CHAPTER 41 ■ MECHANISMS OF ACTION OF ANTISEIZURE MEDICATION

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Antiseizure medications (ASMs) protect against seizures through interactions with a variety of cellular targets. By affecting the functional activity of these targets, ASMs suppress abnormal hypersynchronous activity in brain circuits, leading to protection against seizures. The actions on these targets can be categorized into five broad groups: (a) modulation of voltage-gated ion channels, including sodium, calcium, and potassium channels; (b) enhancement of γ-aminobutyric acid (GABA) inhibition through effects on $GABA_A$ receptors, the GAT-1 GABA transporter or GABA transaminase; (c) direct modulation of synaptic release through effects on components of the release machinery, including SV2A and α2δ; (d) inhibition of synaptic excitation mediated by ionotropic glutamate receptors (iGluRs), including AMPA receptors; and (e) inhibition of hyperactive mechanistic target of rapamycin (mTOR) intracellular pathway signaling through an action on mTORC1 (Table 41.1). The ultimate effects of these interactions are to modify the bursting properties of neurons and to reduce synchronization in localized neuronal ensembles. In addition, ASMs inhibit the spread of abnormal firing to distant sites. Some seizures, including typical generalized absence seizures, result from thalamocortical synchronization. ASMs effective in these seizure types interfere with the rhythm-generating mechanisms that underlie synchronized activity in the thalamocortical circuit. Rapalogs such as everolimus have antiseizure activity only in epilepsies associated with pathologies of the mTOR pathway suggesting that they act by reversal of pathologic mTOR signaling; how this leads to a reduction in seizures is not understood. In this chapter, we consider each of the targets and discuss how ASMs affect the activity of these targets.

Many ASM targets are ion channels, most notably voltage-gated sodium and potassium channels and $GABA_A$ receptors. It is interesting to note that certain idiopathic epilepsy syndromes are believed to be the result of mutations in these same ion channels (see Chapter 5).

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. Neuronal depolarizations by a few millivolts, ordinarily as a result of synaptic activation of glutamate receptors (mainly AMPA receptors), activate sodium channels, causing opening of the channels and influx of sodium. The channels then inactivate within milliseconds. Influx of sodium ions during the brief time that sodium channels are open generates the depolarizing component of the action potential. Although the bulk of sodium channels inactivate, about 1% of the sodium current is noninactivating 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, sustaining repetitive firing, and enhancing depolarizing synaptic currents (1). Some ASMs, most notably phenytoin, inhibit I_{NaP}, which is believed to contribute to their efficacy (2).

Voltage-gated sodium channels are multimeric protein complexes, composed of a large α subunit that forms four subunit-like homologous domains (designated I to IV) and one or more smaller β subunits (3). The ion-conducting pore is contained within the α subunit, as are the elements of the channel that mediate its fundamental physiologic properties including rapid inactivation. There are nine voltage-gated sodium channels, designated $Na_v1.1$ to $Na_v1.9$. $Na_v1.2$ is the most abundant sodium channel α-subunit in brain neurons, comprising approximately 70% of the total brain α-subunit pool, but $Na_v1.1$ and $Na_v1.6$ are also expressed in brain. $Na_v1.1$ is the primary voltage-gated sodium channel in several classes of GABAergic inhibitory interneurons, whereas $Na_v1.6$ is the main sodium channel subunit in the somatodendritic compartments of principal (relay) neurons. Mutations in each of these channels have been associated with various genetic epilepsies (4). $Na_v1.1$ (encoded by the *SCN1A* gene) is of particular relevance to genetic epilepsies, including Dravet syndrome. Mutations in $Na_v1.6$ (encoded by the *SCN8A* gene) have also been associated with epileptic encephalopathies. Increased persistent and resurgent sodium current—caused by an unusual form of gating in which sodium channels reopen following an action potential, thus promoting the firing of another action potential—occurs with certain $Na_v1.6$-associated encephalopathies (5,6).

