Mechanisms of Action of Antiseizure Drugs and the Ketogenic Diet

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Antiseizure drugs (ASDs), also termed antiepileptic drugs, are the main form of symptomatic treatment for people with epilepsy, but not all patients become free of seizures. The ketogenic diet is one treatment option for drug-resistant patients. Both types of therapy exert their clinical effects through interactions with one or more of a diverse set of molecular targets in the brain. ASDs act by modulation of voltage-gated ion channels, including sodium, calcium, and potassium channels; by enhancement of γ-aminobutyric acid (GABA)-mediated inhibition through effects on $GABA_A$ receptors, the GABA transporter 1 (GAT1) GABA uptake transporter, or GABA transaminase; through interactions with elements of the synaptic release machinery, including synaptic vesicle 2A (SV2A) and α2δ; or by blockade of ionotropic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors. The ketogenic diet leads to increases in circulating ketones, which may contribute to the efficacy in treating pharmacoresistant seizures. Production in the brain of inhibitory mediators, such as adenosine, or ion channel modulators, such as polyunsaturated fatty acids, may also play a role. Metabolic effects, including diversion from glycolysis, are a further postulated mechanism. For some ASDs and the ketogenic diet, effects on multiple targets may contribute to activity. Better understanding of the ketogenic diet will inform the development of improved drug therapies to treat refractory seizures.

Antiseizure drugs (ASDs), often referred to as antiepileptic drugs or anticonvulsant drugs, are administered chronically with the intent of preventing the occurrence of epileptic seizures in a person at risk. Although limited results from animal studies or uncontrolled clinical studies suggest that some ASDs might have antiepileptogenic actions (Kaminski et al. 2014), there is insufficient evidence to conclude that any ASD can produce clinically useful disease modification. The symptomatic relief from seizures that ASDs provide occurs through interactions with a variety of cellular targets. The actions on these targets can be categorized into four broad groups: (1) modulation of voltage-gated ion channels, including sodium, cal-

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Table 1. Molecular targets of clinically used ASDs

Molecular target	ASDs that act on target
Voltage-gated ion channels	
Voltage-gated sodium channels	Phenytoin, fosphenytoin,[a] carbamazepine, oxcarbazepine,[b] eslicarbazepine acetate,[c] lamotrigine, lacosamide; possibly topiramate, zonisamide, rufinamide
Voltage-gated calcium channels (T-type)	Ethosuximide
Voltage-gated potassium channels (K_v7)	Ezogabine
GABA inhibition	
GABA$_A$ receptors	Phenobarbital, primidone, benzodiazepines, including diazepam, lorazepam, and clonazepam; possibly topiramate, felbamate, ezogabine
GAT1 GABA transporter	Tiagabine
GABA transaminase	Vigabatrin
Synaptic release machinery	
SV2A	Levetiracetam
α2δ	Gabapentin, gabapentin enacarbil,[d] pregabalin
Ionotropic glutamate receptors	
AMPA receptor	Perampanel
Mixed/unknown	Valproate, felbamate, topiramate, zonisamide, rufinamide, adrenocorticotrophin

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GABA, γ-Aminobutyric acid; GAT1, γ-aminobutyric acid transporter 1; SV2A, synaptic vesicle protein 2A; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate.

[a]Fosphenytoin is a prodrug for phenytoin.
[b]Oxcarbazepine serves largely as a prodrug for licarbazepine, mainly *S*-licarbazepine.
[c]Eslicarbarbazepine acetate is a prodrug for *S*-licarbazepine.
[d]Gabapentin enacarbil is a prodrug for gabapentin.

cium, and potassium channels; (2) enhancement of γ-aminobutyric acid (GABA) inhibition through effects on GABA$_A$ receptors, the GAT1 GABA transporter or GABA transaminase; (3) direct modulation of synaptic release through effects on components of the release machinery, including synaptic vesicle protein 2A (SV2A) and the α2δ subunit of voltage-gated calcium channels; and (4) inhibition of synaptic excitation mediated by ionotropic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors (Table 1). The result of the interactions at these diverse targets is to modify the intrinsic excitability properties of neurons or to alter fast inhibitory or excitatory neurotransmission. These actions reduce the probability of seizure occurrence by modifying the bursting properties of neurons (reducing the capacity of neurons to fire action potentials at high rate) and reducing synchronization in localized neuronal ensembles. In addition, ASDs inhibit the spread of abnormal firing to adjacent and distant brain sites. This occurs through strengthening of the inhibitory surround, a function predominantly of GABA released from interneurons acting on GABA$_A$ receptors, and by inhibition of glutamate-mediated excitatory neurotransmission within the network of principal (relay) neurons. Some seizures, including typical generalized absence seizures, result from elevated thalamocortical synchronization. ASDs effective against these seizure types interfere with the rhythm-generating mechanisms that underlie synchronized activity in the thalamocortical circuitry. In this review, we consider each of the principal targets of ASDs and discuss how, to the extent known, these agents affect the activity of these targets. The goal of epilepsy therapy is the complete elimination of seizures. This objective is not achievable for many patients. When ASDs are not able to control seizures, some patients

may benefit from dietary therapies. The ketogenic diet is the most widely used and best validated such treatment approach. Here, we review a variety of theories to explain the action of the ketogenic diet in the treatment of epilepsy ranging from the view that the antiseizure activity relates to ketone bodies or the production of inhibitory mediators, such as adenosine, to the hypothesis that it is a result of shifts in intermediary metabolism.

VOLTAGE-GATED ION CHANNELS
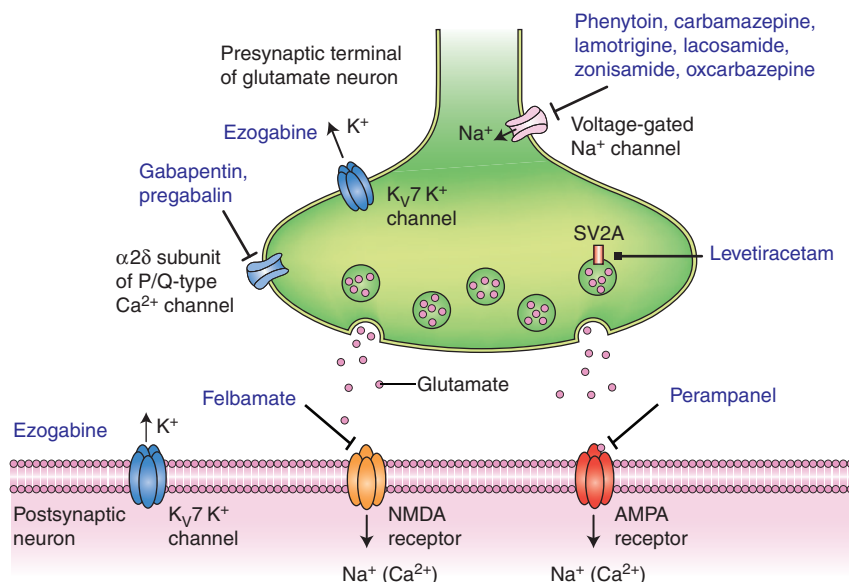
Voltage-Gated Sodium Channels

Voltage-gated sodium channels play an essential role in the initiation and propagation of action potentials in neurons (Mantegazza et al. 2010). Neuronal depolarizations by only a few millivolts, which ordinarily result from activation of synaptic glutamate receptors (mainly AMPA receptors, but also N-methyl-D-aspartate [NMDA] receptors), cause sodium channels to open and enable influx of sodium along its electrochemical gradient. These channels then inactivate within milliseconds. The influx of sodium ions during the brief time that sodium channels are open generates the depolarizing component (upstroke) of the action potential. Although nearly all sodium channels inactivate on depolarization, ∼1% of the sodium current is non-inactivating, resulting in a small persistent sodium current (I_{NaP}), which is carried by the same channels as the fast transient current. I_{NaP} facilitates epileptic burst firing by reducing the threshold for action potential generation, sustained repetitive firing, and augmentation of depolarizing synaptic currents (Stafstrom 2007). Some ASDs, most notably phenytoin, inhibit I_{NaP}, an action that is believed to contribute to their efficacy (Mantegazza et al. 2010).

Voltage-gated sodium channels are multimeric protein complexes, composed of a large α-subunit that forms four subunit-like homologous domains (designated I–IV) and one or more smaller β subunits (Meldrum and Rogawski 2007). The ion-conducting pore is contained within the α-subunit, as are the elements of the channel that mediate its fundamental physio-logical properties, including rapid inactivation. The α subunits alone are able to form a functional sodium channel, but β subunits (β1–β4 are known) can modulate the kinetics and trafficking of the channel (Patino and Isom 2010). Of the 10 known α subunits, using the channel and receptor nomenclature of Alexander et al. (2013), $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_V1.6$ are the four most abundantly expressed subunits in the brain (Yu and Catterall 2003; Vacher et al. 2008). Mutations in voltage-gated sodium channels have been associated with various genetic forms of epilepsy (Oliva et al. 2012).

