivative whose antiseizure effect was discovered by its protective activity against audiogenic seizures in susceptible mice and other nonstandard models, as opposed to the conventional maximal electroshock and chemoconvulsant screening tests (Gower et al., 1992). Levetiracetam is used to treat partial seizures and is also probably effective against myoclonic and primary generalized seizures and also absence seizures.

SV2A is the molecular target in brain of levetiracetam (Rogawski and Bazil, 2008; Kaminski et al., 2010). It has been known for some time that the drug binds to a specific saturable and stereoselective site in brain membranes (Noyer et al., 1995). More recently, this binding site was identified as SV2A, which is a 12-transmembrane segment integral membrane glycoprotein that is ubiquitous in synaptic vesicles (Lynch et al., 2004). SV2A is a member of a family of related proteins that exist in three isoforms. SV2A is the most widely distributed, being nearly ubiquitous in the central nervous system; SV2B is also brain specific, with a widespread but not ubiquitous distribution; SV2C is a minor isoform in brain (Buckley and Kelly, 1985; Bajjalieh et al., 1994). Levetiracetam has affinity only for the SV2A isoform. The physiological role of SV2A in epilepsy has not been defined. However, it is interesting that homozygous SV2A knockout mice experience severe seizures and die between postnatal period P12 and P23; heterozygous animals are also susceptible to seizures but have nearly normal survival (Kaminski et al., 2009). SV2A appears to play a role in vesicle dynamics: an absence of SV2A results in a decrease of the readily releasable pool of vesicles (Xu and Bajjalieh, 2001). SV2 may prime vesicles in quiescent neurons; it may also play a role in low-frequency neurotransmission (Custer et al., 2006). Indeed, SV2 proteins are also known to modulate the formation of soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) complexes that are essential for vesicle fusion (Lezzi et al., 2005). In sum, SV2A almost certainly plays a role in regulating neurotransmitter release but how levetiracetam interacts with this protein and how it exerts its antiseizure action is, at present, obscure.

**Rufinamide**

Rufinamide is a triazole derivative, approved for the adjunctive treatment of seizures associated with the Lennox–Gastaut syndrome. The drug may also be effective against partial seizures (Brodie et al., 2009; Elger et al., 2010). The mechanism of action of rufinamide is poorly understood. The drug is orally active in the maximal electroshock test (ED$_{50}$ 24 mg/kg and 6 mg/kg in mice and rats, respectively) and also protects against pentylenetetrazol-induced seizures (ED$_{50}$ 46 mg/kg). In addition, it is effective in suppressing afterdischarges in amygdala-kindled cats and reduces seizure frequency in rhesus monkeys with chronic alumina foci in the motor cortex (Cheng-Hakimian et al., 2006). Rufinamide decreases the sustained repetitive firing of sodium-dependent action potentials (EC$_{50}$ 3.8 µmol/L). However, the extent to which an effect on sodium channels accounts for its antiseizure activity has not been defined (Arroyo, 2007).

**Tiagabine**

Tiagabine is a selective GABA reuptake blocker designed for use as an antiseizure drug. Unfortunately, it has low efficacy and relatively fewer patients achieve
seizure freedom in comparison with other agents (Bauer and Cooper-Mahkorn, 2008). It also has a high rate of adverse effects and may induce nonconvulsive status epilepticus (Koepp et al., 2005).