ASMs that protect against seizures through an interaction with voltage-gated sodium channels are commonly referred to as "sodium channel blockers." They are among the most frequently used drugs in the treatment of focal and primary generalized tonic–clonic seizures and include phenytoin, carbamazepine, lamotrigine, oxcarbazepine (as well as its active metabolite licarbazepine), and lacosamide. ASMs that interact with voltage-gated sodium channels exhibit a characteristic "use-dependent" blocking action so that they inhibit high-frequency trains of action potentials much more potently than they inhibit individual action potentials or firing at low frequencies. Because they also exhibit a "voltage dependence" to their blocking action, sodium channel blocking ASMs are more potent at inhibiting action potentials that ride on a depolarized plateau potential as characteristically occurs in

TABLE 41.1

MOLECULAR TARGETS OF CLINICALLY USED ASMS

Molecular Target	ASMs 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	Ethosuximide
Voltage-gated potassium channels	Ezogabine
GABA inhibition	
$GABA_A$ receptors	Phenobarbital, primidone, benzodiazepines including diazepam, lorazepam, and clonazepam; possibly, topiramate, felbamate
GAT-1 GABA transporter	Tiagabine
GABA transaminase	Vigabatrin
Synaptic release machinery	
SV2A	Levetiracetam, brivaracetam
α2δ	Gabapentin, gabapentin enacarbil,[d] pregabalin
Ionotropic glutamate receptors	
AMPA receptor	Perampanel
mTORC1 signaling	Everolimus
Mixed/unknown	Valproate, felbamate, topiramate, zonisamide, rufinamide, adrenocorticotrophin, cannabidiol

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

seizures. Thus, sodium channel blocking ASMs 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 ASMs inhibit the release of diverse neurotransmitters including glutamate; whether this is responsible for the therapeutic activity of the drugs is uncertain (7).

The binding site on sodium channels for sodium channel blocking ASMs is believed to overlap 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. Sodium channel blocking ASMs are highly structurally diverse. However, as a general rule, the molecules are electroneutral and contain a nonionizable polar group at one end of the molecule and an aromatic moiety at the other end. The molecules are believed to trap a sodium ion in the selectivity filter of the channel pore and the ion–drug complex blocks the permeation pathway by electrostatic and steric mechanisms (8). Phenytoin, carbamazepine, and lamotrigine are considered "classical" sodium channel blocking ASMs. Such sodium channel blocking ASMs act in a state-dependent fashion according to the "modulated receptor hypothesis" in which they bind with lowest affinity to the closed state of the channel, higher affinity to the open state, and maximal affinity to the inactivated state. 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 exhibit. Lacosamide also is believed to exert its therapeutic effects by interacting with sodium channels (9). Unlike other sodium channel blocking ASMs, lacosamide does not inhibit high-frequency repetitive spike firing on the time scale of 100s of milliseconds. It does, however, inhibit spike firing in long trains of spikes on the time scale of 1 to 2 seconds. The very slow action of lacosamide is due to channel block on a much slower time scale than other sodium channel ASMs (10). Like other sodium channel blocking ASMs, lacosamide blocks the sodium permeation pathway, but it binds to the open (and preopen) states of the channel. Lacosamide does not bind to the inactive state and it does not exhibit voltage dependence. Interestingly, I_{NaP} is particularly susceptible to block by lacosamide, which could be a major factor in its clinical activity. Moreover, the unusually slow development of block produced by lacosamide during high-frequency activity could allow it to better discriminate between seizure-like pathologic firing and normal network activity, thus resulting in a favorable tolerability profile.

T-Type Voltage-Gated Calcium Channels

Low voltage-activated (T-type) calcium channels play a role in the intrinsic thalamocortical oscillations that underlie the spike–wave discharges of generalized absence seizures (11–13). There are three T-type Ca^{2+} channel isoforms encoded by separate genes, denoted as $Ca_v3.1$ (α1G), $Ca_v3.2$ (α1H), and $Ca_v3.3$ (α1I). All three T-type calcium channel isoforms are expressed in thalamocortical circuits (14). $Ca_v3.1$ is prominently expressed in thalamic relay neurons in the dorsal thalamus, which plays a key role in absence seizures; $Ca_v3.2$ and to a lesser extent $Ca_v3.3$ are prominently expressed in thalamic reticular neurons. All three T-type calcium channel isoforms are expressed in the cortex, with $Ca_v3.2$ mainly localized to layer V. In non-REM sleep, including during delta waves, sleep spindles, and k-complexes, the thalamocortical circuit switches from a tonic to oscillatory mode of firing, but in absence epilepsy, this switching can occur inappropriately, even during wakefulness (15,16). Studies in rodent models of absence epilepsy suggest that spike–wave discharges originate from a restricted region of the cerebral cortex, including layer 5/6 neurons of the primary somatosensory cortex, and that these types of seizure—despite the conventional view that they begin everywhere in the brain simultaneously—can be considered to have a "focus" (17). T-Type calcium channels in the thalamus and cortex contribute to the abnormal behavior of the thalamocortical circuit. These channels generate low-threshold spikes, leading to burst firing and oscillatory behavior (18). GABAergic neurons of the thalamic reticular nucleus are also critically involved in absence seizures as they hyperpolarize thalamic relay neurons, which deinactivate T-type calcium channels allowing the channels to generate burst firing and the propagation of spike–wave discharges in