ASDs that protect against seizures through an interaction with voltage-gated sodium channels are commonly referred to as "sodium channel blockers" (Fig. 1). They are among the most frequently used drugs in the treatment of both focal and primary generalized tonic–clonic seizures. Such drugs include phenytoin, carbamazepine, lamotrigine, oxcarbazepine (as well as its active metabolite licarbazepine), rufinamide, and lacosamide. ASDs that interact with voltage-gated sodium channels also show a characteristic "use-dependent" blocking action so that they inhibit high-frequency trains of action potentials much more potently than they attenuate individual action potentials or firing at low frequencies. Because they also show a "voltage-dependence" to their blocking action, sodium-channel-blocking ASDs are more potent at inhibiting action potentials superimposed on a depolarized plateau potential as characteristically occurs with seizures. Thus, importantly, sodium-channel-blocking ASDs preferentially inhibit seizure discharges in relation to normal ongoing neural activity. By virtue of their ability to inhibit the action potential invasion of nerve terminals, sodium-channel-blocking ASDs inhibit the release of diverse neurotransmitters, including glutamate; whether this is responsible for the therapeutic activity of the drugs remains uncertain (Waldmeier et al. 1995).

The binding site for sodium on sodium-channel-blocking ASDs is believed to overlap with the binding site of local anesthetics, which is within the pore of the channel and is formed by the S6 segments of domains I, II, and IV.

Figure 1. Diverse molecular targets for antiseizure drugs (ASDs) at excitatory glutamatergic synapses. Seizure protection can be conferred by effects on voltage-gated sodium channels, M-type voltage-gated potassium channels (K_v7), and voltage-gated calcium channels located in presynaptic terminals. Additional presynaptic targets include the synaptic vesicle protein SV2A and the $\alpha2\delta$ accessory subunit of voltage-gated calcium channels. These presynaptic targets may act to diminish glutamate release. Postsynaptic targets include ionotropic glutamate receptors of the *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) types.

Sodium-channel-blocking ASDs bind with higher affinity to this site when the channel is in the inactivated state, and, when such a drug is bound, the channel is stabilized in the inactivated state (Mantegazza et al. 2010). When neurons are depolarized and firing rapidly, sodium channels spend a greater amount time in the inactivated state and are able to accumulate bound drug so that they become trapped in the inactivated state. This accounts for the use- and voltage-dependent blocking action that they show. Phenytoin, carbamazepine, and lamotrigine are considered "classical" sodium-channel-blocking ASDs. Lacosamide is also believed to induce its therapeutic effects by interacting with sodium channels (Rogawski et al. 2015). However, unlike other sodium-channel-blocking ASDs, lacosamide does not inhibit high-frequency repetitive spike firing on the time scale of hundreds of milliseconds. It does, however, inhibit spike firing in long trains of spikes on the time scale of 1−2 sec. It has been

proposed that the very slow action of lacosamide is caused by an enhancement of a distinct and poorly understood form of inactivation, referred to as "slow inactivation." An alternative explanation is that lacosamide binds more slowly to fast-inactivated sodium channels than do the other sodium-channel-blocking ASDs. In any case, the unusually slow development of block produced by lacosamide during high-frequency activity could allow lacosamide to better discriminate between seizure-like pathological firing and normal network activity.

T-Type Voltage-Gated Calcium Channels

Low-voltage-activated (T-type) calcium channels play a major role in the intrinsic thalamocortical oscillations that underlie the spike-wave discharges of generalized absence seizures (Avoli et al. 2001; Huguenard 2002; Lambert et al. 2014). There are three T-type Ca^{2+} channel isoforms encoded by separate genes, denoted as

Ca$_v$3.1 (α1G), Ca$_v$3.2 (α1H), and Ca$_v$3.3 (α1I). All three T-type calcium channel isoforms are expressed in thalamocortical circuits (Talley et al. 1999). Ca$_v$3.1 is prominently expressed in thalamic relay neurons in the dorsal thalamus, which plays a key role in absence seizures; Ca$_v$3.2—and to a lesser extent Ca$_v$3.3—are prominently expressed in thalamic reticular neurons. All three T-type calcium channel isoforms are expressed in the cortex, with Ca$_v$3.2 mainly localized to layer V. In non-REM sleep, including when δ waves, sleep spindles, and K-complexes occur, the thalamocortical circuit switches from a tonic to oscillatory mode of firing, but in absence epilepsy, this switching can occur inappropriately, even during wakefulness (Crunelli et al. 2014; Powell et al. 2014). T-type calcium channels in the thalamus and cortex contribute to the abnormal behavior of the circuit. These channels generate low-threshold spikes, leading to burst firing and oscillatory behavior (Suzuki and Rogawski 1989). GABAergic neurons of the thalamic reticular nucleus are also critically involved in absence seizures as they hyperpolarize thalamic relay neurons, which de-inactivate T-type calcium channels, allowing the channels to generate burst firing and the propagation of spike-wave discharges in the thalamocortical circuit (Danober et al. 1998).

Ethosuximide, which is highly efficacious in the treatment of absence seizures—but not other seizure types—is thought to act by inhibition of T-type calcium channels in the thalamocortical circuit (Coulter et al. 1989; Broicher et al. 2007; Gören and Onat 2007). At clinically relevant concentrations ($20-40$ µg/ml), some but not all investigators have observed a partial ($20\%-30\%$) reduction of T-type calcium current by ethosuximide. Notwithstanding this discrepancy, studies with recombinant T-type calcium channels have confirmed that ethosuximide blocks all three channel types (Gomora et al. 2001). The block increases when the current is activated from more depolarized potentials and when T-type calcium channels are inactivated, as occurs especially during high-frequency activation, so that the drug has selectivity for pathological behavior in the tha-

lamocortical circuit, which is associated with neuronal depolarization and inactivation of T-type calcium channels. Effects on other membrane currents, including I$_{NaP}$, calcium-activated potassium current (Broicher et al. 2007), and inward rectifier potassium current (Huang and Kuo 2015), may contribute to the efficacy of ethosuximide in absence epilepsy. Remarkably, results in animal models indicate that early treatment with ethosuximide can have disease-modifying (i.e., antiepileptogenic) effects, causing a persistent reduction in seizures and mitigation of behavioral comorbidities (Blumenfeld et al. 2008; Dezsi et al. 2013). These actions may be caused by epigenetic modifications. A study showing that children with absence epilepsy who receive ethosuximide are more likely than those who receive valproate to achieve long-term remission is consistent with the disease-modifying actions observed in animal studies (Berg et al. 2014).

The efficacy of some other ASDs may also depend, at least in part, on actions at T-type calcium channels. Zonisamide, in addition to effects on voltage-gated sodium channels, may also block T-type voltage-gated calcium channels (Powell et al. 2014), thus accounting for its likely efficacy in absence epilepsy (Hughes 2009). Similarly, there is evidence that valproate, a drug of choice in absence epilepsy, may also inhibit T-type calcium channels (Broicher et al. 2007).

K$_v$7 Voltage-Gated Potassium Channels

Voltage-gated potassium channels open in response membrane depolarization, permitting efflux of potassium ions, thus driving the membrane potential toward a hyperpolarized level. This serves to repolarize depolarizing events (such as action potentials and synaptic potentials) and cause a generalized reduction in excitability. In 1998, the first genes for a human idiopathic epilepsy were identified (Charlier et al. 1998). These genes, designated *KCNQ2* and *KCNQ3*, encoded novel brain potassium channel subunits, K$_v$7.2 and K$_v$7.3, respectively, which are homologous to a previously identified cardiac potassium channel K$_v$7.1, encoded

by *KCNQ1* (*LQT1*). These brain potassium channels mediate the M current, a potassium current that increases as the membrane potential in neurons approaches action potential threshold. K_v7 channels, together with hyperpolarization-activated cyclic nucleotide-gated potassium (HCN) channels and small-conductance calcium-activated potassium (K_{Ca}/SK) channels, generate the medium after-hyperpolarization, which is elicited by a burst of action potentials and serves to limit further firing (Gu et al. 2005). K_v7 potassium channels also serve to counteract the spike after-depolarization generated by recruitment of I_{NaP}, which can lead to bursting (Yue and Yaari 2006). K_v7 potassium channels, therefore, act as a "brake" on epileptic burst firing. The K_v7 family of potassium channels is now known to contain five members, including $K_v7.1$, which is expressed predominantly in the heart and $K_v7.2$–$K_v7.5$, which are expressed exclusively in the nervous system (Brown and Passmore 2009). Of these K_v7 family members, $K_v7.2$ and $K_v7.3$ are highly expressed in neurons relevant to epilepsy, including principal (pyramidal) cells of the hippocampus and neocortex. Kv7.2 and Kv7.3 compose heterotetrameric channels in which four subunits are arranged around a potassium selective pore. $K_v7.5$ channels may also contribute to M current and to neuronal after-hyperpolarization, for example, in the CA3 area of the hippocampus (Tzingounis et al. 2010).

Studies of the localization of $K_v7.2$ and $K_v7.3$ potassium channels by immunohistochemical techniques have indicated that the channels are present at highest density in axons and their terminals (Vacher et al. 2008). In myelinated fibers, they are present at nodes of Ranvier and the channels are also expressed at axon initial segments. In addition, the channels are expressed at lower levels in the somata of principal neurons and in some GABAergic neurons. Physiological studies in CA1 pyramidal neurons indicate that K_v7 channels are functionally active in the perisomatic region (Hu et al. 2007) and possibly also on distal dendrites (Yue and Yaari 2006).