Tiagabine is active against various chemoconvulsant-induced seizures, including those elicited by pentylenetetrazol, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), picrotoxin, and bicuculline. It is also active against audiogenic seizures, amygdala and hippocampal kindled seizures, and the seizures in genetically epilepsy-prone rats (Nielsen et al., 1991; Suzdak and Jansen, 1995; Brodie, 1995; Smith et al., 1995; Morimoto et al., 1997). Tiagabine is a potent and specific GABA uptake inhibitor (Gram, 1994). There are four distinct types of GABA transporters, termed GAT-1, GAT-2, GAT-3, and BGT-1, that are responsible for GABA reuptake into both neurons and glial cells (Borden et al., 1994). Tiagabine has specificity for the GAT-1 isoform (Borden et al., 1994), the predominant GABA transporter in the forebrain (cortex, striatum, hippocampus) (Meldrum and Chapman, 1999). GAT-1 is predominantly localized to GABAergic terminals. Inhibition of GABA uptake leads to prolongation of the synaptic effects of GABA as assessed by prolongation of inhibitory postsynaptic potentials and inhibitory postsynaptic currents, thus resulting in a net increase in synaptic inhibition (Suzdak and Jansen, 1995). In addition, the drug causes a marked increase in regional brain GABA levels (Fink-Jensen et al., 1992; Dalby, 2000), which may cause activation of extrasynaptic GABA_A receptors.

**Topiramate**

Topiramate is a structurally novel antiseizure agent; it is a sulfamate-substituted fructose-related monosaccharide. Topiramate has a broad spectrum of activity, with efficacy in partial and generalized tonic–clonic seizures as well as in various specific epilepsy syndromes including the Lennox–Gastaut syndrome. The drug exhibits protective activity in the maximal electroshock test, in amygdala-kindled seizures, and in sound-induced seizures. It is ineffective against clonic seizures induced by pentylenetetrazol, bicuculline, or picrotoxin. Therefore, its spectrum of activity in animal models is similar to sodium channel-blocking antiseizure agents and also ionotropic glutamate receptor antagonists. However, topiramate has several properties that are atypical for sodium channel-blocking drugs: it is effective in a rat genetic model of absence epilepsy and can also raise the threshold for clonic seizures induced by intravenous pentylenetetrazol in mice (White et al., 1997). Nevertheless, the relatively restricted profile in animal models is surprising given topiramate’s broad clinical antiseizure activity.

Several cellular targets have been proposed to be relevant to the therapeutic activity of topiramate: (1) voltage-gated sodium channels; (2) high-voltage-activated calcium channels; (3) GABA_A receptors; (4) AMPA/kainate receptors; and (5) carbonic anhydrase isoenzymes (Macdonald and Rogawski, 2008). Effects of topiramate on sodium channels occur at relatively low, therapeutically relevant concentrations (Zona et al., 1997; McLean et al., 2007). There is evidence that topiramate may alter the activity of the various receptors and ion channels with which it interacts by affecting their phosphorylation state rather than through direct binding, as is typically the case for antiseizure drugs (Mikael et al., 2005). Given its profile in animal models, an action on sodium channels could contribute to the clinical efficacy of topiramate. Indeed, the effect of topiramate on sodium channels is comparable to that of other sodium channel-blocking antiseizure agents except that recovery from the block of fast transient sodium currents may be more rapid. In addition to its action on fast inactivating sodium currents, topiramate, like phenytoin, blocks persistent sodium currents at low concentrations which, as noted previously, is a potentially important action for seizure protection. Although topiramate may block high-voltage-activated calcium channels (Zhang et al., 2005), the relevance of this action to the clinical activity of the drug is uncertain (White, 2005; Macdonald and Rogawski, 2008).

Although the profile of topiramate in animal models is not characteristic of antiseizure agents that act on the GABA system, the effect of the drug in an absence model and on pentylenetetrazol seizure threshold is compatible with an action on GABA_A receptors. Indeed, topiramate has been found to produce variable effects on GABA receptor currents. A recent study has suggested that the variability relates to the drug’s specific actions on different heteromeric GABA_A receptor isoforms (Simeone et al., 2006b). In particular, topiramate potentiated GABA_A receptors containing β2 or β3 subunits whereas receptors containing the β1 subunit could be enhanced or inhibited. Recently, topiramate was found to enhance muscimol and synaptically-evoked GABA_A receptor currents in basolateral amygdala principal (pyramidal) neurons (Braga et al., 2009). The timecourse of the effect was compatible with the possibility that topiramate acts through a direct action on GABA_A receptors. Overall, the clinical activity of topiramate appears to reside, at least in part, in an action on GABA_A receptors. Interestingly, topiramate therapy has been found to be associated with elevations in GABA concentrations in diverse brain regions (Petroff et al., 1999, 2001; Kuzniecky et al., 2002), but the clinical significance of these changes is unclear.