the thalamocortical circuit (19). While the roles of each T-type calcium channel type in the generation of absence seizures is yet to be defined, it has been shown that $Ca_v3.2$ T-type calcium channels in thalamic reticular neurons are required (20).

Ethosuximide, which is highly efficacious in the treatment of absence seizures but not other seizure types, may act by inhibition of T-type calcium channels in the thalamocortical circuit (21–23). At clinically relevant concentrations (20 to 40 μg/mL), some but not all investigators have observed a partial (20% to 30%) reduction of T-type calcium current by ethosuximide. Studies with recombinant T-type calcium channels have confirmed that ethosuximide blocks all three channel types (24). The block increases when the current is activated from more depolarized potentials and when T-type calcium channels are inactivated as especially occurs during high-frequency activation, so that the drug has selectivity for pathologic behavior in the thalamocortical circuit, which is associated with neuronal depolarization and inactivation of T-type calcium channels. Effects on other membrane currents, including I_{NaP} and calcium-activated potassium current, have also been proposed as the basis for the efficacy of ethosuximide in absence epilepsy (21,23). However, it has been demonstrated that selective inhibition of T-type calcium channels suppresses absence seizures in a rodent model of absence epilepsy (25). Remarkably, result in animal models indicates that early treatment with ethosuximide can have disease-modifying (antiepileptogenic) effects, causing a persistent reduction in seizures and mitigation of behavioral comorbidities (26–29). A study showing that children with absence epilepsy who receive ethosuximide are more likely than those who receive valproic acid to achieve long-term remission is consistent with the disease-modifying actions observed in animal studies (30).

The efficacy of some other ASMs may also depend, at least in part, on actions at T-type calcium channels. Zonisamide, in addition to effects on voltage-activated sodium channels, may also block T-type calcium channels (16), thus accounting for its likely efficacy in absence epilepsy (31). Similarly, there is evidence that valproate, a drug of choice in absence epilepsy, may also inhibit T-type calcium channels (21).

K_v7 Voltage-Gated Potassium Channels

Voltage-gated potassium channels are a diverse and evolutionarily ancient group of ion channels that serve a variety of key functions in the nervous system. Opening of potassium channels drives the membrane potential toward a hyperpolarized level, which 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 (32). These genes, designated *KCNQ2* and *KCNQ3*, encoded novel brain potassium channel subunits, $K_v7.2$ and $K_v7.3$, respectively, that are homologous to a previously identified cardiac potassium channel $K_v7.1$, encoded by *KCNQ1* (*LQT1*). The novel brain potassium channels mediate the M current, a potassium current that increases as the membrane potential in neurons approach action potential threshold. K_v7 channels, together with HCN (hyperpolarization-activated cyclic nucleotide-gated potassium channels) and KCa2/SK (small conductance calcium-activated potassium channels), generate the medium afterhyperpolarization, which is elicited by a burst of action potentials and serves to limit further firing (33). K_v7 potassium channels therefore contribute to spike-frequency adaptation and can be considered to serve as a "brake" on epileptic 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 $K_v7.2$ to $K_v7.5$, which are expressed exclusively in the nervous system (34). In addition to mediating benign familial neonatal seizures (32) (Singh et al., 1998), certain mutations in *KCNQ2* are associated with an early-onset epileptic encephalopathy (35,36).