Ezogabine (retigabine), which is efficacious in the treatment of focal seizures, acts as a pos-itive modulator of the nervous system K_v7 potassium channels ($K_v7.2$–$K_v7.5$), but does not affect the cardiac member of the family ($K_v7.1$) (Fig. 1). Of particular relevance to the antiseizure action of ezogabine is its action on the M current, which is predominantly carried by channels composed of $K_v7.2$ and $K_v7.3$, although $K_v7.5$ alone or in combination with $K_v7.3$ also contributes (Rogawski 2006; Gunthorpe et al. 2012). Ezogabine causes a hyperpolarizing shift in the activation of K_v7 channels such that more M current is generated near the resting potential. It also causes a change in the kinetics of single KCNQ channels to favor channel opening, thus increasing the macroscopic M current; ezogabine, nevertheless, does not alter the single channel conductance of individual K_v7 channels (Tatulian and Brown 2003). As noted, many K_v7 channels in the brain are believed to be $K_v7.2$/$K_v7.3$ heteromers, and these are highly sensitive to ezogabine (EC_{50}, 1.6 μM) (Gunthorpe et al. 2012). Peak plasma levels of ezogabine range from 354 to 717 ng/ml (1.2–2.4 μM) (Hermann et al. 2003), and plasma protein binding is 80% so that free plasma concentrations are estimated to be ∼0.2–0.5 μM; brain concentrations are expected to be similar. Therefore, therapeutic concentrations likely only modestly potentiate the most sensitive K_v7 channels and do not affect less sensitive channels. The binding site for ezogabine in $K_v7.2$/$K_v7.3$ heteromers is in a pocket formed by the pore-lining S5 membrane segment of one subunit and the pore-lining 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.

Several experimental approaches support the role of K_v7 potassium channels in the antiseizure activity of ezogabine. Mice with a genetic defect in these channels show reduced sensitivity to the antiseizure effect of ezogabine (Gunthorpe et al. 2012). Furthermore, the KCNQ inhibitor, XE-991, partially blocks the antiseizure effect of ezogabine in an electrical seizure test (Gunthorpe et al. 2012). However, the precise way in which activation of K_v7 chan-

nels leads to seizure protection remains to be elucidated. Consistent with the presynaptic localization of many K_v7 channels, ezogabine has been found to inhibit the release of various neurotransmitters, including GABA and probably also glutamate (Martire et al. 2004). Inhibition of glutamate release is expected to confer seizure protection. The impairment of GABA release or the direct inhibition of inhibitory interneurons (Lawrence et al. 2006; Grigorov et al. 2014) would be expected to enhance circuit excitability. Indeed, in rodents, retigabine shows proconvulsant activity at doses (≥ 61.9 mg/kg) that are approximately 10-fold greater than those associated with seizure protection (U.S. Food and Drug Administration 2010). Paradoxically, and in contrast to biochemical observations, physiological studies have found that ezogabine "increases" glutamate-mediated synaptic transmission in the hippocampus through a presynaptic action by reducing the inactivation of voltage-gated sodium channels so that sodium-dependent action potentials are elicited with greater probability (Vervaeke et al. 2006). This action could contribute to the proconvulsant effects of the drug. If ezogabine does confer seizure protection through effects on excitatory neurons, postsynaptic actions to inhibit somatic excitability likely predominate.

In addition to its effects on K_v7 potassium channels, ezogabine has been reported to interact with the GABA system. At high concentrations (>10 μM), ezogabine was shown to potentiate GABA-mediated inhibitory transmission by acting as a positive allosteric modulator of $GABA_A$ receptors (Rundfeldt and Netzer 2000; Otto et al. 2002). Recent evidence that inhibitory effects of the drug on seizure-like activity in hippocampal neurons persist in the presence of blockade of K_v7 channels has bolstered the view that positive modulation of $GABA_A$ receptors could be key to its antiseizure activity (Treven et al. 2015). Thus, at lower concentrations than are required for effects on synaptic $GABA_A$ receptors, ezogabine selectively enhances extrasynaptic $GABA_A$ receptors that contain the δ-subunit (Treven et al. 2015). Ezogabine has also been reported to increase GABA synthesis (Kapetanovic et al. 1995); the mecha-

nism and implications of this effect are not known. In sum, various lines of evidence raise the possibility that effects of ezogabine on GABA mechanisms could be of importance in its antiseizure activity.

GABA INHIBITION

GABA, the neurotransmitter of local inhibitory interneurons, acts through $GABA_A$ and $GABA_B$ receptors. $GABA_A$ receptors, which are Cys loop-type ligand-gated chloride channels, represent an important target for ASDs and will be considered here. $GABA_B$ receptors, which are heterodimeric G-protein-coupled receptors that activate potassium channels and inhibit calcium channels, are distinct in structure and function from $GABA_A$ receptors and are not a target of any ASD. Although only about one in five cortical neurons is GABAergic (Sahara et al. 2012), these neurons play a critical role in controlling the firing rate and timing of principal (excitatory) neurons. In addition, they synchronize local neuronal ensembles and restrain the generation of abnormal epileptic behavior. Consequently, enhancement of GABAergic inhibition is a key mechanism of ASD action.

$GABA_A$ Receptors

$GABA_A$ receptors are heteropentameric protein complexes localized to the postsynaptic membrane of inhibitory synapses (Fig. 2) where they mediate fast neuronal inhibition on a millisecond timescale. They are also located extrasynaptically where they respond to ambient GABA in the extracellular milieu and confer tonic (long-term) inhibition. There are 19 known $GABA_A$ receptor subunits (α1−6, β1−3, γ1−3, δ, ε, θ, π, and ρ1−3) (Olsen and Sieghart 2009). However, the bulk (60%) of synaptic $GABA_A$ receptors are believed to have the α1β2γ2 configuration, and a considerable fraction of the remainder (15%−20%) has α2β3γ2. Among the receptor subtypes that contribute to tonic signaling in brain regions relevant to epilepsy are α4βxδ receptors, which are believed to mediate the tonic current in dentate granule cells and thalamocortical neurons, and α5-contain-
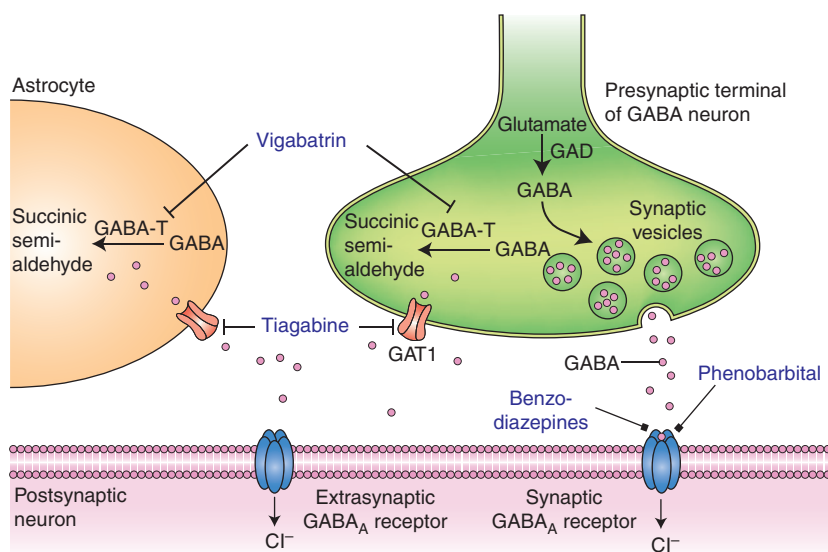
Figure 2. Diverse molecular targets for antiseizure drugs (ASDs) at inhibitory γ-aminobutyric acid (GABA)ergic synapses. Seizure protection can be conferred by effects on synaptic or extrasynaptic GABA$_A$ receptors or on GABA transaminase (GABA-T) or GABA transporter 1 (GAT1). Furthermore, some ASDs (e.g., valproate) have been shown to increase the activity of the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD), thereby increasing GABA turnover. Astrocytes contain elements, including GABA transporters, which influence the dynamics of GABA, thereby affecting the excitability of the postsynaptic neuron.

ing GABA$_A$ receptors in CA1 pyramidal cells (Walker and Kullmann 2012).

Benzodiazepines, such a diazepam, lorazepam, and clonazepam, and barbiturates, such as phenobarbital (Löscher and Rogawski 2012), are ASDs that act on GABA$_A$ receptors as positive allosteric modulators (Fig. 2). At higher concentrations, barbiturates can directly activate GABA$_A$ receptors in the absence of GABA (Rho et al. 1996), whereas benzodiazepines cannot. Benzodiazepines are specific for synaptic GABA$_A$ receptors containing the γ2 subunit and act to allosterically modulate these receptors to increase the channel-opening frequency, resulting in enhanced synaptic inhibition. This confers a broad-spectrum antiseizure action. In most epilepsy syndromes, the specific cellular types that are involved in the antiseizure activity of benzodiazepines are not known. However, in the case of absence epilepsy, it is believed that benzodiazepines desynchronize the thalamocortical oscillations underlying generalized spike-wave discharges by specific effects on α3-containing GABA$_A$ receptors in

the thalamic reticular nucleus (Sohal et al. 2003). Barbiturates, presumably because they are not specific for α3-containing GABA$_A$ receptors, are not active in absence epilepsy and may even aggravate absence seizures. In contrast to benzodiazepines, barbiturates do not appear to increase the frequency of GABA-induced chloride channel opening, but instead increase the channel open time. In addition to effects on GABA$_A$ receptors, barbiturates modulate other ion channel systems, including calcium and sodium channels, and these actions may contribute to therapeutic activity (ffrench-Mullen et al. 1993).

GAT1 GABA Transporter

The action of the neurotransmitter GABA is terminated by uptake into neurons and glial cells by membrane-bound GABA transporters, of which there are four types, termed GAT1, BGT1, GAT2, and GAT3. GAT1 (encoded by the *SLC6A1* gene), the predominant form in the forebrain (including the neocortex and hip-

pocampus), is localized to GABAergic terminals, as well as to glial processes near GABA synapses (Fig. 2). Tiagabine is a highly selective inhibitor of GAT1 in neurons and glia (Schousboe et al. 2014). Inhibition of GAT1 by tiagabine suppresses the translocation of extracellular GABA into the intracellular compartment, thus raising extracellular GABA levels. Functionally, tiagabine prolongs GABA-mediated inhibitory synaptic responses (Thompson and Gähwiler 1992) and the marked elevation in extracellular GABA it produces may lead to activation of extrasynaptic GABA receptors.