In addition to its actions on GABA_A receptors, topiramate influences glutamate-mediated excitatory neurotransmission. Therefore, like felbamate, topiramate seems to have dual actions on inhibitory and excitatory neurotransmission. There is no evidence that topiramate
affects NMDA receptors. However, topiramate was reported to inhibit responses to kainate (an agonist of AMPA and kainate receptors) and AMPA in cultured neurons (Gibbs et al., 2000; Poulsen et al., 2004). More recently, the drug was found to selectively inhibit pharmacologically isolated GluK1 (GluR5) kainate receptor-mediated postsynaptic currents, whereas AMPA receptor-mediated currents were only modestly reduced (Gryder and Rogawski, 2003). GluK1 kainate receptors are a type of ionotropic glutamate receptor within the class of kainate non-NMDA receptors. The drug’s effect on GluK1 kainate receptors has also been demonstrated in recordings from basolateral amygdala interneurons, which, like principal neurons, express GluK1 kainate receptors (Braga et al., 2009). Topiramate provided protection against seizures induced by intravenous infusion of the selective GluK1 kainate receptor agonist ATPA, but was less effective against seizures induced by AMPA or NMDA, indicating that the selective interaction of topiramate with GluK1 kainate receptors is relevant to its anticonvulsant properties (Kaminski et al., 2004b). Therefore, topiramate is the first antiseizure agent with a relatively specific action on ionotropic glutamate receptors of the kainate type. The action of topiramate on GluK1 kainate receptors evoked GABA release from interneurons through an action on presynaptic GluK1 receptors (Braga et al., 2009).

The action of topiramate on carbonic anhydrase isoenzymes may contribute to the drug’s side-effects, including its propensity to cause metabolic acidosis and calcium phosphate kidney stones (Vega et al., 2007). Carbonic anhydrase inhibition is presumably related to the sulfamate structure, which includes a sulfonamide group common to acetazolamide and zonisamide (see below). Topiramate selectively inhibits cytosolic (type II) and membrane associated (type IV) forms of carbonic anhydrase (Dodgson et al., 2000). Carbonic anhydrase inhibition is expected to reduce bicarbonate. GABA_A receptors are permeable to bicarbonate, and activation of GABA_A receptors ordinarily causes an inward (depolarizing) bicarbonate current owing to efflux of bicarbonate. Therefore, inhibition of carbonic anhydrase would reduce the tendency of GABA_A receptors to produce excitation and allow for a greater net outward (inhibitory) current. This inhibition could be one of the mechanisms underlying the topiramate-induced enhancement of the amplitude of GABA_A receptor-mediated inhibitory synaptic responses. However, a recent study failed to provide support for this hypothesis (Braga et al., 2009). Tolerance develops to the anticonvulsant action of carbonic anhydrase inhibitors such as acetazolamide; these drugs, therefore, have limited utility in chronic epilepsy therapy. In contrast, topiramate exhibits continuing effectiveness. Thus, carbonic anhydrase inhibition is unlikely to be a major factor in its clinical activity.

Vigabatrin

Vigabatrin (γ-vinyl-GABA) is an irreversible, enzyme-activated (suicide) inhibitor of 4-aminobutyrate aminotransferase (GABA transaminase; GABA-T), the enzyme that catalyzes the conversion of GABA and 2-oxoglutarate into succinic semialdehyde and glutamate. Vigabatrin is a racemate; the S-enantiomer is responsible for the pharmacological activity whereas the R-enantiomer is inert. Although GABA itself does not cross the blood–brain barrier, vigabatrin enters the brain and can be recovered in the cerebrospinal fluid (Rogawski and Porter, 1990). Vigabatrin is effective in the treatment of partial seizures and infantile spasms but is not useful in the treatment of other primary generalized seizure types. Use of vigabatrin has been curtailed because of various toxicities including irreversible retinal damage. In addition, the drug causes reversible hyperintensities on magnetic resonance imaging in selected brain regions in children (Pearl et al., 2009); such changes have not been seen in adults.