Ezogabine, which is efficacious in the treatment of partial seizures, acts as a positive modulator of the nervous system K_v7 potassium channels ($K_v7.2$ to $K_v7.5$) but does not affect the cardiac member of the family ($K_v7.1$). 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 (37,38). Ezogabine causes a hyperpolarizing shift in the activation of K_v7 channels such that more M current is generated near 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 does not alter the single channel conductance of individual K_v7 channels (39). Recently, it has been proposed that the predominant effect of ezogabine at clinically relevant concentrations is to preferentially target K_v7 channels in the open state and stabilize them in that state so as to produce a persistent hyperpolarization of the resting membrane potential that reduces overall excitability (40). Many K_v7 channels in brain are believed to be $K_v7.2/K_v7.3$ heteromers, which are highly sensitive to ezogabine (EC_{50}, 1.6 μM) (37). Peak plasma levels of ezogabine range from 354 to 717 ng/mL (1.2 to 2.4 μM) (41), and plasma protein binding is 80% so that free plasma concentrations are estimated to be about 0.2 to 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.

GABA INHIBITION

GABA, the neurotransmitter of local inhibitory interneurons, acts through $GABA_A$ receptors and $GABA_B$ receptors. $GABA_A$ receptors, which are Cys loop-type ligand-gated chloride channels (42), represent an important target for ASMs 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 ASM. Although only about one in five cortical neurons is GABAergic (43), 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 ASM action.

$GABA_A$ Receptors

$GABA_A$ receptors are heteropentameric protein complexes localized to the postsynaptic membrane of inhibitory synapses

where they mediate fast neuronal inhibition on a millisecond time scale. 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). However, the bulk (60%) of synaptic $GABA_A$ receptors are believed to have the α1β2γ2 configuration and a considerable fraction of the remainder (15% to 20%) are 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-containing $GABA_A$ receptors in CA1 pyramidal cells (44).

Benzodiazepines, such a diazepam, lorazepam, clonazepam, and clobazam, and barbiturates, such as phenobarbital, are ASMs that act on $GABA_A$ receptors as positive allosteric modulators. At higher concentration, barbiturates can directly activate $GABA_A$ receptors in the absence of GABA (45), 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 anticonvulsant action. All benzodiazepines used in the treatment of epilepsy have a 1,4-diazepine core with the exception of clobazam, which is unique in having a 1,5-benzodiazepine structure. The potency and efficacy of conventional 1,4-benzodiazepines such as clonazepam is similar irrespective of whether the $GABA_A$ receptor contains α1, α2, or α3-subunits; the same is true of clobazam and its major metabolite *N*-desmethylclobazam, which has similar potency and efficacy as a positive allosteric modulator of $GABA_A$ receptors as the parent (46). 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 (47). 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 (48).

Stiripentol, which is approved for the treatment of seizures associated with Dravet syndrome, is a positive allosteric modulator of $GABA_A$ receptors with properties similar to that of barbiturates (49,50). In studies with recombinant $GABA_A$ receptors, stiripentol caused modest enhancement of $GABA_A$ receptor responses. Interestingly, α3-containing $GABA_A$ receptors, which are prominent in the developing brain, exhibited a larger response than those composed of other α-subunits, perhaps accounting for stiripentol's preferential clinical utility in childhood epilepsy syndromes. Stiripentol was highly active at α3β3δ-containing receptors, indicating that it enhances tonic $GABA_A$ receptor signaling; the relevance of this action to antiseizure activity is uncertain. Stiripentol is often used in conjunction with benzodiazepines, most commonly clobazam. Stiripentol does not act via the benzodiazepine recognition site on $GABA_A$ receptors and is able to independently positively modulate $GABA_A$ receptors in the presence of benzodiazepines including clobazam (51). Thus, it has been demonstrated that stiripentol and clobazam or *N*-desmethylclobazam exhibit additive effects on synaptic-type (α3β3γ2L) $GABA_A$ receptors. Stiripentol has drug–drug interactions that complicate the interpretation of its antiseizure mechanism. Importantly, it causes an approximately twofold increase in plasma concentrations of clobazam and an approximately threefold increase in *N*-desmethylclobazam, which during chronic dosing is present at ninefold greater concentration than the parent (52). This occurs because stiripentol modestly inhibits the conversion of clobazam to *N*-desmethylclobazam by cytochrome P450 2C19 (CYP 2C19) and also markedly inhibits the conversion of *N*-desmethylclobazam to its metabolite 4′-hydroxy-*N*-desmethylclobazam, which also occurs via CYP 2C19. As noted above, clobazam and *N*-desmethylclobazam have similar potency and efficacy as positive allosteric modulators of $GABA_A$ receptors. Stiripentol can therefore be considered a "booster" of clobazam by its metabolic actions. While stiripentol is approved for the treatment of seizures associated with Dravet syndrome only in patients taking clobazam, the effect on clobazam metabolism is unlikely to be the only way that stiripentol contributes to clinical efficacy. Effects on synaptic and perhaps also extrasynaptic $GABA_A$ receptors likely play a role as well.