GABA Transaminase

4-Aminobutyrate aminotransferase (GABA transaminase), an enzyme that catalyzes the conversion of GABA and 2-oxoglutarate into succinic semialdehyde and glutamate, is responsible for the metabolic inactivation of GABA (Fig. 2). Inhibition of GABA transaminase with vigabatrin (γ-vinyl GABA), an irreversible suicide inhibitor of the enzyme, leads to marked increases in brain GABA levels (Petroff et al. 1996). Although the antiseizure action of vigabatrin is believed to reflect inactivation of GABA transaminase, how this occurs is not straightforward and does not appear to be caused by an enhancement of inhibitory synaptic transmission. In contrast to the action of tiagabine, vigabatrin does not elicit larger or more prolonged GABA$_A$ receptor-mediated synaptic responses (Overstreet and Westbrook 2001; Wu et al. 2003). Rather, preincubation of brain slices with vigabatrin irreversibly inhibited miniature and evoked inhibitory postsynaptic currents. Additional experiments suggested that the paradoxical effect resulted from a reduction in the GABA content of synaptic vesicles caused by GABA transaminase inhibition. In contrast to the effect on GABA-mediated synaptic transmission, vigabatrin caused an increase in tonic (nonsynaptic) GABA$_A$ receptor current. This steady current is believed to be mediated by the action of GABA in the extracellular milieu acting on extrasynapic GABA$_A$ receptors. High levels of intracellular GABA cause a reversal of GABA transporters, resulting in a marked elevation in extracellular GABA, which is likely responsible for the increase in tonic GABA$_A$ receptor current. It can be concluded that vigabatrin causes divergent effects on synaptic and extrasynaptic GABA-mediated inhibition with seizure protection resulting from a predominance of the extrasynaptic action. Interestingly, in the early period after administration of vigabatrin to animals, there is a reduction in seizure threshold, whereas the antiseizure actions become evident only later (Löscher et al. 1989; Stuchlík et al. 2001). Thus, vigabatrin has a biphasic action with proconvulsant effects likely related to suppression of synaptic GABAergic neurotransmission and antiseizure effects as a result of spillover of GABA into the extracellular space and activation of extrasynaptic GABA$_A$ receptors. Interestingly, individuals with a rare genetic deficiency of GABA transaminase experience refractory seizures, supporting the view that inhibition of GABA transaminase is, in fact, the proconvulsant mechanism of vigabatrin (Medina-Kauwe et al. 1999).

SYNAPTIC RELEASE MACHINERY

SV2A

Multiple lines of evidence support the conclusion that SV2A, a membrane glycoprotein found in the secretory vesicles of neurons and endocrine cells and possibly immune cells, is the molecular target for levetiracetam (Kaminski et al. 2012; Li et al. 2013). There is a strong correlation between the affinity of levetiracetam analogs for binding to SV2A and the potency of the analogs in several animal seizure models. Moreover, seizure protection conferred by levetiracetam and other SV2A ligands strongly correlates with the degree of SV2A occupancy in vivo. Finally, the antiseizure efficacy of levetiracetam (but not valproate, which does not interact with SV2A) is reduced in SV2A$^{+/-}$ mice that have one copy of SV2A disrupted by gene targeting. The precise way in which binding of levetiracetam to SV2A leads to seizure protection is not understood.

Indeed, the function of SV2A itself is obscure. Among the various functions proposed

are roles in calcium-dependent exocytosis, neurotransmitter loading/retention in synaptic vesicles, and synaptic vesicle priming, as well as transport of vesicle constituents. SV2A is one of three homologous SV2 proteins that belong to the major facilitator superfamily of 12-transmembrane domain transporters. Despite substantial effort, no transport function of these proteins has yet been identified, although studies with protein tomography have found that SV2A can adopt two alternate conformations consistent with a transporter role (Lynch et al. 2008). Interestingly, however, levetiracetam binding does not cause a large-scale conformational change in SV2A, or lock a specific conformational state of the protein, as would an inhibitor of transport. Apparently, the drug has a more subtle effect on the protein. Although the function of SV2A is still poorly defined, $SV2A^{-/-}$ knockout mice show a lethal seizure phenotype demonstrating that SV2A, in some way, serves to restrain seizure activity.

A series of recent studies has examined the impact of levetiracetam on synaptic transmission in brain slice recordings (Meehan et al. 2011, 2012). Although the drug had no effect on synaptic physiology with low frequency activation, levetiracetam did reduce the synaptic release of both excitatory (glutamate) and inhibitory (GABA) neurotransmitters during high-frequency activation. The frequency dependence is compatible with the selective suppression of epileptic activity. Modulation of synaptic release is a common mechanism of many ASDs, including sodium-channel blockers, which indirectly inhibit release at both excitatory and inhibitory synapses by inhibiting action potential firing. It appears that drugs that suppress inhibition and excitation can effectively protect against seizures, and they are not often proconvulsant. However, it is noteworthy that in some instances ASDs (notably, phenytoin) can have proconvulsant effects, particularly at high doses.

With several seconds of heavy use, as likely occurs during epileptic activity, excitatory synapses undergo a form of short-term plasticity that is dependent on the supply of new vesicles referred to as "supply-rate depression" (García-Pérez et al. 2008). This form of synaptic depression is distinct from that mediated by depletion of the readily releasable pool of vesicles and is not believed to come into play during ordinary ongoing activity. It may, however, limit synaptic transmission during sustained high-frequency firing and thus serve as an endogenous mechanism to restrain epileptic activity. It is, therefore, of interest that levetiracetam has been shown to cause a small acceleration of supply rate depression (García-Pérez et al. 2015). This effect was observed in experiments with mouse hippocampal CA1 neurons activated by Schaffer collateral stimulation in brain slices and also in hippocampal neurons in culture, but not in cultured neurons from mice lacking SV2A, indicating that an interaction of levetiracetam with SV2A is likely to account for the phenomenon. Thus, work in various laboratories raises the possibility that levetiracetam confers seizure protection through an interaction with SV2A by reducing synaptic release in a use-dependent fashion that requires high-frequency activation for prolonged periods.

With respect to the specificity of levetiracetam's effect on SV2A, it is important to note that several other cellular and molecular effects of this ASD have been reported at therapeutically relevant concentrations, including inhibition of high-voltage–gated calcium channels, inhibition of calcium release from intraneuronal stores, effects on the metabolism and turnover of GABA in discrete brain regions, reduction in the firing activity of GABAergic neurons in substantia nigra pars reticulata in vivo, and reversal of zinc-induced inhibition of $GABA_A$ receptors in epileptic tissue (Löscher et al. 1996; Lyseng-Williamson 2011; Wakita et al. 2014). The relevance of any of these various pharmacological actions to the antiseizure effect of levetiracetam remains to be defined.

α2δ-1

The gabapentinoids gabapentin and pregabalin act by binding to the α2δ-1 protein, which is an accessory subunit of voltage-gated calcium channels (Dolphin 2013; Stahl et al. 2013). α2δ-1 is located heterogeneously in the brain,

particularly, at presynaptic sites on excitatory (glutamatergic) neurons. Dense expression is observed in areas relevant to epilepsy, including in excitatory hippocampal mossy fibers and in the neocortex and amygdala. In contrast, α2δ-1 has minimal expression in the thalamus and it is noteworthy that gabapentinoids are not active in absence seizures, which, as discussed above, are dependent on this brain structure. Four α2δ subunits have been identified, but gabapentinoids only bind to α2δ-1 and α2δ-2 owing to the presence of an RRR motif containing a critical arginine that is required for binding. In knock-in mutant mice bearing a mutation in this motif (RRR mutated to RRA) in α2δ-1, which eliminates gabapentin and pregabalin binding, the analgesic and anxiolytic-like activity of pregabalin is eliminated (Field et al. 2006; Lotarski et al. 2011). A corresponding mutation in α2δ-2, which also eliminates binding, has no similar effect on the anxiolytic-like activity of pregabalin demonstrating that α2δ-1 and not α2δ-2 is relevant for its pharmacological activity; studies to determine whether binding to α2δ-1 and not α2δ-2 is necessary and sufficient to account for the antiseizure activity of gabapentinoids have not been reported. Interestingly, deletion of α2δ-1 or α2δ-2 in mice is associated with absence epilepsy or enhanced seizure susceptibility (Ivanov et al. 2004; Davies et al. 2007).

The precise manner in which binding of gabapentin and pregabalin to the α2δ-1 protein results in its pharmacological activity, including seizure protection, is not well understood (Rogawski and Bazil 2008). Although some studies have found that the drugs inhibit calcium channel currents, most have not and it is generally believed that calcium channel inhibition is not the mechanism of action of gabapentinoids (Stefani et al. 1998; van Hooft et al. 2002; 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, and this may account for the antiseizure activity (Dooley et al. 2007). There is some evidence that gabapentinoids cause internalization of calcium channels by reducing trafficking to the cell membrane (Hendrich et al. 2008; Weissmann et al. 2013). Whether this action could account for the rapid antiseizure effects of gabapentinoids in animal models is uncertain.