Vigabatrin has an unusual profile of activity in animal models. It is inactive in the maximal electroshock test (unless focally injected into certain brain regions) and is also inactive against pentyleneetetrazol seizures. Intravenous vigabatrin protects against bicuculline-induced myoclonic activity, strychnine-induced tonic seizures, isoniazid-induced generalized seizures, and amygdala-kindled seizures (Ångehagen et al., 2003; Vinogradova et al., 2005). Interestingly, the duration of seizure protection matches the inhibition of GABA-T, which can persist for many days because recovery requires resynthesis of enzyme and not the presence of the drug itself in the brain.

GABA-T is a pyridoxal 5'-phosphate-dependent enzyme. Vigabatrin inactivates GABA-T by two mechanisms involving the pyridoxal 5'-phosphate cofactor (Wang and Silverman, 2004). Although GABA-T is expressed in both neurons and glia (Chan-Palay et al., 1979), the increase in brain GABA levels produced by vigabatrin is predominantly due to inhibition of GABA-T in neurons (Sarhan and Seiler, 1979). Marked increases in brain GABA levels have been demonstrated in laboratory animals (Jung et al., 1977) and in humans (Petroff et al., 1996; Ben-Menachem et al., 1988, 1989).

The antiseizure action of vigabatrin almost certainly reflects inactivation of GABA-T. Nevertheless, the
precise way in which inhibition of GABA-T results in seizure prevention is obscure. Paradoxically, vigabatrin does not elicit larger GABA<sub>A</sub> receptor-mediated synaptic responses (Overstreet and Westbrook, 2001). Rather, the drug has generally been found to inhibit spontaneous and evoked synaptic GABA currents, an effect that would be expected to promote rather than suppress seizures. There are several alternative actions of vigabatrin that could explain its antiseizure action. One is enhancement of tonic GABA-mediated inhibition, owing to an increase in ambient extracellular GABA. High levels of intracellular GABA cause a reversal of GABA transporters, resulting in a marked elevation in extracellular GABA; this elevation could activate extrasynaptic GABA<sub>A</sub> receptors. An alternative hypothesis is that vigabatrin alters the dynamics of inhibitory synaptic function, reducing the fading of inhibitory GABA<sub>A</sub> receptor responses during repetitive activation of interneurons (Jackson et al., 2000). Such fading, which is dependent on GABA<sub>B</sub> receptors, may be an important factor that permits focal epileptiform activity to develop into a full-blown seizure. Reduction in this fading could account for the selective suppression of seizures by vigabatrin.

Zonisamide

Zonisamide is a structurally novel antiseizure agent that consists of a benzisoxazole aromatic ring with a sulfonamide side chain. Interestingly, topiramate also has a sulfonamide group in a sulfamate configuration. These two drugs are the only marketed sulfur-containing antiseizure agents; their structural similarity may account for some of their common pharmacological actions, including carbonic anhydrase inhibition. Both zonisamide and topiramate are associated with weight loss and rarely kidney stones and oligohydrosis. Whether these actions are related to the common sulfamate group is not known.

Zonisamide is useful to treat a variety of epileptic conditions, including simple and complex partial seizures, generalized tonic-clonic seizures, myoclonic epilepsies, seizures in the Lennox–Gastaut syndrome, and infantile spasms. Zonisamide is also effective in the progressive myoclonic epilepsy syndrome that is intractable to most antiepileptic drugs (Kyllerman and Ben-Menachem, 1998).