GAT-1 GABA Transporter

The action of neurotransmitter GABA is terminated by uptake into neurons and glial cell by membrane-bound GABA transporters, of which there are four types, termed GAT-1, BGT-1, GAT-2, and GAT-3. GAT-1 (encoded by the *SLC6A1* gene), the predominant form in the forebrain (including the neocortex and hippocampus), is localized to GABAergic terminals as well as to glial processes near GABA synapses. Tiagabine is a highly selective inhibitor of GAT-1 in neurons and glia (53,54). Inhibition of GAT-1 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 and the marked elevation in extracellular GABA it produces may lead to activation of extrasynaptic $GABA_A$ receptors (55). However, the extent to which activation of extrasynaptic $GABA_A$ receptors contributes to the antiseizure activity of tiagabine remains to be determined.

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. Inhibition of GABA transaminase with vigabatrin (γ-vinyl GABA), an irreversible suicide inhibitor of the enzyme, leads to marked increases in brain GABA levels. 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 due to 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 (56,57). 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 nonsynaptic tonic $GABA_A$ receptor current. This steady current is believed to be mediated by the action of GABA in the extracellular milieu acting on extrasynaptic $GABA_A$ 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 anticonvulsant actions become evident only later (58,59). Thus, vigabatrin has a biphasic action with proconvulsant effects likely related to suppression of synaptic GABAergic neurotransmission and anticonvulsant effects due to 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 (60).

SYNAPTIC RELEASE MACHINERY

SV2A

A variety of 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 (61,62). 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 anticonvulsant 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 of SV2 proteins that belong to the major facilitator superfamily (MFS) of 12-transmembrane domain transporters. Despite substantial effort, no transport function of these proteins has been identified, although studies with protein tomography have found that SV2A can adopt two alternate conformations consistent with a transporter role (63). 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 exhibit a lethal seizure phenotype demonstrating that SV2A in some way serves to restrain seizures.

A series of recent studies has examined the impact of levetiracetam on synaptic transmission in brain slice recordings (64). 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) neurotransmitter during high-frequency activation. It has further been proposed that the effect of levetiracetam is to interfere selectively with the replenishment of vesicles during the abnormally frequent activation that occurs during incipient seizures while sparing function during normal synaptic use (65). The frequency dependence is compatible with the selective suppression of epileptic activity. Modulation of synaptic release is a common mechanism of many ASMs, including sodium channel blockers that indirectly inhibit release at both excitatory and inhibitory synapses by inhibiting action potential firing. It seems 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, ASMs (notably phenytoin) can have proconvulsant effects.

Brivaracetam, the analog of levetiracetam with an *n*-propyl substituent at the 4-position of the pyrrolidine ring, like levetiracetam, is believed to confer seizure protection by virtue of binding to SV2A. However, brivaracetam has 15- to 30-fold greater affinity for SV2A than levetiracetam (66). Brivaracetam is also more potent at conferring seizure protection in animal seizure models than levetiracetam and has activity in the pentylenetetrazol and maximal electroshock models, albeit at high doses, in contrast to levetiracetam, which is notably inactive in these models (67,68). Studies in brain slice recordings demonstrated brivaracetam to be substantially more potent than levetiracetam at inducing synaptic depression with high-frequency stimulation (69). Whether there are qualitative differences between the two drugs and not simply differences in potency in their interactions with SV2A remains to be determined. Studies investigating binding of radiolabeled levetiracetam and brivaracetam to recombinant human SV2A expressed in heterologous cells have suggested that there could be differences in how the two analogs interact with SV2A, but the relevance of these differences to antiseizure activity has not been demonstrated (70,71).

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 (72,73). 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 (74) and it is noteworthy that gabapentinoids are not active in absence seizures, which as discussed above are dependent upon this brain structure. Indeed, gabapentin can precipitate absence and myoclonic status epilepticus (75). 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. Seizure

protection conferred by gabapentinoids is eliminated in mice bearing a mutation in this motif (RRR mutated to RRA) in α2δ-1, demonstrating that α2δ-1 and not α2δ-2 is relevant for pharmacologic activity. Interestingly, deletion of α2δ-1 or α2δ-2 in mice is associated with absence epilepsy or enhanced seizure susceptibility (76) (Ivanov et al., 2004), but it has been argued that this is not due to an alteration in the function of T-type calcium channels (75).