Gabapentin and pregabalin were initially developed as lipophilic, blood–brain barrier permeable forms of GABA (3-alkylated GABA analogs), but it is generally accepted that effects on GABA do not contribute to the pharmacological action of these drugs (Taylor et al. 2007). However, the gabapentinoids do activate the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD) (Silverman et al. 1991) and elevate GABA turnover at antiseizure doses in rats (Löscher et al. 1991) and patients with epilepsy (Petroff et al. 1996). Interestingly, the activating effects of the gabapentinoids on GAD and GABA turnover were similar to those reported for valproate (Löscher 1989; Silverman et al. 1991; Taylor et al. 1992).

AMPA RECEPTORS

Of the synapses in the brain, 80%–90% are excitatory (Hassel and Dingledine 2012). At these synapses, which are often between the presynaptic terminal of an excitatory neuron and a dendritic spine of the postsynaptic neuron, neurotransmitter glutamate released from the presynaptic terminal acts on ionotropic (ion flux conducting) and metabotropic (nonion flux conducting, G-protein-coupled) glutamate receptors localized to the postsynaptic density. Ionotropic glutamate receptors mediate a fast (millisecond timescale) depolarization of the postsynaptic neuron, referred to as the excitatory postsynaptic potential (EPSP). AMPA receptors, one type of ionotropic glutamate receptor, generate the bulk of the EPSP and are therefore the principal ion channel responsible for fast synaptic excitation. It has been long appreciated that cascading excitation within networks of synaptically connected neurons is a key mechanism of epileptic synchronization, at least in the hippocampal CA3 subfield and, possibly, in other brain areas (Rogawski 2013). Epileptic activity emerges from the network when GABA-mediated inhibition is deficient and, indeed, chronic alterations in inhibition

represent a leading hypothesis to explain some forms of epilepsy (Houser 2014). AMPA receptors have a special role in epileptic activity as epileptic synchronization cannot occur when AMPA receptors are blocked. In contrast, kainate receptors, which are ionotropic glutamate receptors that have a similar structure to AMPA receptors, do not have a similarly essential role as kainate receptor knockout does not interfere with seizure generation (Fritsch et al. 2014). NMDA receptors are thought to contribute to epileptiform activity, but the blockade of NMDA receptors is insufficient to abolish epileptiform discharges in many seizure models (Neuman et al. 1988; Löscher 1998). Moreover, NMDA blockers may exacerbate seizures in humans (Sveinbjornsdottir et al. 1993; Kasteleijn-Nolst Trenité et al. 2015). Pharmacological blockade of AMPA receptors has broad spectrum antiseizure activity in in vitro and animal seizure and epilepsy models (Yamaguchi et al. 1993; Rogawski 2011; Twele et al. 2015). In neocortical and hippocampal tissue removed from patients with focal epilepsy, there is evidence that AMPA receptor density is increased (Mathern et al. 1998; Zilles et al. 1999; Eid et al. 2002). Thus, it has been proposed that the hyperexcitability in focal epilepsy, in part, relates to strengthened excitatory circuits because of increased expression of AMPA receptors. AMPA receptors may therefore be a pivotal pharmacological target for the control of seizures.

Perampanel, a potent noncompetitive AMPA receptor antagonist, is the first and at present only antiseizure agent available for clinical use that selectively targets AMPA receptors (Fig. 1). Perampanel does not affect NMDA receptor responses and has no known effects on other ion channels or molecular targets at therapeutically relevant concentrations (Hanada et al. 2011; Chen et al. 2014). Therapeutic blood levels are expected to result in brain concentrations that would produce only low levels of inhibition of AMPA receptors (Rogawski and Hanada 2013). However, such low-level block of AMPA receptors is apparently sufficient to exert a clinical antiseizure action. Perampanel has a relatively low therapeutic window. Adverse central nervous system effects, such as dizziness,

irritability, and somnolence, are common, particularly at higher doses, emphasizing the importance of AMPA receptors in brain function. Although AMPA receptor antagonists have powerful antiseizure effects, they are not antiepileptogenic in animal models (Rogawski et al. 2001; Twele et al. 2015).

Secondarily generalized tonic–clonic seizures and, probably, also primary generalized tonic–clonic seizures engage distant regions in the cerebral cortex, as well as subcortical structures including thalamus, cerebellum, and basal ganglia (Blumenfeld et al. 2009). Glutamatergic neurons are responsible for the spread of excitation to these structures and also for thalamocortical projections that synchronize distant cortical sites. As is the case at glutamate synapses, generally, AMPA receptors generate the bulk of the excitatory current during fast synaptic transmission in these pathways (Turner and Salt 1998). The involvement of AMPA receptors in long cortical–cortical and cortical–subcortical pathways, which mediate the spread of excitation in generalized seizures, is likely the basis for the effectiveness of AMPA receptor antagonists in the treatment of secondarily generalized (Steinhoff et al. 2013) and primary generalized tonic–clonic seizures, and also in suppressing the generalized photoparoxysmal electroencephalography (EEG) response in patients with photosensitive epilepsy (Kasteleijn-Nolst Trenité et al. 2015).

MIXED TARGETS

Valproate

Although valproate is one of the most widely prescribed ASDs, the mechanism by which it protects against seizures is poorly understood. Valproate has multiple pharmacological actions (Rogawski and Porter 1990; Löscher et al. 2002). Because it has been difficult to relate any one mechanism to the drug's broad spectrum of clinical activity, it has been proposed that combined actions on several targets could account for its therapeutic properties. Although the actions of valproate on GABA systems are not straightforward, among the various pharmacological effects that have been described, those

related to GABA mechanisms are among the most likely to be relevant to valproate's antiseizure activity. For example, valproate increases the turnover of GABA in a regionally selective manner (Löscher 1989) and this might be associated with enhanced synaptic or extrasynaptic inhibition. At high concentrations, valproate affects voltage-gated sodium channels, but recent studies in brain slice recordings have failed to provide support for sodium-channel block as a relevant mechanism to explain clinical activity (Englund et al. 2011). Similarly, despite efficacy in absence epilepsy, there is little support for effects on T-type calcium channels. It is clear that valproate has pharmacological actions relevant to its antiseizure activity that remain to be elucidated.

Felbamate

Felbamate has been shown to act both as a positive modulator of GABA$_A$ receptors and also to inhibit NMDA receptors (Rho et al. 1994). Felbamate potentiates GABA responses via an interaction with a site on the GABA$_A$ receptor that is distinct from the benzodiazepine recognition site. This action may be of relevance to felbamate's clinical activity. As discussed above, NMDA receptors have not been validated as a target to treat focal or generalized seizures. Therefore, it is uncertain whether the NMDA-receptor-blocking activity of felbamate is relevant to its clinical antiseizure activity.

Topiramate

Topiramate shows a variety of pharmacological actions, but the extent to which any of the actions are relevant to its broad-spectrum antiseizure activity is uncertain. Targets potentially relevant to seizure protection include voltage-gated sodium channels, GABA$_A$ receptor subtypes, AMPA or kainate receptors, and types II and IV carbonic anhydrase isoenzymes. Unlike other ASDs, the effects on ion channels are not likely to occur through direct modulation of channel gating. Rather, the pharmacological actions of topiramate seem to be mediated indirectly, possibly through effects on channel phosphorylation (Shank et al. 2008).

The effects of topiramate on sodium channels occur at relatively low, therapeutically relevant concentrations and could be similar to the effects of other sodium-channel-blocking ASDs (Avoli et al. 1996). In addition to effects on fast sodium currents, topiramate, like phenytoin, blocks I$_{NaP}$ at low concentrations (Sun et al. 2007). Effects of topiramate on GABA$_A$ receptors could contribute to the broad spectrum activity of topiramate. Topiramate is not active in animal models, such as the pentylenetetrazol (PTZ) test, which are typically sensitive to drugs that positively modulate GABA$_A$ receptors. Nevertheless, the drug does have activity in an absence of epilepsy models (Rigoulot et al. 2003) and can affect pentylenetetrazol threshold, which is consistent with effects on GABA$_A$ receptors. There is evidence that topiramate may preferentially modulate a subset of GABA$_A$ receptors and that drug sensitivity is dependent on the β-subunit type (Simeone et al. 2011).

Several investigators have suggested that actions on fast glutamate-mediated excitatory neurotransmission could contribute to topiramate's antiseizure activity. In cultured neurons, the drug has been reported to inhibit responses to kainate, an agonist of AMPA and kainate receptors, leading to the conclusion that topiramate could be an antagonist of either AMPA or kainate receptors (Gibbs et al. 2000). Recently, kainate receptors have been found to be an unlikely target for an antiseizure agent (Fritsch et al. 2014). Whether actions of topiramate on glutamate-mediated neurotransmission contribute to its antiseizure activity remains to be determined.

It has been assumed that the topiramate's inhibition of carbonic anhydrase does not contribute to its clinical efficacy because there is no cross-tolerance to the antiseizure activity of topiramate when tolerance occurs to the classical carbonic anhydrase inhibitor acetazolamide in mice. However, a recent review left open the possibility that carbonic anhydrase inhibition could, in part, play a role (Shank and Maryanoff 2008).

Topiramate is well recognized to cause adverse cognitive effects, including impairment of working memory and verbal fluency. A blood-

concentration-dependent decline in phonemic generative fluency has been shown, but the basis for this effect is not understood (Ahmed et al. 2015).

Zonisamide

There are some similarities between topiramate and zonisamide as they both contain a sulfur atom and both inhibit carbonic anhydrase. In addition, like topiramate, zonisamide may act on voltage-gated sodium channels (Biton 2007). Electrophysiological studies, however, do not support an action on GABA$_A$ receptors. Unlike topiramate, there are reports that zonisamide can inhibit T-type voltage-gated calcium channels (Matar et al. 2009), which as stated above may account for its activity in absence epilepsy.