Zonisamide is protective in the maximal electroshock test but inactive against subcutaneous pentylentetrazol seizures in both mice and rats; it is also inactive against seizures induced by bicuculline and picrotoxin (Vazda, 2000). Zonisamide therefore exhibits a profile similar to that of sodium channel-blocking antiseizure agents. In addition, zonisamide has activity in various kindling models; it is also effective against sound-induced seizures in susceptible animals and has been reported to suppress focal epileptiform discharges in cortical injury models (Masuda et al., 1998; Macdonald and Rogawski, 2008).

Some of the pharmacological targets of zonisamide that have been considered relevant to its antiseizure actions are (1) voltage-dependent sodium channels, (2) low-threshold T-type voltage-gated calcium channels, and (3) carbonic anhydrase isoenzymes (Victor, 2007; Matar et al., 2009). Various studies have reported effects of zonisamide on benzodiazepine and muscimol binding to GABA<sub>A</sub> receptors. However, even at high concentrations (30 mg/mL), zonisamide did not affect GABA receptor responses; thus, the antiseizure action of zonisamide appears to be independent of GABA<sub>A</sub> receptors (Rock et al., 1989). Similarly, zonisamide failed to influence responses to glutamate. A recent study has demonstrated that zonisamide (30 μM) reversibly activates large-conductance (BK) calcium-activated potassium channels in a hippocampal neuron-derived cell line (Huang et al., 2007); the relevance of this action to the antiseizure effects of zonisamide is uncertain.

Like other sodium channel-blocking antiseizure agents, zonisamide inhibits sustained repetitive firing of action potentials induced by depolarizing current pulses (Rock et al., 1989). In voltage-clamped *Myxicola* (annelid worm) giant axons, zonisamide was found to retard recovery from fast and slow sodium inactivation (Schauf, 1987). Whether the effect on slow inactivation is similar to that of lacosamide and is relevant to the antiseizure action requires further study. The effect of zonisamide on sodium channels is believed to result in inhibition of excitatory neurotransmitter (glutamate) release (Rogawski, 2002).

Zonisamide affects low-threshold T-type voltage-dependent calcium channels – as do the succinimides, including ethosuximide (Suzuki et al., 1992; Kito et al., 1996). A recent study has demonstrated that zonisamide is selective for the Ca<sub>3.2</sub> subtype of T-type calcium channels and is a less potent blocker of Ca<sub>3.1</sub> and Ca<sub>3.3</sub> subtypes (Matar et al., 2009). The blockade of Ca<sub>3.2</sub> channels by zonisamide is not use dependent or voltage dependent. Zonisamide does not affect L-type voltage-gated calcium channels. Zonisamide does not have activity in the pentylentetrazol clonic-seizure model, suggesting that it would not protect against absence seizures, since other antiabsence agents (except lamotrigine) are effective in this model. However, zonisamide does appear to have clinical antiabsence activity, which is compatible with its succinimide-like effects on T-type calcium channels (Hughes, 2009).

Like the potent carbonic anhydrase inhibitor acetazolamide, zonisamide has a sulfonamide side chain, which, like topiramate, presumably accounts for its activity as a carbonic anhydrase inhibitor. Zonisamide inhibits type IX membrane-associated carbonic anhydrase
with high affinity ($K_i$ 5.1 nM) and also very weakly inhibits the membrane-associated isoenzyme XII (Nishimori et al., 2005). However, the overall potency of zonisamide may not be sufficiently high for carbolic anhydrase inhibition to be relevant to the antiseizure activity of the drug (Masuda et al., 1980; Thone et al., 2008). Further, 7-methylated zonisamide, a derivative of zonisamide with carbolic anhydrase-inhibiting properties similar to those of zonisamide, is not effective in animal seizure models.

In addition to its action on traditional antiseizure drug targets, extensive literature supports the view that zonisamide influences the release and metabolism of monoaminergic neurotransmitters, including dopamine, serotonin, and acetylcholine (Okada et al., 1995, 1999). The relevance of these effects to the drug's antiseizure action is uncertain.

REFERENCES


Crullen V, Leresche N (2002). Block of thalamic T-type Ca^{2+} channels by ethosuximide is not the whole story. Epilepsy Curr 2: 53–56.