The precise way in which binding of gabapentin and pregabalin to the α2δ-1 protein confers 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) (77). Whether this action could account for the rapid antiseizure effects of gabapentinoids in animal models is uncertain.

AMPA RECEPTORS

Perampanel is the first selective AMPA receptor antagonist approved for epilepsy treatment. Whereas $GABA_A$ receptors mediate fast synaptic inhibition, AMPA receptors are cation channels that serve as the main mediators of fast (millisecond time scale) 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 region and possibly in other brain areas (78). 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.

Fast synaptic excitation is elicited by the exocytotic release of glutamate from excitatory principal neurons, which diffuses across the synaptic cleft and interacts with iGluRs of the AMPA and NMDA types to generate excitatory postsynaptic potentials (EPSPs). Summation of EPSPs leads to the firing of action potentials by the postsynaptic neuron. 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 iGluRs 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 (79). 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 (80). Pharmacologic blockade of AMPA receptors has broad-spectrum anticonvulsant activity in in vitro and animal seizure models.

Perampanel is a potent noncompetitive antagonist of AMPA receptors that does not affect NMDA receptor responses and has no known affects on other ion channels or molecular targets at therapeutically relevant concentrations (81). Therapeutic blood levels are expected to result in brain concentrations that would produce only low levels of inhibition of AMPA receptors. 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 dose levels, emphasizing the importance of AMPA receptors in brain function.

MTOR SIGNALING PATHWAY INHIBITOR

In epilepsies caused by a specific genetically defined abnormality, it should be feasible to prevent seizures and possibly also treat associated comorbidities with a therapy that functionally reverses the molecular defect. The first and only example of such a therapeutic approach that has received regulatory approval to date is everolimus, which is used for the treatment of focal seizures associated with tuberous sclerosis complex (TSC).

Malformations of cortical development are a common cause of epileptic encephalopathies and pharmacoresistant seizures. Many of these epileptic encephalopathies are believed to be due to dysfunction in the mTOR signaling cascade (82). mTOR is one member of a family of six atypical serine/threonine protein kinases, referred to as PIKKs (PIK3-related kinases) (83). mTOR forms the catalytic subunit of the cytosolic protein complex mTORC1, which acts as a central controller of cell growth. Drugs that inhibit mTORC1, such as rapamycin (sirolimus) and the rapalog everolimus, have various clinical roles including prevention of organ transplant rejection and slowing cancer growth and spread. Rapamycin and everolimus bind to the cyclophilin protein FKBP12, a peptidyl-prolyl isomerase (84). The rapamycin–FKBP12 complex then allosterically inhibits mTORC1 by binding to mTOR (when it is associated with the adaptor protein raptor and MLST8).

The prototypic epilepsy related to mTOR signaling is tuberous sclerosis caused by loss-of-function mutations in the *TSC1* gene encoding the protein hamartin or in the *TSC2* gene encoding tuberin. The mutations lead to constitutive mTOR activation, resulting in abnormal cerebral cortical development with multiple focal structural malformations (85). The substrate for the development of epilepsy is believed to be cortical tubers and perituberal cortical tissue with dysmorphic neurons, giant cells, reactive astrocytes, and disturbed cortical layering (82). The precise basis for epileptogenesis in the presence of these diverse cellular abnormalities is not understood. However, the recognition that mTOR signaling pathway hyperactivity is the basis for the seizure disorder in TSC led to the investigation of mTOR inhibitors everolimus and sirolimus in clinical trials with favorable results (86). Apart from TSC, mTOR dysregulation has been implicated in a large spectrum of genetic and acquired epilepsies, particularly those associated with malformations of cortical development (82,87). However, to date, there is evidence for effects on seizure frequency in only one other symptomatic epilepsy, the rare developmental disorder known as Pretzel syndrome that is associated with loss-of-function in the STRADA gene, an upstream inhibitor of mTORC1.

MIXED/UNKNOWN ACTIONS

Valproate

Although valproate is one of the most valuable ASMs, the mechanism by which it protects against seizures is poorly understood. Valproate has multiple pharmacologic actions

(88,89). Since it has been difficult to relate any one mechanism to the drug's broad spectrum of 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 pharmacologic 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 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 (90). Similarly, despite efficacy in absence epilepsy, there is little support for effects on T-type calcium channels. It is likely that valproate has pharmacologic actions relevant to its antiseizure activity that remain to be elucidated.