Rufinamide

The unique spectrum of clinical activity of rufinamide in the treatment of Lennox–Gastaut syndrome (a highly medically intractable epileptic encephalopathy) suggests that it has a distinct mechanism of action (Rogawski 2006). However, to date, rufinamide has only been shown to interact with voltage-gated sodium channels and the effects are subtle. Relevant concentrations of the drug may, at least for some subunit isoforms, cause a depolarization in the activation voltage and slowing of recovery from inactivation, which would be expected to reduce neuronal excitability (Gilchrist et al. 2014). Clearly, the effects on sodium channels cannot explain the special clinical profile of rufinamide.

Adrenocorticotrophin

The mechanism of adrenocorticotrophin (ACTH) in the treatment of infantile spasms is not well understood (Stafstrom et al. 2011). ACTH stimulates glucocorticoid (cortisol) synthesis and release from the zona fasciculata of the adrenal cortex. The cortisol could produce an anti-inflammatory action or have some other action in the brain to influence infantile spasms. Indeed, glucocorticoids are well recognized themselves and have therapeutic activity in the treatment of infantile spasms; whether ACTH is truly superior remains to be shown conclusively. One possible additional action of ACTH that could contribute to an enhanced action is through stimulation of neurosteroid synthesis. In addition to its actions with respect to glucocorticoids, ACTH also stimulates deoxycorticosterone (DOC) release from the zona glomerulosa of the adrenal cortex. DOC is, in part, converted to the antiseizure neurosteroid tetrahydro-DOC, which is a positive allosteric modulator of GABA$_A$ receptors (Reddy and Rogawski 2002). It has been hypothesized that the tetrahydro-DOC could, at least in part, contribute to the ability of ACTH to terminate infantile spasms.

KETOGENIC DIET

The use of dietary manipulations to treat epilepsy—in particular, controlling seizures through sustained fasting—dates back to the time of Hippocrates (Lennox and Cobb 1928; Wheless 2008). In the early 1920s, investigators realized that a diet composed principally of fats could be as effective as fasting and yield long-term suppression of seizures without severe caloric deprivation. Thus was born the ketogenic diet whose hallmark feature is the production of ketone bodies (β-hydroxybutyrate, acetoacetate, and acetone) by the liver (Peterman 1924; Freeman and Kossoff 2010). In an era before the availability of safe and effective ASDs, the ketogenic diet quickly became popular in large medical centers. However, with the advent of phenytoin in 1938, the ketogenic diet quickly fell out of favor mainly because the oral medication was simpler than the strict and exacting dietary regimen. Beginning in the mid-1990s, there was a resurgence of interest in the ketogenic diet as it was increasingly recognized that the diet has utility in the treatment of drug-resistant epilepsy (Freeman and Kossoff 2010). Today, the ketogenic diet is an established therapy for difficult-to-treat epilepsies, particularly in children, and is increasingly being studied for therapeutic efficacy in a number of other neurological disorders (Gasior et al. 2006; Neal et al. 2008; Stafstrom and Rho 2012).

Scientific interest in the mechanisms by which the ketogenic diet exerts seizure control has evolved rapidly in parallel with the growth in clinical use of the diet over the past 20 years (Masino and Rho 2012). Effectiveness of the ketogenic diet has been amply shown in animal models of seizures and epilepsy. Still, at present, the basic mechanisms of ketogenic diet action remain incompletely understood. There is substantial interest in defining these mechanisms. Dietary therapy is not feasible for many patients, but the insights gained from study of the diet may provide clues to the creation of more effective ASDs to address pharmacoresistant epilepsy. Since the 1930s, numerous mechanistic hypotheses have been advanced to explain the antiseizure effects of the ketogenic diet; the key mechanisms are discussed below and summarized in Table 2.

Direct Effects of Ketone Bodies

The earliest demonstration that ketone bodies have antiseizure properties was given in the 1930s. Acetoacetate was shown to protect against thujone-induced seizures in rabbits (Keith 1933). Further, acetone was shown to exert antiseizure activity in several animal models (Likhodii et al. 2003; Gasior et al. 2007). Recently, chronic administration of the major ketone, β-hydroxybutyrate, was shown to block spontaneous seizures in epileptic mice (Kim et al. 2015). Interestingly, the in vivo evidence for the antiseizure effects of ketone bodies has been discordant with earlier in vitro studies. Application of acetoacetate or β-hydroxybutyrate in acute hippocampal brain slices from normal rats failed to reveal effects on $GABA_A$ receptors and ionotropic glutamate receptors, and on synaptic transmission (Thio et al. 2000). However, more recent data indicate that the antiseizure activity of ketone bodies in vitro may require long-term incubation and not simply acute exposure (Kim et al. 2015). Collectively, it appears that ketone bodies afford broad spectrum antiseizure activity in animal models and, hence, the possibility that these metabolites contribute to the therapeutic efficacy of the diet cannot be discounted. Although the

molecular targets through which ketone bodies act to protect against seizures have not yet been clearly identified, there is strong evidence to suggest that mitochondria are critically involved (Masino and Rho 2012). Also, given that the ketogenic diet is mainly used in younger patients, enhanced efficacy in this population could reflect age-dependent changes in the expression of monocarboxylate transporters, which import ketone bodies across the blood–brain barrier from the systemic circulation (Morris 2005; Pierre and Pellerin 2005).

Effects on GABA and Glutamate Metabolism

It has been proposed that increased synthesis or levels of the inhibitory neurotransmitter GABA or decreased synthesis or levels of the excitatory neurotransmitter glutamate could explain the antiseizure activity of the ketogenic diet. The implication of such findings is uncertain inasmuch as it has not been shown that these manipulations influence neural circuit excitability, either through synaptic or nonsynaptic actions. Nevertheless, we summarize the results of some of these studies to illustrate the kinds of observations that have been reported.

The influence of the ketogenic on GABA has been proposed to originate in changes in glutamate metabolism (Yudkoff et al. 2005). Glutamate is cleared from the synaptic space by astrocytes, which convert glutamate to glutamine through the action of the glial enzyme glutamine synthetase. Glutamine is then exported to neurons where it is hydrolyzed to glutamate and can then either be converted to GABA or transaminated to aspartate in a reaction that requires oxaloacetate. Because the ketogenic diet induces metabolic changes that require available oxaloacetate to condense with acetyl-CoA for incorporation into the tricarboxylic acid (TCA) cycle, the production of aspartate from glutamate is reduced. This may result in enhanced flux through GAD to increase the synthesis of GABA.

Experimental support for this biochemical hypothesis is mixed. In the study proposing the hypothesis, which used a mouse model of ketosis, changes in levels of aspartate were found,

Table 2. Putative mechanisms of the antiseizure effects of the ketogenic diet

Mediator/physiological change	Basis	Mechanism of antiseizure effects
Ketone bodies (acetone, acetoacetate, and β-hydroxybutyrate have antiseizure activity; further, acetoacetate, and β-hydroxybutyrate provide resistance to oxidative stress)	Chronic ketosis as a result of elevated FFAs	Unknown ? Inhibit presynaptic release of glutamate by competing with Cl^- for allosteric activation of vesicular glutamate transporter ? Activate K_{ATP} and $GABA_B$ receptors (reduced ATP also activates K_{ATP}) ? Inhibit HDAC, leading to increased resistance to oxidative stress ? Inhibit mitochondrial permeability transition
Increased GABA synthesis (flux through GAD)	Brain converts ketone bodies to acetyl-CoA; increased flux through TCA cycle, consumes oxaloacetate, which is less available to the aspartate aminotransferase reaction; less glutamate is converted to aspartate and relatively more glutamate becomes available to the glutamine synthetase and GAD reactions	? Enhanced GABA-mediated inhibition
Adenosine	Levels of ATP elevated leading to increased conversion to adenosine in neurons and astrocytes	Activation of adenosine A1 receptors on excitatory neurons
Increased mitochondrial function and biogenesis	Unknown	Increase ATP production and enhanced energy reserves
Nrf2	Ketogenic diet initially produces mild oxidative and electrophilic stress, activating Nrf2 via redox signaling	Reversal of chronically low GSH in epilepsy Induction of genes encoding protective proteins; improvement of the mitochondrial redox state
Reduced mitochondrial ROS	Enhanced expression of UCPs by fatty acids acting on PPAR and FOX	Increased UCPs diminish $\Delta\Psi$ leading to reduced ROS
Anaplerosis	In ketogenic diet, there is reduction in glycolysis and increase in oxidation of FA and ketone bodies (glycolytic restriction/diversion)	Correct glutamate and GABA deficiencies in brain Enhanced neuronal ATP production Reduce expression of proepileptic BDNF and TrkB through NRSF binding to NRSE; decrease in cytosolic and nuclear levels of NADH

Continued

Table 2. *Continued*

Mediator/physiological change	Basis	Mechanism of antiseizure effects
PUFAs	Ketogenic diet enhances mobilization of PUFAs from adipose tissue to liver and brains	PUFAs directly affect ion channels Activation of PPARα and PGC-1α (coactivator) leads to changes in transcription of genes linked to energy, amino acid, and neurotransmitter metabolism Boost activity of UCPs
Medium-chain triglycerides	Exogenous administration of in the medium-chain triglyceride ketogenic diet	Unknown (similar action to valproate)
FFA3	Activated by short-chain fatty acids and β-hydroxybutyrate	Inhibit N-type voltage-gated calcium channels, leading to reduced glutamate release at synapses