Felbamate

Felbamate, at concentrations within the therapeutic range, has been shown both to act as a positive modulators of $GABA_A$ receptors and also to inhibit NMDA receptors (91). 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. Although drugs that block NMDA receptors can exert antiseizure effects in certain animal models, there is doubt whether blockade of NMDA receptors is a useful strategy to treat epilepsy (92). Therefore, it is uncertain whether the NMDA receptor blocking activity of felbamate is relevant to its clinical antiseizure activity.

Topiramate

As is the case for valproate and felbamate, the broad-spectrum anticonvulsant activity of topiramate is likely to result from mixed effects on several targets (Shank et al., 2008). Among topiramate's diverse pharmacologic actions, effects on voltage-activated sodium channels, $GABA_A$ receptor subtypes, AMPA or kainate receptors, and types II and IV carbonic anhydrase isoenzymes are potentially relevant to seizure protection. Unlike other ASMs, the effects on ion channels are unlikely to occur through direct modulation of channel gating. Rather, the pharmacologic actions of topiramate seem to be mediated indirectly, possibly through effects on channel phosphorylation.

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 ASMs (93). In addition to effects on fast sodium currents, topiramate, like phenytoin, blocks I_{NaP} at low concentrations. Effects of topiramate on $GABA_A$ receptors could contribute to the broad spectrum of activity of topiramate. Topiramate is not active in animal models, such as the pentylenetetrazol test, that are typically sensitive to drugs that positively modulate $GABA_A$ receptors. Nevertheless, the drug does have activity in an absence epilepsy model 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 upon the β-subunit type (94).

Several authors 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 (95). Recently, kainate receptors have been found to be an unlikely target for an antiseizure agent (79). Whether actions of topiramate on glutamate-mediated neurotransmission contribute to its anticonvulsant activity remains to be determined.

The action of topiramate on carbonic anhydrase has been assumed not to contribute to its clinical efficacy because there is no cross-tolerance to the anticonvulsant 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 (96).

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-dependent sodium channels (97). Physiologic studies do not support an action on $GABA_A$ receptors. Unlike topiramate, there are reports that zonisamide can inhibit T-type voltage-gated calcium channels (98), which may account for its activity in absence epilepsy.

Rufinamide

The unique spectrum of clinical activity of rufinamide in the treatment of the Lennox–Gastaut syndrome suggests that it has a distinct mechanism of action (38). 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 (99). Clearly, the effects on sodium channels cannot explain the special clinical activity of rufinamide.

Adrenocorticotrophin

The mechanism of adrenocorticotrophin (ACTH) in the treatment of infantile spasms is not understood (100). ACTH stimulates glucocorticoid (cortisol) synthesis and releases 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 to themselves have therapeutic activity in the treatment of infantile spasms; whether ACTH is truly superior remains to be demonstrated 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 anticonvulsant neurosteroid tetrahydro-DOC, which is a positive allosteric modulator of $GABA_A$ receptors (101). It has been hypothesized that the tetrahydro-DOC could, at least in part, contribute to the ability of ACTH to terminate infantile spasms.

Cannabidiol

Cannabidiol (CBD), a nonpsychoactive phytocannabinoid found in *Cannabis sativa*, exhibits broad-spectrum antiseizure activity in animal seizure models, although the overall potency is weak (102). Unlike the structurally related cannabinoid Δ^9-tetrahydrocannabinol (THC), which acts as an agonist of CB1 (central nervous system) and CB2 (immune system) cannabinoid receptors, CBD is not a CB1 or CB2 receptor agonist. Accordingly, it has been shown that the antiseizure activity of CBD is not due to an action on brain CB1 receptors (103). The basis of the antiseizure activity of CBD is unknown. Among the targets that have been proposed are G-protein–coupled receptor GPR55, transient receptor potential cation channel TRPV1, $Na_v1.6$ voltage-activated sodium channels, and equilibrative nucleoside transporter ENT1. CBD is an antagonist of GPR55 (IC_{50}, 0.4 μM), which is an orphan G-protein–coupled receptor activated by endocannabinoids and some plant-derived and synthetic cannabinoid ligands (104). Interestingly, deletion of GPR55 in mice produces no conspicuous gross phenotypic, behavioral, or pathologic changes, and there have been no mention of changes in seizure susceptibility, which would be expected if inhibition of GPR55 is an antiseizure mechanism (105,106). Nevertheless, GPR55 is expressed in brain regions relevant to epilepsy, including the dentate gyrus and other regions of the hippocampus where it is present in both interneurons and excitatory neurons (107,108). CBD has demonstrated clinical efficacy in the treatment of seizures associated with Dravet syndrome, which is often caused by mutations in the $Na_v1.1$ voltage-activated sodium channel that is predominantly expressed in inhibitory interneurons. Reduced sodium current in interneurons and impaired inhibitory function is believed to be the fundamental pathogenic mechanism in Dravet syndrome. CBD (albeit at high doses) protects against thermally induced seizures (modeling febrile seizures) in a *Scn1a*$^{+/-}$ mouse model of Dravet syndrome (108). Moreover, evidence has been presented that CBD increases action potential generation in hippocampal GABAergic interneurons in *Scn1a*$^{+/-}$ mice, which in turn increases the frequency of inhibitory events in dentate granule cells. An antagonist of GPR55 occluded the beneficial effects of CBD on inhibitory function, raising the possibility (but by no means providing conclusive evidence) that CBD may exert an antiseizure action in Dravet syndrome through effects on GPR55.

CBD has also been reported to be an agonist of TRPV1, a nonselective cation channel, which is predominantly expressed in nociceptive neurons of the peripheral nervous system but may also be expressed in brain regions relevant to epilepsy including the dentate gyrus of the hippocampus (109). CBD was found to activate and rapidly desensitize TRPV1 and to reduce epileptiform activity in hippocampal brain slices. A link between the agonist effect on TRPV1 and antiseizure activity was not established. Indeed, TRPV1 activation with capsaicin enhanced excitatory transmission in the dentate gyrus of mice with experimental temporal lobe epilepsy, suggesting that TRPV1 activation could be proepileptic (110). Moreover, knockout of TRPV1 did not markedly impact chemoconvulsant seizures in neonatal mice (111).

$Na_v1.6$ sodium channels, which are an abundantly expressed sodium channel isoform in the somatodendritic compartment of brain relay (excitatory) neurons, generate particularly robust resurgent sodium current, which could play a role in some forms of seizure activity. Recently, evidence has been presented that inhibition of $Na_v1.6$ may be a potential therapeutic strategy for the treatment of Dravet syndrome (112). It is therefore of interest that CBD preferentially inhibits resurgent current generated by $Na_v1.6$ (6).

An effect on adenosine dynamics is among the most plausible mechanisms proposed to explain the antiseizure activity of CBD. In studies of cannabinoid actions on immune function, it was found that CBD potently inhibits (IC_{50}, 0.12 μM) ENT1, one isoform of the most abundant family of mammalian plasma membrane transporters of nucleosides including adenosine (113). ENT1, which acts as an equilibrative bidirectional transporter, is widely distributed throughout the body and is present in the brain. There is evidence that expression of ENT1 is increased in the temporal neocortex of patients with temporal lobe epilepsy (114). Block of ENT1 by CBD could theoretically enhance extracellular adenosine. Inasmuch as adenosine is well recognized to inhibit seizure mechanisms, this is a reasonable hypothesis to explain the antiseizure activity of CBD but no supporting evidence has as yet been presented. However, it is noteworthy that the specific ENT1 inhibitor nitrobenzylthioinosine does appear to inhibit excitatory neurotransmission and to have an antiseizure action (114,115).

BASIS OF COMBINATIONAL TREATMENT

All clinically used ASMs protect against seizures in animal models as single agents. Studies with early ASMs suggested that the seizure protection drug conferred by drug combinations is simply additive (116). Since the use of more than one agent compounds the risk of side effects, these and other observations led to the recommendation that ASMs should be tried sequentially in monotherapy before combining agents. More recent experimental data suggest that combining drugs with complementary mechanisms of action might lead to synergism for efficacy (117). Observational studies of results obtained in clinical practice have shown that combining newer ASMs with different mechanisms of action appears to have greater effectiveness (a combination of efficacy and tolerability) than combining drugs with similar mechanisms of action (118). Consequently, an understanding of mechanism may impact clinical decision-making in regard to the choice of drug combinations.

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