FFAs, Free fatty acids; HDAC, histone deacetylase; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; Nrf2, NF E2-related factor 2; TCA, tricarboxylic acid; GSH, glutathione; ROS, reactive oxygen species; UCPs, uncoupling proteins; PPAR, peroxisome proliferator-activated receptor; FOX, forkhead box; FA, fatty acid; NRSF, neural restrictive silencing factor; NRSE, neuron restrictive silencing element; PUFAs, polyunsaturated fatty acids; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor; NADH, nicotinamide adenine dinucleotide (reduced).

along with the expected increases in acetyl-CoA, but there were no changes in the levels of GABA or glutamate (Yudkoff et al. 2005). The same study did show increases in glutamine and GABA on infusion of the nitrogen donors alanine or leucine. Rats fed the ketogenic diet for 3 wk showed a reduction in brain glutamate levels, but also no change in GABA (Melo et al. 2006). However, in children fed the ketogenic diet, cerebrospinal fluid levels of GABA have been shown to increase, but without a change in glutamate concentrations (Dahlin et al. 2005). Another study in rats fed the ketogenic diet for 3 wk found "increased" levels of glutamate and glutamine in the hippocampus (Bough et al. 2006). Further, using both mild caloric restriction (90% of daily energy requirements) and an isocaloric ketogenic diet, investigators found significant increases in the messenger RNA (mRNA) expression of both isoforms of GAD (GAD65 and GAD67) in several brain regions that were independent of ketogenic effects (Cheng et al. 2004); the functional implications are unclear given that GABA levels were not increased.

Recently, it has been proposed that the ketogenic diet could suppress neuronal excitability by inhibiting the presynaptic release of glutamate. The ketone bodies β-hydroxybutyrate and acetoacetate were shown to diminish glutamate release by directly competing with Cl$^-$ for allosteric activation of vesicular glutamate transporters (Juge et al. 2010). In the same study, application of the potassium channel blocker 4-aminopyridine to rat brain in vivo evoked seizures with concurrent secretion of glutamate, and these effects were blocked by acetoacetate. The relevance of this study is further supported by the recent observation that β-hydroxybutyrate does indeed induce antiseizure activity (Kim et al. 2015).

K$_{ATP}$ Channels and GABA$_B$ Receptors

It has been proposed that activation of K$_{ATP}$ channels and GABA$_B$ receptors could underlie the action of the ketogenic diet (Ma et al. 2007). In brain slice recordings, acetoacetate and β-hydroxybutyrate were found to reduce the spontaneous firing rate of GABAergic neurons in the substantia nigra pars reticulata, a putative subcortical seizure gate, and this action was dependent on K$_{ATP}$ channels and GABA$_B$ receptors. Apart from the fact that K$_{ATP}$ channels and

GABA$_B$ receptors have been previously shown not to be germane antiseizure targets except in special circumstances (Meldrum and Rogawski 2007), most problematic for the hypothesis is the fact that the ketogenic diet and ketone bodies actually increase ATP production, which would tend to close K$_{ATP}$ channels and enhance instead of inhibit neuronal excitability (Masino and Rho 2012). In any case, the same group has shown that the open probability of K$_{ATP}$ channels in the hippocampus in vitro is enhanced in the presence of β-hydroxybutyrate (Tanner et al. 2011).

Adenosine

Recent studies have implicated the potent inhibitory modulator adenosine, which is well recognized to have antiseizure activity (Dunwiddie and Masino 2001), in the action of the ketogenic diet (Masino et al. 2012). Adenosine produces antiseizure effects through activation of inhibitory adenosine A$_1$ receptors on excitatory neurons. Adenosine is synthesized by hydrolysis of ATP in neurons and astrocytes. Because levels of ATP are elevated by the ketogenic diet, it is plausible that adenosine synthesis and release could also be enhanced. Targeted heterozygous ($A_1R^{+/-}$) or homozygous ($A_1R^{-/-}$) deletion of A$_1$ receptors or increased expression of adenosine kinase (Adk-Tg), an enzyme that enhances clearance of adenosine, causes spontaneous electrographic seizures in mice (Masino et al. 2011). A 3-wk treatment with the ketogenic led to decreased electrographic seizures in Adk-Tg and $A_1R^{+/-}$ mice, but not in animals entirely missing A$_1$R receptors ($A_1R^{-/-}$), supporting the concept that adenosine may be an important mediator of the ketogenic diet's antiseizure effects.

Bioenergetic and Mitochondrial Changes

Pathological changes in mitochondrial energy metabolism and reactive oxygen species (ROS) production are known to occur with epileptogenesis, and the ketogenic diet has been found to profoundly affect these processes (Rowley and Patel 2013). In addition to enhancing ener-gy reserves, ATP levels, and the expression of many enzymes involved in mitochondrial metabolism, the ketogenic diet has also been shown to increase mitochondrial biogenesis in the hippocampus (Bough et al. 2006) and to reduce oxidative stress through multiple cellular mechanisms, including those involving mitochondria, such as through increases in reduced glutathione (GSH). The effect on GSH is of particular interest because depletion of GSH is known to occur in epilepsy (Mueller et al. 2001). Increased GSH was shown to correlate with increased activity of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH biosynthesis, and enhanced expression of the GCL catalytic subunit, GCLC, and modulatory subunit, GCLM, in rats fed the ketogenic diet (Jarrett et al. 2008).

The increase in GSH and associated changes were subsequently shown to involve NF E2-related factor 2 (Nrf2), a redox-sensitive transcription factor that is activated by cellular stress and induces a diverse array of genes, including the GSH antioxidant pathway (Suzuki et al. 2013). The ketogenic diet resulted in elevated levels of Nrf2 for 3 wk, and this was associated with increased activity of NAD(P)H:quinone oxidoreductase, a prototypical Nrf2 target (Milder et al. 2010). Interestingly, a recent study found that increasing Nrf2 expression in a rat model of temporal lobe epilepsy decreased spontaneous seizures (Mazzuferi et al. 2013).

Acute application of the ketone bodies β-hydroxybutyrate and acetoacetate in hippocampal slices enhanced catalase activity in response H$_2$O$_2$ (Kim et al. 2010) and decreased oxidation of carboxy-2′,7′-dichlorodihydrofluorescein diacetate, a dye often used as an indicator of intracellular ROS (Maalouf and Rho 2008). In isolated mitochondria, β-hydroxybutyrate and acetoacetate have been shown to decrease ROS levels in response to glutamate by enhancing oxidation of NADH (Maalouf et al. 2007). Additionally, β-hydroxybutyrate and acetoacetate reduced mitochondrial ROS both basally and in response to the ATP synthase inhibitor oligomycin (Kim et al. 2007).

A possible mechanism mediating the decrease in mitochondrial ROS production with

the ketogenic diet is enhanced expression of un-coupling proteins (UCPs). Increased activity of UCPs can diminish the mitochondrial membrane potential ($\Delta\Psi$), resulting in a decrease in ROS production, and this has been associated with increased resistance to kainate-induced seizures (Sullivan et al. 2003). Additionally, fatty acids can induce increases in UCP expression possibly through enhanced activity of transcription factors, such as the peroxisome proliferator-activated receptor (PPAR) and the forkhead box (FOX) family of transcription factors (Azzu and Brand 2010). In mice fed the ketogenic diet, UCP activity was enhanced and this was associated with increased levels of UCP2, UCP4, and UCP5 in the hippocampus (Sullivan et al. 2004). Additionally, ROS production—assessed in the presence of oligomycin to maximize $\Delta\Psi$—was reduced in mice fed the ketogenic diet (Sullivan et al. 2004).

Recently, β-hydroxybutyrate was shown to be an inhibitor of class I histone deacetylases (HDACs) in vitro and in vivo, and this activity was associated with increased resistance to oxidative stress (Shimazu et al. 2013). Specifically, β-hydroxybutyrate increased acetylation of histone H3 lysine 9 (H3K9) and histone H3 lysine 14 and enhanced transcription of genes regulated by FOXO3A, including the antioxidant enzymes manganese superoxide dismutase and catalase. Further, β-hydroxybutyrate (administered in vivo for 24 h via an osmotic pump) decreased protein carbonylation and 4-hydroxynonenal and lipid peroxides in the kidney. Although the investigators did not report such effects in neuronal tissue or cells, it is possible that direct inhibition of HDACs and the ensuing transcriptional changes may mediate some of the antioxidant effects known to occur in the brain with the ketogenic diet.

Glycolytic Restriction/Diversion

It has been proposed that increased neurotransmission caused by the hyperexcitability in neural networks in chronic epilepsy leads to depletion of TCA cycle intermediates, including α-ketoglutarate, which is a precursor for glutamate and GABA (Borges and Sonnewald 2012).

Seizure susceptibility is further augmented by the reduction in these neurotransmitters and reduced energy (ATP) production as a result of diminished acetyl-CoA. A key feature of the ketogenic diet is a relative reduction in glycolysis and an increase in nonglucose sources of fuel through the oxidation of fatty acids and ketone bodies, which ultimately feed the TCA cycle through a process known as anaplerosis (i.e., the replenishing of depleted metabolic cycle intermediates). Anaplerosis is believed to correct the neurotransmitter (glutamate and GABA) deficiencies and enhance neuronal ATP production, which ultimately leads to a reduction in seizure susceptibility. Glycolytic restriction is thought to be an important mechanism mediating the antiseizure properties of the ketogenic diet (Masino and Rho 2012). Indeed, caloric restriction has antiseizure, and possibly antiepileptogenic, effects (Mantis et al. 2004). The earliest clinical observation supporting this notion is the rapid reversal of seizure control on ingestion of carbohydrates or glucose in patients on the ketogenic diet (Huttenlocher 1976). Additionally, studies in animals using labeled metabolic precursors have shown that the ketogenic diet reduces glycolysis (Yudkoff et al. 2005; Melo et al. 2006).

Recently, attempts have been made to mimic the antiseizure activity of the ketogenic diet using glycolytic inhibitors. In vitro application of 2-deoxy-D-glucose (2-DG), an inhibitor of phosphoglucose isomerase, reduced epileptiform bursts induced in hippocampal slices by bicuculline, 4-aminopyridine, and increased extracellular K^+ (Stafstrom et al. 2009). Additionally, in vivo administration of 2-DG provides protection against audiogenic and 6-Hz stimulation-induced seizures in mice and also produced an antiseizure and antiepileptogenic effect in a rat kindling model (Garriga-Canut et al. 2006; Stafstrom et al. 2009; Gasior et al. 2010). The antiseizure effects of 2-DG may be partially mediated by changes in the expression of genes encoding brain-derived neurotrophic factor and its receptor TrkB, both of which are regulated by the activity of the transcription factor neural restrictive silencing factor (NRSF), which represses transcription by binding to the

neuron restrictive silencing element (NRSE) in promoter regions (Garriga-Canut et al. 2006). NRSF transcriptional repression was enhanced by 2-DG and this was associated with a reduction in acetylation and an increase in methylation of H3K9 at the NRSE promoter, epigenetic modifications associated with suppression of gene transcription. NRSF-mediated repression required an interaction with the transcriptional corepressor carboxyl-terminal binding protein that was disrupted with increasing concentrations of NADH, a cofactor that is elevated on increased glycolytic flux. This suggests that the antiseizure actions of 2-DG may be mediated by a decrease in cytosolic and nuclear levels of NADH and subsequent influences on histone modifications.

Fructose-1,6-bisphosphate (FBP) also inhibits glycolysis by diverting the metabolism of glucose to the pentose phosphate pathway and affords antiseizure effects in vivo. FBP was found to protect against pilocarpine, kainate, and PTZ-induced seizures in rats (Lian et al. 2007) and also affected kindling acquisition (Ding et al. 2010). FBP had broader activity than valproate and 2-DG, whereas the ketogenic diet was not active in the models. These results reinforce the view that diversion of metabolism away from glycolysis is a promising antiseizure strategy.

Triheptanoin, the triglyceride of the C7 fatty acid heptanoate, is an anaplerotic substrate that restores levels of TCA intermediates (Hadera et al. 2014). Oral administration of triheptanoin has been shown to have antiseizure effects in some animal models, providing support for the anaplerosis hypothesis (Willis et al. 2010; Kim et al. 2013). A recent report suggests that triheptanoin may also have anti-ictal activity in humans (Pascual et al. 2014).

Fatty Acid Oxidation and Polyunsaturated Fatty Acids

Intake of a high-fat diet, such as the ketogenic diet, increases the rate of fatty acid oxidation. This leads to changes in the levels and types of polyunsaturated fatty acids (PUFAs) in the circulation, liver, and brain. In particular, it has been show that there is enhanced mobilization of PUFAs from adipose tissue to liver and brain (Taha et al. 2005). Large quantities of PUFAs are not a typical component of the ketogenic diet, thus increases in PUFAs must be the result of endogenous production and export into the circulation (Fraser et al. 2003). PUFAs affect diverse ion channels, including voltage-gated sodium and potassium channels (Vreugdenhil et al. 1996), and it has been speculated that actions of PUFAs on such ion channels could mediate the antiseizure effects of the ketogenic diet (Michael-Titus and Priestley 2013). An additional mechanism whereby PUFAs could affect seizure susceptibility is through activation of peroxisome proliferator-activated receptor-α (PPARα), a nuclear receptor that regulates the transcription of numerous genes linked to energy, amino acid, and neurotransmitter metabolism. Recently, it was shown that fenofibrate, a PPARα ligand, exerts anticonvulsant activity comparable to the ketogenic diet in adult rats (Porta et al. 2009). The specific genes relevant to this effect remain to be determined.

In pediatric patients with epilepsy, 3–4 wk of ketogenic diet treatment led to elevations in circulating levels of β-hydroxybutyrate, cortisol, and free fatty acids (FFAs) (Fraser et al. 2003). Most PUFAs were also increased in the serum, including the triglyceride and phospholipid forms of linoleic acid (LA), arachidonic acid (AA) and docosahexaenoic acid (DHA), triglyceride levels of stearic acid, and palmitic acid in phospholipids. Additionally, these alterations in fatty acids were associated with seizure reduction in 78% of the children. Interestingly, seizure control was correlated with circulating AA levels, but not EEG changes.

Whether PUFA ingestion can render antiseizure effects remains controversial. Oral supplementation with 5 g of omega-3 PUFA for 6 mo appeared to reduce the frequency and severity of seizures in a small observational study (Schlanger et al. 2002). However, in a 12-wk randomized, placebo-controlled parallel group study in adults with epilepsy, administration of eicosapentaenoic acid (EPA) and DHA (1 g EPA, 0.7 g DHA daily) reduced seizure frequency for the first 6 wk of treatment, but this effect did not persist in spite of sustained increases in

DHA and EPA and decreases in AA and LA in the plasma (Yuen et al. 2005). In an additional randomized, blinded trial of PUFA supplementation (EPA plus DHA, 2.2 mg/d in a 3:2 ratio over 12 wk) in adults with uncontrolled epilepsy, there was a lack of clear efficacy when compared with placebo (Bromfield et al. 2008).

In rats fed the ketogenic diet, there were marked reductions of PUFA levels in plasma and adipose tissue, but enhanced mobilization of the PUFA AA and DHA to the liver and brain (Taha et al. 2005). This was accompanied by an initial increase in plasma levels of β-hydroxybutyrate, followed by a reduction by day 10 of the diet. Following a calorie-restricted ketogenic diet, mRNA expression of the rate-limiting enzyme for ketone body production, HMG-CoAS2, was found to increase in both the liver and brain, in contrast to feeding with an isocaloric standard chow, which resulted in enhanced expression only in the liver (Cullingford et al. 2002). Collectively, these and other animal studies suggest that diet-induced changes in brain content and metabolism of PUFAs may be important contributors to antiseizure effects of the ketogenic diet (Taha et al. 2010).

Medium-Chain Triglycerides

The medium-chain triglyceride ketogenic diet is as efficacious as the classic ketogenic diet in which the primary fat source is long-chain triglycerides (Huttenlocher et al. 1971; Neal et al. 2009; Liu and Wang 2013). This diet increases the plasma levels of medium straight-chain fatty acids. Recently, medium-chain triglycerides of various structural classes have been shown to reduce the frequency of epileptiform discharges in in vitro slice models and to inhibit behavioral and EEG seizures in a rat status epilepticus model; modest activity was also reported in various in vivo models used to screen for antiseizure activity (Chang et al. 2012, 2013, 2015). Valproate is a short-branched-chain fatty acid. Certain structurally novel medium-chain fatty acids have been identified that have greater activity than valproate in seizure models and that lack the HDAC activity of valproate, which has been proposed as a basis for its teratogenicity.

Whether administration of a specific medium-chain triglyceride in the presence of a normal diet could represent an epilepsy treatment approach remains to be determined.

Free Fatty Acid Receptor 3

Free fatty acid receptor 3 (FFA3) is a G-protein coupled receptor whose endogenous ligands may include short-chain fatty acids, such as acetate and propionate. A study in dissociated sympathetic neurons recently showed that β-hydroxybutyrate is also an agonist of FFA3, causing inhibition of N-type voltage-gated calcium channels (Won et al. 2013). A prior study also found the ketone body to interact with FFA3, but in this case it was an antagonist (Kimura et al. 2011); the basis for the discrepancy is not apparent. As of yet, the relevance of these actions to brain function remains to be determined.

CONCLUSION

In recent years, there have been dramatic advances in our understanding of how ASDs prevent seizures. ASDs interact with a wide variety of molecular targets. There are unique aspects to the mechanism of each ASD so that it is rarely the case that two ASDs have what could be considered the same mechanisms of action (gabapentin and pregabalin are perhaps exceptions). Although some ASDs have a single cognate target, for many of the drugs, clinical activity is likely the result of actions on several targets. This makes the definition of "mechanism of action" challenging, but it is perhaps not surprising that control of epilepsy, which is a functional disorder of neural networks, would benefit from therapeutic interventions at various points in the network.

Although ASDs provide control of seizures for many people with epilepsy, a disappointingly large number do not achieve seizure freedom with medications. The ketogenic diet is an alternative approach that may provide seizure control in cases of drug-resistant epilepsy. There are still many open questions regarding the mechanisms of the ketogenic diet. However, as

reviewed here, effects of the diet on intermediary metabolism are likely to be central to its therapeutic activity. These mechanisms are fundamentally different from those believed to be relevant to ASDs. Consequently, study of the ketogenic diet may open avenues to the creation of entirely novel treatment strategies for pharmacoresistant epilepsies. Such treatment approaches may improve on the efficacy of the diet and be more convenient and palatable. There is, therefore, strong justification for continued investigation of ketogenic diet mechanisms.

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Mechanisms of Action of Antiseizure Drugs and the Ketogenic Diet

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TOC Blurb: The ketogenic diet is a treatment option for epilepsy patients who are resistant to antiseizure drugs. These forms of therapy exert their clinical effects via distinct mechanisms and molecular targets.